Synergistic And Cytotoxic Effect Of Rosuvastatin, Celecoxib, And Colchicine On Lung Cancer A549 Cell Line

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KEYWORDS
Lung Cancer, Toxicity, Public Health.

ABSTRACT:

Introduction: Lung cancer is a major type of cancer that causes a large scale of mortality. Despite the side effects of high doses cost and side effects of chemotherapy, the resistance of cancer cells became an important issue. The researchers develop a variety of methods to overcome these challenges. The use of drug combinations to modulate the apoptosis and autophagy of cancer cells was one of the new methods to overcome the anti-cancer resistance challenge.

Objectives: The study was designed to find the best drug combination at lower therapeutic doses of routinely used drugs to reduce the public health toxicity issue of humans suffering from lung cancer. The current study investigates three FDA-approved drugs and four combinations' effects in modulating cancer cells using lung cancer cell line A549 and evaluating the IC50, Synergistic effect of the combinations.

Methods: Three drugs of Rosuvastatin (Ro), Celecoxib (Ce), and Cholchicine (Co), and four combinations (Ro-Ce, Ro-Co, Ce-Co, and Ro-Ce-Co) were exposed to cell line for 72 hours was the cytotoxicity was evaluated using MTT assay, IC50 calculated using GraphPad Prism depending on the optical densities, and the combination synergism and Combination Index was evaluated by Compusyn Software.

Results: Results showed that all treatments had a cytotoxic effect in a dose-dependent manner. The combination showed variable degrees of synergism according to the doses and type of drug. The combination of Ro-Ce-Co had the highest effect, while the Ce-Co showed antagonism.

Conclusions: The study concludes that the combination of the drugs in the current study is beneficial in lowering cancer cell proliferation and could reduce the public health issues associated with higher chemotherapy doses. Except for the combination of Ce-Co that could induce unfavorable results. The combination doses should calculated and administrated precisely to reach the maximum effect.

1. Introduction

Cancer is defined as the abnormal and uncontrollable growth of cells associated with the integrity of genetic makeup caused by several internal and environmental factors [1]. Lung cancer is the most incidence type with the highest mortality rate in males and the second in both sexes [2]. Adenocarcinoma lung cancer of alveolar basal epithelial is a wild type of lung cancer, researchers developed the A549 cell lines from this type of cancer to study the characteristics of this type of cancer. The new drugs and chemotherapies developing also frequently use the A549 cell line as a routine cell line in researches that aim to reduce the growth and proliferation of lung cancer [3], [4]. The chemotherapy resistance of cancer cells became an important section of cancer therapy researchers were focused on new strategies like developing a different drug combination that could be used by

the patient normally in chronic or acute cases of diseases that interfere with the vital mechanisms of cancer proliferation and metastasis [5], [6]. This strategy narrows the time and cost required to FDA approve for new drug reduces the cost of therapy, and lowers the doses of chemotherapy required for treatment, which is important for the reduction of unnecessary toxicity to the body [7].

The combination is not just a mixing of two drugs, the process should tested on different scales starting from the cell lines and experimental animals to exploring and evaluating the effects and side effects of this combination the result should induce a synergistic effect more than the single drug alone with lower toxicity to body tissues [8]. Rosuvastatin is a lipid-lowering drug classified in a group of drugs called Statins used to treat patients suffering from hyperlipidemia and cardiac diseases acting by inhibiting the HMG-CoA enzyme [9].

Rosuvastatine investigated by different researchers in the treatment of different types of cancer either as a single drug or combined with other types of drugs, Rosuvastatine regulates different autophagy and apoptotic pathways like AMPK/Akt/mTOR and p53 resulting in different effects depending on the type of cancer cell [10], [11].

Celecoxib is a lipid-soluble and very low water-soluble anti-inflammatory drug that acts by inhibiting of COX-2 enzyme also has an anti-cancer effect by inducing autophagy at low doses and inducing apoptosis at higher doses through different pathways especially the AMPK/Akt/mTOR and NF-κB pathways [12], [13].

Colchicine is an Alkaloid drug used in the treatment of gout acting by attaching microtubules to the beta-tubulin site preventing the polymerization of tubules and blocking the autophagy process inducing rapid apoptosis. Colchicine also stops the movement of leukocyte and muscle fiber extensions [14], [15].

Researchers also showed that Colchicine and Rosuvastatine induce senescence in cancer cells and this feature could increase the efficiency of other drugs that both drugs combined with [16], [17]. Chou and other researchers explain and make a mathematical model and software to find the Combination Index that calculates precisely the relation of two combined drugs with each other at different points of doses to understand the relation of two or more drugs relation [18], [19]. The current study aims to evaluate the effect of the combinations of Rosuvastatin, Celecoxib, and Colchicine on Lung Cancer using the A549 cell line as a model, and finding the best synergistic CI value for a wide range of points (nine concentrations).

2. Objectives

The main objective is to find the best combination that serves public health in the section of cancer aid therapy.

The second objective is to evaluate each combination's cytotoxicity in comparison with single drugs and a control group of treatment.

The third objective is to provide a wide range of drug combinations that are used in large-scale chronic and acute diseased patients.

3. Methods

Drugs were weighted and dissolved in 0.26 % DMSO to prepare nine concentrations (200 µM, 100 µM, 50 µM, 25 µM, 12.5 µM, 6.25 µM, 3.12 µM, 1.56 µM, 0.78µM) and control ($y = 400e^{-0.693x}$) of eight groups of treatments. Groups 1, 2, 3, 4, 5, 6, 7, 8 of Rosuvastatin (Ro), Celecoxib (Ce), Colchicine (Co), Rosuvastatin-Celecoxib (Ro-Ce), Rosuvastatin- Colchicine (Ro-Co), Celecoxib- Colchicine (Ce-Co), Rosuvastatin-Celecoxib-Colchicine (Ro-Ce-Co), and control group respectively. Cell line A549 was cultured using Minimum Essential Media (MEM) for 24 h at 37 C^0 and 5% Co2 before the monolayer was diluted and subcultured in new media containing 10% Bovine Serum. Cells (50\µl) were exposed to treatments and incubated for 72h [20], [21]. The cytotoxic effect was determined by using an MTT assay [22–24]. The IC50, graphs and statistics analysis was made using Graphpad Prism version 8. The Combination synergism and combination index were calculated by using CompuSyn software [18].

4. Results

Results showed that all treatments had a cytotoxic effect on lung cancer cell line A549, and the combination of drugs was more effective than single drugs Figure (1-1), Figure (1-2), Figure (1-3). The results also showed that the single drugs and combinations had a significant ($p< 0.05$) dose-dependent effect. The cytotoxic effect at

higher doses accordingly was Ro-Ce-Co, Ce-Co, Ro-Ce, Ro-Co, Co, Ce, and Ro groups of treatment, while at the lower doses Ro-Co, Co, Ro, Ro-Ce-Co, Ce-Co, Ce, and Ro-Ce groups respectively had the highest cytotoxic effect. The IC50 value was 2.22, 2.7, and 5.296 for Co, Ro, and Ce. The lowest combination index (CI) value that indicates the degree of max synergistic dose for the combination was 0.407, 0.773, and 0.886 for Ro-Co, Ro-Ce, and Ro-Ce-Co combinations respectively Table (1-1). All the combinations induced a synergistic effect at three of ten concentrations (points) localized within the Isobologram triangle (or under the CI line of Combination Index Plot) except the Ce-Co combination, which showed antagonistic characteristic Figure (1-3). Within each group of treatment, the three groups of Ro-Ce-Co, Ro, and Co have induced the most significant stable shafting in response accompanied by an increased dose of treatment, while the group of Ce-Co showed a sudden shafting between the lower and higher doses that also induce non-significant changes between most of the doses.

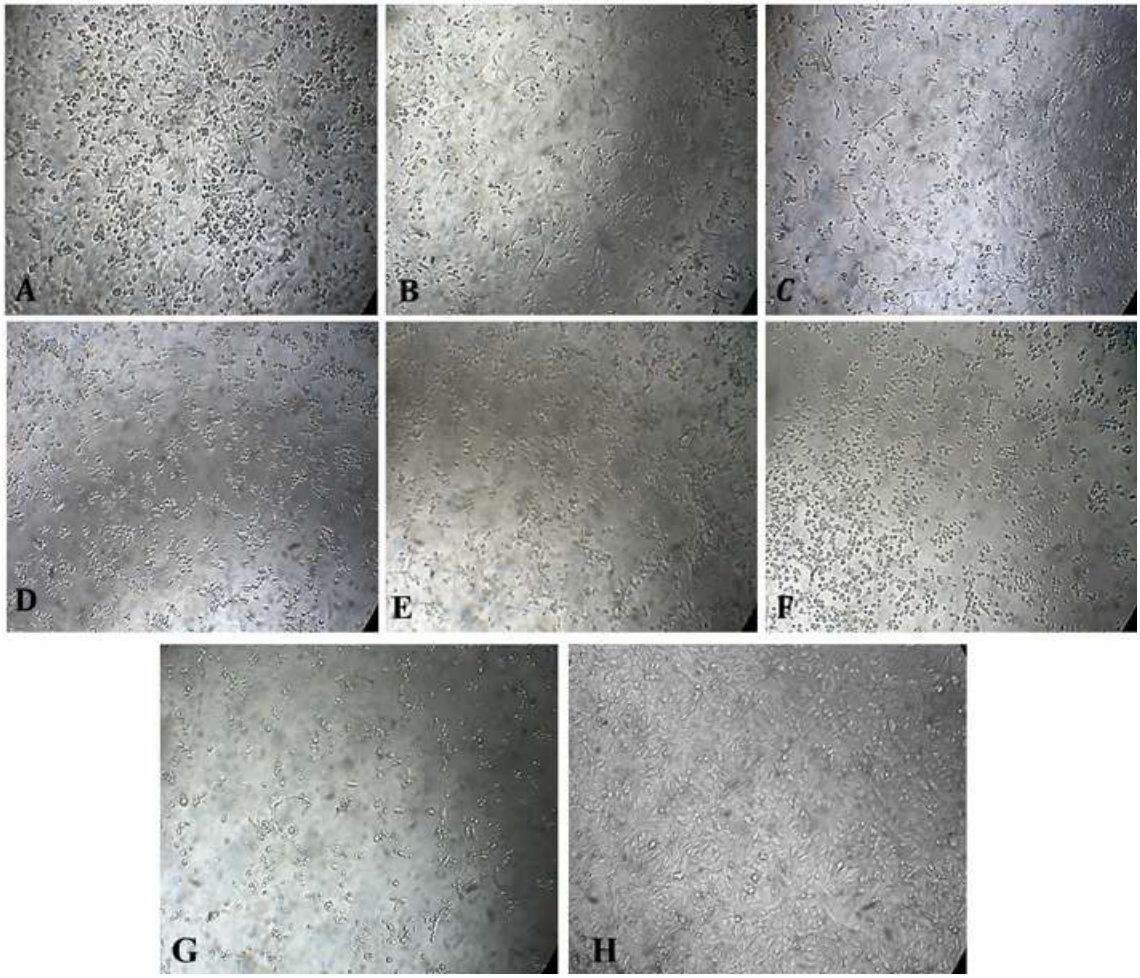

Figure 1-1: Cytotoxic effect of single and combination of drugs on cancer cell line A549 proliferation, A, C, D, E, F, G, and H were Ro, Ce, Co, Ro-Ce, Ro-Co, Ce-Co, and Ro-Ce-Co groups respectively.

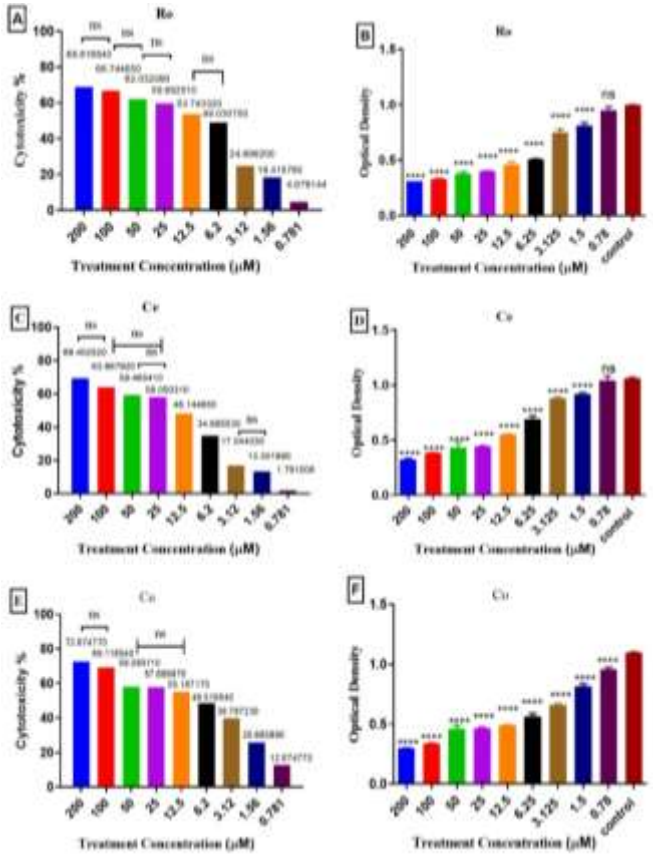

Figure 1-2: Cytotoxic effect of single drugs on cell line A549, A, C, and E were Ro, Ce, and Co groups cytotoxicity percentage, B, D, and F were the Significances of each group concentration based on optical densities of MTT assay, P ns=non-significant, *= P<0.05, **= P<0.01, ***= P<0.001, ****= P<0.0001.

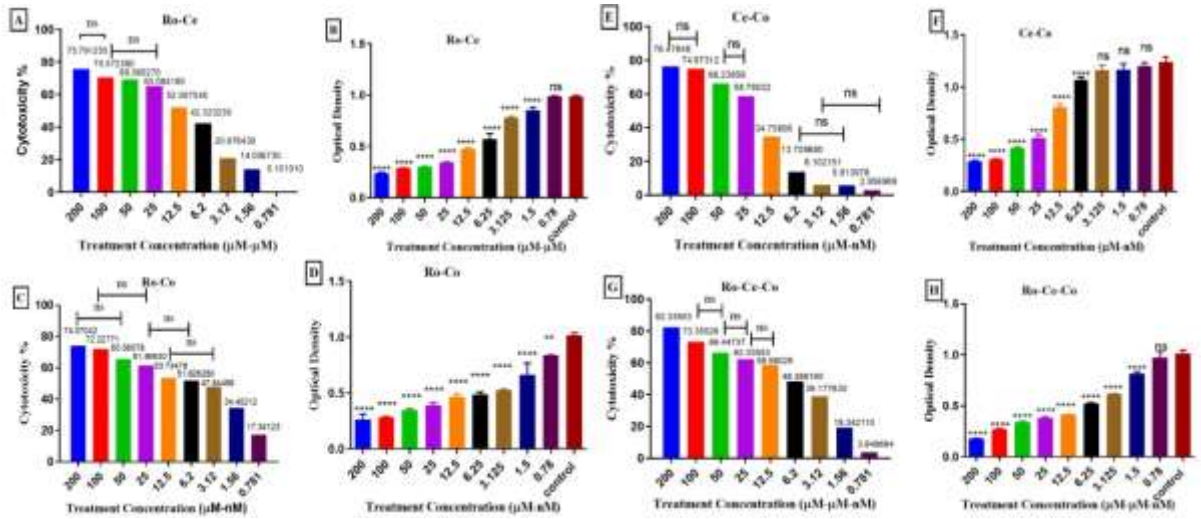

Figure 1-3: Cytotoxic effect of drug combinations on cell line A549, A, C, E, and G were Ro-Ce, Ro-Co, Ce-Co, and Ro-Ce-Co respectively of cytotoxicity percentage. B, F, D, and H were the Significance of each group concentration based on optical densities of MTT assay, P ns=non-significant, *= P<0.05, **= P<0.01, ***= P<0.001, ****= P<0.0001.

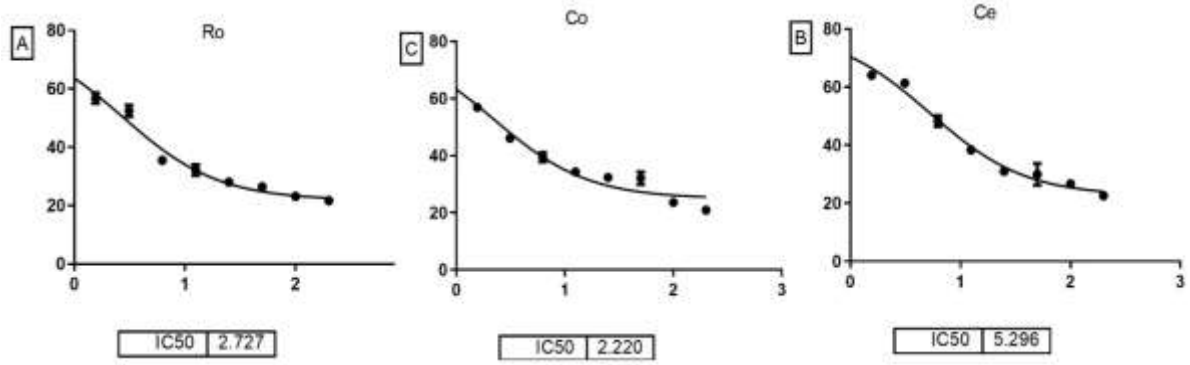

Figure 1-4: The IC50 values of single drugs on cell lines A549, A, B, and c were Ro, Co, and Ce groups.

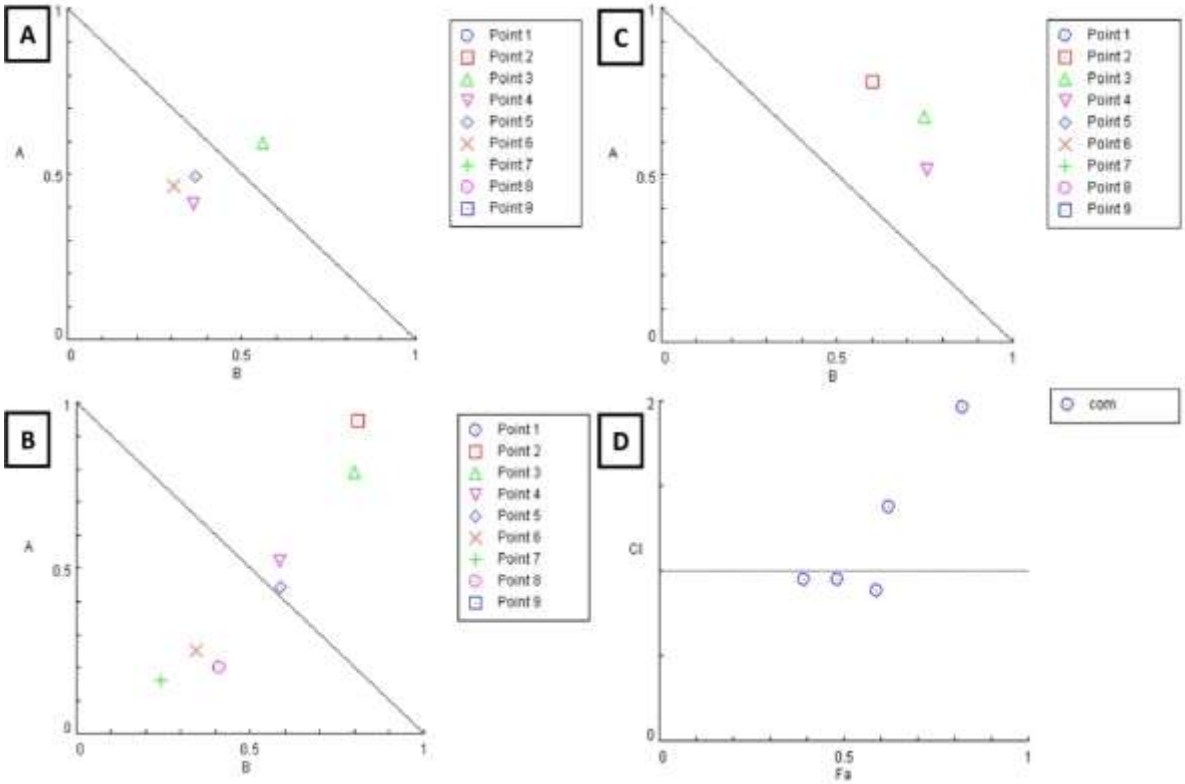

Figure 1-5: Synergistic effect demonstrated by Isobologram (A, B, and C) and Combination Index Plot (D) of drugs combinations on cell line A549; A, B, C, and D were Ro-Ce, Ro-Co, Ce-Co, and Ro-Ce-Co groups respectively, Point 1-9 were the concentration of each treatment 200 µM -0.78µM.

Table 1-1 the combination Index value for each concentration (point), 1, 2, 3, 4, 5, 6, 7, 8, and 9 were 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78 µM

Point	Rosuvastatin	Celecoxib	Colchicine	CI	Synergism
1	+	+	-	+	-
2	+	+	-	+	-
3	+	+	-	+	-
4	+	+	-	+	+
5	+	+	-	+	+
6	+	+	-	+	+
7	+	+	-	+	-
8	+	+	-	+	-
9	+	+	-	+	-
1	+	-	+	+	-
2	+	-	+	+	-

3	+	-	+	+	-
4	+	-	+	+	-
5	+	-	+	+	-
6	+	-	+	+	+
7	+	-	+	+	+
8	+	-	+	+	+
9	+	-	+	+	-
1	-	+	+	+	-
2	-	+	+	+	-
3	-	+	+	+	-
4	-	+	+	+	-
5	-	+	+	+	-
6	-	+	+	+	-
7	-	+	+	+	-
8	-	+	+	+	-
9	-	+	+	+	-
1	+	+	+	+	-
2	+	+	+	+	-
3	+	+	+	+	-
4	+	+	+	+	-
5	+	+	+	+	+
6	+	+	+	+	+
7	+	+	+	+	+
8	+	+	+	+	-
9	+	+	+	+	-

5. Discussion

Data suggested that Colchicine as a single drug induced the highest level of cytotoxicity at low and high concentrations (Figure 1-2), this effect was due to the rapid and rigid attachment of Colchicine at the tubulin site leading to de-polymerization of cytoskeleton microtubules [25]. Which was enhanced when combined with Rosuvastatin (Figure 1-5) at low doses (points 4,5, and 6) because of the active regulation of AMPK/Akt/mTOR pathway and other pathways by Rosuvastatine that enhance the effect of Colchicine [26], [27]. At high doses, Rosuvastatine lowers the lipid environment and reduces the solubility of lipid-soluble drugs, but Rosuvastatine has a low bioavailability so its action requires more doses and a longer duration to induce its effect so the antagonism only appears at high doses [28].

The Colchicine antagonized with all points of Celecoxib (Table 1-1), (Figure 1-5) because the Colchicine already had the lowest IC50 value (Figure 1-4) with max single drug cytotoxic effect, and it requires a more potent drug for induction a synergistic effect, while the Celecoxib had the highest IC50 value. The researchers also showed that Celecoxib and Colchicine interact with each other producing inert metabolite persist in cells (Bonate et al., 1998; Kim & Moon, 2012; Otani et al., 2020; Xiao et al., 2024; Yin & Wang, 2016).

Researchers also showed that Celecoxib and Colchicine worked by different means and there was no relation between COX-2 and tubulin site directly [25], [33], [34].

Rosuvastatin which had a lower IC50 value than Celecoxib (Figure 1-4) was the key factor in the synergistic effect of the combination of Rosuvastatin-Celecoxib-Colchicine, although the effect was reduced by one point from the Colchicine-Rosuvastatine combination (Table 1-1). The effect of Rosuvastatin in the three-drug combinations played a major role because of the enhancement of Celecoxib's lower cytotoxic effect. The cytoskeleton modulation by Colchicine regulates the PI3K/Akt/mTOR pathway preventing the Celecoxib from expressing its cytotoxic response decreasing its efficiency and reducing its response [13], [15], [35–40].

The combination of Rosuvastatie-Celecoxib showed a synergistic effect at three concentration points (4, 5, and 6) because the combination induced a significant cytotoxic effect at these points more than the single stat of

each drug alone. The synergistic effect appears because Rosuvastatine senescence the cancer cells at low concentrations enhancing the activity of the drug that is combined with [17].

Celecoxib at lower doses works as an autophagy inducer reducing the apoptotic pathway activation so the cytotoxic effect does not appear at lower concentrations, but this process shafted and apoptotic pathway activated with increased doses because of ROS produced as a result of Celecoxib metabolism that affects the mitochondrial integrity [41].

Rosuvastatin prevents the production of ROS inhibiting lipid production and lowering the lipid solubility of Celecoxib that antagonizes the effect of Celecoxib at lower doses [42], [43]. The antagonism effect does not appear at very low concentrations (1, 2, and 3) because of Rosuvastatin bioavailability and mode of action which require more doses and longer duration than Celecoxib [42–45].

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