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EFFECTS OF CHROMIUM AND TAMOXIFEN ON FEMALE SEX HORMONES, BLOOD LIPID PROFILE AND UTERINE REACTIVITY IN HYPERLIPIDEMIC RATS

Salah A. Ghareib, Hekma A. Abd El-Latif * and Hassan M. El-Fayomi

Department of Pharmacology and Toxicology, Faculty of Pharmacy,
Zagazig and Cairo* Universities - Egypt

ABSTRACT:

In the present study the effects of chromium and tamoxifen given alone and in combination on female sex hormones, lipid profile and uterine contractility to oxytocin were studied in female rats in estrous stage. The drugs alone and their combinations were given orally by gavage to rats for 30 days. Results of this investigation showed that hyperlipidemic rats treated with the solvent exhibited some sort of changes in the plasma levels of female sex hormones. Hyperlipidemia per se decreased estradiol (E_2) and Luteinizing hormones (LH) , while increased the FSH and cortisol blood levels. Hypercholesterolemic diet significantly increased the plasma levels of Total cholesterol (TC), Triglycerides (TG), Low-density lipoprotein cholesterol (LDL-C) and Very-low density lipoprotein cholesterol (VLDL-C). However, the diet decreased the High-density lipoprotein cholesterol (HDL-C). It dramatically decreased the oxytocin-induced uterine contractions of the isolated rat uterus in the estrous stage compared to the normolipidemic rats. Chromium decreased the E_2 and cortisol, while increased the progesterone and LH levels. Tamoxifen and its combination with chromium increased the E_2, LH and Follicle stimulating hormone (FSH), and decreased the progesterone blood levels. Cortisol levels were reduced by the combination only but did not affected by tamoxifen alone. Chromium decreased, while its combination with tamoxifen increased the oxytocin-induced uterine contractions, compared to the hyperlipidemic control. Chromium , tamoxifen and their combination decreased the plasma levels of TC, TG, LDL-C and VLDL-C , while they increased the levels of HDL-C.

It could be concluded that, in spite of their capability to reduce the plasma levels of total cholesterol and other cholesterol containing particles, both tamoxifen and chromium exert different effects on hormonal blood levels. Chromium decreased while tamoxifen increased both estradiol and cortisol levels. Chromium increased progesterone while, tamoxifen decreased it. Tamoxifen, which decreased the levels of cholesterol, increased the hormonal blood levels and steroidogenesis, while, chromium decreased both. The effect of chromium on female sex hormones may be correlated to its cholesterol lowering capacity and its antihyperlipidemic effect, however, there is no correlation in the effects of tamoxifen. The reducing effect of chromium on the oxytocin-induced uterine contractions should be taken into consideration, especially in pregnant women, because it may decrease the uterine activity and contractions during labor.

INTRODUCTION:

Chromium, as a micronutrient, is known to be involved in regulation of blood glucose and has been proposed as a part of glucose tolerance factor regulating insulin sensitivity [1]. In diabetic patients, it decreases the elevated blood glucose levels, improves glucose tolerance and increases insulin sensitivity [2]. In addition, some studies have demonstrated changes in the blood lipid profile following chromium supplementation in man [3,4]. It significantly decreased the blood levels of VLDL-C and LDL-C [5-7] while increased that of the HDL-C [8].Chromium produces a dramatic regression of cholesterol – induced atherosclerotic plaques[9]. Chromium supplementation is associated with lowering serum TG and plasma non esterified fatty acids [10, 11]

Tamoxifen, a compound with antiatherogenic activity, is used as an adjuvant therapy in the treatment of postmenopausal estrogen-receptor-positive breast cancer [12]. It is not considered as a true antiestrogen but it produces a mixture of antiestrogenic and estrogenic effects [13]. Despite tamoxifen possesses antiestrogenic activity, however, many studies showed that tamoxifen treatment has some beneficial cardiovascular and cardioprotective effects in patients with breast cancer [14]. The mechanism of this effect may be due to the reduction of plasma total cholesterol and the other proportions of plasma cholesterol present in VLDL and LDL, and the increased levels of HDL [15]. Moreover, tamoxifen was reported to have a direct inhibitory effect on the de-novo synthesis of cholesterol [12], inhibiting its biosynthesis[16]. It inhibits the lanosterol dimethylation step in cholesterol synthesis pathway [17], on a step beyond HMG-COA reductase, resulting in, a 50-folds increase in the [8] cholesterol in plasma and downregulation of cholesterol synthesis[16]. These effects are neither mediated via the classic estradiol receptors nor related to the mechanism by which estradiol reduces the LDL- cholesterol levels[12].

Since cholesterol is a precursor in the biosynthesis of steroid hormones, in theory, the inhibition of cholesterol biosynthesis could have a potentially negative effect on gonadal functions and sex hormones biosynthesis. Statins, the HMG-COA reductase inhibitors, were reported to have an effect on testicular steroidogenesis and spermatogenesis [18] and may induce hypospermia [19]. Chromium was reported to markedly decrease the testosterone and cortisol serum levels [7,20] . Moreover, it suppress the release of corticotrophic hormone (CRH) from hypothalamus and adrenocorticotrophic hormone (ACTH) from the anterior pituitary. Abdel-Aziz et al., [7] reporetd that there are a correlation between the chromium-induced reduction in testosterone, cortisol, and cholesterol levels. It also decreases the levels of gonadotrophic releasing hormones (GnRH) from hypothalamus, which is responsible for the release of both FSH and LH. Recently, in spite of the wide use of chromium among women either before- or after the

menopause, the information and literatures concerning its effects on female sex hormones and steroidogenes are scarce. Also there is not enough available data for the effect of its combination with the antiestrogenic-anticancer, tamoxifen, which is commonly used among obese females with breast cancer. The present study was designed in a trial to find if there is a correlation between the effects of chromium and tamoxifen on cholesterol and lipoproteins blood levels and their effect on sex hormones and steroidogenesis in females. The effect of chromium and tamoxifen, each alone and in combination on estradiol, progesterone, FSH and LH as well as on adrenal glucocorticoids, lipid profile and uterine contractility in female rats in estrous stage was studied.

MATERIAL AND METHODS:

Animals:

Mature albino female rats weighing 150-200 gm were used in this study. Animals were housed on a 12-hours light – dark cycle with a constant ambient temperature (25°C). The rats have access to rodent chow and water *ad libitum*.

Materials:

Chromium (Hi-Chrom® –Amon, Pharm. Co., Egypt), Tamoxifen (Tamoxifen®, El-America Pharm., Co., Egypt). Propylene glycol, Cholesterol and cholic acid. NaCl, $MgSO_4$, $7H_2O$, KH_2PO_4, $NaHCO_3$, glucose and $CaCl_2$ (EL-Nassr, Chem. Co., Egypt). Thiouracil (BDH, England). Estradiol, progesterone, FSH, LH and cortisol kits (Diagnostic systems Laboratories Inc., and Santa Monica, By; Pantex and Gamma trad. Co. Inc. Egypt). Stanbio-Kits were used for determination of TC, TG and HDL-C were obtained from Inter-Trade Co., Egypt. Diethylestilbosterol and Oxytocin (Schering, Germany)

Experimental design:

I- In vivo studies:

Rats were made in estrous stage by subcutaneous injection of 0.1 mg/ kg diethylstilbosterol ,two days before use. Animals were divided into two main groups: ***Group I:*** Rats of this group (n = 8) were in the estrous stage, fed on normal diet and were used as a reference control. They received the solvent (water : propylene glycol 1:1) all-over the course of the experiment (45 +30 days).

Group II: Animals of this group were left on a hypercholesterolemic diet for 45 days for induction of hyperlipidemia in rats. Rats were then injected with diethylstilbosterol in a dose of 0./ kg in corn oil, and then were divided into four subgroups as follows:

Subgroup A: Animals (n = 10) received vehicle (water: propylene glycol 1:1) orally by a gavage for four weeks and were left as a hyperlipidemic control.

Subgroup B: Rats (n = 10) were given Chromium picolinate in a dose of 20 µg / kg/ day orally by a gavage for 30 successive days.

Subgroup C: Animals (n = 10) received Tamoxifen in a dose of 3 mg / kg / day orally for 30 days.

Subgroup D: Rats (n = 10) received both Chromium (20 µg / kg / day) and tamoxifen (3 mg / kg / day) orally for 30 days.

Blood samples were collected from the retro-orbital sinus of rats (in estrous stage), on ethylenediaminetetraacetic acid (EDTA), before- and 30 days after solvent or drugs administration. The collected blood samples were rapidly centrifuged at 4000 rpm for 15 min. Plasma was separated and one part was used for determination of lipids and the remaining part was kept at – 20°C for the hormonal assays.

II- In vitro experiments:

In this part of the study, rats were divided similarly to that of the in vivo part, but n = 5 -6 for each group or subgroup. At the end of the treatment period, on the 28th day, rats were injected with diethylstilbosterol in a dose of 0.1 mg / kg in corn oil. After one day, rats were killed by stunning and cervical dislocation between 9.00 AM and 12.00 noon [21,22]. Vaginal smears were taken to allow subsequent microscopic examination to check the cycle stage and get sure that rats were in the estrous stage. The abdomen was opened and both uterine horns were carefully removed and separated from connective tissue. They were placed in a Petri dish containing modified Kreb's Henseleit solution with the following composition, (in mM): NaCl- 118, KCl- 4.7, $MgSO_4.7H_2O$ – 1.1, KH_2PO_4- 1.18, NaHCO3- 25, glucose –11.66, CaCl- 1.9. One horn was mounted in a 25 ml organ bath containing warm (at 32°C), aerated modified Krebs` Henseleit solution and bubbled with carbogen (5% CO_2 and 95% O_2). The preparations were setup under a resting force of 0.5g. The preparation usually takes 15-30 mins. to settle down before giving regular responses. The uterine contractions were recorded on smoked drum using electrically motored kymograph with a very low speed (Bio-Science, England). Oxytocin was allowed to act for 30 seconds only in each time. Oxytocin was added to the bath solution after dilution (10 IU/1ml ampoule, 10 IU= 17.2 µg oxytocin, diluted in 10 ml of physiological solution) and added to the bath as 0.1, 0.2. 0.4 and 0.8 ml, which corresponds to 6.88, 13.72, 27.52. and 55.04 µg / L in each corresponding addition respectively.

The contractile responses of oxytocin were measured in mm. The mean responses for each dose was plotted versus the concentration of oxytocin and the area under the curves were calculated by the trapezoidal method and compared together.

Biochemical and hormonal assays:-

Plasma levels of total cholesterol [23] and triglycerides, [24] were measured colorimetrically using the Bio- analytical kits. High-density lipoprotein cholesterol levels were measured according to the method of warnick et al, [25] using the Stanbio

analytical kits. The low-density lipoprotein fraction was computed using a standard formula [26]: LDL = TC- [HDL + TG/5]. The very-low-density lipoprotein cholesterol (VLDL-C) fraction was similarly computed as the residual fraction of the total cholesterol. VLDL = TC – (HDL + LDL).

For hormonal assays the frozen plasma were used and the levels of estradiol, progesterone, FSH, LH and cortisol were determined by the radioimmunoassy quantitative assay technique using the Kits of Diagnostic system Laboratories Inc., Webster..

Statistical analysis:

Results were expressed as the mean ± SEM and were analyzed within each group using paired Student's "t" test. The one- way ANOVA test was used to compare the significance between the effects of drugs and that of the control groups at the same period. The two – ways ANOVA test was used to test the significance between the responses of oxytocin on isolated rat uteri of different treatment groups at the same concentration. The unpaired Student's "t" test was used to test the significance of the AUC of the dose response curves of oxytocin between the different treatment groups. Significance was assigned at $P < 0.05$.

RESULTS

I- Effect of the hypercholesterolemic diet on lipid profile, sex hormones and uterine contractions:-

A-Changes in female sex hormones of rats under the hypercholesterolemic diet:

Rats fed this diet for 45 days showed only an increase in estradiol serum levels by 22% of control value. (Table 1).

B- Changes in lipid profile of female rats:

Female rats subjected for this diet showed an increase in the serum levels of TC (+29%), TG (35%), LDL (75%), and VLDL (45%). The serum level of HDL was significantly decreased by 36%. (Table 2).The atherogenic indexes, (LDL-C / HDL-C and TC/ HDL-C),were also increased by 175% and 102% of the normal rats (Table 3).

C-Changes in the contractile responses of uterus to oxytocin of rats under the hypercholesterolemic diet:

Induction of hyperlipidemia in rats induced dramatic and significant reduction in the contractile responses of the isolated rat uterus (Fig1 and 2). The contractile effect of oxytocin on the uterine muscles isolated from hyperlipidemic rats was greatly reduced (-84.6% compared with that of the normal rats), the mean area under the response curve was 532 $mm.\mu g.L^{-1}$ Vs 3452 $mm.\mu g.L^{-1}$ of normo-lipidemic rats, (Table 4).

II- Effect of chromium, tamoxifen and their combination on lipid profile of female rats.

Both chromium and tamoxifen significantly ($P<0.05$) reduced the serum levels of TC (-20% and -16.9%), TG (-21% and – 16.7%), LDL (-32% and – 32.4%) and the VLDL-C (-34.7% and – 18%) of values before administration (Fig3,4,5 and 6). Concurrent administration of chromium and tamoxifen similarly reduced TC (-18.9%), TG (-23%), LDL (-36%) and VLDL-C (-24%). Chromium, tamoxifen and their combination significantly ($P<0.05$) increased the HDL-C levels by 33%, 47% and 40% of values before administration respectively (Fig 7).

Table (1): Effect of the hypercholesterolemic diet on lipid profile of mature female rats in the estrous stage.

Parameters	Before diet	After 45 days on diet		
	X ± S.E	X ± S.E	% of the initial value	% Change
TC (mg / dL)	93 ± 4.13	129 ± 7.93*	129	+ 29
TG (mg /dL)	80 ± 6.39	110 ± 8.11*	137.5	+ 37.5
LDL-C (mg /dL)	44 ± 3.19	77 ± 4.29*	175	+ 75
HDL-C (mg/dL)	33 ± 2.15	21 ± 1.18*	63.64	- 36.36
VLDL-C (mg/dL)	20 ± 1.33	29 ± 1.98*	145	+ 45

* Significantly different from the initial value before the diet at $P< 0.05$.

Table (2): Effect of the hypercholesterolemic diet on steroidal hormones of mature female rats in the estrous stage.

Parameters	Before diet	After 45 days on diet		
	X ± S.E.	X ± S.E.	% of the initial value	% Change
Estradiol (nmol/L)	0.219 ± 0.027	0.312 ± 0.023*	142	+ 42
Progesterone (nmol/L)	0.813 ± 0.043	0.88 ± 0.067	108.2	+ 8.2
FSH (mIU/ml)	7.8 ± 0.504	9.11 ± 0.89	116.8	+ 16.8
LH (mIU/ml)	8.6 ± 0.38	9.8 ± 0.78	113.9	+ 13.9
Cortisol (nmol/L)	430 ± 24.9	470 ± 39.96	109.3	+ 9.3

* Significantly different from the initial value before the diet at $P< 0.05$.

Table (3): The atherogenic indexes in normal, hyperlipidemic or hyperlipidemic- female rats treated with chromium, tamoxifen and their combinations.

Treatment		Before treatment		After treatment	
		LDL-C / HDL-C X ± S.E	TC / HDL-C X ± S.E	LDL-C / HDL-C X ± S.E	TC / HDL-C X ± S.E
Normal rats		1.33 ± 0.9	2.82 ± 0.8	1.46 ± 0.09	2.93 ± 0.23
Hyperlipidemic rats treated with	Solvent	3.66 ± 0.21*	7.71 ± 0.31*	4.61 ± 0.33*	6.90 ± 0.48*
	Chromium (20 ug/kg)	3.25 ± 0.31*	5.20 ± 0.43*	1.65 ± 0.08**	3.28 ± 0.25**
	Tamoxifen (3mg/kg)	4.05 ± 0.33*	6.21 ± 0.49*	1.86 ± 0.07**	3.50 ± 0.29**
	Chromium + Tamoxifen	3.24 ± 0.28*	5.08 ± 0.46*	1.42 ± 0.19**	2.94 ± 0.21**

* Significantly different from the values of the normal rats at P< 0.05.
** Significantly different from the values before treatment at P< 0.05.

Table (4): The total area under the concentration response curves of oxytocin on the isolated uterus of normal, hyperlipidemic or hyperlipidemic- female rats treated with chromium, tamoxifen and their combinations.

	Normal rats	Hyperlipidemic rats			
		Solvent	Chromium	Tamoxifen	Chromium + Tamoxifen
The AUC (mm . µg . L^{-1}) (X ± S E)	3452 ± 129	532 ± 23.3*	416 ± 120**	2500 ± 241**Ŧ	2279 ± 131**Ŧ
% Effect of normal rats	100	15.4	12	72.4	66
% Effect to hyper-lipidemic rats	------	100	78.19	469.9	428.4
% Change from hyperlipidemic rat		----	- 21.81	+ 369.9	+ 328.4

* Significantly different from the values of the normal rats at P< 0.05.
** Significantly different from the values hyperlipidemic control rats at P< 0.05.
Ŧ Significantly different from the values of chromium treated rats at P< 0.05

Moreover, chromium, tamoxifen and their combinations significantly (P<0.05) reduced the atherogenic indices .The LDL-C / HDL-C index was reduced by 49.2% , 54% and 56% while the TC/ HDL-C index was reduced by 36.9% , 43.6% and 42% respectively (Table 3).

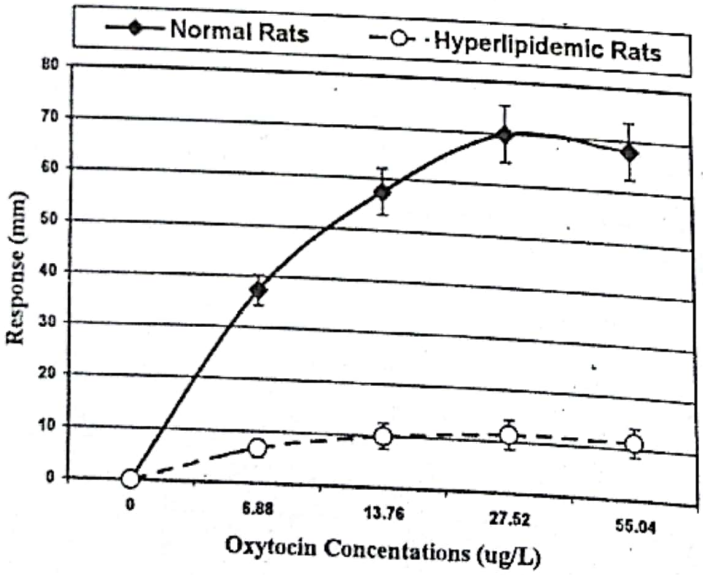

Fig (1): Dose response curves of the contractile effects of oxytocin on rat uteri dissected from normal and hyperlipiodemic rats.

III- Effect of chromium, tamoxifen and their combination on steroid hormones of female rats in estrous stage.

As shown in Figs (8 - 12), chromium significantly (P< 0.05), reduced the serum levels of estradiol (-21.7%) and cortisol (-23.4%), while increased the level of progesterone and LH by 52.5% and 38.8% of the values before administration respectively. Tamoxifen increased the levels of estradiol (+ 103%), FSH (+24%) and LH (+45%), while decreased the progesterone blood level by 23.6%. The concomitant administration of chromium and tamoxifen modulated the effect of each other. They significantly (P<0.05), increased the plasma levels of estradiol (51.7%), FSH (20.8%) and LH (37%), while decreased the levels of both progesterone (-19.2%), and cortisol (-29%). These results showed that tamoxifen reversed the effect of chromium on estradiol, progesterone and FSH, while chromium reversed the effect of tamoxifen on cortisol only.

IV- Effect of oxytocin on smooth muscle contractions of uterus isolated from rats-pretreated with chromium, tamoxifen and their combinations.

Oxytocin in concentrations of 6.88, 13.72, 27.52 and 55.04 µg / L (10^{-} M/L) induced a dose-dependent increase in contractions of uteri isolated from the normal rats in estrous stage (Fig 13 and 14). The recorded area under the drug response curve was 3452 ± 129 mm. µg. L^{-1}(Table 4). Chromium pretreatment did not significantly decrease the contractile effect of oxytocin on rat uterus by -21.7% of the hyperlipidemic rats (Fig 13a). However, tamoxifen significantly increased the effect of oxytocin-induced contractions on the isolated uterus and the recorded AUC was 2500 mm. µg. L^{-1} (+ 369.9%) compared to the hyperlipidemic control (Fig 3b). On the other hand, when given in combination with tamoxifen, chromium induced partial reduction in the contractile response of oxytocin on the isolated rat uterus and the AUC was 2279 mm. µg. L^{-1} (+ 328.4%), (Fig 13c). The recorded percentage changes in the AUC in case of the combination compared to tamoxifen alone was -8.84% and to chromium alone was + 447.5% (Fig 13d).

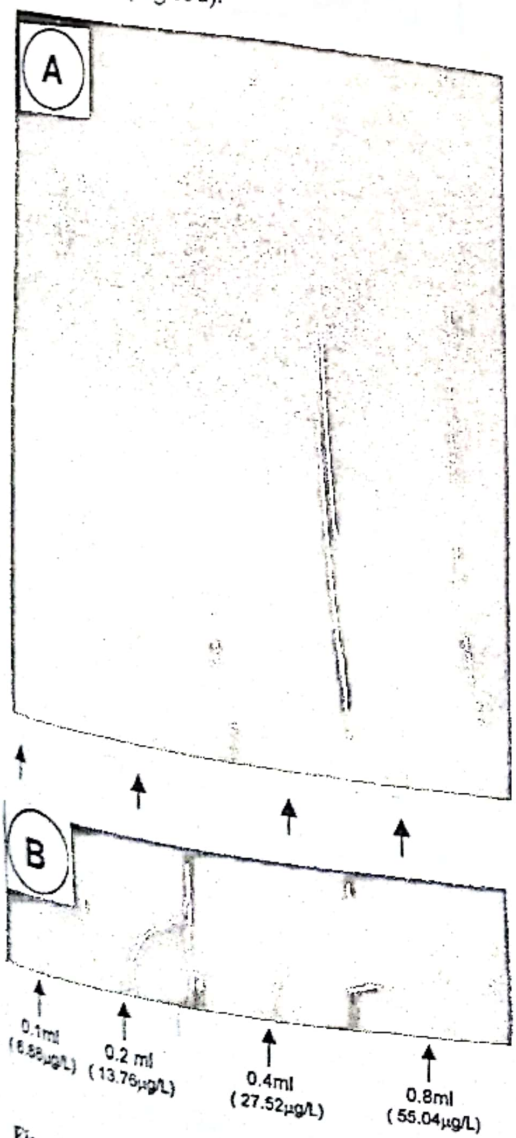

Fig (2): The tracing of the contractile effects (recorded on a smoked drum), of oxytocin on rat uteri dissected from Normal rats (A) and hyperlipidemic rats (B).

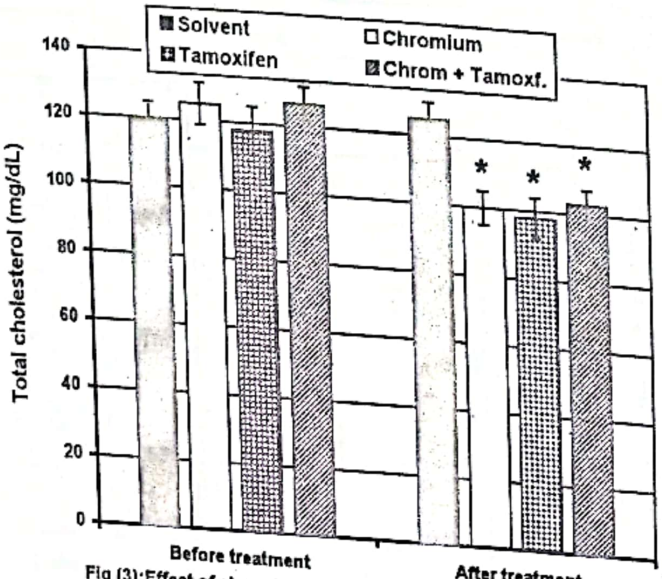

Fig (3):Effect of chromium (20 ug/kg/day), tamoxifen (3mg/kg/day) and their combination, given orally for 30 days, on total cholesterol blood levels of hyperlipidemic mature female rats.

* Significantly different from the values before treatment at P< 0.05.

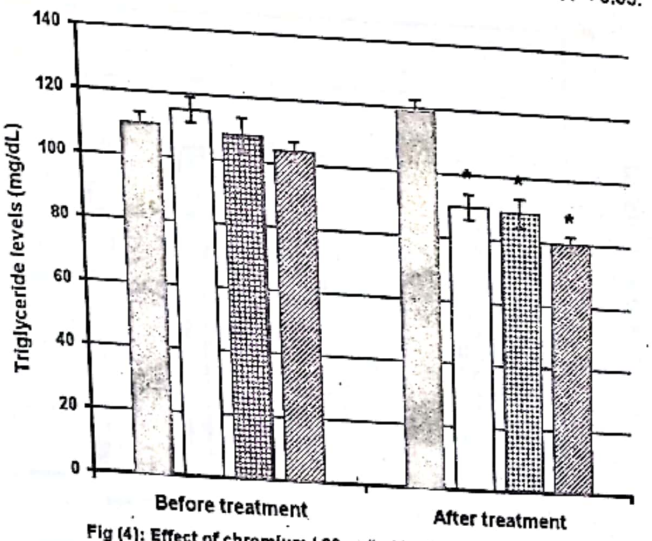

Fig (4): Effect of chromium (20 ug/kg/day), tamoxifen (3mg/kg/day) and their combination, given orally for 30 days, on triglyceride blood levels of hyperlipidemic mature female rats.

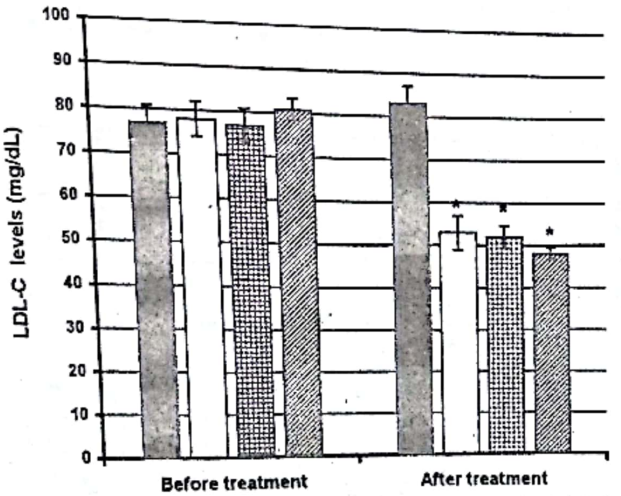

Fig (5): Effect of chromium (20 ug/kg/day), tamoxifen (3mg/kg/day) and their combination, given orally for 30 days, on low-density lipoprotein cholesterol (LDL-C) blood levels of hyperlipidemic mature female rats.

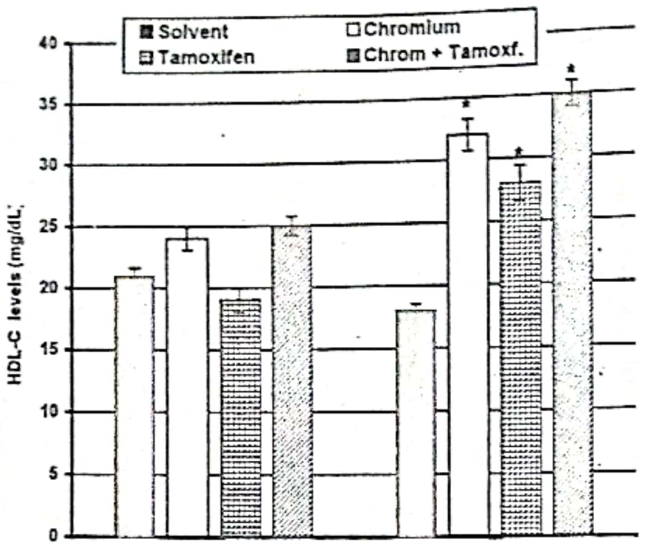

Fig (6): Effect of chromium (20 ug/kg/day), tamoxifen (3mg/kg/day) and their combination, given orally for 30 days, on high-density lipoprotein cholesterol (HDL-C) blood levels of hyperlipidemic mature female rats.

* Significantly different from the value before treatment at P< 0.05.

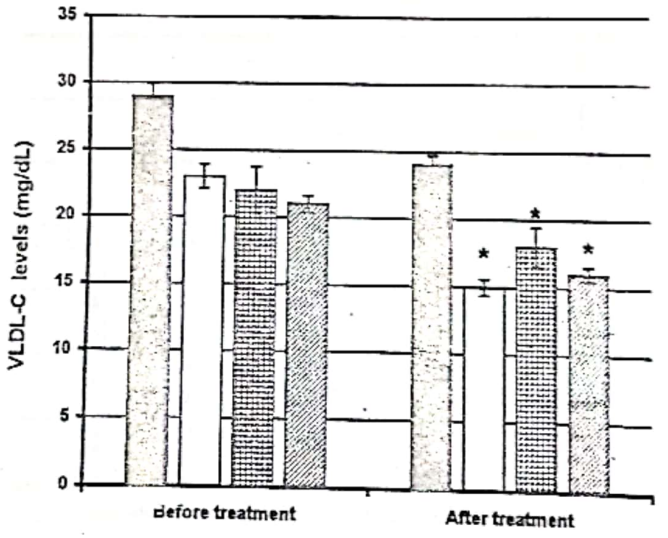

Fig (7): Effect of chromium (20 ug/kg/day), tamoxifen (3mg/kg/day) and their combination, given orally for 30 days, on very low-density lipoprotein cholesterol (VLDL-C) blood levels of hyperlipidemic mature female rats.

* Significantly different from the value before treatment at P< 0.05.

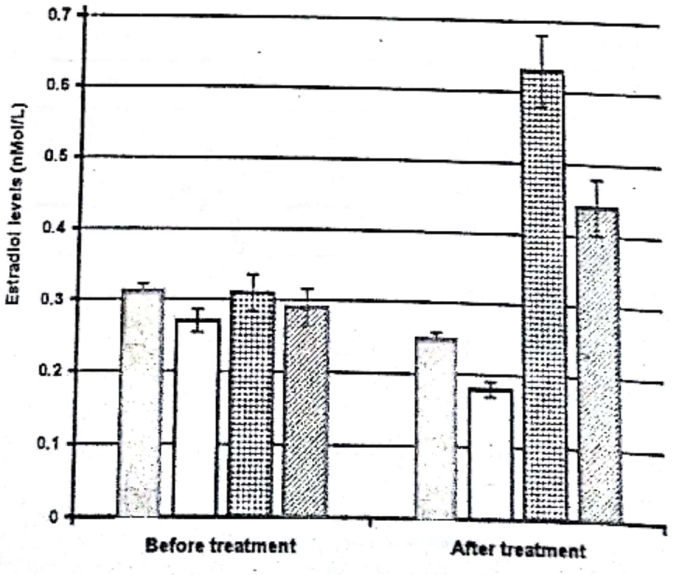

Fig (8): Effect of chromium (20 ug/kg/day), tamoxifen (3mg/kg/day) and their combination, given orally for 30 days, on estradiol blood levels of hyperlipidemic mature female rats.

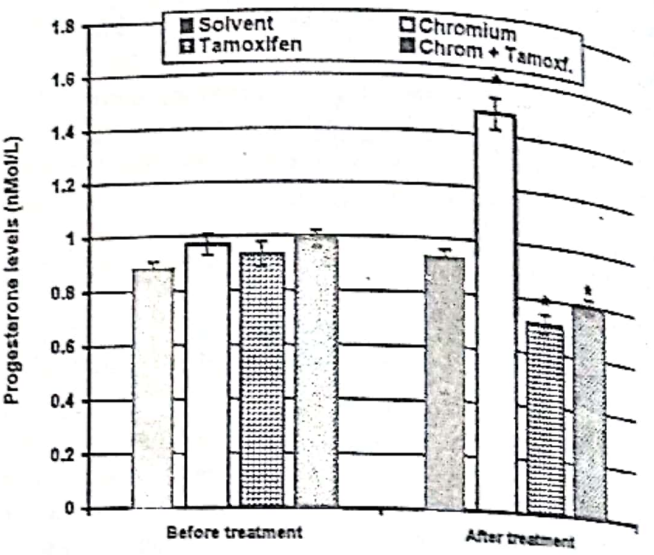

Fig (9): Effect of chromium (20 ug/kg/day), tamoxifen (3mg/kg/day) and their combination, given orally for 30 days, on progesterone blood levels of hyperlipidemic mature female rats.

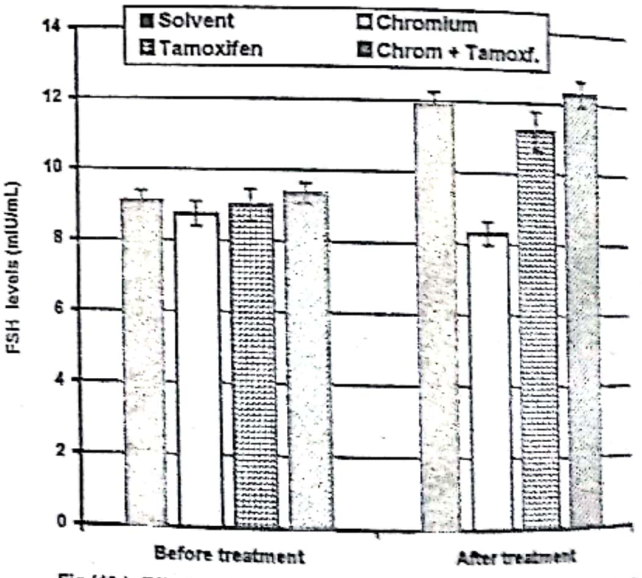

Fig (10): Effect of chromium (20 ug/kg/day), tamoxifen (3mg/kg/day) and their combination, given orally for 30 days, on FSH blood levels of hyperlipidemic mature female rats.

* Significantly different from the value before treatment at P< 0.05.

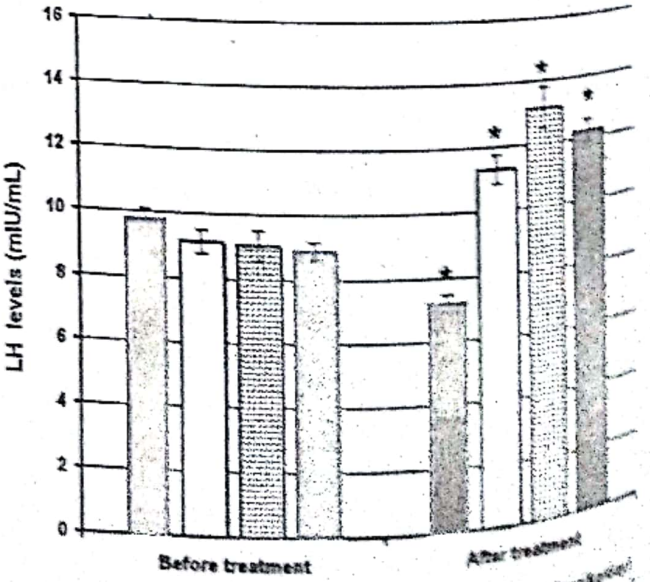

Fig (11): Effect of chromium (20 ug/kg/day), tamoxifen (3mg/kg/day) and their combination, given orally for 30 days, on LH blood levels of hyperlipidemic mature female rats.

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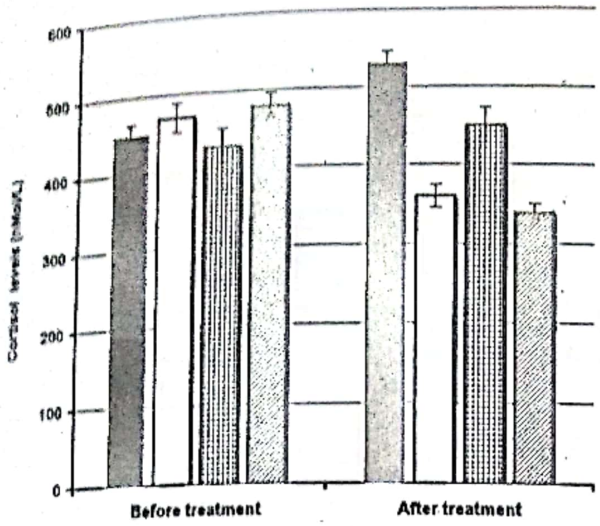

Fig (12): Effect of chromium (20 ug/kg/day), tamoxifen (3mg/kg/day) and their combination, given orally for 30 days, on cortisol blood levels of hyperlipidemic mature female rats.

*** Significantly different from the value before treatment at P< 0.05.**

DISCUSSION

Tamoxifen is a nonsteroidal antiestrogenic medication containing triphenylethylene. It is like estrogen (E2), it can bind to the cytoplasmic estrogen receptors and the formed complex is transferred to the nucleus, resulting in a reduced number of free estrogen receptors and competitive inhibition with the endogenous estrogen. Tamoxifen directly induces steroidogenesis in the ovaries of premenopausal women [27].

In the present study, tamoxifen given for one month significantly ($P < 0.05$) increased the estrogen plasma level by 103% of the value before administration . Moreover, levels of both FSH and LH were increased, whereas, progesterone levels were decreased. These findings are in accordance with that previously reported by many authors [27,28]. They reported that tamoxifen increased the E2 blood levels up to 2,500 pg/ml. Despite the elevated levels of E2, the unexpected effects of tamoxifen are the elevation of the levels of both FSH and LH [28]. These effects may be due to blocking of the negative feedback mechanism and the agonist - antagonist action of tamoxifen [29]. Our results showed that tamoxifen increased the contractile response of oxytocin in the isolated rat uterus dissected from tamoxifen-pretreated hyperlipidemic rats. This effect may be due to increased sensitivity and the number of oxytocin receptors in the myometrium that may result from the increased E2 production coupled with the estrogenic action of tamoxifen. Batra et al., [30] reported that the plasma progesterone / estrogen ratio was decreased in women in labor compared to that in women not in labor. In addition, the estrogen - dominanted uterus is more sensitive to oxytocin than the depreviated one . Moreover, under the influence of estradiol, the uterine muscles become more sensitive and their action potential become more active, excitable and more frequent [31]. Estrogen was reported to activate the uterine contractions and increases the muscle content of contractile proteins [32]. Estrogens up-regulate endometrial estrogen and progestin receptors , while progestins down-regulate these receptors [33]. Tamoxifen, by its estrogenic action, may up-regulate the estrogenic receptors in the endometrium and its treatment is associated with an increased incidence of proleferartive changes in endometrium [34]. Estrogen was reported to increase the production of nitric oxide (NO) [35] and decrease the formation and production of the superoxide oxygen radical species(O^-_2) [36]. Estrogen as a molecule acts as antioxidant and free radical scavenger [37]. It decreased the LDL-C oxidation and increased the HDL levels [38]. These effects of the increased estradiol levels coupled with the estrogenic actions of tamoxifen may increase the viability of the myometrium , the formation of the contractile elements of the muscle and mRNA expression of some receptors such as oxytocin receptors.

In the present study, chromium significantly decreased the plasma levels of estradiol and LH, while increased the progesterone levels. These results coupled with the ability of chromium to decrease the oxytocin- induced uterine contractions (-21.7% of hyperlipidemic control). These findings support the above assumption because the chromium-induced reduction of estradiol levels makes the uterus-estrogen deprivated for longer time. This effect may be enough to reduce the estrogen-mediated effect through the genomic cytosolic receptors. The capability of chromium to decrease the plasma levels of estrogen, may reduce the amount of the contractile elements in uterine smooth muscle. Also chromium significantly increased the plasma levels of progesterone, the latter was reported to block the uterine contractions [32].

Further investigations should be carried out to test the in-vitro effects of chromium on smooth muscle contractions to clarify whether, its inhibitory effect on uterus is mediated through estrogen-cytosolic receptors or not, and does it have an inhibitory action on the calcium - gated channels ?

On the other hand, some in vitro studies reported that estradiol relaxes the smooth muscle cells at high concentrations [39]. They found that estrogen suppressed not only the spontaneously generated burst discharges and muscle contractions but also that evoked by electric stimulation [40]. It suppressed the voltage-dependent calcium current and may inhibit the Ca 2+ channels in smooth muscle cells by a mechanism similar to- [41] or different from-[39, 42] , organic Ca2+ channel blockers. This means that the effect of estradiol on smooth muscles is a concentration-dependent. Estradiol at these high concentrations may stimulate some receptor sites other than the cytosolic estrogen receptors. Farhat et al., [43] and Ogata et al., [44] suggested that in addition to those genomic activities, some effects of estrogen in the vasculature involve fast, direct membrane

interactions with ion channels and some cell membrane receptors rather than the cytosolic receptors. This will rise another postulation that tamoxifen may block these inhibitory cell membrane receptors rather than the cytosolic ones. Leaving the latter receptors free for estrogen to induce its slow and prolonged effects on the contractile elements of the smooth muscle. This is supported by the finding that, tamoxifen (1 uM in vitro) reversibly inhibited the outward current (voltage-dependent K^+ current (K. Okabe and Y. Inoue; unpublished observations) [39]. On the other hand, tamoxifen, by its estrogenic property, may stimulate these cytosolic estrogen receptors upon prolonged treatment and enhances the uterine contractions induced by oxytocin.

The induction of hyperlipidemia in rats decreased estradiol and LH and increased cortisol. Moreover, hyperlipidemia itself decreased the oxytocin-induced uterine contractions. The ability of hyperlipidemia to induce this effect on uterus may be due in part to the reduction of estrogen and increased cortisol plasma levels. Moreover, in hyperlipidemic rats there is an increase in plasma levels of LDL-C, and decreased HDL-C which is accompanied by the increased OxLDL-C [45]. The increased production of OxLDL-C increased the production of O^-_2, [46] which oxidizes NO [47] and decreases its levels below that one which is essential for the actin-myosin interaction of the uterine muscles. Moreover, the increased O^-_2 increased lipid peroxidation in cell membranes [48]. The increased synthesis of cortisol may play an important role in regulation of the production of some pituitary and ovarian hormones. Glucocorticoids inhibit LH release in response to LHRH [49], whereas, enhances the FSH release in-vitro and increase the pituitary content of FSH by selectively increasing the FSHB messenger RNA (mRNA) [50].

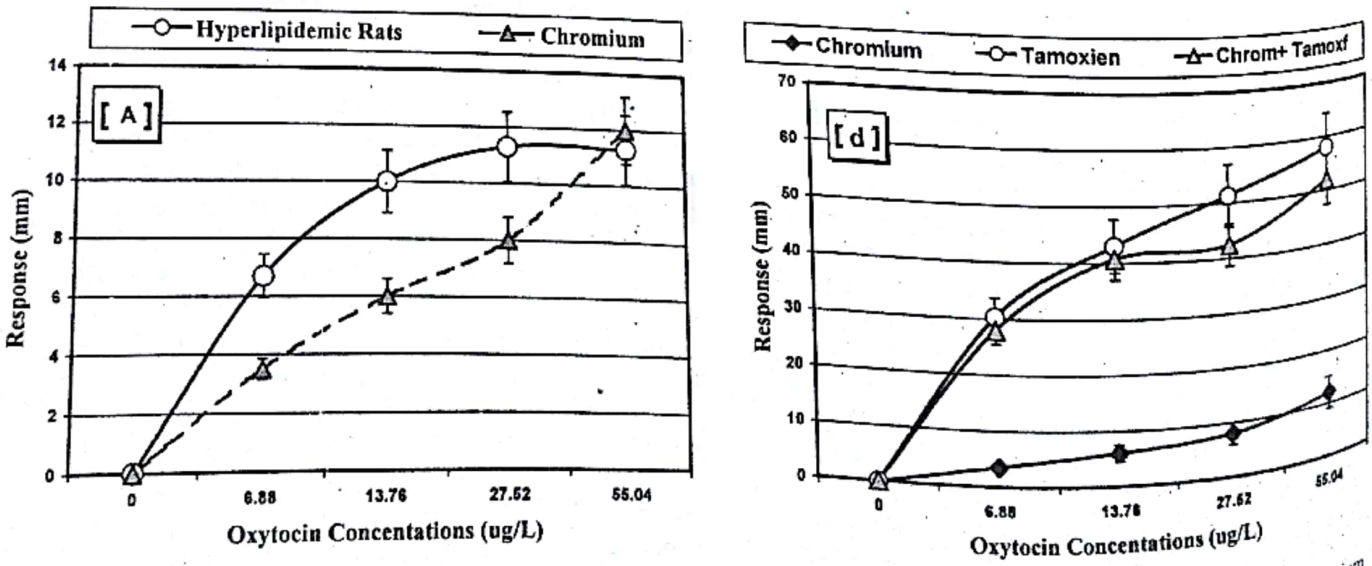

Fig (13): Dose-response curves of the contractile effects of oxytocin on rat uteri dissected from rats pretreated with chromium (A), Tamoxifen (B), and chromium plus tamoxifen (C and D). Rats were in the estrous stage. Each point is a mean of at least five successful experiments ± S.E.M.

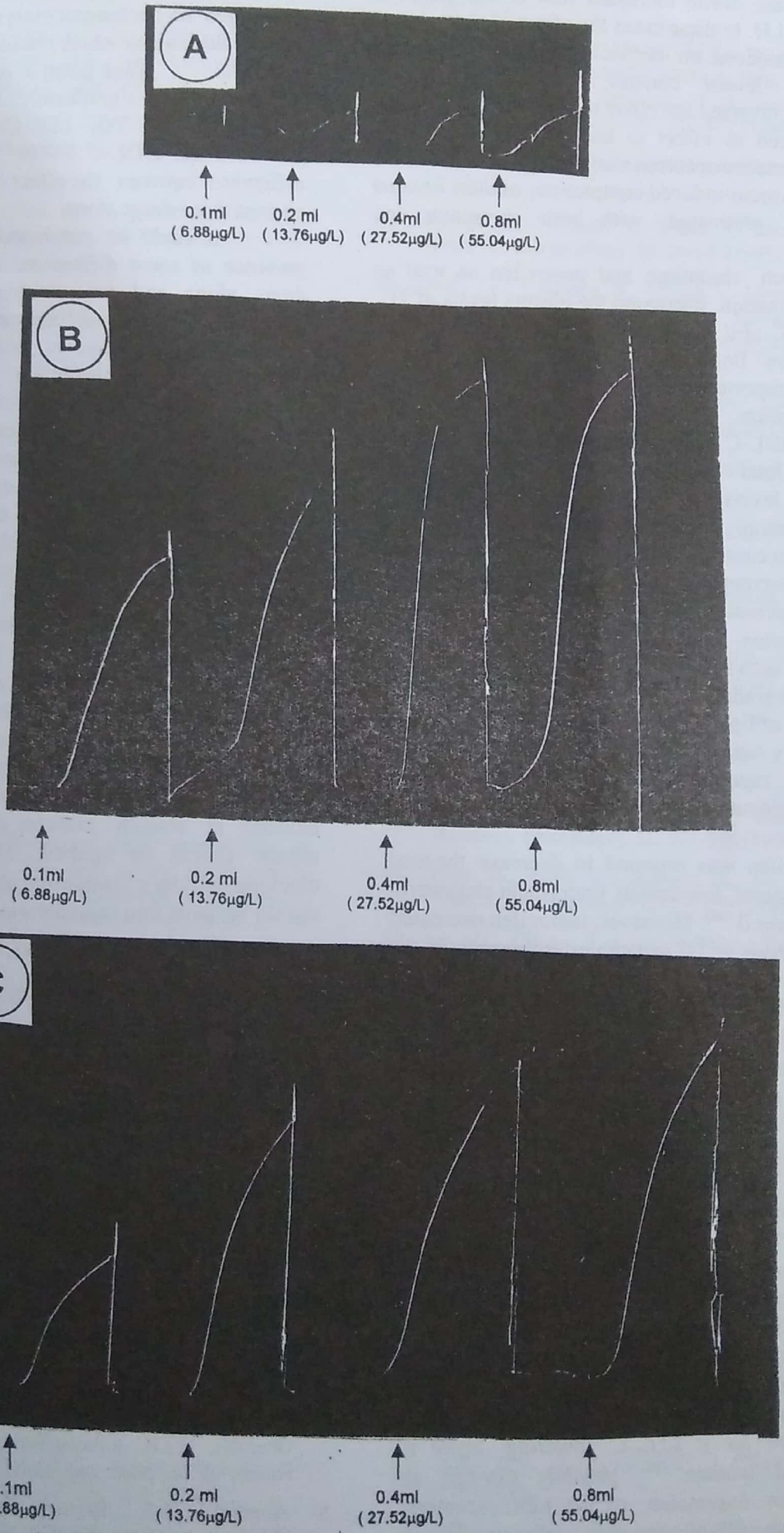

Fig (14): The tracing of the contractile effects (recorded on a smoked drum), of oxytocin on rat uteri dissected from rats pretreated with chromium (A), Tamoxifen (B), and chromium plus tamoxifen (C). Rats were in the estrous stage.

The combination of tamoxifen and chromium significantly reduced the levels of cortisol and progesterone, while increased that of the estradiol, FSH and LH. In these cases the effect of chromium is more pronounced on cortisol. Since chromium alone decreased leveof cortisol and estradiol. Here chromium reversed the effect of tamoxifen on cortisol and reduced its effect to increase estradiol levels. These effects are accompanied with the reduced AUC of the oxytocin-induced contractions of uteri isolated from rats pretreated with both chromium and tamoxifen.

Both chromium and tamoxifen as well as their combination decreased the plasma levels of TC, TG, LDL-C and VLDL-C, while increased that of HDL. These findings are in consistent with that previously reported [8]. They showed that chromium supplementation significantly increased the plasma levels of HDL-C with a decrease in the LDL-C, VLDL-C, total cholesterol [4] and TG [51]. The increased levels of HDL-C after chromium supplementation is related to the possible role of chromium in controling of atherosclerosis. Chromium induced an incremental decrease in TG concentrations and this decrease is likely the result of increased insulin secretion induced by chromium from β-cells [51]. Insulin activates the lipoprotein lipase, leading to enhanced degradation of TG [52] and concurrent suppression of lipolysis, resulting in decreased supply of free fatty acids required for TG biosynthesis. Mirsky, [53] reported a 50% decrease of plasma FFA in STZ diabetic rats treated with chromium.

Tamoxifen was reported to decrease the total serum cholesterol, low density lipoprotein cholesterol , apolipoprotein B [34]. Moreover, tamoxifen decreased the plasma levels of TC and cholesterol present in the most TG-rich particles of VLDL and LDL, while elevated the HDL levels in postmenopausal women and men with atherosclerosis [54]. The modulation of lipoprotein profile may be one of the possible mechanism of the cardioprotective effects of tamoxifen [14]. Tamoxifen is more effective than pravastatin and simvastatin as a lipid lowering agent in men with atherosclerosis [55]. A direct effect of tamoxifen on the biosynthesis of cholesterol has been suggested [16]. They found, in addition to the reductions in LDL-C in patients treated with tamoxifen, the serum levels of $?^{8}$ cholesterol were increased by 50 folds in these patients. Tamoxifen may inhibit the conversion of $?^{8}$ cholesterol to lathosterol, leading to down-regulation of cholesterol synthesis. Moreover, since tamoxifen has been shown to be effective as a LDL-C lowering agent in postmenopausal women [56], possibly through an increase in the expression of the LDL-receptors. Tamoxifen inhibits P-glycoprotein transport [57] and $?^{24}$ reductase activities [12] and causes an accumulation of sterol precursors. This effect of tamoxifen on cholesterol biosynthesis is not mediated via the classic estradiol receptor mechanisms and does not act by a receptor-mediated mechanism to reduce the cholesterol synthesis. Indicating that the effects of tamoxifen on cholesterol biosynthesis is not related to the mechanism by which estradiol promotes the LDL-C levels. When given in combination, chromium and tamoxifen significantly decreased the plasma levels of TC, TG, LDL-C and VLD-C, while increased the HDL-C levels. There is no significant difference between the effect of the combination and each of these drugs alone.

It could be concluded that, in spite of the presence of some differences in the effect of these drugs alone and in combination on female sex hormones, there is no difference in their effects on TC and lipoproteins. There is some correlation between the capability of chromium to reduce the total cholesterol and the cholesterol-containing lipoproteins and the process of steroidogenesis. As well chromium did not affect the tamoxifen-induced elevation in female sex hormones such as, estradiol, FSH and LH, that may be considered as a core in its anticancer effect. On the other hand, the reducing effect of chromium on the oxytocin-induced uterine contractions should be taken into consideration, especially in pregnant women, because it may prolong the gestation, decrease the uterine activity and contractions during labor. The effect of chromium on the oxytocin-induced uterine contractions of uterus isolated from pregnant rats should be the point of further research. Also, the effects of both chromium and tamoxifen on the mobilization of the cytosolic calcium in smooth muscles of blood vessels and uterus should be studied. The calcium channel blocking activity of tamoxifen on smooth muscle cells should be taken into considerations.

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تأثير الكروميوم و التوماكسفن علي الهرمونات الأنثوية - مستويات دهون الدم - و نشاط الرحم في الجرذان المصابة بارتفاع في مستويات الدهون

صلاح غريب – حكمة عبد التواب عبد اللطيف* - حسن محمود الفيومي

قسم الفارماكولوجي و السموم – كلية الصيدلة – جامعة الزقازيق و القاهرة* – مصر.

في هذا البحث تمت دراسة تأثير الكروميوم و التوماكسفن عند إعطائها منفصلة أو مجتمعة معا علي مستويات الهرمونات الأنثوية و دهون الدم و نشاط الرحم في إناث الجرذان البالغة المصابة بارتفاع في مستويات الدهون بالدم و قد تم تجريع الأدوية عن طريق الفم لمدة ثلاثون يوما متتالية.

و قد أشارت النتائج إلي أن الإصابة بارتفاع مستويات دهون الدم قد أدت إلي انخفاضا ملموسا ذات دلالة إحصائية في مستويات الاستراديول بينما زادت هرمون الـ FSH و الكورتيزول و كذلك أدت إلي ارتفاع مستويات الكولسترول – الجلسرينات الثلاثية و البروتينات المدهنة الخفيفة و البروتينات الدهنية الخفيفة جداً بينما خفضت من مستوي البروتينات المدهنة الثقيلة و كذلك أدت إلي النقصان الشديد في تأثير الاوكستوسين القابض لعضلات الرحم المفصول من هذه الجرذان.

كذلك اثبتت النتائج أن الكروميوم يقلل من مستوي الاستراديول و الكورتيزول بينما زاد من مستوي البروجستيرون و الـ LH ، أما التوماكسفن معطي منفصلاً أو مجتمعا مع الكروميوم قد زاد من مستويات الاستراديول ، LH، FSH و قد قللا من مستويات البروجستيرون – و أن الدواءين مجتمعان و ليس الفوماكسن منفرداً قد قللا فقط من مستويات الكورتيزول .

و أن الكروميوم وحده قد قلل بينما مع التوماكسفن قد زاد من تأثير الأوكستوين القابض لجدار الرحم بالمقارنة بالجرذان المصابة بارتفاع مستويات الدهون في الدم. أن الكروميوم و الفوماكس كل علي حدة أو مجتمعان أنقصوا جميعا من مستويات الدهون في الدم ماعدا البروتينات المدهنة الثقيلة فقد زاد مستواها.

و في الخلاصة أنه علي الرغم من قدرتهم علي خفض مستويات الكوليسترول في الدم و الدهون الأخرى المحتوية علي الكوليسترول فإن الكروميوم و التوماكسفن قد أحدثا تأثيراً متفاوتاً علي مستويات الهرمونات الأنثوية في دم الجرذان – فالكروميوم أنقص بينما التوماكسفن زاد من مستويات الاستراديول و الكورتيزول ، و الكروميوم زاد من مستويات البروجسترون و التوماكسفن أنقص ، و أن التوماكسفن الذي أنقص مستوي الكوليسترول قد رفع مستويات الهرمونات و كذلك عملية تصنيعها – بينما الكروميوم أحدث نقصاناً واضحاً في مستويات جميع الهرمونات و الدهون و الكولوستيرول) و أن تأثير الكروميوم علي مستويات الهرمونات الأنثوية مرتبطاً مع تأثيره الخافض لمستويات الكولستيرول و الدهون في الدم – و أن التوماكسفن ليس هناك علاقة بين تأثيره علي مستويات الهرمونات في الدم و قدرته علي خفض مستويات الكولستيرول و الدهون في الدم.