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EFFECT OF PREGNANCY ON THE HYPOTENSIVE ACTION OF ANGIOTENSIN - CONVERTING ENZYME INHIBITORS IN FEMALE RATS

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ABSTRACT

The effect of angiotensin-converting enzyme (ACE) inhibitors versus alpha methyldopa on the arterial blood pressure of pregnant adult female rats were studied. Captopril, enalapril, benazepril and perindopril were injected intraperitoneally and used as representative for the commonly used antihypertensive ACE inhibitors. The effects of ACE inhibitors and alpha methyldopa on estradiol, porgesterone, prolactin, cortisol, cholesterol, glucose, potassium and calcium blood levels were studied. Results showed that pregnancy significantly increased both the intensity and duration of the hypotensive effect of ACE inhibitors in comparison with the non pregnant rats. This difference in the effect was not observed in case of alpha methyldopa. Pregnancy per se significantly increased the blood levels of estradiol, progesterone, prolactin, cortisol and glucose . Both estradiol and progesterone were increased directly after occurrence of pregnancy. While cortisol, prolactin and glucose levels were increased after 12 days of starting of pregnancy. Alpha methyldopa significantly increased the blood levels of estradiol, progesterone and prolactin while decreased that of cortisol. Perindopril significantly increased the blood levels of both estradiol and progesterone after 18 days of gestation . Benazepril induced significant reduction of both estradiol and prolactin while increased that of progesterone. Both alpha methyldopa and benazepril significantly increased the glucose blood levels. Captopril, enalapril , and benazepril significantly increased the potassium blood levels after 12 days of starting of gestation, however, both alpha methyl dopa and perindopril did not. While captopril significantly decreased the calcium blood level, alpha methyldopa significantly increased it.

In conclusion, pregnancy per se potentiates the hypotensive effect of ACE inhibitors but not that of alpha methyldopa. This may be, in part due to elevation of female sex hormones and refractoriness to angiotensin II (Ang II) that occur during pregnancy .

INTRODUCTION

Normal pregnancy usually, is accompanied with an increase in plasma renin concentration, renin activity, renin substrate, angiotensin II (Ang II), and aldosterone[1]. As pregnancy is associated with an increased maternal placental estrogen level, the the latter may drive the renin- angiotensin-aldosterone system (RAAS), by increasing angiotensinogen substrate [2] .

In spite of the early increasing levels of circulating Ang II in normal pregnancy, there is a fall in the arterial blood pressure [3] . This fall in the blood pressure despite the elevated levels of Ang II may be due to refractoriness to Ang II. This type of refractoriness may occur at the level of the resistance vessels and it appears that there is a loss in refractoriness in women destined to develop pregnancy - induced hypertension [4] .

It has been reported that intravenous administration of Ang II causes a decrease in blood pressure of pregnant compared with no pregnant women [5]. **Matsuura et al.** [6] found that normal pregnancy in human and a number of other species is associated with a loss in vascular response to the pressor effects of exogenously administered Ang II . The blunted pressor reponses to intravenous Ang II in pregnancy might be attributed to altered reflex sensitivity or altered Ang II receptor function in cardiac, renal, adrenal or central nervous tissues [7] or may be secondary to increased circulating levels of endogenous Ang II [8] . On the other hand , in case of pregnancy - induced hypertension, there is a significant increase in the concentration of ACE [9] , decrease in protacyclin levels leaving a relative imbalance with increased thromboxane A_2 [10], and rise in the circulating Ang II levels [11]. This type of hypertension complicates 6-10% of all pregnancies and is responsible for increased maternal and prenatal mortality and morbidity [12].

ACE inhibitors are reported to reduce the arterial blood pressure in both renal and essential hypertension accompanied by uncomplicated diabetes mellitus [13], chronic obstructive pulmonary disease[14] and congestive heart failure [15]. They act mainly by inhibition of renin - angiotensin aldosterone system [16] resulting in reduction of Ang II levels in both plasma and tissues[17]. ACE inhibitors were reported to increase insulin sensitivity and glucose disposal rate [18,19] Update reports recommended that these agents should be avoided during pregnancy unless indicated where other drugs can not be given or ineffective. It was reported that prolonged treatment as well as long - term infusion of ACE inhibitors in pregnant animals is associated with a reduced or may be loss of refractoriness to exogenously administered Ang II [20].

The present study aimed at studying the effect of pregnancy and the resultant refractoriness to Ang II on the hypotensive effect of ACE inhibitors. And if any, is there a correlation between the increased plasma levels

of female sex hormones that occurs during pregnancy and these changes ? . As well as do these changes will be for ACE inhibitors only or can be extended to other antihypertensive agents like alpha methyl dopa?

MATERIALS AND METHODS

Adult fertile female rats aged 14 - 16 weeks were used in the present study and were obtained from the animal house of the National Information and Documentation Center (NIDOC), Dokki, Cairo, Egypt . Rats were left for more than one week for accommodation in our laboratory and were left free on excess food and water *ad libitum*.

Preparation of pregnant rats :

Pregnancy was induced in female rats following the methods described by [21,22]. Female rats , distinct to become pregnant , were caged individually with an adult male rat for at least 24 to 48 hrs. and in the early morning , the surface of the external genitalia of each female was examined and the detection of sperm plug was designated as the gestation day number zero. The percentage induction of pregnancy was 80% of the rats subjected to this method and these rats did delivery within 21± 1 days.

Experimental protocol :

A-Effect of the given drugs on arterial blood pressure:

This part of the work was carried out to study the effect of the given drugs on the arterial blood pressure of both pregnant and non pregnant female rats . Rats were divided into two major groups, six subgroups of pregnant and six non pregnant females. Each drug was intraperitoneally (IP) injected in two subgroups , one for non pregnant and the second for pregnant rats according to the following protocol:

1- Pregnant rats :

A- Subgroup (i) received alpha methyldopa (MSD) in a dose of 2.5 mg/kg . IP.

B-Subgroups (ii, iii, iv, & v) , received enalapril (MSD,0.5 mg/kg , benazepril (Ciba-Geigy, 0.5 mg/kg), captopril (Squibb, Egypt, 1 mg /kg) and perindopril (Servier , 0.5 mg/kg) respectively.

C- Subgroup (vi) is used as pregnant control .

2-Non pregnant rats :

This group was consists also of six subgroups of non pregnant female rats and rats received drugs and solvent by the same consequence that mentioned before incase of pregnant rats. Drugs and their solvents were injected intraperitoneally in a single dose on the day 18th of pregnancy and the blood pressure was recorded before and after injection and the recording continued for 3 hours at different time intervals. Non pregnant females were manipulated by the same manner and were kept under the same conditions as the pregnant ones.

Determination of arterial blood pressure :

On the 18th day of pregnancy, rats were anaesthetized with pentobarbitone in a dose of 30mg/kg . The carotid artery was cannulated with a polyethylene 50, filled with heparinized glucose, which was connected to a calibrated blood pressure transducer PT400 which was connected to an Oscillograph (MD 4C , Bioscience , Washington) through FC -137 coupler . Blood pressure was recorded before (0.00 min) and after administration of drugs or their solvents, at 15, 30 , 60 , 90, 120, 150 and 180 minutes.

B- Effect of given drugs on female sex hormones and some biochemical parameters:

Pregnant female rats were divided into seven groups (6 rats each) and were subjected for drug administration according to the following protocol :

a) **group I** : received alpha methyldopa in a dose of 25 mg/kg .

b) **groups II, III, IV and V** : received ACE inhibitors, enalapril (2mg/kg), benazepril (2mg/kg), and perindopril (1mg/kg) respectively.

c) **group VI** : received solvent and was used as control for pregnant rats .

d) **group VIII** : received solvent and was used as control for non pregnant rats.

All drugs and their solvent were given orally by gavage in the form of two doses per day all over the time of pregnancy and 3 days postpartum.

Collection of blood samples and preparation of plasma for analysis :

Blood samples were taken from the orbital sinus of each rat before and on the 6th , 12th and 18th day of gestation and on the 3rd day postpartum. Blood samples were centrifuged at 4000 rpm for 15 min and plasma was separated . One ml of plasma was used for determination of glucose , potassium , calcium and cholesterol. The remainder portion of each sample was kept at -20°C for hormonal assays .

Assay and determinations :

1- Biochemical analysis

Blood glucose level was determined by glucose oxidase method [23] using Bio-analytical kits. Potassium level was measured turbidimetrically following the method of [24] , using QCA, kits. Diamond Diagnostic kit was used for the colorimetric determination of serum

calcium levels , according to previous method (25). Total cholesterol was measured enzymatically according to the method of **Allain et al.** (26).

2- Hormonal assay :

Blood hormone levels weredetermined only in plasma of those rats that became pregnant, whereas that did not , there plasma were discarded and results of biochemical analysis were neglected. Estradiol, progesterone, prolactin and cortisol blood levels were determined by the fluoroimmunoassay method using DELFIA immunoassay kits and pharmacia fluorometer (LKB, Wallac, Model, No. 1230).

Statistical analysis :

Data of the present study are expressed as the mean ± SEM . The 95% confidence level was considered to be statistically significant in the all analysis . The obtained data of each individual drug treated group were compared to that of control and subjected for statistical analysis using Student "t" test . ANOVA was used for comparing the effects of any ACE inhibitor with that of alpha methyldopa and control especially in case of hormonal analysis .

RESULTS

1-Effects on the arterial blood pressure :

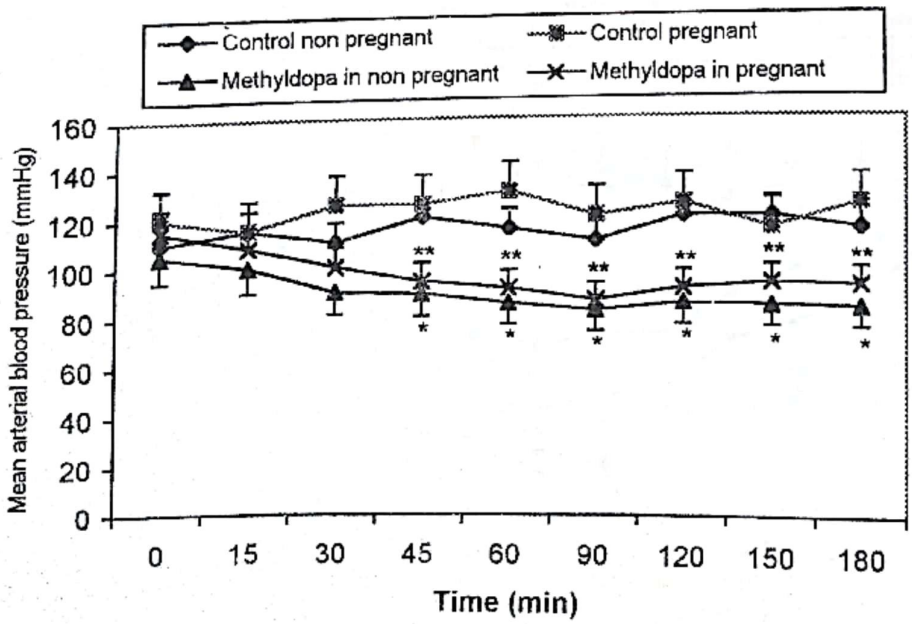
a- Effect of alpha methyldopa:

As shown in Fig. (1), alpha methyldopa significantly reduced the arterial blood pressure of both pregnant and non pregnant rats . This effect started after 30 minutes and lasted for more than 180 minutes of drug administration. In pregnant rats the effect of methyldopa was less than that produced in case of non pregnant rats , but the difference was not significant.

b-Effect of ACE inhibitors :

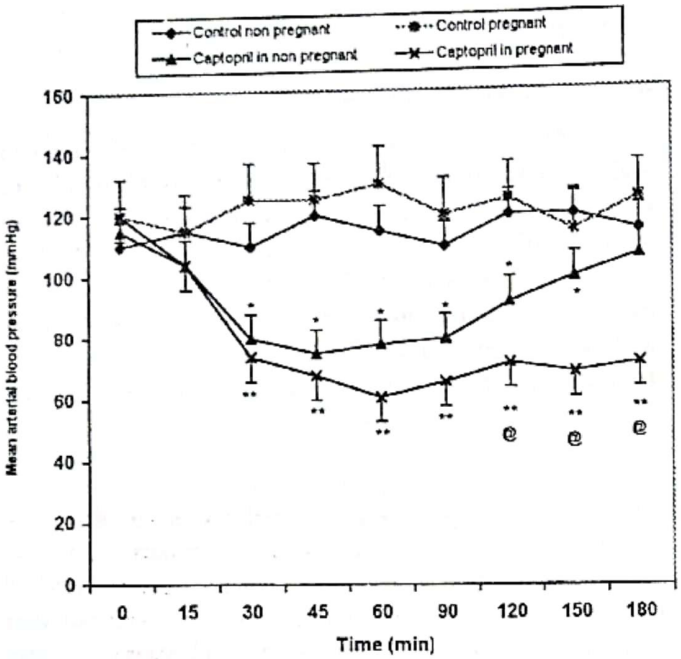
All ACE inhibitors that used in this study induced a significant reduction in the arterial blood pressure in both pregnant and non pregnant rats . This effect started after 30 minutes in case of captopril (Fig.2), enalapril (Fig. 3) and benazepril (Fig.4) and after 15 minutes in each of perindopril injection (Fig. 5) in both pregnant and non pregnant rats. The hypotensive effect of the tested ACE inhibitors lasted for more than 180 minutes, in case of both pregnant and non pregnant rats .

The effect of captopril lasted after 150 minutes in case of non pregnant rats and for more than 180 minutes in case of pregnant rats . In pregnant rats, the intensity and duration of the hypotensive action of all the tested ACE inhibitors were significantly greater than that produced in non pregnant ones. This effect started after 120 minutes after injection of captopril (Fig. 2), 60 minutes after injection of enalapril (Fig.3) , 45 minutes after injection of benazepril (Fig.4) and 90 minutes after injection of perindopril (Fig.5) and lasted for more thatn 180 minutes of injection of all the tested ACE inhibitors. This indicates that pregnancy per se increases the intensity and duration of the hypotensive effect of the injected ACE inhibitors .



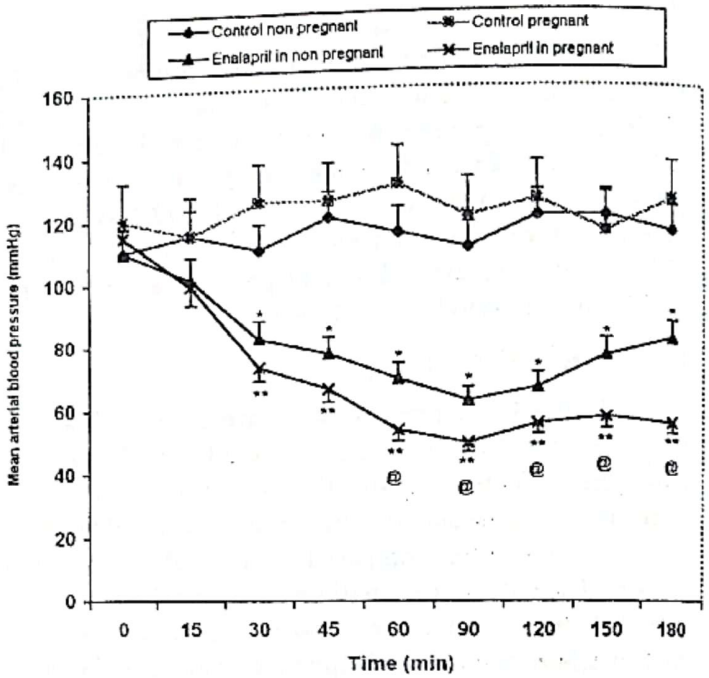
Fig(1): Effect of a single dose of methyldopa (2.5mg/kg, IP) on the arterial blood pressure in pregnant (after18 days of gestation) and non pregnant femal rats.

* Significantly different from control value of non pregnant rats at $P < 0.05$.
** Significantly different from control value of pregnant rats at $P < 0.05$.



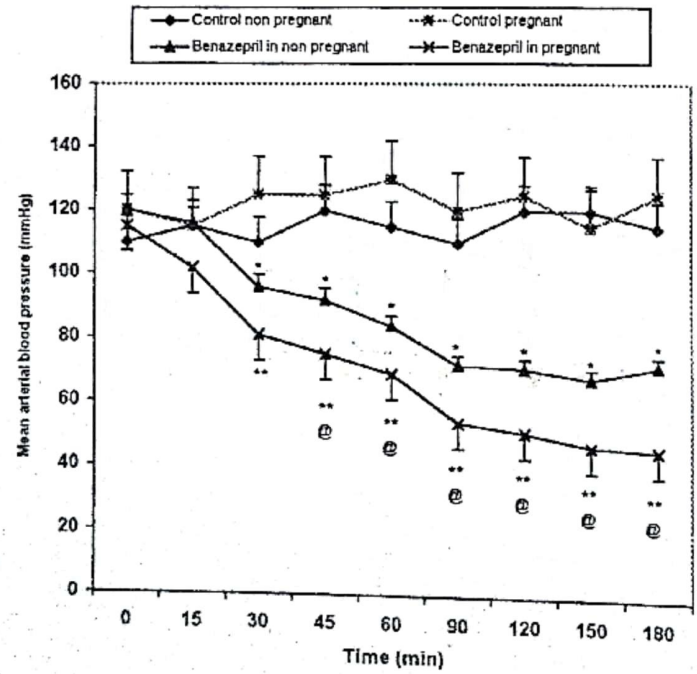
Fig(2): Effect of a single dose of captopril (1mg/kg, IP) on the arterial blood pressure in pregnant (after 18 days of gestation) and non pregnant female rats.

* Significantly different from control value of non pregnant rats at P < 0.05.
** Significantly different from control value of pregnant rats at P < 0.05.
@ Significantly different from the value of non pregnant treated rats at P < 0.05.



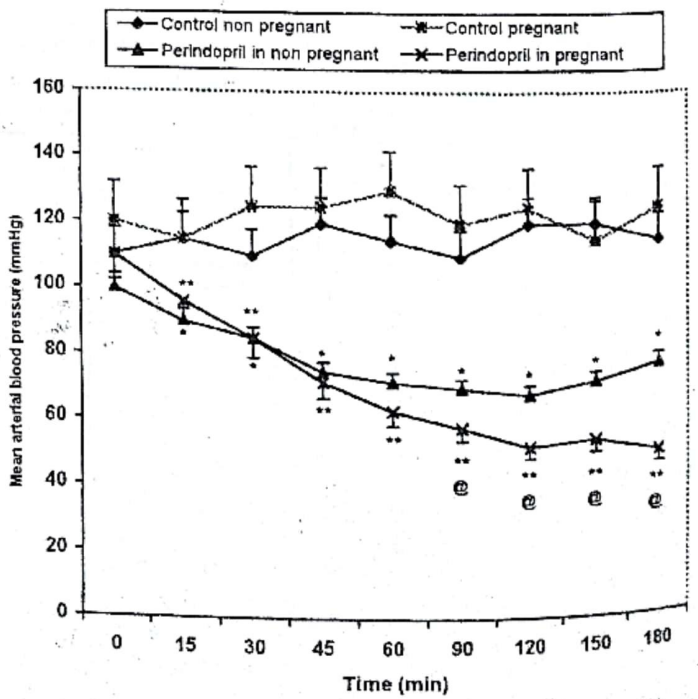
Fig(3): Effect of a single dose of enalapril (0.5mg/kg, IP) on the arterial blood pressure in pregnant (after 18 days of gestation) and non pregnant female rats.

* Significantly different from control value of non pregnant rats at P < 0.05.
** Significantly different from control value of pregnant rats at P < 0.05.
@ Significantly different from the value of non pregnant treated rats at P < 0.05.



Fig(4): Effect of a single dose of benazepril (mg/kg, IP) on the arterial blood pressure in pregnant (after 18 days of gestation) and non pregnant female rats.

* Significantly different from control value of non pregnant rats at P < 0.05.
** Significantly different from control value of pregnant rats at P < 0.05.
@ Significantly different from the value of non pregnant-treated rats at P < 0.05.



Fig(5): Effect of a single dose of perindopril (0.5mg/kg, IP) on the arterial blood pressure in pregnant (after 18 days of gestation) and non pregnant female rats.

* Significantly different from control value of non pregnant rats at P < 0.05.
** Significantly different from control value of pregnant rats at P < 0.05.
@ Significantly different from the value of non pregnant-treated rats at P < 0.05.

Zagazig J. Pharm. Sci., June 1997
Vol.6 , No.1 , pp. 77-90

2- Effects on female sex hormones :

a-In pregnant control rats :

Pregnancy in rats significantly increased the blood levels of estradiol, progesterone, prolactin and cortisol (Fig.6) . The blood levels of both estradiol and progesterone were increased directly after begining of pregnancy and the effect remain all over its time course. Six day after starting of pregnancy, prolactin blood level was decreased , whereas, after 12 and 18 days the levels of both prolactin and cortisol were increased.

b-In pregnant treated rats :

i-Effect on estradiol :

Alpha methyldopa significantly increased the blood levels of estradiol recording 52% , 65% , 44% and 143% of control value after 6,12 and 18 days of treatment during pregnancy and postpartum respectively (Table 1). While perindopril significantly increased the blood level estrogen by 23% of control only after 18 days of treatment. However, benazepril significantly reduced in its levels by 16.5% and 16.3% of control value after 6 and 12 days of treatment respectively. Neither captopril nor enalapril alter the estradiol blood levels in prgnant rats (Table 1).

ii-Effect on progesterone :

The given results in table (2) showed that alpha methyldopa significantly increased the progesterone blood levels by 70% , 110% and 119% after 6,12 and 18 days of treatment during pregnancy respectively and by 47% three days postpartum. Like that of alpha methyldopa, both benazepril and perindopril significantly increased the levels of progesterone. Benazepril induced 14%, 23% and 35% increase during the time course of pregnancy respectively, however, perindopril induced an increase only after 18 days of pregnancy, recording 17% of control. Both captopril and enalapril did not alter the progesterone blood levels during pregnancy and postpartum.

iii-Effect on prolactin :

Alpha methydopa significantly increased the prolactin blood level of pregnant rats during both gestation periods and postpartum, recording 75%, 84% and 79% after 6,12 and 18 days of treatment during pregnancy and 69% during postpartum (Table 3). Benazepril was the only one of the tested ACE inhibitors that decreased the blood level of prolactin during pregnancy, recording 20%, 17.6% and 24% reduction of control after 6,12 and 18 days of treatment respectively.

Table (1) : Effect of oral administration of alpha methyldopa, enalapril, enazepril, captopril and perindopril on estradiol blood levels before and after 6,12 and 18 days of gestation and 3 days postpartum in pregnant female rats.

Treatment	Estradiol blood level (nmol/L)				
	Before gestation and administration	After administration during pregnancy and postpartum			
		After 6 days	After 12 days	After 18 days	Postpartum
Control	0.23 ± 0.012	8.14 ± 0.54	40 ± 2.41	73 ± 4.41	0.16 ± 0.013
-Methyl dopa	0.19 ± 0.015	12.4 ± 0.33 *	66 ± 2.55 *	105 ± 5.66 *	0.39 ± 0.009 *
Enalapril	0.21 ± 0.022	8.59 ± 0.49	49 ± 3.23	81 ± 5.64	0.14 ± 0.012
Benazepril	0.25 ± 0.019	6.8 ± 0.22 *	33.5 ± 2.01 *	71 ± 3.69	0.13 ± 0.01
Captopril	0.27 ± 0.025	7.82 ± 0.45	38 ± 2.09	75 ± 4.15	0.15 ± 0.011
Perindopril	0.22 ± 0.022	8.98 ± 0.51	44 ± 2.62	89 ± 4.56 *	0.18 ± 0.012

* Significantly different from the corresponding control value at P<0.05
Values are expressed as mean ± S.E.M. , (n=6).

Table (2): Effect of oral administration of alpha methyldopa, enalapril, benazepril, captopril and perindopril on progesterone blood levels before and after 6, 12 and 18 days of gestation and 3 days postpartum in pregnant female rats.

Treatment	Progesterone blood level (nmol/L)				
	Before gestation and administration	After administration during pregnancy and postpartum			
		After 6 days	After 12 days	After 18 days	postpartum
Control	0.72 ± 0.054	110 ± 6.13	195 ± 10.61	430 ± 16.07	0.89 ± 0.043
-Methyl dopa	0.98 ± 0.075	187 ± 13.26 *	409 ± 15.26 *	940 ± 21.63 *	1.31 ± 0.067 *
Enalapril	0.78 ± 0.061	111 ± 5.29	200 ± 13.19	470 ± 14.23	0.91 ± 0.063
Benazepril	0.87 ± 0.077	125 ± 6.92	240 ± 12.13 *	580 ± 15.91 *	0.99 ± 0.058
Captopril	0.65 ± 0.064	98 ± 4.16	180 ± 9.36	400 ± 13.98	0.82 ± 0.087
Perindopril	0.81 ± 0.056	115 ± 5.51	210 ± 10.12	501 ± 15.87 *	0.93 ± 0.077

* Significantly different from the corresponding control value at P< 0.05
Values are expressed as mean ± S.E.M. , (n=6).

Table (3): Effect of oral administration of alpha methyldopa, enalapril, benazepril, captopril and perindopril on prolactin blood levels before and after 6 ,12 and 18 days of gestation and 3 days postpartum in pregnant female rats.

Treatment	Prolactin blood level (ng/ml)				
	Before gestation and administration	After administration during pregnancy and postpartum			
		After 6 days	After 12 days	After 18 days	postpartum
Control	56.7 ± 4.26	50 ± 2.93	125 ± 5.14	145 ± 4.06	65 ± 5.11
-Methyl dopa	65.1 ± 5.11	87.5 ± 4.69 *	230 ± 8.19 *	260 ± 8.11 *	110 ± 7.63 *
Enalapril	71.4 ± 6.99	54.5 ± 4.23	131 ± 5.13	130 ± 4.28	85 ± 6.99
Benazepril	59.8 ± 4.75	40 ± 2.36 *	103 ± 6.19 *	110 ± 3.36 *	47 ± 4.31
Captopril	61.6 ± 4.32	52.5 ± 3.13	141 ± 6.77	160 ± 5.12	67 ± 5.98
Perindopril	81.6 ± 7.14	65 ± 6.91	114 ± 4.63	135 ± 3.89	57 ± 4.69

* Significantly different from the corresponding control value at P< 0.05
Values are expressed as mean ± S.E.M. , (n=6).

Table (4) : Effect of oral administration of alpha methyldopa, enalapril, benazepril, captopril and perindopril on cortisol blood levels after 6, 12 and 18 days of gestation and postpartum in pregnant female rats.

Treatment	Cortisol blood level (nmol/L)				
	Before gestation and administration	After administration during gestation and postpartum			
		After 6 days	After 12 days	After 18 days	postpartum
Control	413 ± 22.62	410 ± 34.13	490 ± 35.19	510 ± 43.14	450 ± 36.46
-Methyl dopa	460 ± 13.91	400 ± 26.32	360 ± 25.18 *	340 ± 21.66 *	310 ± 23.52 *
Enalapril	492 ± 35.55	512 ± 42.19	503 ± 24.98	499 ± 35.96	418 ± 34.57
Benazepril	510 ± 25.66	530 ± 45.29	500 ± 32.91	530 ± 38.88	480 ± 26.59
Captopril	463 ± 24.69	480 ± 34.21	489 ± 41.74	490 ± 31.85	450 ± 33.18
Perindopril	472 ± 22.91	498 ± 22.17	481 ± 31.16	501 ± 47.38	430 ± 32.86

* Significantly different from the corresponding control value at P< 0.05
Values are expressed as mean ± S.E.M. , (n=6).

iv-Effect on cortisol :

As presented in table (4), alpha methyldopa significiantly reduced the blood level of cortisol recording 22% and 33% after 12 and 18 days after treatment during pregnancy respectively and 26% during postpartum. All the tested ACE inhibitors did not significantly change the cortisol blood level during pregnancy and postpartum.

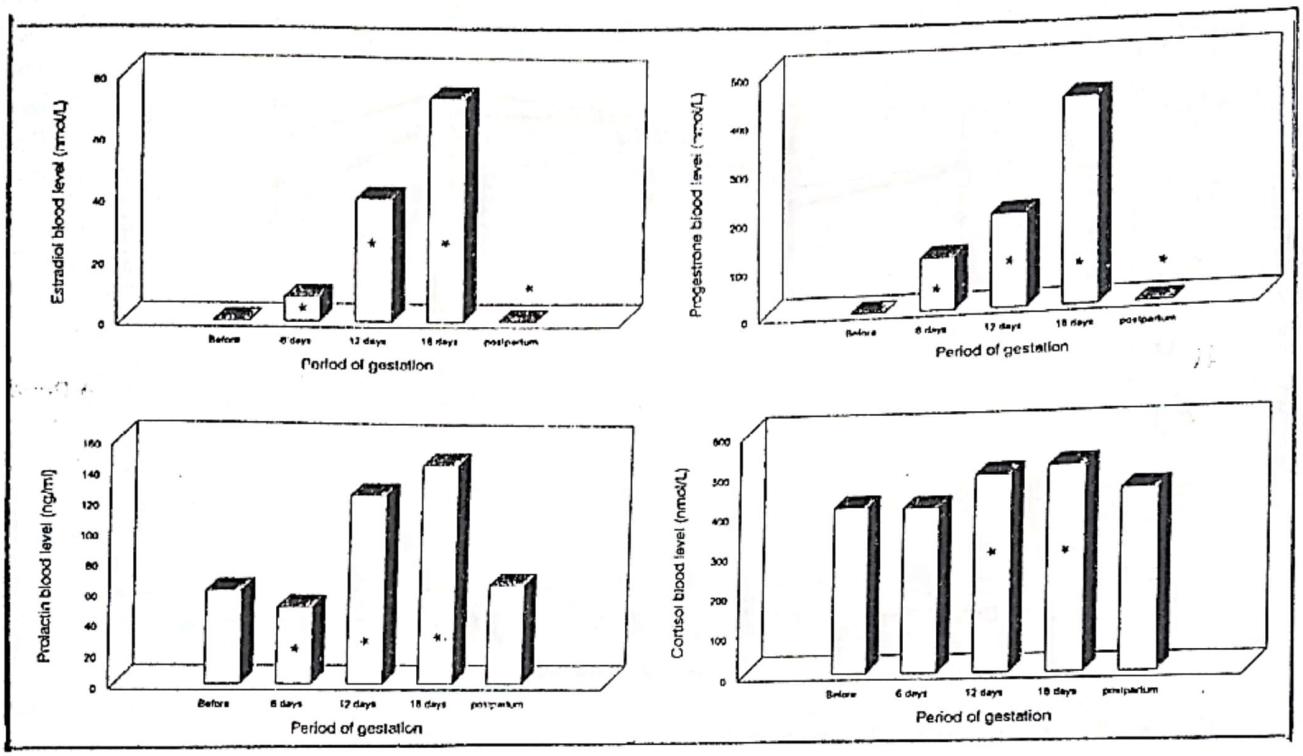
3-Effects on blood glucose, cholesterol, potassium and calcium levels :

Pregnancy in rats significantly increased the blood glucose levels only after 12 and 18 days of gestation. Serum cholesterol, potassium and calcium did not significantly changed during pregnancy (Fig.7). Alpha methyldopa significantly increased the blood glucose level, recording 45% and 50% increase of control value after 12 and 18 days of treatment during pregnancy respectively (Fig.8) . Benazepril was the only of the tested ACE inhibitors that increased the blood levels by 25% and 26% of control value after 12 and 18 days of treatment during pregnancy.

All the tested ACE inhibitors did not significantly change the cholesterol blood levels during both gestation and postpartum periods. Alpha methyldopa decreased the cholesterol blood level after 18 days of treatment and postpartum, recording 25% and 20% reduction respectively (Fig. 9).

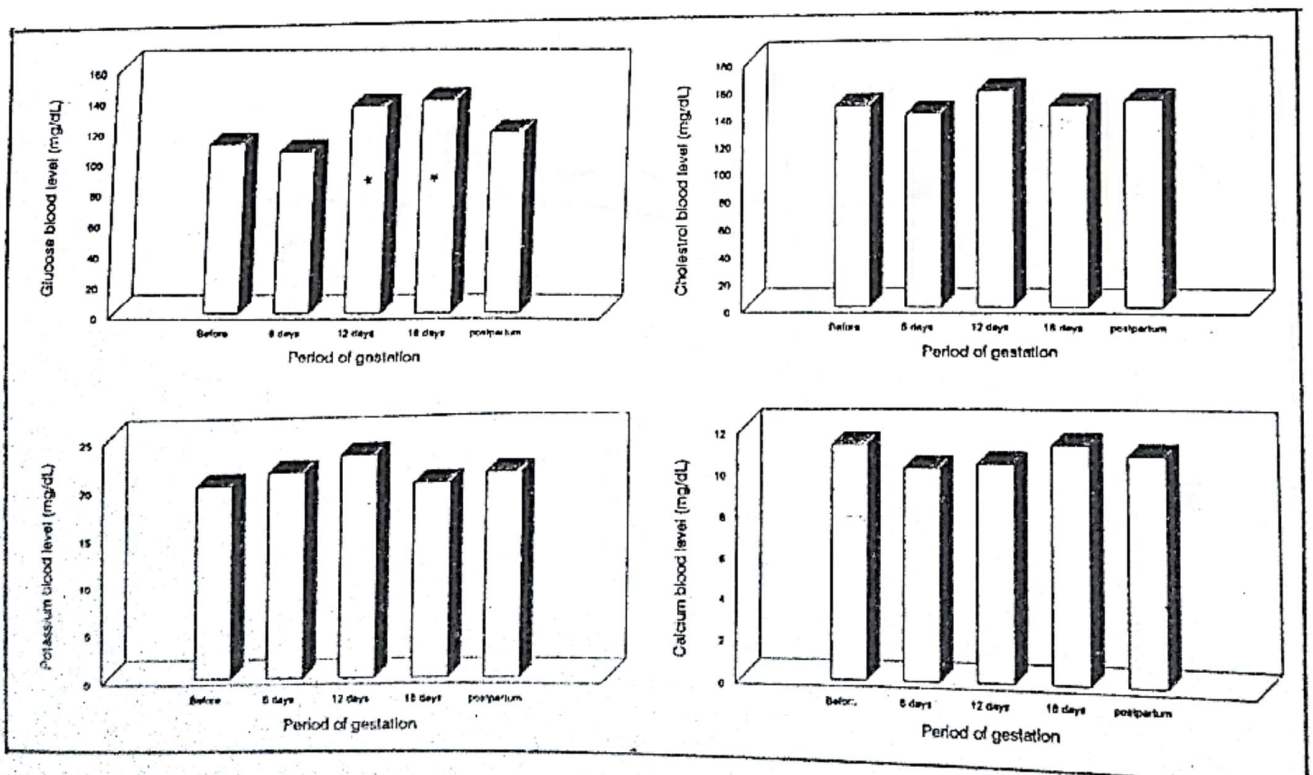
Captopril, enalapril and benazepril significantly increased the potassium blood level after treatment . The effect of enalapril appeared only during postpartum recording 26% increase, however, the effects of both captopril and benazepril started after 12 days of treatment and lasted for postpartum (Fig.10) Benazepril recorded 37%, 44% and 39% increase but captopril recorded 24% and 24% increase in potassium blood level. Both perindopril and alpha methyldopa did not affect potassium blood level. Fig (11) showed that alpha methyldopa significantly increased the calcium blood level by 35% , 42% , 57% and 48% after 6 , 12 and 18 days of treatment during pregnancy and postpartum respectively. Captopril significantly decreased the calcium blood levels by 15% , 10% and 15% after 12 and 18 days of treatment and postpartum respectively.

Zagazig J. Pharm. Sci., June 1997
Vol.6 , No.1 , pp. 77-90



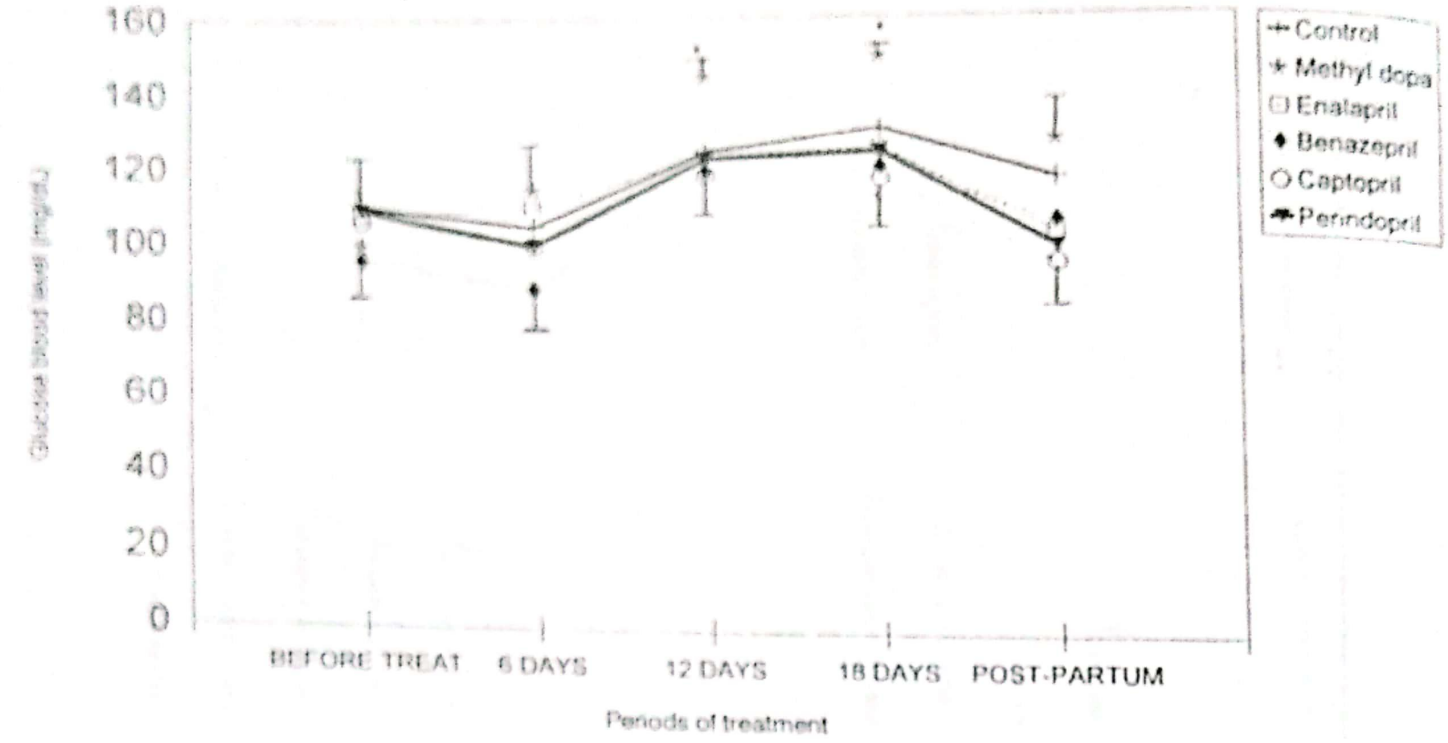
Fig(6): Changes in oestrogen, progesterone, prolactine and cortisol blood levels in female rats before , during and after gestation.

* Significantly different from the value before gestation at P<0.05



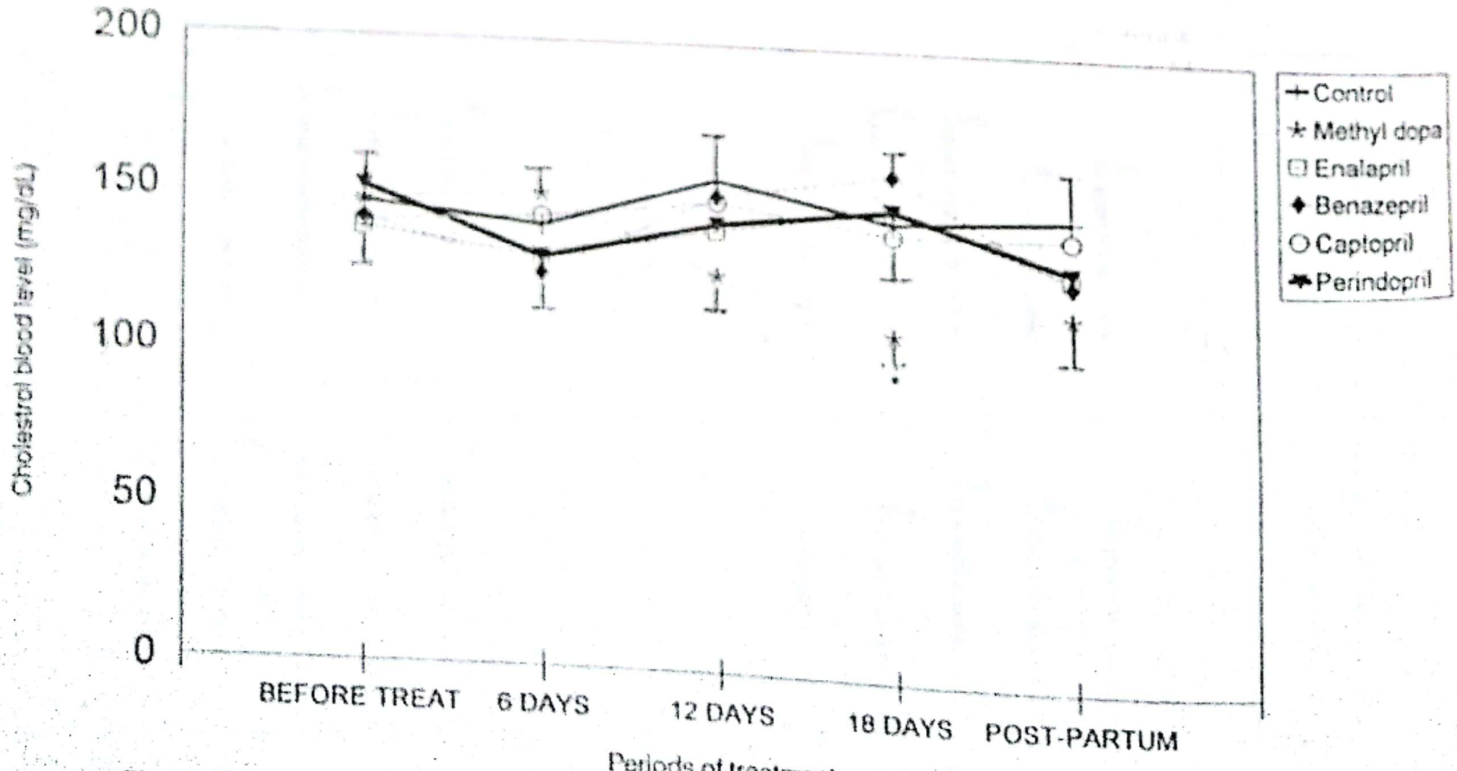
Fig(7): Changes in glucose, cholesterol, potassium and calcium blood levels in female rats before, during and after gestation.

* Significantly different from the value before gestation at P<0.05



Fig(8): Effect of oral administration of alpha methyl dopa, enalapril ,benazepril ,captopril and perindopril on glucose blood levels after 6, 12, and 18 days during gestation, and postpartum in pregnant female rats

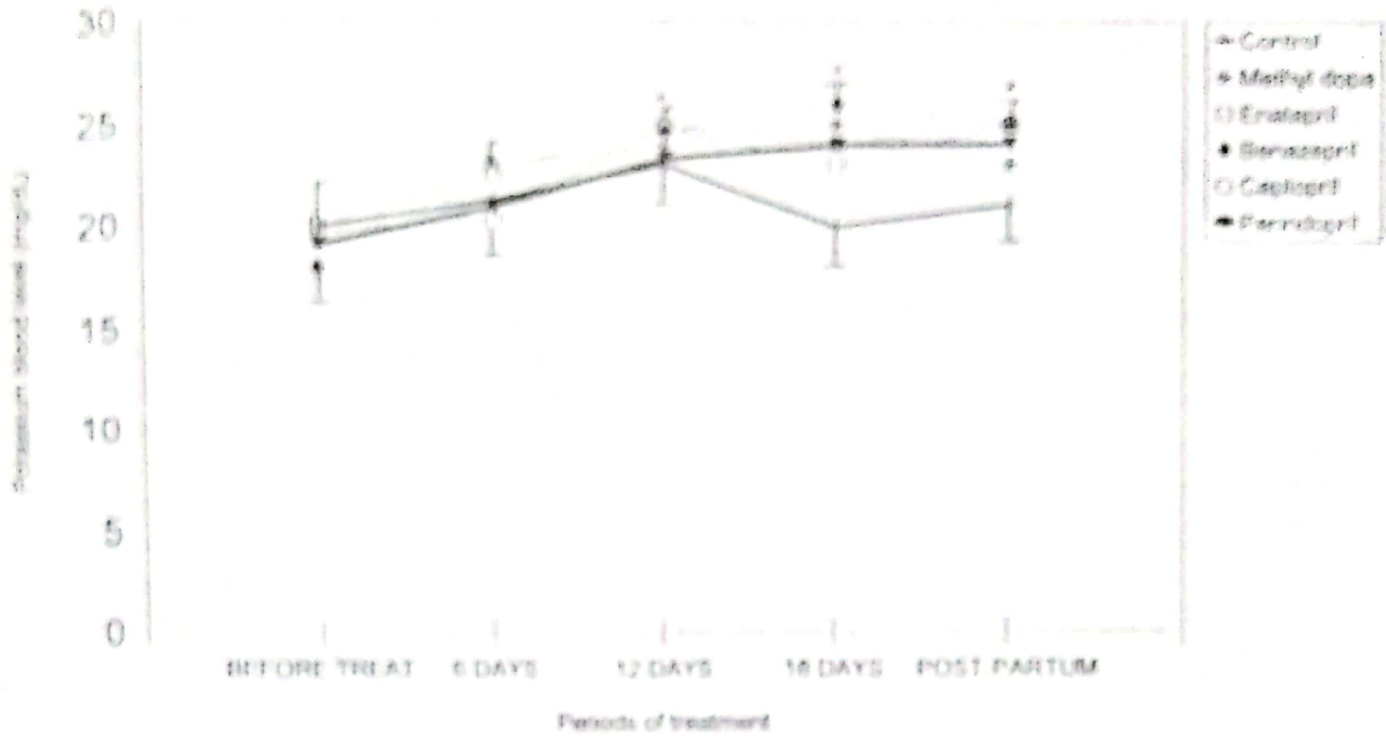
* Significantly different from control value at P<0.05



Fig(9): Effect of oral administration of alpha methyl dopa, enalapril, benazepril, captopril and perindopril on cholesterol blood levels after 6, 12, and 18 days during gestation, and postpartum in pregnant female rats.

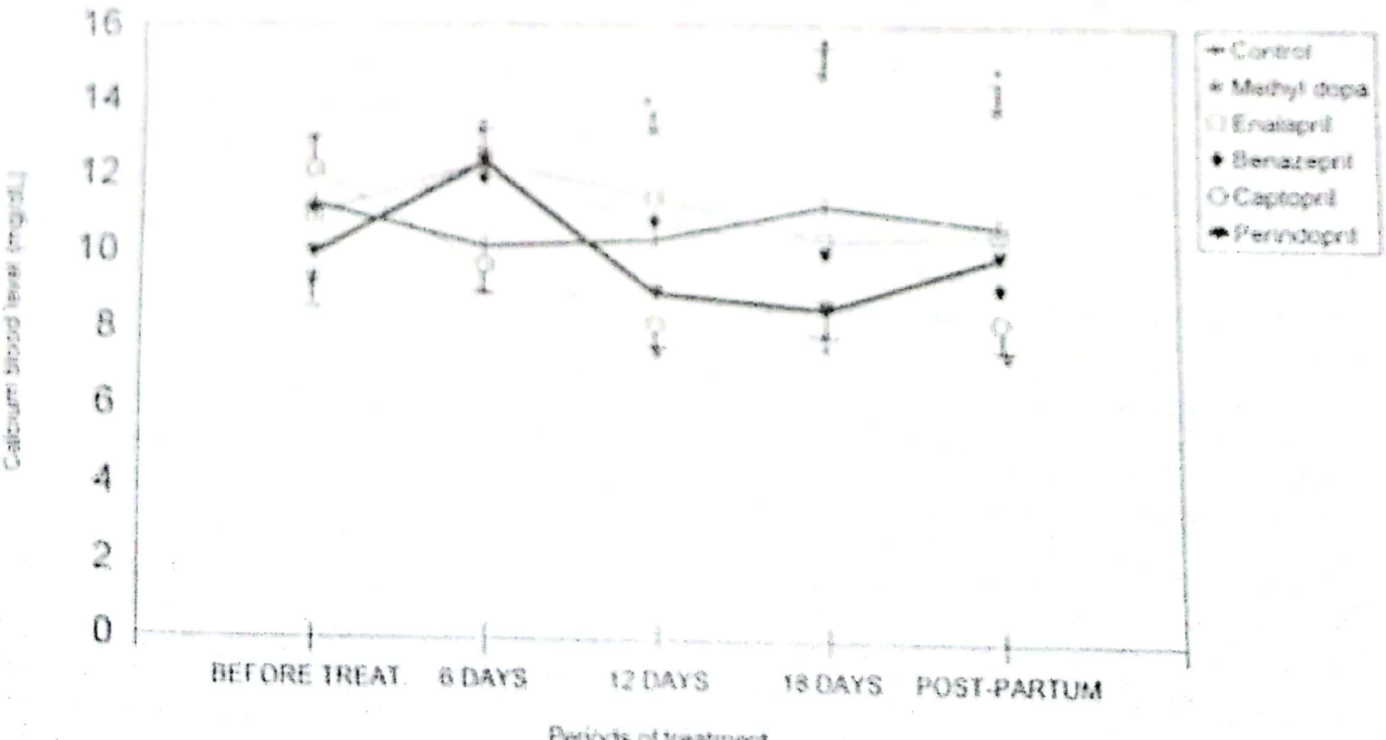
* Significantly different from control value at P<0.05

Egyptian J. Pharm. Sci., June 1997
Vol. 6, No 1, pp. 77-86



Fig(10): Effect of oral administration of alpha methyl dopa, enalapril ,benazepril, captopril and perindopril on potassium blood levels after 6, 12 and 18 days during gestation, and postpartum in pregnant female rats.

* Significantly different from control value at P<0.05



Fig(11): Effect of oral administration of alpha methyldopa, enalapril, benazepril,captopril and perindopril on calcium blood levels after 6, 12, and 18 days during gestation, and postpartum in pregnant female rats.

* Significantly different from control value at P<0.05

DISCUSSION

During normal pregnancy, there is an increase in plasma renin concentration , renin activity, renin substrate, Ang II and aldosterone[11,27] . The circulating levels of Ang II rises early in pregnancy [3] , despite a fall in the arterial blood pressure . It was previously reported that , in normal pregnant women , the pressor response to Ang II before delivery was less than that after delivery and the pregnant women was blunted compared to that in non pregnant ones [5] . This refractoriness to Ang II is due to neither to changes in plasma renin activity nor to a baroreceptor response, but more likely the result of alterations in the characteristics of the vessels wall [6]. This refractoriness to infused Ang II was lost in pregnancies complicated by pregnancy - induced hypertension [28].

Results of the present study showed that captopril, enalapril, benazepril and perindopril produced significant reduction in the arterial blood pressure of both pregnant and non pregnant rats. The hypotensive action of all ACE inhibitors was significantly greater, in both the intensity and duration, in case of pregnant compared with the non pregnant rats. This difference in the effect between the pregnant and non pregnant rats did not occur after treatment with alpha methyl dopa .

These results are not consistent with that reported before [20] , who found that the responses to Ang II were increased during both short - and long - term (24 hours) infusion of ACE inhibitors in pregnant ewes. They also reported that the increased sensitivity to Angll in presence of ACE inhibitors may be due to the increased number of functional Ang II receptors in vascular smooth muscles during the long - term infusion. In other world, there is an increase in the availability of existing Ang II receptors that have been freed from their substrate because of the decrease in the endogenous levels of Ang II production after the exposure to ACE inhibitors [20] .

The main reason for this difference in our finding and that of those authors is the dose regimen, since ACE inhibitors in this study were given in the form of a single dose but they gave it by continuous intravenous infusion for 24 hours. They found that the effect of long-term infusion was more pronounced than the short - term one. Thus due to the fact that Ang II receptors in vascular smooth muscle will be decreased or down - regulated during pregnancy [6] . One would not expect that the short-term infusion [20] or single IP dose of ACE inhibitors , will be associated with a concomitant increase in Ang II receptor availability since this needs at least 24 hours for regeneration [29] . However, long - term infusion of ACE inhibirors induces long - term inhibition of Ang II synthesis and reduction of its plasma levels , resulting in supersensitivity of the existed Ang II receptors or increasing of their availability in vascular tissues [6].

According to results of the present study, the possible suggested mechanisms of the pronounced hypotensive action of acute and single dose of ACE inhibitors in pregnant rats are :

a- Pregnancy is associated with a specific reduction in the pressor response to Ang II, however, responses to norepinephrine are not altered [8]. This observation is consistent with the concept of decreased receptor availability rather than a modification in vascular reactivity. The decrease in receptor availability may be secondary to prior receptor occupancy [30], decreased receptor affinity [31] or a decline in the total receptor population [32] . The reduction of the number and availability of Ang II receptors in vascular tissues and their down regulation [20] may be due to the increased concentrations of circulating Ang II during pregnancy [8].

Moreover, there is a possibility that pregnancy pr se may modulate the proportionality of the Ang II receptors subtypes , by increasing the number of the vasodilating Ang II subtype 2 receptors . Ang II was reported to bind to at least two specific receptors , Ang II receptor subtype 1 (AT1) and subtype 2 (AT2)[33] . Actions of Ang II on the blood pressure , involved, vasoconstriction, stimulation of catecholamine release and aldosterone secretion are mediated through binding to AT1 receptors [34] . Ang II can induce reduction in the arterial blood pressure of rats after losartan - induced AT1 receptor blokade [35] , therefore the hypotensive effect is probably mediated by stimulation of AT2 receptors. The sudden reduction of Ang II levels after single dose of ACE inhibitors [36] in presence of the down-regulated Ang II receptors and their refractoriness to Ang II [6], may be responsible for this difference in the effect of ACE inhibitors on the blood pressure between the pregnant and non pregnant rats. Since the length of time required for regeneration of Ang II receptors is approximately 24 hours [29]. The support of this possibility is that the tested ACE inhibitors but not alpha methyldopa, produced this significant difference in reduction of blood pressure reduction during pregnancy. The blunted pressor response to Ang II during pregnancy is specific to it, since the pressor responses to norepinephrine are similar in the pregnant and non pregnant state [8] . This means that Ang II receptor refractoriness and low responsiveness to Ang II is mainly responsible for this difference in the effect of ACE inhibitors in pregnant compared to non pregnant rats.

b- The elevated levels of both estrogen and progesterone that occurred during pregnancy in rats significantly increased the plasma levels of estrogen, progesterone and prolactin, this effect started after one week of pregnancy and continues till delivery. These results are in accordance with that previously reported [37] whom found that normal pregnancy increases the estradiol blood levels to 20-40 fold. Estrogen was reported to drive the renin- angiotensin system, by increasing angiotensinogen substrate[10]. The increased substrate may lead to an increase in Ang II but the progressive increase in angiotensinase protects the mother from the effect of Ang II [5]. The pressor response to the infused Ang II was suppressed by administration of estradiol [37]. It has been reported that there is an increase in cardiac output and vasodilatation after infusion of estradiol both of which may modulate the vascular reactivity to some extent and offset the refractoriness to Ang II [38]. The remarkable increase in estrogen blood levels during normal pregnancy may be related to the mechanism of refractoriness obtained physiologically by modifying the vascular reactivity to Ang II [37]. Moreover, the increased progesterone levels during pregnancy, may play a major role in the developed refractoriness to Ang II. Progesterone treatment in human blunted the renal responses to Ang II, but does not affect the overal pressor response [39]. The 5-alpha dihydroprogesterone, a progesterone metabolite, can modulate the Ang II responsiveness either through prostaglandin (PG) production or a steroid action which is independent on prostaglandin action [40]. Although our results and the data mentioned by other authors are conflecting, we can not ignore the possible complicated role of female sex hormones in the hypotensive action of ACE inhibitors in pregnant rats. This will increase the possiblity of involvement of Ang II receptors availability in this potentiation, since alpha methyl dopa does not affect the RAAS and Ang II levels.

c-Increased production and delayed breakdown of the endothelin - derived - relaxant factor (EDRF), nitiric oxide (NO). It was previously reported that drugs that inhibit angiotensin - converting enzyme exert much of their therapeutic effect through enhancement of nitric oxide production, which in turn prevents the metabolism of bradykinin and increases its tissue concentration [41]. Moreover,bradykinin is a potent activator of constitutive nitic oxide synthetaze (NOS) [42] that in turns can increase the synthesis and production of NO[43]. Sulphahydral containing ACE inhibitors scavenge superoxide anions, thereby potentiating the EDRF by protecting it from superoxide- mediated destruction [44]. Additionally, sulphahydral as well as non sulphahydral ACE inhibitors reduce myocardial infarct size and activate the L-arginine and nitric oxide pathway, which protects the vasculature by improving local blood flow, prevnting platelet activation and exerting antiproliferative effects [45]. It is possible that there is an increase in the production of NO and inhibition of NO degradation during pregnancy that may be responsible for the existed hypotension which occurs during pregnancy inspite of the increased circulating Ang II levels.

d-The increased levels of bradykinin, a potent vasodilator, and prostaglandlins, that occur after treatment with ACE inhibitors[36]. These localhormones play a role in the hypotensive effect of these drugs [46], since administration of indomethacin, a cyclooxygenase inhibitor, significantly decreased both the hypertensive and the antihypertensive action of ACE inhibitors Bradykinin sites in a balance with Ang II and it is a competitive inhibitor of Ang I conversion. Bradykinin potentiating factors inhibit the destruction of bradykinin and block the conversion of Ang II [10]. An imbalance in this system may then explain the increase in sensitivity of the arterioles to Ang II and the clinical presentation of pregnancy - induced hypertension. The infusion of PGs lowers Ang II sensitivity and PGI 2 was found to be decreased in pregnancies associated with pregnancy-induced hypertension. The linkage of this system to PGs was provided by the fact that indomethacin decreased the refractoriness to Ang II in pregnant dogs [37] and normal pregnant women [10] and increased sensitivity to exogenous Ang II [6].

Administration of AGE inhibitors significiantly increased potassium blood levels, this effect started after two weeks of treatment. These results are in accordance with that previously reported [47]. There is no comment on the role of hyperkalemia in the existed refeactoriness to Ang II and the potentiation of the hypotensive action of ACE inhibitors during pregnancy, because these drugs induced hyperkalemia only after two weeks of treatment, while their effect on the arterial blood pressure was studied after single acute dose and the effect of this dose on potassium blood level was not determined.

In respect to the effect of ACE inhibitors on glucose, cortisol and cholesterol blood levels during pregnancy. Our resuls showed that non - of the given ACE inhibitors significantly change the blood levels of glucose, cortisol and cholesterol during pregnancy. These results are in confelection with that reported before [19] in case of blood glucose. This discrepancy may due to that they tested the effect of these drugs in non pregnant women and animals, however, our study was carried out in pregnant rats. In another word, we found that pregnancy per se induced hyperglycemia, at the time in which ACE inhibitors can reduce the blood

glucose levels and increase the glucose disposal rate[48]. So with caution, we can say that the effect of ACE inhibitors on blood glucose may be masked by the pregnancy - induced hyperglycemia.

In conclusion, acute intraperitoneal injection of ACE inhibitors in a single dose produced significant reduction in the arterial blood pressure in both pregnant and non pregnant rats. The intensity and the duration of the hypotensive effect of these drugs was significantly greater in case of pregnant compared with the non pregnant rats. The main reason for this difference is the presence of refractioriness to Ang II during pregnancy, that results from the sustained elevated levels of Ang II, as well as the modulation of the number and availability of Ang II receptors. This refractoriness may be the result of the elevated levels of female sex hormones, increased production of NO and accumulation of bradykinin that occur during pregnancy.

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تأثير الحمل على الانخفاض فى ضغط الدم المستحدث بالأدوية المثبطة للانزيم المحول للانجيوتنسين فى اناث الجرذان

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تمت دراسة تأثير عقار الكابتوبريل ، الانالابريل ، البنزابريل ، البربندوبريل والفا ميثيل دوبا ، بعد حقنها داخل البريتون، على ضغط الدم فى الجزذان الحوامل وذلك بالمقارنه مع مجموعة الجرذان الضابطة والغير حوامل ، وكذلك تمت دراسة تأثير هذه الأدوية بالمقارنه بالفا ميثيل دوبا على مستوى الهرمونات الأنثوية فى الدم مثل الاستراديول ، البروجستيرون ، البرولاكتين وكذلك تأثيرها على الكورتيزول ، الكوليستيرول ، الجلوكوز ، البوتاسيوم والكالسيوم.

وقد أشارت النتائج إلى أن أحداث الحمل فى اناث الجرذان ادى إلى زيادة ذات دلالة احصائية فى قوة وفترة التأثير الخافظ لضغط الدم للادوية المثبطة للانزيم المحول للانجيوتنسين وذلك بالمقارنه بمجموعة الجرذان الضابطة ، بينما لم يحدث ذلك فى حالة المعالجة بالفا ميثيل دوبا . ومن ناحية أخرى فإن الحمل فى الجرذان قد أدى إلى زيادة ذات دلالة احصائية فى مستوى الاستراديول ، البروجستيرون ، البرولاكتين والكورتيزول . ومن ناحية تأثير الأدوية على مستوى الهرمونات فإن حقن الفا ميثيل دوبا قد أدى إلى ارتفاع مستوى الاستراديول ، البروجستيرون والبرولاكتين فى الدم بينما إدى إلى نقص مستوى الكورتيزول، وقد بدأ هذا التأثير بعد اليوم السادس من الحمل واستمر حتى بعد الولادة بثلاثة أيام بالمقارنه بالمجموعة الضابطة . كما أن حقن البريندوبريل أدى إلى ارتفاع مستوى كل من الأستراديول والبروجستيرون وذلك بعد ١٨ يوماً من بداية الحمل بينما إدى عقار البنزابريل إلى خفض مستوى الاستراديول والبرولاكتين ورفع مستوى البروجستيرون فى الدم وقد بدأ هذا التأثير من اليوم السادس من الحقن وأن الفا ميثيل دوبا والبنزابريل قد أدبا إلى ارتفاع مستوى السكر فى الدم ارتفاعاً ملحوظاً وهذا التأثير قد بدأ من اليوم الثانى عشر من بداية الحمل والمعالجة. اضافة إلى ماسبق فإن الكابتوبريل ، الانالابريل والبنزابريل قد احدثوا زيادة فى مستوى ايون البوتاسيوم فى الدم وفى الوقت الذى أدى فيه الكابتوبريل إلى انخفاظ مستوى الكالسيوم فى الدم فإن الفا ميثيل دوبا احدث زيادة ملحوظة .

نستخلص من ذلك أن الحمل فى الجرذان بعضد من التأثير الخافض لضغط الدم والحادث بالحقن البريتونى للادوية المثبطة للانزيم المحول للانجيوتنسين وأن هذا التأثير لم يحدث فى حالة حقن الفا ميثيل دوبا . وأن المبكانيكية المحتملة لهذا التأثير قد ترجع ولو جزئياً إلى الزيادة الحادثة فى الهرمونات الأنثوية فى الجرذان أثناء الحمل ، والتى بدورها قد تؤدى إلى الزيادة فى مستوى الانجيوتنسين فى الدم والذى قد يترتب عليه عدم استجابة مستقبلات الانجيوتنسين -٢ ، وكذلك ربما تؤدى إلى التغير فى اتاحة وعدد ونوع المستقبلات الخاصة بالانجيوتنسين -٢ والموجودة اساساً فى الأوعية الدموية.