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Article

# Effect of P-glycoprotein inhibitor (Carvedilol) on pharmacological and hematological effects of its substrate (methotrexate) on pregnant and lactating rat mothers

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**Abstract**

Sixty albino rats (40 female rats and 20 males) were allocated into four dosing groups administered orally carvedilol (T1) 0.72mg/kg, methotrexate (T2) 0.36mg/kg, combined doses (T3) carvedilol + methotrexate and control group (Distilled water) for 2 months in male and 2 weeks in female rats before mating and after copulation and approval of pregnancy, dosing continued in female groups during pregnancy and lactation periods. Half of the animal groups were euthanized one day before parturation to determine P-gp concentration in the placenta and liver of pregnant mothers. In contrast, the other half was left for parturition and lactation to study the effects of carvedilol (P-gp inhibitor) on methotrexate (P-gp substrate) when given alone and in combination with some pharmacological, hematological, and biochemical parameters in lactating mothers and pups at the end of lactation. The pharmacological results showed the highest significant antinociceptive responses both in the early and late phase in the T3 (CV+MTX) followed by the T2 (MTX) group. In contrast, the T1 (Cv) animal group showed nearly resembling results as that of the control group in the early and late phases of the formalin test. The written test also recorded the same result as the Formlin test, indicating a significantly higher analgesic effect in T3 followed by T2 in comparison to T1 and control groups. The TNF alpha results supported the anti-inflammatory effect, recording a significant decline mainly in the T3 and T2 groups. The hematological results recorded significantly more reduction in WBC levels in mother and their pups in comparison with T1 and control groups. The serum electrolyte levels (Na+, K+, Ca+2) recorded in all treated groups and pups showed a significant increase in potassium levels in the T1 and T2 mother rat groups. The P-glycoprotein (P-gp) concentration measured in liver and placenta of euthanized pregnant mother before delivery recorded potent inhibition of P-gp in T1 & T3 groups which might altered pharmacokinetic and pharmacodynamic effects of substrate by carvedilol that considered a potent P-gp inhibitor drugs, this might explain our results of increase in pharmacological effect of methotrexate and their side effect on electrolyte concentration and hematology in combined group of CV+ MTX.

**Keywords:** Carvedilol, Methotrexate, p-gp inhibitor, Anti-inflammatory, Hematology, Electrolyte.

## Introduction

ATP-binding cassette (ABC) efflux transporters encompass 49 members in humans that are primarily implicated in the movement of endogenous substances, drugs, xenobiotics and metabolites across cell membranes [1,2]. The ABCB1 gene, or the multidrug resistance 1 gene, encodes P-gp (MDR1). A wide variety of substrates with various ionization states, acid/base characteristics, hydrophobicities, or amphipathic qualities can be transported by P-gp. P-gp is the most thoroughly researched drug transporter widely expressed in human and animal bodies [3]. Intestinal epithelium, liver hepatocytes, kidney proximal tubular epithelium, brain microvessel endothelial cells, and placental syncytiotrophoblasts all have significant levels of P-gp expression on their apical membranes. [4,5], therefore, plays an essential role in the renal clearance, absorption, biliary elimination, brain, and distribution of drugs and xenobiotics [6,28]. P-gp can also restrict fetal exposure to drugs and xenobiotics due to its expression and activity in the placental barrier [7]. Methotrexate (MTX) is an antifolate metabolite that inhibits DNA synthesis, repair, and cellular replication. It was first used as one of the essential treatments for pediatric leukemia [8,9]. According to previous studies, MTX has also been used to treat rheumatoid arthritis (R.A.) and psoriasis as an anti-inflammatory and immunomodulatory agent [10] Methotrexate, as one of the alternative pharmacological steroid-sparing immunosuppressive agents, is becoming more and more popular as the preferred treatment in several autoimmune conditions requiring long-term immunosuppression [11] P-gp confers resistance to some hydrophobic MTX-related antifolates, such as trimetrexate, which enter cells by passive diffusion [12]
Carvedilol is an adrenergic receptor (A.R.) antagonist blocking several A.R. types. Specifically, it acts as an inverse agonist at the β1- and β2-ARs and blocks α1ARs in the vasculature, thereby causing vasodilatation (Lymperopoulos et al., 2015). Carvedilol is a potent inhibitor of this plasma membrane transporter. This leads to several potentially significant drug-drug interactions of carvedilol, such as with cyclosporine and digoxin, two major P-glycoprotein substrates [12]
Essential clinical evidence is that interaction between methotrexate and carvedilol for p.gp may occur in pregnant humans who suffer from chronic diseases treated with such drugs with possible pharmacological consequences that might occur, so we tried to explore such effect and to modulate the role of P-gp. Inhibitors induce such effects through their combined

## Materials and methods:

Animals: A total of sixty (60) Albino rats consisting of forty females and twenty males were used to perform different studies. They aged over 14-16 weeks and weighed (200- 250) grams. They were fed standard pellets and drank tap water adlibitum. The animals were left in special cages with optimal conditions two weeks before the experiment. There maintained the standard conditions at a 12/12 hour light–dark cycle (20-25$^{o}$C) in an air-conditioned room. The bed was wood shaves that continuously changed, and the cages were cleaned twice per week. The female was separated from the male one month before the study for acclimatization and synchronization. After that, the animal kept a 2:1 female-to-male rate ratio in each cage. These studies were performed under the rules and ethics for management laboratory animals submitted by the University of Baghdad and under the supervision of the side ethical committee in the College of Veterinary Medicine.

Pharmacological study: A total number of sixty Albino rats (40 female and 20 male rats treated orally with therapeutic doses of carvedilol, methotrexate and in combination) for two months in males and 2 weeks in the females before mating and after conception of the pregnant female rats divided equally according to treatment regimen into four groups (T1, T2, T3 and control group) each group consist of ten female rats as following

T1 group (Cv): Pregnant female administered oral therapeutic dose of Carvedilol (Cv) 0.72 mg/kg B.W. during pregnancy and continued dosing during lactation period [13].

T2 group (MTX): An oral therapeutic dose of Methotrexate (MTX) administered at the same regimen as in T1, at 0.36mg/kg B.W./week [14].

T3 group: (Cv + MTX) Same dosing regimen as in T1 and T2 but in combination.

C: Control group Same regimen dosing with distilled water.

Half of the animals were euthanized by anesthesia in each group one day before delivery for prenatal study, while the other half were kept to study the postnatal effect in their pups group during the lactation period.

Parameters of study:

1. Anti-inflammatory effect in lactating mothers at end study

A. Formalin test

Formalin (2.5%) was administered subcutaneously (S.C.) into the rats' dorsal surface of the right hind paw using a sterile insulin syringe. The responses were measured for two distinct phases ( No. of licking and flicking of injected paw ), the initial 5 min. After formalin injection, it is known as an acute or early phase of formalin-induced pain and lasts between 15 and 40 min as the chronic or late phase [15]. Then, the dose of Methotrexate in T2 was compared for the analgesic and anti-inflammatory effect of carvedilol alone (T1) and their combination group (T3) compared with that of control one at the end of the lactation period.

B. Writhing test

The method performed according to that of [16,29] was used in this test. The study was performed at the end of the lactation period for all four treated groups (five [5] female rats in each group). The first group was treated with methotrexate at a dose of 0.36mg/kg/week P.O. (T2) compared with the alone dose of the carvedilol group (T1) and their combined group (T3) compared with the control group. Each rat from the treated groups was injected with 10 ml/kg of an aqueous solution of 0.7% acetic acid i.p. and placed in a transparent glass box. The number of writhes/stretches was counted for 30 min.

2. Blood sample: Blood samples were collected from all rats (mothers and pups) at the end of the experiment. The blood was collected from cardiac puncture from anesthetized rats by intramuscular injection of ketamine (90mg/kg B.W.) and xylazine (40mg/kg B.W.) [17]. The collected blood samples were kept in two tubes, one with EDTA anticoagulant in order to study the following hematological parameter (Hb, PCV, RBC count and WBC counts), while the other collected tubes without anticoagulant used to measure both serum electrolyte levels (Ca+2, Na+, K+) and tumor necrotic factor alpha(TNF-α) (mother only) for each treated groups after separating serum of blood samples by centrifuge at 250 rpm for 15 minute and then serum samples were put in escrow tube and serum stored at -20°C until analysis [18,19].

3. Serum electrolyte: The absorbance of Ca+2, Na+, and K+ in standards and sample solutions was measured by a flame atomic absorption spectrophotometer (FAAS).

4. Serum TNF alpha of mother at the end of lactation According to the sandwich method applied by (Bioassay Technology Laboratory China company).

5. P-glycoprotein in the placenta and liver of pregnant rat groups euthanized the day before parturition.

Liver and placenta homogenate preparation

The liver and placenta samples are used to prepare their tissue homogenate after animal euthanization and after surgical removal of the liver and a placenta from euthanized pregnant rats one day before parturition and washing the organs with phosphate buffer saline\ pH 7.4 (PBS) placed in a Petri dish in order to remove excess blood thoroughly, after that a weight of (5) grams from liver and (4) grams of placenta were put in screw tube after cutting to small pieces by using the scalpel and transferred them to glass homogenizer with mince tissue and homogenize with PBS on ice for few minutes, then transferred the homogenized tissue to glass tubes and freezing at −20 °C, were thawed at 2-8°C followed by centrifugation at 3000 RPM for approximately 20 minutes, then isolated the supernatant. The hemogenate samples were stored frozen at −20 ◦C until use [20].

## Results

*Determination of analgesic and anti-inflammatory effects of the used drug in lactating mother groups:*

A. Formalin test results:

The results of the formalin test listed in Table (1) showed that there was a significant decline ($p<0.05$) in nociceptive response (No of licking/min.) between different treated groups (T1, T2, T3) and control one, Also between acute and chronic phase for all treated groups, especially in combined T3 group (MTX+ Cv) that showed a significant decline than T2 group (MTX) and control one. In contrast, T1 showed no significant difference in response level from the control one at the early phase of the experiment (0-5 minutes). The same pattern was noticed in the late phase (15-45 min) with a significantly ($p<0.05$) higher increase than early phase responses (number of licking and flicking between treated groups of two phases. The T3 group (MTX+Cv) showed the highest significant antinociceptive responses in the early and late phases, followed by the T2 (MTX) group. In contrast, the T1 (Cv) animal group showed nearly resemblant results to the control group in the early and late phases of the formalin test.

| **Groups**<br><br>**Phases** | **Control**<br><br>**M±S.E** | **T1**<br><br>**M±S.E** | **T2**<br><br>**M±S.E** | **T3**<br><br>**M±S.E** |
|---|---|---|---|---|
| Early phase (First 5 min.) | **21.4±1.63**<br>**B a** | **20±1.84**<br>**B a** | **17±1.30**<br>**B b** | **15.4±0.81**<br>**B b** |
| Late phase (15-45min.) | **50±1.37**<br>**An a** | **50±1.14**<br>**An a** | **39.8±0.86**<br>**A b** | **34.8±1.74**<br>**A c** |
| LSD | **2.75** | | | |

**Table 1. The formalin test analgesic and anti-inflammatory effect (No licking/min.) of different treatments with Carvedilol, MTX alone and combination in mother rat at the end lactation period.**

**Control: The control group administered distilled water.**

**T1: 0.72mg/kg B.w Carvedilol orally.**

**T2: 0.36mg/kg B.w Methotrexate orally.**

**T3: 0.72mg/kg B.w Carvedilol + 0.36mg/kg B.w Methotrexate.**

**-Different capital letters donate significant group differences (P<0.05).**

**-Different small letters donate significant differences between groups(P<0.05).**

### Writhing test results

The results of the writhing test listed in Table (2) showed less pain reflex writhing/30 minutes. In the group of an animal dosed with combination drugs (MTX+Cv) which was significantly lesser than that of T2- MTX (23±1.14), while the T1- Cv group showed nearly the same effect as the control but significant differences were recorded with that of T2 and T3 groups results.

| Groups | Control<br>M±S.E | T1<br>M±S.E | T2<br>M±S.E | T3<br>M±S.E |
|---|---|---|---|---|
| NO. of writhes Within 30 min | 28±1.78<br>A | 29.8±1.85<br>A | 23±1.14<br>B | 18.2±1.39<br>C |
| LSD0.05 | 4.711 | | | |

**Table 2. The analgesic effect of different treatments with carvedilol alone and in combination with methotrexate at the end of the lactation period on induced writhe No/30 min. by acetic acid in mother rat.**
**Control: The control group administered distilled water.**

**T1: 0.72mg/kg B.w Carvedilol orally.**

**T2: 0.36mg/kg B.w Methotrexate orally.**

**T3: 0.72mg/kg B.w Carvedilol + 0.36mg/kg B.w Methotrexate.**

***-Different capital letters donate significant differences between groups(P<0.05).***

*Hematology*

WBC, RBC, Hb, PCV levels in mothers of experimental groups.

The results listed in Table (3) showed that there was a significant decline (P<0.05) in WBC level numbers of combined doses of the mothers' group as compared with those of the control one and T1 group. The highest significant reduction was noticed in the T3 group that recorded (4.39±0.93x$^{10\text{^}3/\ ml}$) of WBC, followed significantly (p<0.05) T2 (6±0.50) and T1 (7.54±0.22), while T1 WBC level recorded nonsignificant differences than the control group.

The result of the Dosed mother groups' RBC, Hb, and PCV levels recorded no significant differences between all treated groups and the control one at the end of the lactation period.

| Groups | WBC<br>10^3/ml<br>M±S.E | RBC<br>10^6/ml<br>M±S.E | Hb<br>g/dl<br>M±S.E | PCV<br>%<br>M±S.E |
|---|---|---|---|---|
| Control D.W. | 6.94±1.03<br>AB | 5.006±0.63<br>A | 9.02±1.77<br>A | 26.2±5.13<br>A |
| T1 0.72mg/kg | 7.54±0.22<br>A | 4.62±0.28<br>A | 10.66±0.43<br>A | 32.66±0.73<br>A |
| T2 | 6±0.50A | 5.57± 0.32 | 10.86±0.34 | 32±1.29 |

| | | | | |
|---|---|---|---|---|
| **0.36mg/kg** | BC | A | A | A |
| **T3 0.72mg/kg+0.36mg/** | 4.39±0.93 C | 4.91±0.68 A | 9.26±1.127 A | 29.2±0.95 A |
| **$LSD_{0.05}$** | **2.251** | **1.325** | **3.254** | **9.550** |

**Table 3. WBC level (10^3/ml), RBC level (10^6/ml), Hb level (g/dL), PCV level (%) of mother rats treated orally carvedilol, MTX alone and combined at the end of the lactation period**

**-Different capital letters donate significant differences between groups(P<0.05).**

WBC, RBC, Hb, PCV levels in pups of experimental groups:

The results listed in Table (4) showed that there was a significant decline (P<0.05) in WBC levels of the pups group of T3 and T2 as compared with those of the control and T1 group. The highest significant decline was noticed in the T3 group, recorded (at 3.70±0.74 $x^{10^3/ml}$) of WBC, followed significantly by T2 (4.68±0.21) and T1 (10.1±0.33), while T1 WBC level recorded nonsignificant differences than the control group.

In the RBC level of the dosed pups group, the results showed a nonsignificant difference (P<0.05) in the RBC level number of pups of treated groups (T1, T2, and T3) in comparison with the control.

The Hb level results showed a significant increase (P<0.05) in Hb of T3 and T2 groups pups compared with the control alone and T1. In contrast, the PCV level showed a significant increase (P<0.05) in the PCV level of T3 and T2 compared withT1, while the T1 pup's PCV level recorded nonsignificant differences from the control one.

| **Groups** | **WBC 10^3/ml M±S.E** | **RBC 10^6/ml M±S.E** | **Hb g/dl M±S.E** | **PCV % M±S.E** |
|---|---|---|---|---|
| **Control D.W.** | 8.78±0.39 A | 4.57±0.20 A | 5.16±1.29 B | 16.85±3.95 A |
| **T1 0.72mg/kg** | 10.1±0.33 A | 4.57±0.23 A | 4.80±0.17 B | 14.42±0.53 A |
| **T2 0.36mg/kg** | 4.68±0.21 B | 4.38± 0.26 A | 8.42±0.27 A | 23.24±0.98 A |
| **T3 0.72mg/kg+0.36mg/** | 3.70±0.74 B | 5.07±0.11 A | 10.08±0.26 A | 21.5±1.14 A |
| **$LSD_{0.05}$** | **2.251** | **1.543** | **3.254** | **9.550** |

**Table 4. WBC level (10^3/ml), RBC level (10^6/ml), Hb level (g/dL), PCV level (%) of pups of treated rat mother at the end of lactation period.**

**-Different capital letters donate significant differences between groups(P<0.05).**

*Serum electrolyte:*

Potassium, Sodium, and Calcium serum levels in mothers of experimental groups:

The results listed in Table (5) showed that there was a significant increase ($P<0.05$) in the Potassium level of mother groups of T1 and T2 as compared with those of T3 and the control group.

The sodium serum level of the dosed mother in the T2 and T3 groups showed a significant decline ($P<0.05$) compared to the control one. The highest significant reduction was noticed in the T2 group that recorded (94.47±1.76) of sodium level, followed by T3 (114±1.26) and T1 (118.7±1.78).

The results of serum calcium level recorded nonsignificant differences in calcium level in treated mothers of T3, T2 and T1 groups when compared with control one.

| **Groups**<br><br>**N=5** | **Potassium**<br>**mmol/L**<br>**M±S.E** | **Sodium**<br>**mmol/L**<br>**M±S.E** | **Calcium**<br>**mg/dl**<br>**M±S.E** |
|---|---|---|---|
| **Control**<br>**D.W.** | 6.95±0.19<br><br>B | 125.11±4.27<br><br>A | 8±0.11<br><br>A |
| **T1**<br>**0.72mg/kg** | 10 ±0.10<br><br>A | 118.7±1.78<br><br>AB | 6±0.59<br><br>A |
| **T2**<br>**0.36mg/kg** | 9.42±0.42<br><br>A | 94.47± 1.76<br><br>C | 8.49±0.16<br><br>A |
| **T3**<br>**0.72mg/kg+0.36mg/kg** | 7.41±0.69<br><br>B | 114±1.26<br><br>B | 6.64±0.50<br><br>A |
| **LSD** | **1.242** | **9.952** | **3.810** |

**Table 5. Potassium level (mmol/L), Sodium level (mmol/L), and Calcium level (mg/dl) of treated mothers at the end of the lactation period.**

**-Different capital letters donate significant differences between groups($P<0.05$).**

Potassium, Sodium, and Calcium serum levels in pups:

The results of critical essential metals (Potassium, Sodium, and Calcium serum levels**)** listed in Table (6) showed that there was a significant decrease ($P<0.05$) in the Potassium level of pups groups of T3 and T2 as compared with those of T1 and control group that showed nearly similar levels.

While sodium and calcium serum levels of pups treated groups showed nonsignificant differences($P<0.05$) in sodium and calcium levels of pups of T3, T2, and T1 groups in comparison with the control alone.

| Groups<br>N=3 | Potassium<br>mmol/L<br>M±S.E | Sodium<br>mmol/L<br>M±S.E | Calcium<br>mg/dl<br>M±S.E |
|---|---|---|---|
| **Control**<br>**D.W.** | 9.66±0.26<br>A | 110±3.33<br>A | 8.49±0.21<br>A |
| **T1**<br>**0.72mg/kg** | 9.98 ±0.42<br>A | 111.5±5.02<br>A | 9.27±0.38<br>A |
| **T2**<br>**0.36mg/kg** | 6.20±0.37<br>B | 113.2± 3.72<br>A | 8.83±0.22<br>A |
| **T3**<br>**0.72mg/kg+0.36mg/kg** | 6.85±1.33<br>B | 105.42±4.19<br>A | 8.74±0.26<br>A |
| **LSD** | **2.208** | **12.341** | **1.546** |

**Table 6. Potassium level (mmol/L), Sodium level (mmol/L), and Calcium level (mg/dl) of pups of treated mother rats at the end of the lactation period.**

**-Different capital letters donate significant differences between groups(P<0.05).**

*Serum TNFalpha and P-glycoprotein (ng/L) in liver and placenta homogenates in pregnant rats groups treated with Cv, MTX alone and combined administration*

The results of P-glycoprotein concentration in liver homogenate liver sample of dosed mother rats showed significant decrease ($p<0.05$) in the p-glycoprotein concentrations in T1 and T3 treated groups as compared with control one and T2 as listed in table (7) with significant differences between them recording (5.81±0.34) and (6.3±0.49) (ngL) for T3 and T1 respectively, while T2 and control groups recorded nearly identical results.

The Placental tissue homogenate of all experimental groups recorded a significant decline in P-glycoprotein concentration (ng/L) in the placental tissue of T3 and T1 groups as compared with T2 and control groups, without significant differences. While serum TNFa at the end of the experiment in the mother group serum sample showed a significant decrease ($p<0.05$) in TNFalpha concentration in the T3 group –combined and T2 groups- MTX as compared with T1- Cv group and control. Only the T3 group showed a significantly higher decline in TNF-alpha concentration as compared with the T2 group.

| Groups<br><br><br>N=5 | P-glycoprotein concentration in tissue homogenate liver sample (ng/L)<br>M±S.E | P-glycoprotein concentration in tissue homogenate placenta sample (ng/L)<br>M±S.E | TNFalpha level( ng/L)<br><br>M±S.E |
|---|---|---|---|
| **Control**<br>**D.W.** | **8.63± 0.67** | **9.96±0.32** | **480±4.97**<br><br>A |

| | | | |
|---|---|---|---|
| | **A** | **A** | |
| **T1**<br>**0.72mg/kg** | **6.3±0.49**<br>**B** | **8.22±0.24**<br>**B** | **476±5.05**<br>**A** |
| **T2**<br>**0.36mg/kg** | **9.38 ± 0.29**<br>**A** | **10.54± 0.42**<br>**A** | **390±7.28**<br>**B** |
| **T3**<br>**0.72mg/kg+0.36mg/kg** | **5.81± 0.34**<br>**B** | **8±0.39**<br>**B** | **276±5.38**<br>**C** |
| **$LSD_{0.05}$** | **1.427** | **0.978** | **17.25** |

**Table 7. Serum TNFalpha and P-glycoprotein concentration (ng/L) in liver and placenta experimental treatment of mother rat groups after administration of carvedilol and methotrexate alone and combined at prenatal study.**

**-Different capital letters donate significant differences between groups(P<0.05).**

## Discussion

Expression of p-gp decreases gradually towards the end of pregnancy, being highest during the first trimester [21]. BCRP is another protective placental transporter against xenobiotics [22]. The pharmacological results showed that the lowest pain perception was recorded in the combined (MTX+ CV) group, followed by the MTX group. At the same time, there was no change between the control and T1 group in the early phase of the formalin test. The same pattern was noticed for the result of the late phase that indicates the analgesic and anti-inflammatory effect of the used drug, in which the combined group followed by methotrexate showed the highest analgesic and anti-inflammatory effect in comparison with the T1 and control group, which showed nearly the same result. The sopprutity of analgesic and anti-inflammatory resulted from the inhibition of P-gp presented in the intestine, liver and kidney of the treated mother group by carvedilol which might affect the kinetic of methotrexate in the body of T3 treated mothers group leading to accumulation of the drug at the pain receptor site causing increase analgesic and anti-inflammatory effect in T3 group in comparison with methotrexate alone treated group (T2) that showed the effect of drug without the inhibion of P-gp by carvedilol. Methotrexate has been reported to cause inhibition of monocytic and lymphocytic cytokines by considering IL1 and TNFa, which are cytokines with a central role in the inflammatory process and which are mainly produced by monocytes/macrophages at the level of the R.A. synovial tissue, MTX inhibits IL1 production in vivo during methotrexate interferes directly with the binding of IL1 to its receptor and thereby inhibits the cellular responses to IL1 [23].

Prostaglandins and leucotrienes are intensely involved in the inflammatory reaction and analgesic effect. In particular, prostaglandins are essential mediators of joint destruction in R.A. A. Most studies investigated the effects of MTX on cyclooxygenase (COX) metabolism by evaluating the prostaglandin E2 (PGE2) synthesis in cultured human rheumatoid synoviocytes. The results showed a

dose-dependent decrease of IL1-induced PGE2 production by cultured R.A. synoviocytes that was determined by MTX treatment [24].

The hematological result showed that the T3 group (combined) recorded a significantly higher reduction in WBC levels in mothers and their pups in comparison with the T1 group and control, possibly due to inhibition of P-gp (transmembrane transporter) in the T3 group by carvedilol causing higher pharmacological or side effect of methotrexate due to its accumulation in the body. Methotrexate is inflammatory is a highly selective competitive inhibitor of the enzyme dihydrofolate reductase, leads to reduced production of thymidylate and purine biosynthesis, and DNA synthesis eventually halts, and cells can no longer replicate [25]. This present study is in agreement with [16] who have reported that methotrexate combined with leflunomide, used for most therapeutic regimes for rheumatoid arthritis, causes severe leukopenia as a result of its primary pharmacological action. The serum electrolyte levels (Potassium, Sodium, Calcium) were measured in the current study and recorded in all treated female groups and pups. A significant increase in Potassium levels in mother rat groups of T1 and T2 groups may be due to its pharmacological effects by various mechanisms such as suppression of aldosterone secretion from the adrenal cortex and a decrease in cellular uptake of potassium by beta-blocking, complete blocking of these receptors by beta-blocker leads to a decrease in voltage gate sodium-potassium adenosine triphosphate pump activity resulting in a decrease in cellular uptake of potassium [26]. The current study agrees with [27], who reported that carvedilol-induced hyperkalemia in patients with chronic kidney disease during beta-adrenergic agonists, such as albuterol, are used in treating hyperkalemia.

The P-glycoprotein (P-gp) concentration measured in the liver and placenta of a euthanized pregnant mother before delivery recorded potent inhibition of P-gp in T1 & T3 groups, which might alter pharmacokinetic and pharmacodynamic effects of the substrate by carvedilol that considered a potent P-gp inhibitor drugs (impairing P-gp mediated uptake or efflux) involving many mechanisms that modulate the membrane transporter by direct inhibition of binding sites that block the transport of substrates or may be due to ATP binding inhibition, ATP hydrolysis, or coupling of ATP hydrolysis to the translocation of the substrate [12]. This might explain our results of an increase in the pharmacological effect of methotrexate and their side effect on electrolyte concentration and hematology in the combined group of CV+ MTX.

## Conclusions

The current study aimed to study the effect of the P-gp inhibitor drug (carvedilol) that may be used in a pregnant woman as an antihypertensive drug on the pharmacological effect of methotrexate (p-gp substrate), which is commonly used in the treatment of neoplasias and autoimmune diseases like rheumatoid arthritis and psoriasis, therefore we studied their possible pharmacological outcome when used together with carvedilol before and during gestation and lactation periods. ABC transporters are efflux transporters, and if localized in the apical membrane of the syncytiotrophoblast, they have a protective role by expelling chemical molecules out from the syncytiotrophoblast back to the maternal circulation. Expression of placental transporters varies throughout pregnancy.

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