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Some Biochemical Alternations Associated with Oral Contraceptives And Atherosclerosis in Albino Rats.

E,A. Khalil* and M. H. Affifi**
*National Organization For Drug Control And Research,
BO Box 29 Cairo, Egypt
**Faculty of Veterinary Medicine Cairo University.

Abstract

An excess intake of vitamin D_2 can result in mobilization of calcium in the skeleton and increase the serum calcium level. This calcium is taken up by soft tissues such as arteries. The risk of calcium builds up in arteries, a significant component of atherosclerotic plaque. Many researches clarify the relationship between oral contraceptives and atherosclerosis. This study aims to evaluate the changes in some biochemical parameters as well as the histopathological examination of liver and aorta following the administration of hormonal oral contraceptives (O .C_S) with different concentrations of estrogen (ethinyle-estradiol) (E.E) and progestogen DL-norgestrel (norethindrone) (NOR.) to the atherosclerotic rats. In addition to this, the study clarifies the role of low dose oral contraceptives. 48 adult female albino rats were divided into six comparable groups of 8 animals each. Group I (Gr.1) was considered as control, group II (Gr. II) was intramuscularly (i.m.) injected with vit. D_2 350.000 IU /kg B.W., group III (Gr. III) administered O.C (35µ g E.E/0.5 mg NOR.), group IV (Gr. IV) received (vit. D_2 350.000 I.U vit. D_2 /kg B.W plus O.C 35µ g E.E/0.5 mg.), group V(Gr.V) received (vit. D_2 350.000 I.U/kg B.W. plus O.C 35µ g E.E/1mg NOR.), group VI (Gr.VI) received (vit. $D_2$350.000 I.U /kg B.W.plus O.C 70µ g E.E/0.5 mg NOR.) daily for an experimental period eight weeks.Serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), and serum alkaline phosphatase (ALP) displayed significant increase in the following groups (higher progestogen concentrations in O.Cs plus vit. D_2 treated group, at low-dose O.C plus vit. D_2 treated group, at higher estrogen dose within O.C plus vit. D_2 treated group, vit. D_2 treated,and at low-dose O.C treated group). Serum triglycerides recorded significant increase in group treated with higher estrogen dose within O.C plus vit. D_2 treated group, low-dose O.C plus vit. D_2 treated group , higher progestogen concentrations in O.Cs plus vit. D_2 treated group, vit. D_2 treated group,and at low-dose O.C treated group respectively. Serum total cholesterol increased significantly in higher progestogen concentrations in O.Cs plus vit. D_2 treated group, at low-dose O.C plus vit. D_2 treated group, vit. D_2 treated group, low-dose O.C treated group and at higher estrogen dose within O.C plus vit. D_2 treated group at 8 weeks in comparison with control. Histopathological studies of livers showed severe at the higher progestogen concentration in O.C plus vit. D_2 treated group and at low dose O.C plus vit. D_2 treated group .Liver displayed moderate degenerative changes in higher estrogen dose within O.C plus vit. D_2 treated group, in vit. D_2 treated group and in low dose O.C treated group. Media calcinosis in aorta was more obvious at the higher progestogen concentrations in O.Cs plus vit. D_2 treated group. Also it develops at low dose O.C treated group.

In conclusion atherosclerosis may develop at low dose O.C due to progestogen content.

Keywords: Hypervitaminosis vit. D_2, O.Cs, Atherosclerosis, Liver, Aorta

Introduction:

The control of a population–explosion is an important part in our national development plan. Hormones are still the most popular contraceptives for such explosion, however their use proved to be responsible for various adverse reactions.

Krauss et al, (1977) found changes in serum high-density lipoproteins, which played a protective role, in atherosclerosis in women on oral contraceptive drugs.

Al-shebib et al, (1982) concluded that hypercholesterolaemia and atheroma could be induced in rabbits by progesterone injection after longer periods of use. Krauss et al (1983) pointed out that changes in serum lipids were associated with the use of two low-dose estrogen progesten and could occur with use of such agents for a 2-month period.

Many researches clarify the relationship between oral contraceptive and cardiovascular disease such as WHO Collaborative Study1995, WHO Scientific Group1997 ,Suissa et al, (1997), Farley et al (1998), and American Society for Reproductive Medicine 1999.

This investigation aims to evaluate the changes in some biochemical parameters as well as the histopathological examination of liver and aorta following the administration of hormonal oral contraceptives with different proportions of estrogen ethinyle-estradiol and progestogen norethindrone to the atherosclerotic rats. In addition to this, the study clarifies the role of low dose oral contraceptives.

Materials and Methods

Experimental drugs

1 ml ampoules of vitamin D2 (Viosterol 600.000 IU in oil) were obtained from the Memphis Chemical Co.(Cairo) Egypt.

Ethinyle-estradiol and norethindrone were obtained from Chemical Industries Development (CID) Laboratories (Giza) Egypt.

Experimental design

Animals

48 adult female albino rats weighing 120-150gm,were kept under good hygienic conditions and a well balanced diet .The animals were divided into six comparable groups of 8 animals. Group I (**Gr.1**) was considered as control, group II (**Gr. II**)was I/M injected with 350.000vit. D_2 /kg B.W., group III (**Gr. III**) administered 35µ g E.E/0.5 mg NOR, group IV (**Gr. IV**) received (vit. D_2 350.000 I.U vit. D_2 /kg B.W plus O.C 35µ g E.E/0.5 mg.), group (**Gr.V**) received (vit. D_2 350.000 I.U/kgB.W. plus O.C 35µ g E.E/1mg NOR.), group VI (**Gr.VI)** received (vit. $D_2$350.000 I.U /kg B.W.plus O.C 70µ g E.E/0.5 mg NOR.) daily for an experimental period eight weeks.

Body weights

Body weights of rats were recorded before treatment, weekly for 2,4,6and8weeks

Blood sampling

Blood samples were collected from retro-orbital plexus (Schermer, 1967). Serum total cholesterol was determined according to(Waston,1960), serum triglyceride was estimated according to (Wahlefeld,1974),(AST), (ALT) were determined according to (Reitman and Frankle, 1957), ALP was determined according to (Kind and King ,1954).The data were statistically analyzed used student's t-test according to (Sendecor and Coebram, 1969).In addition histopathological studies were carried out by collecting the specimens from liver and aorta. These specimens were fixed in 10% formalin 5 micron

thick paraffin sections were prepared ,stained with hematoxylin & eosin and examined microscopically

Results and Discussion

Serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), and serum alkaline phosphatase (ALP) displayed significant increase in the following groups (higher progest-ogen concentrations in O.Cs plus vit. D_2 treated group, at low-dose O.C plus vit. D_2 treated group, at higher estrogen dose within O.C plus vit. D_2 treated group, vit. D_2 treated,and at low-dose O.C treated group)

at 8 weeks in comparison with control tables(4,5&6). Histopathological studies of livers showed clear changes at the higher progestogen concentration in O.Cs plus vit. D_2 treated group and at low-vit. D_2 treated group .Liver displ-ayed moderate degenerative changes in dose O.C plus higher estrogen dose within O.C plus vit. D_2 treated group, and in vit. D_2 treated group. Also in low dose O.C only. Histopathological exam-ination appeared as dissociation of hepatic cords, dilation and congestion of central vein and portal tract, and fatty inferteration. Moreover necrosis of some hepatic cells figures (10,7,13, 1&4).

Vit.D has a highly toxic effect on liver and elevated (AST), (ALT) and (ALP) Canada de Zunzunegui et al, 1984. O.Cs elevates serum transferase activities and impaired secretory liver function (Nareman, et al 1991).

The accumulation of vit.D and hormonal contraceptives in liver caused marked increase in (AST), (ALT) and (ALP).

The elevation in serum cholesterol and triglycerides in vit. D treated group treated was in harmony with Vijayakumar and Kurup (1974). While the same group gained less weight (Bajwa et al 1971and Takahashi ,1993). An excess intake of vitamin D can result in mobilization of calcium in the skeleton and increases the serum calcium level.This calcium is taken up by soft tissues such as arteries. Vit. D_2 over dosing is mediated through its intermediate ,25-hydroxy –D, rather than its active metabolite 1,25 –dihydr-oxy –D .It means that the toxicity of vit. D_2 is believed to be the result of high circulating 25-OHD levels .Thus calc-ium transport and bone resorption goes on at high and unchecked rate ,giving hypercalacemia (Fraser,1995 & Basu and Tapan1996) .

Concerning our data the increase in the serum total cholesterol and gain-ing body weight by oral contrace-ptives treatment, may be refereed to the effect of progesterone content which serves as precursor to all other steroids (Kutsky, 1973). The World Health Organization (WHO) reported a relati-onship between different progestogens in low estrogens oral contraceptives on venous throm-boembolic disease from 1989-1993in 21 centers globally. Petitti ,et.al 1998 recorded that progestogen type might affect myocardial infract-ions. Our res-ults indicated that, the estrogen part elevated serum triglyc-erides in the absence of increased cholesterol levels and gained less weight than the other groups. This result in agreement with (McGill et. al,1977). Harris and Hughes 1998 reported that O.C use increased circulating levels of 25-hydroxyvitamin D (25OHD) in young women. This finding confirmed our results that medial calcinosis develops at low dose O.Cs Groups treated with vit. D plus O.Cs lead to increase the circulating 25-OHDlevels which lead to increase the formation of medial calcinosis.

Media calcinosis in aorta was more obvious at the higher progestogen concentration in O. Cs plus vit. D_2

treated group and at low-dose O.C plus vit. D_2 treated group. Media calcinosis was moderate at higher estrogen dose within O.C plus vit. D_2 treated group, and at vit. D_2 treated group. Also it develops at low dose O.C treated group.

In conclusion atherosclerosis may develop at low dose O.C due to progestogen content.

Acknowledgment:

The authors thankful to Dr/ R.M. Al-Allawy Prof. Dr. in Biochemistry in NODCAR for his sharing in suggestion the point.

Table(1):Average values ± of body weights (gm) in normal rat groups intramuscularly injected with vit. D 350.000IU/kg BW and administered different concentrations of estrogen, ethinyle estradiol, (E-E) and progesterone, norethindrone, (NOR) and their corresponding controls for 8weeks

Time intervals	Groups					
	Gr. I	Gr. II	Gr. III	**Gr. IV**	**Gr.V**	VI
	Control	Vit.D_2 350.000IU/kg BW	O.C (35ugE.E / 50mgNOR)	Vit.D_2+OC(35 µgE.E/ 50mgNOR)	Vit.D_2+OC (35µgE E. / 1mgNOR)	Vit.D_2+OC(70µ gE.E/ 50mgNOR
Zero-time	129.37 ±1.23	131.25 ±1.61	129 ± 1.1	128.62 ±0.7	127 ± 1	127.5 ±1
Two-weeks	146.25 ±1	140.25 ±2	148 ±1	145.6 ±1	160 ± 3	135 ± 5
Four-weeks	167.5 ±1.43	150.87 ± 1.7	166.26 ±1.4	158 ±0.6	186 ± 2	160 ± 1.5
Six-weeks	183.5 ±2.13	170 ±1.8	185 ±1.2	176.25 ± 1	200 ±3	177.5 ±5
Eight-weeks	215 ± 2	180.37↓ ±3	225* ± 2	216.66 ±3	240** ±5.2	190↓ ±5

Number of rats in each group n=8.

Insignificant difference from the corresponding control at $p>0.05$

*Significant difference from the corresponding control at $p<0.05$

**Highly significant difference from the corresponding control at $p<0.01$

***Very highly significant difference from the corresponding control at $p<0.001$

Table(2):Average values ±of serum total cholesterol(mg%) in rat groups intramuscularly injected with vit. D_2 350.000IU/kg BW and administered different concentrations of estrogen, ethinyle estradiol, (E-E) and progesterone, norethindrone, (NOR) and their corresponding controls for 8weeks

Time intervals	Groups					
	Gr. I	Gr. II	Gr. III	**Gr. IV**	**Gr.V**	VI
	Control	Vit.D_2 350.000 IU/ kg BW	O.C (35 µg E.E / 50mgNOR(	Vit.D_2+OC(35µ g E.E/ 50mgNOR)	Vit.D_2+OC (35µgE E. / 1mgNOR)	Vit.D_2+OC(70µ g E.E/ 50mgNOR
Zero-time	60 ±2.2	59.4 ±1.87	60 ±2	58.5 2±	57.7 ±1.5	58.7 ± 1.5
Two-weeks	60.3 ±1.5	65 ±6	62.6 ±2	63 ±2	63.7 ± 3.4	62 ±2
Four-weeks	59.2 ±2	70 ±5	65.4 ± 3	75 ±5	83.8 ±4	64.3 ±1.5
Six-weeks	59 ±1	74 ±6	68.6 ±2.61	78 ±4	90 ±4	65.33 ±2
Eight-weeks	60 ± 2	80** ± 4	73** ± 2.5	88** ± 4	100*** ± 3	70 ±3

Number of rats in each group n=8 .
Insignificant difference from the corresponding control at p>0.05
*Significant difference from the corresponding control at p<0.05
**Highly significant difference from the corresponding control at p<0.01
***Very highly significant difference from the corresponding control at p<0.001

Table(3):Average values ± of serum triglycerides(mg%) in normal rat groups intramuscularly injected with vit. D_2 350.000IU/kg BW and administered different concentrations of estrogen, ethinyle estradiol, (E-E) and progestogen , Norethindrone, (NOR) and their corresponding controls for 8weeks

Time intervals	Groups					
	Gr. I	Gr. II	Gr. III	**Gr. IV**	**Gr.V**	VI
	Control	Vit.D_2 35 0.000IU/ kg BW	O.C (35µg E.E / 50mgNOR(	Vit.D_2+OC(35 µg E.E/ 50mgNOR)	Vit.D_2+OC 35)µgE E. / 1mgNOR)	Vit.D_2+OC(70 µg E.E/ 50mgNOR
Zero-time	33.67 ±5	34 ±1	35 ±1	35 ±1.5	34 ±2	34 ±1.5
Two-weeks	33.8 ± 1	39.5 ±1.8	37 ±1	55 ±2	63 ±3.4	75 ±3
Four-weeks	34 ± 0.8	42.5 ±1.5	37 ±1	65 ±3	81 ±4	92.2 ±4
Six-weeks	34.7 ±1	48.4 ±5	39 ±1.5	80 ±5	120 ±5	150 ±6
Eight-weeks	35 ±1	50** ±5	43** ± 2	100** ±4	150*** ±6	206*** ±10

Number of rats in each group n=8 .
Insignificant difference from the corresponding control at p>0.05
*Significant difference from the corresponding control at p<0.05
**Highly significant difference from the corresponding control at p<0.01
***Very highly significant difference from the corresponding control at p<0.001

Table(4):Average values ± of serum aspartate aminotransferace (SAST) U/L in normal rat groups intramuscularly injected with vit. D_2 3500.000IU/kg BW and administered different concentrations of estrogen, ethinyle estradiol, (E-E) and progestogen , Norethindrone, (NOR) and their corresponding controls for 8weeks

Time intervals	Groups					
	Gr. I	Gr. II	Gr. III	**Gr. IV**	**Gr.V**	VI
	Control	Vit.D_2 350.000 IU/ kg BW	O.C (35 µg E.E / 50mgNOR(	Vit.D_2+OC(35µ g E.E/ 50mgNOR)	Vit.D_2+OC (35µgE E. / 1mgNOR)	Vit.D_2+OC(70µg E.E/ 50mgNOR
Zero-time	23.5 ±1.5	25 ±2	25 ±1.4	22.17 ±1.7	25 ±2	27 ±3
Two-weeks	22.2 ±2	33 ±4	27 ±3	43.3 ±2.7	50 ±4.6	51.8 ±3.4
Four-weeks	23.2 ± 1.5	35 ±5	30 ±3	55 ±5	65 ±4	82.2 ±4
Six-weeks	22 ±2	38 4±	32 ±4	78 ±4	81.4 ±5	92.6 ±5
Eight-weeks	23 ±2	45** ±4	35* ±5	94*** ±4	102*** ± 6	107*** ±7

Number of rats in each group n=8 .
Insignificant difference from the corresponding control at p>0.05
*Significant difference from the corresponding control at p<0.05
**Highly significant difference from the corresponding control at p<0.01
***Very highly significant difference from the corresponding control at p<0.001

Table (5) : Average values ±of serum alanine aminotransferase (U/l) (SALT) in rat groups intramuscularly injected with vit. D_2 350.000IU/kg BW and administered different concentrations of estrogen, ethinyle estradiol, (E-E) and progesterone, norethindrone, (NOR) and their corresponding controls for 8weeks

Time intervals	Groups					
	Gr. I	Gr. II	Gr. III	**Gr. IV**	**Gr.V**	VI
	Control	Vit.D_2 350.000 IU/ kg BW	O.C (35µg E.E / 50mgNOR)	Vit.D_2+OC(35 µg E.E/ 50mgNOR)	Vit.D_2+OC (35µg E E. / 1mgNOR)	Vit.D_2+OC(70µg E.E/ 50mgNOR
Zero-time	15.1 ±2.1	15.5 ±2.5	14.5 ±2.3	15.5 ±2.5	14.5 ±2.3	15 ± 2.4
Two-weeks	15.5 ±1.5	17.5 ±2.7	15.4 ±2.5	19 ± 2.3	20 ±2	22 ±2.3
Four-weeks	16.2 ±2.4	20 ±3	17 ±2	25 ±2.5	30 ±3	33 ±3
Six-weeks	16.5 ±2	25 2.5±	20 ±2.5	30 ±3	41 ±4	40 ±3.1
Eight-weeks	17.2 ±3	35** ±3	25* ±2	40*** ±3.5	45*** ± 3.7	43*** ±2.5

Number of rats in each group n=8 .
Insignificant difference from the corresponding control at p>0.05
*Significant difference from the corresponding control at p<0.05
**Highly significant difference from the corresponding control at p<0.01
***Very highly significant difference from the corresponding control at p<0.001

Table(6):Average values ±of serum alkaline phosphatase U/mL (SALP) groups intramuscularly injected with vit. D 350.000IU/kg BW and administered different concentrations of estrogen, ethinyle estradiol, (E-E) and progesterone, norethindrone, (NOR) and their corresponding controls for 8weeks

Time intervals	Groups					
	Gr. I	Gr. II	Gr. III	**Gr. IV**	**Gr.V**	VI
	Control	Vit.D_2 350.000IU /kg BW	O.C (35ugE.E / 50mgNOR)	Vit.D_2+OC(35µg E.E/ 50mgNOR)	Vit.D_2+OC 35)µgE E. / 1mgNOR)	Vit.D_2+OC(70µgE.E/ 50mgNOR
Zero-time	37.16 ±2.2	37.66 ±1.5	34 ±1.5	35 ±2	33 ±2	35 ±2
Two-weeks	38 ±2	44.16 ±4	40 ±3	54 ±4	60 ±4	49.3 ±3
Four-weeks	34 ±3.2	52 ±4	45.8 ±3	60 ± 5	79 ±5	60 ± 6
Six-weeks	37 ±2.3	59 ±7	50 ±6	66 ±4	84 ±6	65.4 ±4
Eight-weeks	37.3 ±1.5	70.2** ±5	67.5** ±5	78*** ±6	89*** ±5	75*** ± 5

Number of rats in each group n=8 .
Insignificant difference from the corresponding control at $p>0.05$
*Significant difference from the corresponding control at $p<0.05$
**Highly significant difference from the corresponding control at $p<0.01$
***Very highly significant difference from the corresponding control at $p<0.001$

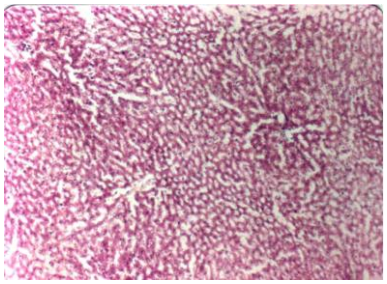


Fig. (1): liver of female rat I/M injected with vit. D_2 350.000 IU /Kg Bw. 8 weeks. Showing congestion, and fatty infilteration. H&E x100

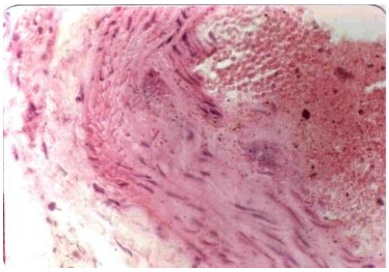


Fig. (2): Aorta of female rat I/M injected with vit. D_2 350.000 IU /KgBw.for 8 weeks. Showing mild degenerative changes in the media. H&E x400

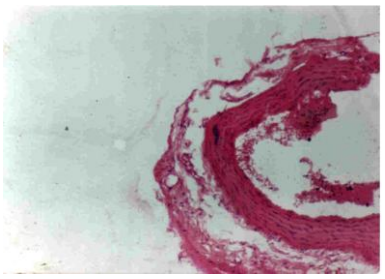


Fig. (3): Aorta of female rat I/M injected with vit. D_2 350.000 IU /KgBw.for 8 weeks. Showing focal calcinosis changes in the media. H&E x100

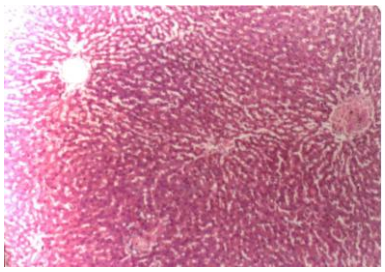


Fig. (4): liver of female rat administered O.C (35µg E. E/ 0. 5mg NOR) for 8 weeks. Showing congestion and fatty infilteration . H&E x100

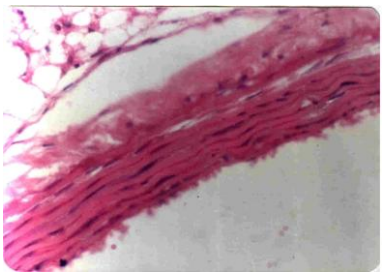


Fig. (5): Aorta of female rat administered O.C (35µg E. E/ 0. 5mg NOR) for 8 weeks. Showing early fibrilar degenerative changes at the media . H&E x 400

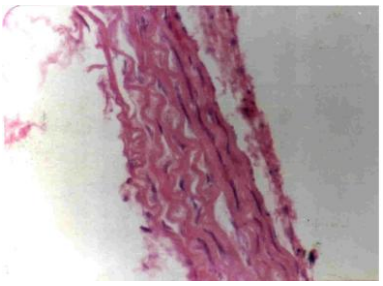


Fig. (6): Aorta of female rat administered O.C (35µg E. E/ 0. 5mg NOR) for 8 weeks. Showing marked fibrilar degenerative changes at the media . H&E x400

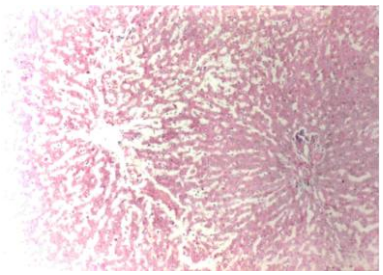


Fig. (7): liver of female rat administered O.C (35µg E. E/ 0. 5mg NOR+ I/M injected with vit. D_2 350.000 IU /KgBw) for 8 weeks. Showing congestion , fatty infilteration and necrotic changes . H&E x 100

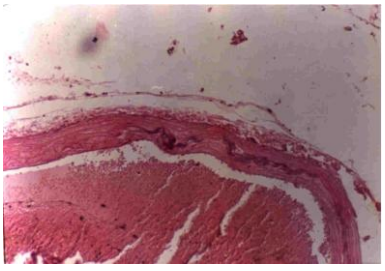


Fig. (8): Aorta of female rat administered O.C (35µg E. E/ 0. 5mg NOR+ I/M injected with vit. D_2 350.000 IU /KgBw) for 8 weeks. Showing moderate calcinosis in the media . H&E x 100

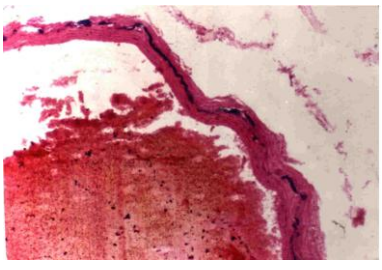


Fig. (9): Aorta of female rat administered O.C (35µg E. E/ 0. 5mg NOR+ I/M injected with vit. D_2 350.000 IU /KgBw) for 8 weeks. Showing medial calcinosis . H&E x 100

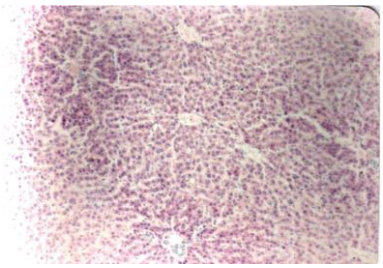


Fig. (10): liver of female rat administered O.C (35µg E. E/ 1mg NOR+ I/M injected with vit. D_2 350.000 IU /KgBw) for 8 weeks. Showing congestion , fatty infilteration and necrotic changes . H&E x 100.

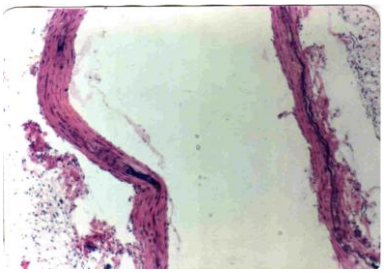


Fig. (14): Aorta of female rat administered O.C (35µg E. E/ 1mg NOR+ I/M injected with vit. D_2 350.000 IU /KgBw) for 8 weeks. Showing medial calcinosis. H&E x 1 00

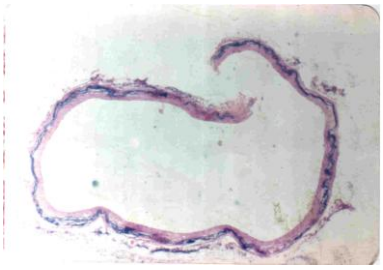


fig.(15): Aorta of female rat administered O.C (35µg E. E/ 1mg NOR+ I/M injected with vit. D_2 350.000 IU /KgBw) for 8 weeks. Showing medial calcinosis. H&E x 100

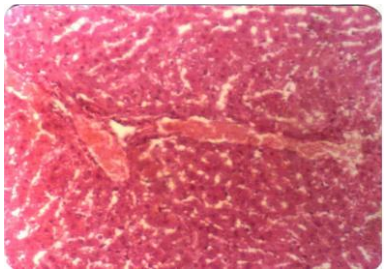


Fig. (13): liver of female rat administered O.C (70µg E. E/ 0.5mg NOR+ I/M injected with vit. D_2 350.000 IU /KgBw) for 8weeks. Showing congestion and fatty infilteration. H&E x 100

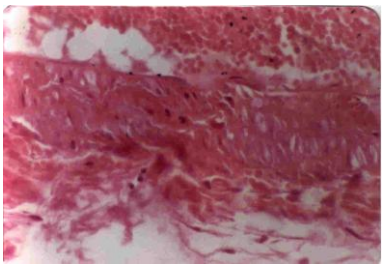


Fig. (11): Aorta of female rat administered O.C (70µg E. E/ 0.5mg NOR+ I/M injected with vit. $D_2$350.000 IU / /KgBw) for 8 weeks. Showing hyalinization (necrobiotic changes) precedes calcinosis. H&E x 400

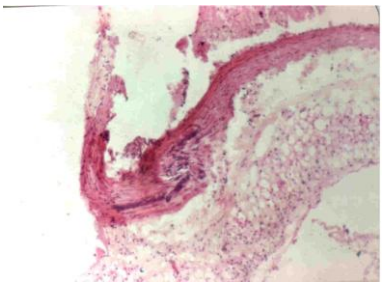
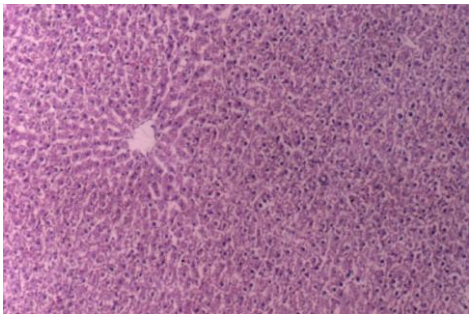
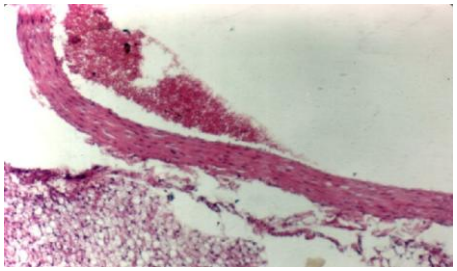


Fig. (12): Aorta of female rat administered O.C (70µg E. E/ 0.5mg NOR+ I/M injected with vit. D_2 350.000 IU /KgBw) for 8 weeks. Showing focal medial calcinosis. . H&E x 100



Fig(13): liver of control female rat at 8weeks
H&E x 100



Fig(14):Aorta of control female rat at 8weeks
H&E x 100

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بعض المتغيرات الكيميائية الحيوية المصاحبة لتناول موانع الحمل التى تستعمل عن طريق الفم ومرض تكلس الشـــرايين (تصلب الشـــرايين) في فئران المهـق

ايناس على مهدى خليل* وممدوح حافظ عفيفى**
الهيئه القومية للرقابه والبحوث الدوائية* -كلية الطب البيطري جامعة القاهره .

ينتج عن ذياده تناول فيتامين د2 ذياده معدل الكالسيوم في الدم ويؤدى ذلك الى ترسيبه فى الانسجه الرقيقه مثل الأوعيه الدمويه . وقد آجـريت أبحاث كثيره وضـحت العلاقه بين تناول موانع الحمل (الهرمونات الأستيرويديه المخلقه) التى تستعمل عن طريق الفم ومرض تكلس الشرايين .
ولهذا فقد تناول البحث تقييم بعض المتغييرات الكيميائيه الحيويه وفحص المتغييرات المرضـيه فـى الكبـد والشـريان الأورطـاالناتجـة عـن تنـاول موانـع الحمـل (مـزيج مـن الأسـتروجين والبروجسـيتيرون)بتركيـزات مختلفـه لفئـران مصـابه بمـرض تكلـس الشرايين عن طريق أعطاء فيتامين د2
بكميـه ذائـدة وأيضـا توضـيح دور موانـع الحمـل التـى تسـتعمل عـن طريـق الفـم ذات الجرعه المنخفضه.
و قد تم تقسيم عدد 48 من إناث الفئران إلى 6 مجموعات كل مجموعه تحتوى على 8 فئران. و تعتبر المجموعة الأولـى مجموعـه ضـابطة و المجموعـة الثانيـة تحقن عضـليا (بجرعة350000فيتامين د$_2$ وحده دوليه / كجم من وزن) الجسم و المجموعـة الثالثـة تتنـاول حبـوب منـع الحمـل (35 ميكـرو جـم مـن الاسـتروجين/0.5ملجـم مـن البروجستوجين) مجموعـة الرابعـة تحقن عضـليا بجرعة(350000 فيتـامين د$_2$ وحده دوليـه / كجـم مـن وزن الجسـم + 35 ميكـرو جـم مـن الاسـتروجين/0.5ملجـم مـن البروجستوجين), المجموعة الخامسة تحقن عضليا(بجرعة 350000 فيتامين د$_2$ وحده دوليـه / كجـم مـن وزن الجسـم + 35 ميكـرو جـم مـن الاسـتروجين/1ملجـم مـن البروجسـتوجين), المحموعـة السادسـة تحقـن عضـليا (بجرعـة 350000 فيتـامين د2 وحده دوليه / كجم من وزن الجسم + 70 ميكرو جم من الاستروجين/0.5ملجم من البروجستوجين)وبعد فتره تتروا ح ثمانية أسابيع تم أخذ عينـات مـن دم الفئـران لدراسـه مستوى بعض الأنزيمات والدهنيات فى الدم وأيضا تم أخذ عينات شمعيه لكل من الكبد والشريان الأورطى لدراسه التغييرات المرضيه بهم.
وأظهرت النتائج ذياده ذو دلالة أحصـائيه فـى مستوى مصـل خمـائر أنزيمـات أمينـوتر انس فيراز ومستوى مصل الفوسفاتاز القلوي فى المجموعات الآتيـه(مجموعـة تعامل بفيتامين د + مانع الحمل ذات الجرعه العاليه من البروجسيترون ، ومجموعـة تعامل بفيتـامين د+مـانع الحمل ذات الجرعـه المنخفضـه ،و المجموعـه المعاملـه بفيتـامين د + مانع الحمل ذات الجرعه العاليه من الأستروجين ، والمجموعـه المعاملـه بفيتـامين د ثـم المجموعه المعامله بمانع الحمل دات الجرعه المنخفضه)، وأيضا الجلسريدات الثلاثيه

فقد سجلت النتائج ذيادة ملحوظه فى المجاميع التاليه(المجموعه المعامله بفيتامين د+ مانع الحمل ذات الجرعه العاليه من الاستروجين ، والمجموعة تعامل بفيتامين د+مانع الحمل ذات الجرعه المنخفضه ، والمجموعه المعامله بفيتامين د ثم المجموعه المعامله بمانع الحمل دات الجرعه المنخفضه)

وبالنسبه للكوليستيرول فقد سجلت النتائج ذيادة ذات معنى أحصائى فى المجموعات التاليه (مجموعة تعامل بفيتامين د + مانع الحمل ذات الجرعه العاليه من البروجسيترون ، ومجموعة تعامل بفيتامين د+ مانع الحمل ذات الجرعه المنخفضه والمجموعه المعامله بفيتامين د ،و المجموعه المعامله بمانع الحمل دات الجرعه المنخفضه ثم المجموعه المعامله بفيتامين د + مانع الحمل ذات الجرعه العاليه من الأستروجين).

وأوضح الفحص المجهرى وجود تغييرات مرضيه فى خلايا الكبد تكون شديده فى المجموعه المعامله بفيتامين د + مانع الحمل ذات الجرعه العاليه من البروجسيترون وأيضا المجموعة تعامل بفيتامين د+ مانع الحمل ذات الجرعه المنخفضه ،وتكون التغييرات المرضيه متوسطه فى المجموعه المعامله بفيتامين د + مانع الحمل ذات الجرعه العاليه من الأستروجين ،و المجموعه المعامله بفيتامين د، و المجموعه المعامله بمانع الحمل دات الجرعه المنخفضه,و أما بالنسبة لتكوين تكلس(ترسيب الكالسيوم) فى الطبقة الوسطى من الأورطى مصاحبا بتغيير مرضى بها فقد كان ظهرا أكثر فى المجموعه المعامله بفيتامين $د_2$ ومحتوى أكبر من البروجستوجين المجموعه المعامله بفيتامين $د_2$ تتناول حبوب منع الحمل بجرعه مخفضه ثم المجموعه المعامله بفيتامين $د_2$ ومحتوى أكبر من الاستروجين والمجموعه المعامله بفيتامين $د_2$فقط وقد ينموأيضا فى المجموعه التى تتناول حبوب منع الحمل بجرعه مخفضه

وقد دلت النتائج أن حبوب منع الحمل ذات الجرعه المنخفضه قادرة لوحدها لاحداث مرض تصلب الشرايين(تكلس الشرايين) ويرجع هذا الى البروجستوجين الموجود بها.