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A comparative study among effects of treatment with nifedipine, verapamil, diazepam or alpha-methyldopa on platelets count and behavior in women with preeclampsia

Atyaf Hasan Mohammed[1], Nawfal A.M. Numan[2], Ghazi F. Al-Temimi[2], Nada N. Al-Shawi[2], Hayder Adnan Fawzi*[3]

[1]Department of Obstetrics and Gynecology, Al-Jadriya Private Hospital, Baghdad, Iraq
[2]Department of Pharmacology and Toxicology, University of Baghdad, Baghdad, Iraq
[3]Department Clinical Pharmacy, Baghdad Medical City, Baghdad, Iraq

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ABSTRACT

This study was conducted to investigate the role of platelets -count and -behavior in the pathogenesis of mild and moderate preeclampsia (PE). Also, the effects of the specific treatment regimen in both cases of preeclampsia investigated. In mild PE only nifedipine had significantly higher platelet count compared to those without treatment, while in moderate PE both verapamil and nifedipine had significantly higher platelet count compared to those without treatment. Concerning platelet response to ADP; in mild PE all drugs show an increase in response compared to those without treatment, a similar finding reported in moderate. While platelet response to collagen, in mild PE both diazepam and verapamil show increased response compared to those without treatment, in moderate PE methyldopa and verapamil show increased response compared to those without treatment. In conclusion, platelets may be involved in the pathogenesis of preeclampsia. Nifedipine and verapamil produced effective enhancement of *ex vivo* platelets aggregation-induced by ADP or collagen and may evolve as antiplatelet agents that able to prevent the *in vivo* activation of platelets and exhaustion cycle, an action which could explain the observed effectiveness of calcium channel blockers for the prevention and treatment of preeclampsia other than diazepam or alpha-methyldopa used in this study.

* Corresponding Author

Name: Hayder A. Fawzi
Phone: +9647722627943
Email: hayder.adnan2010@gmail.com

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INTRODUCTION

Preeclampsia is a clinical syndrome observed after the 20th week of pregnancy, and it is the most common medical complication of pregnancy associated with increased maternal and infant mortality and morbidity. It characterized by the presence of blood pressure of 140/90 mmHg associated with proteinuria and oedema (ACOG, 2002). Evidence for the involvement of circulating platelets in preeclampsia includes a reduction in platelets count, a change in platelets aggregability (Norris *et al.,* 1993). A possible explanation for the increased platelets activation is an intrinsic change in platelets responsiveness or increased consumption and turnover within the microvasculature secondary to hypertensive vasospasm (Barden *et al.,* 1997). Furthermore, the formation of microthrombin in uteroplacental bed and the eventual damage occurs in some patients has been studied by many investigators (Gammill and Roberts, 2007, McKay, 1981). Conflicting reports were obtained; some there is unchanged in the reactivity in proteinuric preeclamptic patients; while, a reduction in platelets reactivity was reported by others (Craici *et al.,* 2014).

The use of potent hypotensive agents can often attain reduction of blood pressure to normal values. An agent which will lower blood pressure on a long-term basis and at the same time improve uteroplacental blood flow is needed. Nifedipine, a calcium channel blocker (CCB) of the dihydropyridine group can influence transmembrane calcium influx. It has a rapid onset of action not longer than 20 minutes orally in a dose of 10mg, and its hypotensive effect lasted 4 hours, with low incidence of side effects such as a headache, palpitation, and cutaneous flushing; they are rarely troublesome (Curtis and Scholfield, 2001, McDonald *et al.,* 1994, Shahad F Obeid *et al.,* 2018). Moreover, nifedipine has been used safely in humans for the treatment of preeclampsia and preterm labor. Data from the Michigan Medicaid Birth Defects Study failed to reveal an association between the use of nifedipine and congenital abnormalities; moreover, Magee LA et al. (1996) revealed that calcium channel blockers could be safely used in pregnancy (Magee *et al.,* 1996). Besides, nifedipine has been used as a tocolytic agent because of its ability to cause uterine relaxation. Some studies have shown it to be as effective as and safer than either terbutaline or magnesium sulfate in the management of preterm labor. The pharmacokinetics of nifedipine has been elucidated in the immediate postpartum period in patients with preeclampsia. Because of the increased plasma clearance of nifedipine in these patients, dosing every three to four hours may be necessary to achieve adequate antihypertensive control (Smith *et al.,* 2000, Barton *et al.,* 1990).

Concerning verapamil, such drug can block voltage-dependent calcium channels. The drug may pass through the placenta to the fetus, but there have been no reports of fetal complications or congenital disabilities due to the use of verapamil (National Collaborating Centre for, 2010). When orally administered, more than 90% of verapamil can be absorbed; but due to high first-pass metabolism, its bioavailability is much lower (10–35%). Furthermore, it is 90% bound to plasma proteins and has a volume of distribution of 3–5 L/kg. It takes 1 to 2 hours to reach peak plasma concentration after oral administration (Barton *et al.,* 1990). Furthermore, Lechner W (1993) demonstrated an excellent uterus relaxing property of such drug in addition to its efficacy as a hypotensive agent in pregnancy-induced hypertension (HTN) (Lechner, 1993).

Diazepam is a long-acting drug of the benzodiazepine group. It has anticonvulsant properties. It has been reported that the anticonvulsant properties of diazepam may be in part to its binding to voltage-dependent sodium channels rather than benzodiazepine receptors. Sustained repetitive firing seems limited by benzodiazepines' effect of slowing recovery of sodium channels from inactivation (Temte *et al.,* 2018). Moreover, the muscle relaxant properties of diazepam are produced via inhibition of polysynaptic pathways in the spinal cord (Date *et al.,* 1984). Diazepam is a positive allosteric modulator of the inhibitory neurotransmitter in the brain, gamma-aminobutyric acid (GABA) type A receptors (GABAA), a ligand-gated chloride-selective ion channels receptors. Binding of diazepam to this receptor complex promotes binding of GABA, which in turn increases the total conduction of chloride ions across the neuronal cell membrane which hyperpolarizes the neuron's membrane potential. As a result, the difference between resting potential and threshold potential is increased, and firing is less likely (Kita *et al.,* 2004).

Authors reported that an increased risk of congenital malformations and other developmental abnormalities associated with the use of benzodiazepine drugs during pregnancy had been suggested, but there may also be non-teratogenic risks associated with the use of benzodiazepines during pregnancy. There have been reports of neonatal flaccidity, respiratory and feeding difficulties, and hypothermia in children born to mothers who have been receiving benzodiazepines late in pregnancy. Furthermore, diazepam has been shown to be teratogenic in mice and hamsters when given orally at daily doses of 100 mg/kg or greater (approximately eight times the maximum recommended human dose [MRHD=1 mg/kg/day] or greater on an mg/m^2 basis). Cleft palate and encephalopathy are the most common and consistently reported malformations produced in these species by administration of high, maternally toxic doses of diazepam during organogenesis. Rodent studies have indicated that prenatal exposure to diazepam doses similar to those used clinically can produce long-term changes in cellular immune responses, brain neurochemistry, and behavior. In general, the use of diazepam in women of childbearing potential, and more specifically during known pregnancy, should be considered only when the clinical situation warrants the risk to the fetus (McElhatton, 1994, Amjed Haseeb Khamees, 2018).

Alpha-methyldopa is a centrally acting antihypertensive agent that results in the reduction of dopaminergic and adrenergic neurotransmission in the peripheral nervous system. This effect lowers blood pressure and causes central nervous systems (CNS) effects such as depression, anxiety, apathy, anhedonia, and parkinsonism (Mah *et al.,* 2009). Because it has been safely and successfully used to treat hypertension during pregnancy, some experts consider it to be the drug of choice for the treatment of non-emergent hypertension during pregnancy (Kattah and Garovic, 2013). This study

was designed to emphasize the role of platelets in this disorder by monitoring platelets -count and -aggregation in preeclamptic women (mild and moderate). Determine the effect after 3-days of treatment with nifedipine, verapamil, diazepam or methyldopa in mild and moderate preeclamptic women on the above parameters.

METHODS

Study design: This study was conducted from August 1995 to April 1996 by the participation of 63 outpatients' pregnant women attending the AbuGraib Hospital; Department of Obstetrics and Gynecology, Baghdad, Iraq. None of the women had cardiac, hepatic or renal dysfunction. None had any obstetrical abnormalities (diabetes, rhesus immunization). None had essential hypertension. After approval from the college of pharmacy/ University of Baghdad committee, written informed consent obtained from all women that participate in the study. The diagnosis was carried out according to WHO criteria (ACOG, 2002), which are based on clinical, laboratory diagnostic measures to detect hypertension and proteinuria in all patients. The levels of blood pressure and protein in urine were determined at the time of sampling. Women were classified into:

1. Fifteen healthy normotensive women in the 3rd trimester of pregnancy served as control group.
2. Twelve pregnant women in the third trimester of pregnancy as a newly-diagnosed preeclamptic control. They classified according to the severity of the disease (ACOG, 2002) into:
   a. Six with mild preeclamptic women
   b. Six with moderate preeclamptic women
3. Thirty-six pregnant women in the third trimester of pregnancy; after blood pressure measurement and protein in urine assessment; this group classified into mild, and moderate cases. They have received a specific treatment as follows:
   a. Mild preeclamptic (17 women):
      1) Six pregnant women; they received diazepam tablet 2 mg twice daily.
      2) Five pregnant women; they received Verapamil 40mg tablet three times daily.
      3) Six pregnant women; they received nifedipine 10mg capsule twice daily.
   b. Moderate preeclamptic (19 women):
      1) Six pregnant women; they received alpha methyldopa 250mg tablet three times daily.
      2) Five pregnant women; they received Verapamil 40 mg tablet three times daily.
      3) Eight pregnant women; they received Nifedipine 10mg capsule twice daily.

Women received their specific treatment until the day of delivery. Blood pressure, weight, fetal heart rate, and other clinical investigation were checked every three days by the gynaecologist. Undesirable side effects of each treatment were recorded. None of them necessitated reduction or cessation of treatment.

Laboratory analysis

A blood sample was taken after the third and six days of starting the treatment, and blood pressure continued to be measured every three days until the day of delivery.

Mid-stream urine was collected from women in a clear plastic tube and utilized to perform a test for protein. Venous blood sample (4.5 ml) were collected and immediately transferred into a plastic tube containing trisodium citrate dehydrate and it is utilized for the assessment of platelet aggregation (O'Brien *et al.,* 1986).

Also, another 5ml of blood was transferred into EDTA tubes for the measurement of haemoglobin (Hb) and platelets count (2003). Additionally, 5.5 ml of blood transferred into a clean disposable plastic tube — samples left at room temperature for the complete clotting. Serum aspirated after centrifugation of the blood. Serum samples were used to measure creatinine, sodium, potassium, and calcium (Zatelli *et al.,* 2012).

Statistical Analysis

Data presented as mean ± standard deviation, unpaired Students t-test was used to examine the difference in the means of the parameters tested. Analysis of variance (ANOVA) used for the comparison among multiple groups, post hoc Tukey U test used for assessment of each pair. A *P*-value less than 0.05 were considered significant.

RESULTS

Systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial blood pressure (MABP), weight, and platelet were significantly higher in moderately preeclamptic (PE) women compared to mild PE and both were significantly higher than normotensive women, as illustrated in table 1.

Ex vivo platelets aggregation of platelets rich plasma (PRP) in response to a different dose of ADP: at 5 μM, 10 μM, 15 μM, and 20 μM platelet aggregation was significantly lower in moderate PE compared to mild PE and normotensive women, additionally mild PE is significantly lower compared to normotensive women, the aggregation in response to 2.5mg/ml of collagen both mild and

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Table 1: Assessment of various variables between normotensive women versus preeclamptic women at baseline

Variables	Normotensive pregnant	Preeclamptic women		p-value
		Mild	Moderate	
Number	15	6	6	-
Age (years)	26±5.6	27.5±3.7	24.5±3.7	0.380
Systolic BP mmHg	117.33±4.5[a]	140.83±2.04[b]	156.7±10.1[c]	<0.001
Diastolic BP mmHg	77.33±4.2[a]	90.83±2.04[b]	106.6±8.2[c]	<0.001
Mean arterial BP mmHg	90.67±3.4[a]	107.5±1.4[b]	123.3±8.7[c]	<0.001
GA at blood sampling (weeks)	31.6±5.4	28.3±5.6	33±6.3	0.148
Urinary protein (g/L)	-	0.15±0.05	1.66±0.82	<0.001
Weight (kg)	65±2.8[a]	75±3[b]	84±2.5[c]	<0.001
Platelets (10^6/ml)	314±30[a]	238±20[b]	187±10[c]	<0.001
Aggregating Agent				
ADP 5 µM	17.5±0.4[a]	12±0.5[b]	6.6±0.6[c]	<0.001
ADP 10 µM	58.5±2.2[a]	14.3±0.34[b]	7.14±1.08[c]	<0.001
ADP 15 µM	60.82±0.9[a]	35.7±0.15[b]	15±0.3[c]	<0.001
ADP 20 µM	61.18±3.2[a]	42.5±1.4[b]	38.6±2.6[c]	<0.001
Collagen 2.5mg/ml	60±9[a]	46.86±6[b]	45.8±7[b]	<0.001

GA: gestational age; Data are shown as Mean ± SD; Variables carrying similar litters indicate no significant difference

moderate PE was significantly lower compared to normotensive women, as illustrated in table 1.

After three days of treatment; diazepam, verapamil, and nifedipine had significantly lower SBP, DBP, and MABP compared to those without treatment, assessment of the difference in blood pressures between the drugs showed that there was significant difference in SBP between nifedipine & diazepam, for DBP there was significant difference between verapamil & diazepam, for MABP there was significant difference between verapamil & diazepam, as illustrated in table 2.

After three days of treatment; the platelet count was significantly higher in nifedipine compared to those without treatment. Additionally, nifedipine had significantly higher platelet count compared to diazepam, as illustrated in table 2.

After 3 days of treatment; where, *ex vivo* platelets aggregation after diazepam was higher compared to both (verapamil and nifedipine) in all doses of ADP. Additionally, *ex vivo* platelets aggregation after verapamil was higher compared to nifedipine in all doses of ADP. Additionally, verapamil was significantly higher compared to nifedipine in all dose of ADP. The aggregation in response to 2.5mg/ml of collagen was significantly higher in diazepam and verapamil compared to those without treatment, as illustrated in table 2.

After 3 days of treatment, methyldopa, verapamil, and nifedipine each drug significantly reduced SBP, DBP, and MABP compared to those without treatment, assessment of the difference in blood pressures between the drugs showed that there was significant difference in SBP between methyldopa compared to both verapamil and nifedipine, for DBP there was significant difference between nifedipine compared to both methyldopa and verapamil, for MABP there was significant difference between verapamil compared to methyldopa and nifedipine, as illustrated in table 3.

After three days of treatment; the platelet count was significantly higher in nifedipine and verapamil compared to those without treatment. Additionally, all the three drugs show significant difference among each other, as illustrated in table 3.

After 3 days of treatment; *Ex vivo* platelets aggregation of platelets rich plasma (PRP) in response to a different doses of ADP: at 5 µM, 10 µM, 15 µM, and 20 µM platelet aggregation was significantly higher all the drugs compared to those without treatment (except at 20 µM ADP there was no significant difference between verapamil and those without treatment). To assess the difference among the individual drugs; at five µM, and 15 µM ADP all drug showed a significant difference when compared to each other, at ten µM, and 20 µM ADP the significant difference was between nifedipine compared to both methyldopa and verapamil. The aggregation in response to 2.5mg/ml of collagen was significantly higher in methyldopa and verapamil compared to those without treatment, as illustrated in table 3.

DISCUSSION

In mild and moderate cases of preeclampsia, there was an elevation in systolic, diastolic, and mean arterial blood pressure levels compared to normotensive control group. In preeclampsia, there are

Table 2: Assessment of the effect of various variables in mild preeclamptic women before and after treatment

Variable	Normotensive pregnant	Without treatment	Diazepam	Verapamil	Nifedipine	p-value
			Before treatment			
Number	15	15	6	5	6	-
Age (years)	26±5.6	27±3.7	28.3±5.2	28.8±3.7	28.5±5.8	0.696
Systolic BP mmHg	117.33±4.5^{a}	140.83±2.04^{b}	140.8±2.04^{b}	138±4.5^{b}	140.83±2.6^{b}	<0.001
Diastolic BP mmHg	77.33±4.2^{a}	90.83±2.04^{b}	90.8±2.04^{b}	90.83±2.04^{b}	91.7±2.85^{b}	<0.001
Mean arterial BP mmHg	90.67±3.4^{a}	107.5±1.4^{b}	107.49±1.4^{b}	106.66±2.4^{b}	108.05±2.2^{b}	<0.001
GA at blood sampling (weeks)	31.6±5.4	28.3±5.6	31±4.7	32±5.1	31±4.7	0.451
			After 3 days pf treatment			
Systolic BP mmHg	117.33±4.5^{a}	140.83±2.04^{b}	126.7±5.2^{c}	120.3±7.1^{ac}	118.3±4^{a}	<0.001
Diastolic BP mmHg	77.33±4.2^{a}	90.83±2.04^{b}	80.83±2.04^{c}	77±4.5^{d}	73.3±5.2^{cd}	<0.001
Mean arterial BP mmHg	90.67±3.4^{a}	107.5±1.4^{b}	96.11±2.5^{c}	91.33±4.5^{d}	88.33±4.1^{cd}	<0.001
Platelets (10^{6}/ml)	314±30^{a}	238±20^{b}	240±30^{b}	267±30^{bc}	290±40^{ac}	<0.001
			Aggregating agent			
ADP 5 µM	17.5±0.4^{a}	12±0.5^{b}	28.5±0.9^{c}	18.5±1.5^{ad}	15±1.5^{e}	<0.001
ADP 10 µM	58.5±2.2^{a}	14.3±0.34^{b}	60±1.9^{c}	48.25±1.7^{ad}	24.6±1.3^{e}	<0.001
ADP 15 µM	60.82±0.9^{a}	35.7±1.5^{b}	74.5±2.9^{c}	59.1±3.4^{ad}	39.79±4.5^{e}	<0.001
ADP 20 µM	61.18±3.2^{a}	42.5±1.4^{b}	77.1±4^{c}	55.94±1.8^{d}	46.25±1.2^{e}	<0.001
Collagen 2.5mg/ml	60±9^{a}	46.86±6^{b}	57.8±4.3^{ac}	58.79±8.6^{ac}	49.5±6.6^{bc}	<0.001

GA: gestational age, Data are shown as Mean ± SD; Variables carrying similar litters indicate no significant difference

some hemodynamic changes in the body characterized by an increase in the extracellular volume (Na^{+} /water retention) due to high levels of estrogen and progesterone (MacGillivray and Campbell, 1980). The increase in extracellular volume usually associated with an increase in cardiac output (COP) and may be associated with the increase in peripheral resistance (PR) which in turn, may lead to increase blood pressure [blood pressure=COP x Total PR] (Mayet and Hughes, 2003).

Oral treatment with Calcium channel blockers (CCBs) (nifedipine 10 mg twice daily; verapamil 40 mg twice daily) to mild or moderate cases of preeclampsia after 3 and 6-days of treatment resulted in a significant reduction in systolic, diastolic, and mean arterial blood pressure levels are reaching the values of normotensive pregnant controls. Calcium channel blockers act by blocking calcium entry into smooth muscle cells, thus interfering with the excitation-contraction coupling (Lullmann and Ziegler, 1987); and they are potent inhibitors of both vascular and extra-vascular muscle contraction (Cohn, 1983). Their action has been reported to improve renal function by decreasing renal vascular resistance and increasing renal blood flow, and they increase urine production by a natriuretic effect at the level of the proximal tubules. Furthermore, CCBs do not give rise to sodium retention (Gould *et al.,* 1982). This may partly be accounted for the lack of effect on the renin-angiotensin-aldosterone system (RAAS).

In this study, treatment of mild preeclamptic women with diazepam showed a significant reduction in systolic, diastolic and meant arterial blood pressure levels. Diazepam through the activation of gamma-aminobutyric acid (GABA) receptors in the CNS may lead to hyperpolarisation and stabilization of cells (Goodchild, 1993), and this may lead to decrease stress (anxiolytic); thus by this effect, diazepam may have beneficial effect in labile or stress hypertension that may occur during pregnancy.

Treatment of moderate preeclamptic women with alpha-methyldopa produced a significant reduction in systolic, diastolic and meant arterial blood

Table 3: Assessment of the effect of various variables in moderate preeclamptic women before and after treatment

Variable	Normotensive pregnant	Without treatment	Methyldopa	Verapamil	Nifedipine	p-value
			Before treatment			
Number	15	15	6	5	6	-
Age (years)	26±5.6	24.5±3.7	29.2±6.6	29±3.6	27.12±5.5	0.250
Systolic BP mmHg	117.33±4.5^{a}	156.7±10.1^{b}	155±8.4^{b}	154±5.5 ^{b}	154.4±8.2 ^{b}	<0.001
Diastolic BP mmHg	77.33±4.2^{a}	106.6±8.2^{b}	102.5±6.1^{b}	102±4.5^{b}	103.7±5.2^{b}	<0.001
Mean arterial BP mmHg	90.67±3.4^{a}	123.3±8.7^{b}	119.9±5.9^{b}	119.33±4.3^{b}	120.6±3.3^{b}	<0.001
GA at blood sampling (weeks)	31.6±5.4	33±6.3	32±4.9	30.4±3.6	32±5.9	0.913
			After 3 days pf treatment			
Systolic BP mmHg	117.33±4.5^{a}	156.7±5.5^{b}	131.7±4.1^{c}	118±4.3^{a}	117.5±5^{a}	<0.001
Diastolic BP mmHg	77.33±4.2^{a}	106.6±2.2^{b}	81.7±2.6^{c}	79±2.24^{c}	72.±2.8^{d}	<0.001
Mean arterial BP mmHg	90.67±3.4^{a}	123.3±5.7^{b}	98.34±4.8^{c}	92±3.8^{d}	87.5±5.5^{c}	<0.001
Platelets (10^{6}/ml)	314±30^{a}	187±10^{b}	206±40^{b}	269±20^{c}	320±50^{a}	<0.001
			Aggregating agent			
ADP 5 µM	17.5±0.4^{a}	6.6±0.6^{b}	31.6±0.4^{c}	14.4±0.3^{d}	36.7±0.7^{e}	<0.001
ADP 10 µM	58.5±2.2^{a}	7.14±1.1^{b}	35±3.2^{c}	33.5±1.9^{c}	63.7±4.4^{d}	<0.001
ADP 15 µM	60.82±0.9^{a}	15±0.3^{b}	32.8±2.5^{c}	28±1.1^{d}	65±3.9^{e}	<0.001
ADP 20 µM	61.18±3.2^{a}	38.6±2.6^{b}	43.3±1.2^{c}	40±0.7^{bc}	57.25±1.1^{e}	<0.001
Collagen 2.5mg/ml	60±9^{a}	46.8±7^{b}	58.1±6.5^{a}	57.57±8.5^{a}	50±6.2^{ab}	<0.001

GA: gestational age, Data are shown as Mean ± SD; Variables carrying similar litters indicate no significant difference

pressure levels. These results are consistent with those of others (Redman, 1976). Alpha-methyldopa acts centrally through the sympathetic nervous system by decreasing sympathetic outflow.

Lower levels of circulating platelets observed in both mild and moderate preeclamptic women compared to the corresponding levels in normotensive pregnant controls. These results are in agreement with those obtained by works of authors, who reported that the reduction of platelets count in preeclampsia may be associated with abnormal activation of the coagulation system, and believed to reflect increased platelets consumption and thus, the suggestion revealed that changes of platelets consumption might be an early marker for preeclampsia (Jaleel and Baseer, 1997, Chauhan *et al.,* 2014). In the present study, the results showed that there is a change in platelets behavior in both cases of preeclampsia compared to normotensive controls. Platelets were less responsive to the stimulatory effects of ADP and collagen, and the percent of maximum aggregation was reduced by 10% with of the aggregating agent. This could be due to lower platelets count. These are consistent with those established by others that platelets aggregation in response to ADP and collagen reduced in moderate preeclampsia and only a slight reduction was observed in mild preeclampsia (Norris *et al.,* 1993). The reduction in *ex vivo* response to ADP and collagen in moderate preeclampsia may be due to increased activation of platelets in microcirculation (O'Brien and Heywood, 1966). Such an increase may lead to abnormal or imbalance between the aggregation and disaggregation of platelets. Furthermore, during the process of *in vivo* activation and aggregation, these platelets become partially exhausted. This exhaustion was depicted by the phenomenon of hypo-aggregation of these platelets when examined (Norris *et al.,* 1993).

In patients with moderate preeclampsia, production of new platelets cannot keep pace with platelets consumption, platelets count decline, and there was a reduction in platelets responsiveness; while in mild preeclampsia, platelets activation appeared to be largely balanced by increased production of young platelets from the bone marrow.

Moreover, the platelets in these patients may contain less degranulated platelets and hence showed only a small decrease in platelets response to the aggregating agents. Regarding the mechanism of activation of platelets during pregnancy, Wallengurg and Rotmans in 1982 reported that enhanced reactivity of platelets-TXA- pathway have been shown to occur in normotensive and hypertensive pregnancies (Wallenburg and Rotmans, 1982). Moreover, it has been suggested that PGI2 reduction in hypertensive pregnancies can make the circulating platelets even vulnerable to aggregation and activation in the retro-placental space. The removal of aggregated platelets might be responsible for the reduction of platelets count as observed in the present study and the studies of others. Additionally, platelets-activating factor (PAF) may modulate blood pressure and platelets function in normal and hypertensive pregnancies; and it has been reported that preeclamptic women showed a decrease in response to the stimulatory effect of PAF on platelets aggregation, and this abnormality of platelets behavior could be reversed by the treatment with the antiplatelet therapy (acetylsalicylic acid) (Askie *et al.,* 2007).

It could be suggested from the current study that each nifedipine or verapamil may act by inhibiting the aggregation of platelets, decreasing the synthesis of thrombogenic stimulators and vasoconstrictors mainly TXA2 in the maternal circulation, and may cause a rise in platelets count and these effects can ameliorate the clinical picture of preeclampsia. Thus, the intended CCBs utilized in this study may have antiplatelet effect; where such agents indicated in women with either mild or moderate preeclampsia can cause an increase in *ex vivo* aggregation following stimulation with ADP or collagen and this increase in platelets response reached that of the normotensive controls. Thus, the results of this study suggested that both CCBs decrease *in vivo* platelets activation and thereby decrease platelets exhaustion revealed by *ex vivo* study. The finding confirms this observed from the work of others; where, the authors utilized acetylsalicylic acid as an antiplatelet agent (Askie *et al.,* 2007).

The mechanisms by which CCBs affected platelets behavior may be proposed as follows: Inhibition of calcium entry into cells and decrease the mobilization of calcium may lead to decrease platelets activation and aggregation (Ware *et al.,* 1986). Calcium channel blockers may inhibit the calcium-dependent enzyme, (phospholipase A2) and hence decrease the release and synthesis of PAF, PGs, and leukotrienes (Sandoo *et al.,* 2010). Calcium channel blockers have a vasodilator effect directly through a decrease in calcium influx as mentioned previously; and indirectly through the activation of PGI2 synthesis by the endothelium layer of blood vessels which has anti-aggregatory effect through an increase of cyclic adenosine monophosphate (cAMP), which in turn may inhibit the mobilization of arachidonic acid from membrane phospholipids (Marcus, 1978). Additionally, accumulation of cAMP may inhibit the conversion of arachidonic acid to TXA2 as well. There is also some evidence that cAMP may prevent the release of calcium from the storage granules (Smith *et al.,* 1980). Calcium channel blockers may act as receptor antagonists for certain vasoconstrictor agents like TXA2 (Marcus, 1978). Verapamil in therapeutic concentration may inhibit the shape change reaction induced by serotonin. Furthermore, such drug may be considered as an antagonist of serotonin receptor type 2 ($5\text{-}HT_2$) of platelets (August *et al.,* 1986).

Concerning diazepam, such drug can act on the central benzodiazepine receptors and peripheral receptors expressed in kidney, heart, and adrenals. Furthermore, peripheral receptors have also been expressed in human uterus, ovary, fallopian tube, and placenta; these receptors may inhibit the contractility of these organs and thus, may lead to decrease the liberation of serotonin or PGs from the placenta (Saano, 1988, Shaw *et al.,* 2010). Chesney et al. at 1987 demonstrated that alprazolam and triazolam (derivatives of benzodiazepines) are potent and specific inhibitors of PAF-induced activation of human platelets *in vivo*; but they have no significant inhibitory effects on platelets activated by ADP, epinephrine, collagen, or other aggregating agents (Chesney *et al.,* 1987).

Ex vivo platelets aggregation in response to different concentrations of ADP and 2.5mg/ml collagen in moderate preeclamptic women treated with alpha-methyldopa showed a significant increase in the % of maximal platelets aggregation compared to the pretreated group. Motulsky et al. in 1983 demonstrated the existence of alpha2-adrenergic receptors in human platelets membrane; furthermore, epinephrine initiated platelets aggregation by binding to such type of receptor on platelets membrane. In preeclampsia, there is an increase in catecholamine levels, and this may participate in the initiation of platelets activation (Motulsky *et al.,* 1980). Alpha-methyldopa acts through stimulation of presynaptic alpha2 receptors (Saboor *et al.,* 2013). Thus, after six days of treatment with alpha-methyldopa, there was an increase in *ex vivo* aggregation of platelets compared to pre-treatment levels.

CONCLUSION

The results of this study suggested that platelets were involved in the pathogenesis of preeclampsia; furthermore, a decrease in platelets reactivity was seen in both mild and moderate preeclampsia

when platelets stimulated by different concentrations of ADP and 2.5mg/ml collagen. Calcium channel blockers (nifedipine and verapamil) produced effective enhancement of *ex vivo* platelets aggregation-induced by ADP or collagen and may evolve as antiplatelet agents that able to prevent the *in vivo* activation of platelets and exhaustion cycle, an action which could explain the observed effectiveness of CCBs for the prevention and treatment of preeclampsia other than diazepam or alpha-methyldopa used in this study.

Conflict of interest

None

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