PROTECTIVE EFFECT OF QUERCETIN AGAINST STATINS INDUCED-HEPATOTOXICITY IN CELL LINE

[1]Mohammad K. El-Anany*,[2] Mohammad M. Badr, [1]Osama A. Mansour

Department of Biochemistry & Molecular Biology, Faculty of Pharmacy (Boys), Al-Azhar University

Department of Biochemistry & Molecular Biology, Faculty of Pharmacy, Almenofia University

*Corresponding Author: Mohamadkamal.202@azhar.edu.eg

ABSTRACT

Objective: Statins are the most prescribed lipid lowering agents and consequently they prevent obstructive cardiovascular events in the world. Severe adverse effects of statins, involving myopathy and hepatotoxicity, sometimes limit their usage as lipid lowering agents. We now investigate the toxicity of statins and prove the protective effect of quercetin against statins toxicity in cell line.

Methods: Human hepatocellular carcinoma cells HepG2 were used in this study were cultured at 37℃ in 5% CO_2, in Roswell Park Memorial Institute medium (RPMI 1640) and were supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 mg/mL streptomycin. HepG2 cells were cultured with some of statins as Atorvastatin, Simvastatin and Rosuvastatin with 10μM concentration with and without pretreatment with 10, 20, 40μM quercetin 4 hours before the addition of statins, then cell line was incubated for 24 and 48 hours. Toxicity of statins was determined by cell viability assay (MTT assay), ALT&AST level assay, lipid peroxidation assay by Malondialdehyde (MDA) and Immunofluorescence staining assay using DAPI.

Results: ALT&AST levels were significantly increased after the addition of statins in HepG2 cells when compared with the control group (DMSO 0.1%), and ALT&AST levels were significantly decreased with pretreatment of the cells with quercetin 4 hours before the addition of statins in a dose-dependent manner when compared with statin group. Cell viability percentage (MTT concentration) was decreased significantly after the addition of statins to HepG2 cells when compared with the control group, and MTT concentration was significantly increased with pretreatment of the cells with quercetin 4 hours before the addition of statins in a dose-dependent manner when compared with statin group. MDA concentrations increased significantly after the addition of statins to HepG2 cells when compared with the control group, and decreased when the cells were pretreated with quercetin 4 hours before the addition of statins in a dose-dependent manner when compared with statin group. Statins cause cellular oxidative damage by liberating reactive oxygen species, and the cellular damage was prevented when the cells pretreated with quercetin 4 hours before addition of statins. **Conclusions:** We found that, quercetin shall protect HepG2 cells from statins-induced hepatotoxicity with 10, 20, 40μM concentrations without significant difference between 20 and 40μM concentrations, and may be developed as a therapeutic agent for possible statins toxicity.

Keywords: Statins, Drug induced liver injury (DILI), Quercetin, MDA, MTT, Immunofluorescence assay, DAPI staining.

INTRODUCTION

Statins (3-hydroxy-3-methylglutaryl co-enzyme-A reductase inhibitors) are the most prescribed agents and the first-line drugs for treatment of hyperlipidemia and consequently they prevent obstructive cardiovascular events in the world. These pharmaceuticals block the mevalonic acid pathway by inhibition of the rate limiting step in the hepatic de novo cholesterol biosynthesis.

Severe adverse effects, involving myopathy and hepatotoxicity, occurred due to statins and sometimes limited their usage as antihyperlipidemic agents (**Beatrice et al., 2008; James K, 2005**).

Many Statins such as Atorvastatin, Simvastatin and Rosuvastatin cause deterioration in liver function tests and liver injury in a predictable and dose-dependent manner, the hepatotoxicity associated with their long-term use remained a significant problem for their clinical use (**Rossana Calderonet et al., 2010**). The use of statins almost associated with biochemical abnormalities of liver functions, moderate elevations of serum transaminases levels (< 3 times the upper limit of the reference range) have been reported following initiation of therapy and are often transient (**Jimmy Jose, 2016**).

Different hepatotoxicity mechanisms proposed, some of which completely explained. However, most hepatotoxicity mechanisms have not been entirely understood and therefore researchers keep conducting studies (**Bensu Karahalil et al., 2017**). The present study focused on statin induced hepatotoxicity and possible hepatotoxicity mechanisms. Many observational studies in North America and Europe have confirmed that that statins increase liver enzymes, risk of myopathy, and risk of diabetes mellitus (**Kubatka et al., 2014**).

Mitochondrial dysfunction is one of the major factors that explain the mechanism of statin-induced hepatotoxicity. Another major reason for statin induced hepatotoxicity is that statins cause apoptotic cell death (**Tolosa et al., 2015**). Statins metabolized by the liver cytochrome P_{450} enzymes which include a huge number of mono-oxygenases and have a critical function for the catabolism of xenobiotic. Mitochondria or cytochrome P_{450}-dependent metabolism act as reactive oxygen species (ROS) generation systems and participate in cell death processes (**Sergio Di Meo et al., 2016**). During statins usage, hepatocytes produce a significant amount of ROS and cause lipid peroxidation leading to a decrease in the mitochondrial membrane potential and promoting cytotoxicity, the highest amount of ROS produced by simvastatin (**Abdoli et al., 2014**).

Flavonoids, and specifically flavonols, have been reported to exhibit a wide range of biological activities. The flavonoid used in this study was quercetin, which is a common dietary flavonol which is well characterized in vitro antioxidant activity (**A. N. Panche et al., 2016**). It is the predominant flavonol found in foods, and intakes of between 6 to 31 mg per day have been reported (**Jennifer et al., 2019**). There are many experimental studies have demonstrated that dietary quercetin supplementation exerts

beneficial effects against a wide range of hepatic toxicants (**Robert Domitrovic et al., 2012**).

This study aims to analyze the hepato-protective effects of quercetin with special emphasis on its mechanisms of action and provide a broad spectrum review on its hepatic properties. HepG2 is a well differentiated transformed cell line that meets all biochemical requirements for the present study and has been widely used in biochemical and nutritional studies because it is considered one of the experimental models that more closely resemble the human hepatocyte in culture (**Lapidot et al., 2002; Narayanan et al., 2001**). This work, therefore, designed to investigate the protective effect of quercetin on HepG2 cells against statins-induced hepatotoxicity.

MATERIALS AND METHODS

HepG2 cells which were used in this work were purchased from (VACSERA, Egypt), the cells were grown in Roswell Park Memorial Institute medium (RPMI 1640) in 75 cm^3 tissue culture flask and were supplemented with 1% of 100 mg/ml of streptomycin, 100 units/ml of penicillin, 2 μmol/ml of L-glutamine, 250 ng /ml Fungizone and 10% of heat-inactivated fetal bovine serum (all from Lonza$^®$, Verviers, Belgium). The cells were incubated in a humidified 5% (v/v) CO$_2$ atmosphere at 37 ℃ and the culture medium was changed every other day and the cells were usually split 1:3 when they reached confluence. The confluent cultures were passaged every four days by trypsinization using 1.5ml trypsin/EDTA solution for 5 min at 37 ℃ in the cell culture laboratory at Faculty of Pharmacy, Al-Azhar University (Boys), Egypt.

The groups design:

In order to fulfill our proposed aims we classified the groups as follows:

1. **Vehicle Control group**: consists of HepG2 cells treated with 0.1% DMSO.
2. **Atorvastatin group;** consists of the following subgroups:
   a) HepG2 cells treated with 10 μM atorvastatin only (Toxic group).
   b) HepG2 cells treated with 10 μM atorvastatin + 10 μM quercetin.
   c) HepG2 cells treated with 10 μM atorvastatin + 20 μM quercetin.
   d) HepG2 cells treated with 10 μM atorvastatin + 40 μM quercetin.
   3. Rosuvastatin group; consists of the following subgroups:
   a) HepG2 cells treated with 10 μM rosuvastatin only (Toxic group).
   b) HepG2 cells treated with 10 μM rosuvastatin + 10 μM quercetin.
   c) HepG2 cells treated with 10 μM rosuvastatin + 20 μM quercetin.
   d) HepG2 cells treated with 10 μM rosuvastatin + 40 μM quercetin.
   4. Simvastatin group; consists of the following subgroups:
   a) HepG2 cells treated with 10 μM simvastatin only (Toxic group).
   b) HepG2 cells treated with 10 μM simvastatin + 10 μM quercetin.
   c) HepG2 cells treated with 10 μM simvastatin + 20 μM quercetin.
   d) HepG2 cells treated with 10 μM simvastatin + 40 μM quercetin.
For all assays, the different concentrations of quercetin were dissolved in dimethylsulfoxide (DMSO 0.1%) and were added in the cell plates 4 hours before the addition of statins.

Determination of Cytotoxicity by cell viability assay (MTT assay): HepG2 cells were seeded in a 96-well plates (5000 cells/well) and then were treated with quercetin at different concentrations (10, 20 and 40μM) 4 hours before the addition of statins. After incubation of the cells for 24 and 48 hours at 37˚C and 5% CO_2, 1ml of MTT solution was added to each well of a micro titer plate and incubated for 2 to 4 hours at 37˚C. MTT assay based on the principle that viable cell converts MTT into formazan crystals (**D.M. Chung et al., 2015**). The resultant formazan product dissolved in 1ml of acidic isopropanol/well, and its concentration was measured at 570nm by a micro plate reader (Beckman[®], UV DU 640 spectrophotometer).

All viability assays were performed in triplicate; its percentage growth inhibition was calculated by the following formula:

Cell viability (%) = (the absorbance of experimental group/ the absorbance of control group) × 100

Determination of ALT & AST: Assay of ALT activity was performed by kinetic method using Spinreact[®] reagent kits (**H. U. Bergmeyer et al., 1986**).

After 24 and 48 hours of incubation, cell culture media from each three wells were collected in eppindorf tube, centrifuged at 3000×g for 10 min and stored at -80°C until assay. Enzymes activities were calculated by the following equation:

Activity (U/L) = factor (1746) × ΔA

Factor =TV × 1000 / 6.3 × SV×P

Lipid peroxidation biomarker (Malondialdehyde MDA): MDA as a marker for lipid peroxidation, it was estimated according to a colorimetric method (**Antonio Ayala et al., 2014**). Briefly, 0.5ml of the supernatant was added to 3ml of 1% O- phosphoric acid and 1ml of 0.6% thiobarbituric acid. The mixture was boiled in a water bath at 95℃ for 30 minutes. After cooling the mixture, 4 ml of n-butanol was added to each tube and mixed vigorously. The n-butanol phase (the upper layer) was separated by centrifugation at 2000 rpm for 10 minutes and the absorbance of the pink color was measured at 534nm. MDA concentration was calculated using the following equation:

Concentration of MDA = (absorbance of sample/absorbance of standard) × concentration of standard

MDA concentration expressed as nmol/ml.

Immunofluorescence assay using DAPI staining: Cellular oxidative stress was quantified by the dichlorofluorescein assay to determine the effect of quercetin on the intracellular generation of reactive oxygen species ROS. After being oxidized by intracellular oxidants, Dichloro-dihydrofluorescein diacetate (DCFH-DA) converted to dichlorofluorescein (DCF) (**Wang and Joseph, 1999**). To assess the expression of lipogenic enzymes by immunofluorescence, cells were seeded on coverslips in 6-well plates in a humidified 5% CO_2 atmosphere at 37 °C for 24 and 48 hours, cells were

treated firstly with 10, 20 and 40µM quercetin for 4 hours, then statins were added (atorvastatin 10µM, rosuvastatin 10µM and simvastatin 10µM). Control samples were treated with media and equivalent concentration of DMSO (0.1%). After incubation for 24 and 48 hours, the wells were incubated with dichlorofluorescen (DCF) for only 30 minutes, then media was removed and cells were washed 3 times in 2 minutes with PBS. Nuclei was stained with 4', 6-Diamidino-2 Phenylindole Dihydrochloride (DAPI) which was added in a concentration of 5µg/ml for only 3 minutes at room temperature, and excess of DAPI was washed with PBS three times (10 minutes for each). Slides were mounted in the mounting medium (Fluoromount®). Microscopic examination was done by LEICA® fluorescence microscope (model: Leica DM5500 B from Leica Microsystems, USA) under red, blue and green channels. Fluometric analysis: fluometric intensity of microscopic fields was measured for each cultured cell using ImageJ/NIH software (National Institute of mental health, Bethesda, Maryland, USA) for statistical analysis and was represented as a ratio [green (fluorescence intensity) / blue (DAPI for nucleus staining)].

Statistical analysis

Statistical analysis was performed using Graphpad prism (version 5) software; all data were expressed as the mean ± standard deviation (SD) of at least three experiments performed in triplicate. Differences between group means were analyzed using one-way analysis of variance **(ANOVA)** followed by **Tukey's test** for multiple comparisons, and statistical significance was established as $p < 0.05$.

RESULTS

Determination of IC_{50} of quercetin in HepG2 cell line was done by Cell Viability Assay and the following concentrations were used (0, 5, 10, 20, 40, 50, 100, 200µM). IC_{50} of quercetin was obtained by computerized Graph Pad Prism-6 program from dose response curve and listed in Table 1.

Table 1: IC_{50} of quercetin after 24 and 48 hours on HepG2 cell line

	After 24 h	**After 48 h**
IC_{50} (µM)	60.45	35.64

Cell viability assay (MTT assay test): MTT assay was used as an indicator of cytotoxicity. In our experiment, treatment of HepG2 cells with atorvastatin, rosuvastatin and simvastatin with 10µM concentrations for 24 and 48 hours incubation evoked decrease in cell viability% by time (fig 1). Pretreatment of HepG2 cell line with 10, 20 and 40µM quercetin for 4 hours reduced cell damage after 24 hours incubation and cell damage was further reduced after 48 hours (fig 1).
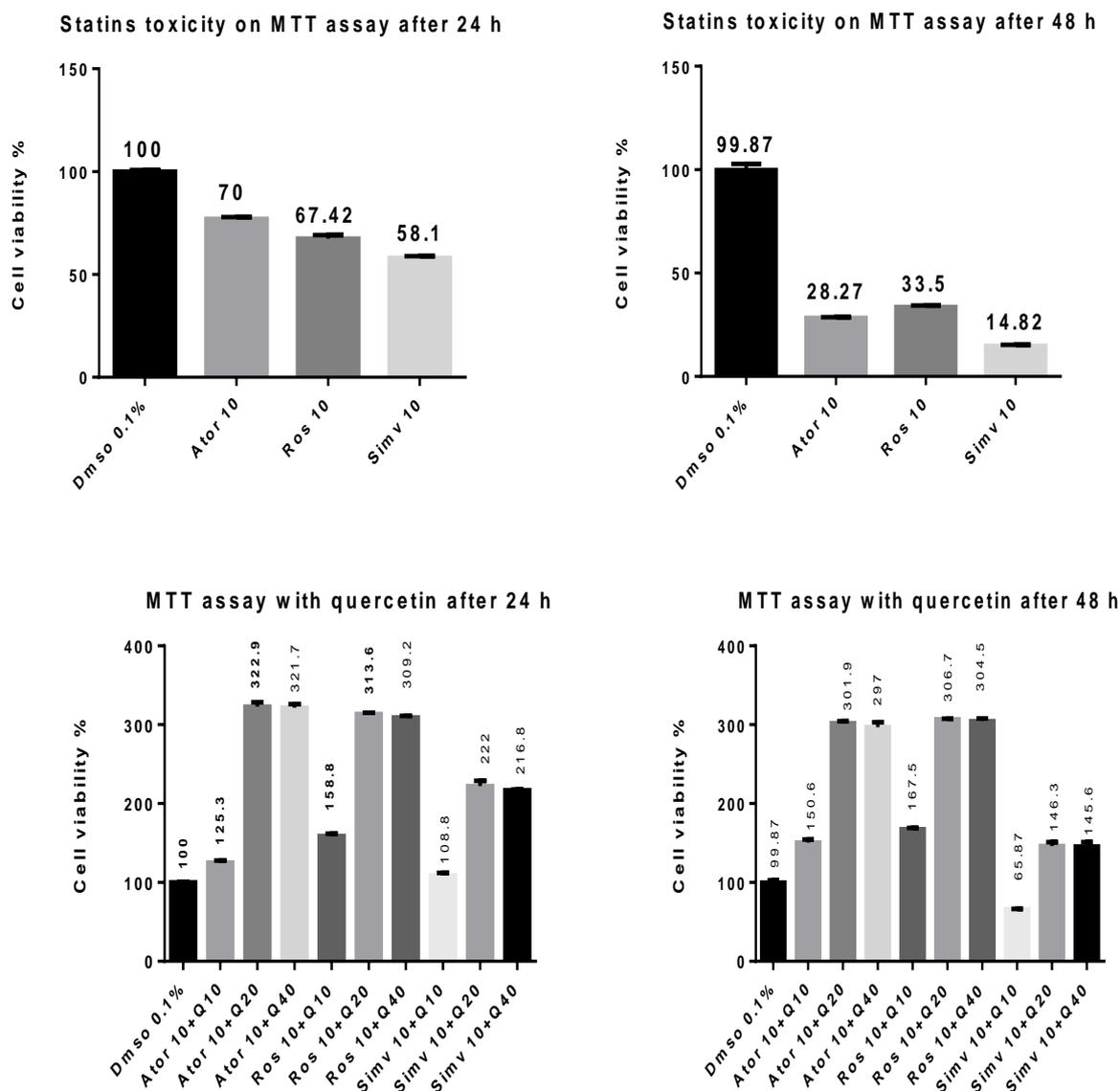
Figure 1: Effect of Statins on cell viability (MTT assay) after cell incubation for 24 and 48 hours and after the addition of 10, 20 and 40µM quercetin; Ator: Atorvastatin, Ros: Rosvastatin, Simv: Simvastatin, Q: Quercetin.

Determination of ALT & AST: Cells were treated with statins for 24 and 48 hours then ALT and AST were measured, the enzyme levels increased. The data shows that toxicity after 48 hours of incubation is higher than the toxicity after 24 hours as shown in figure 2 and figure 3, ALT and AST levels decreased after the addition of 10, 20 and 40µM quercetin in a dose-dependent manner.
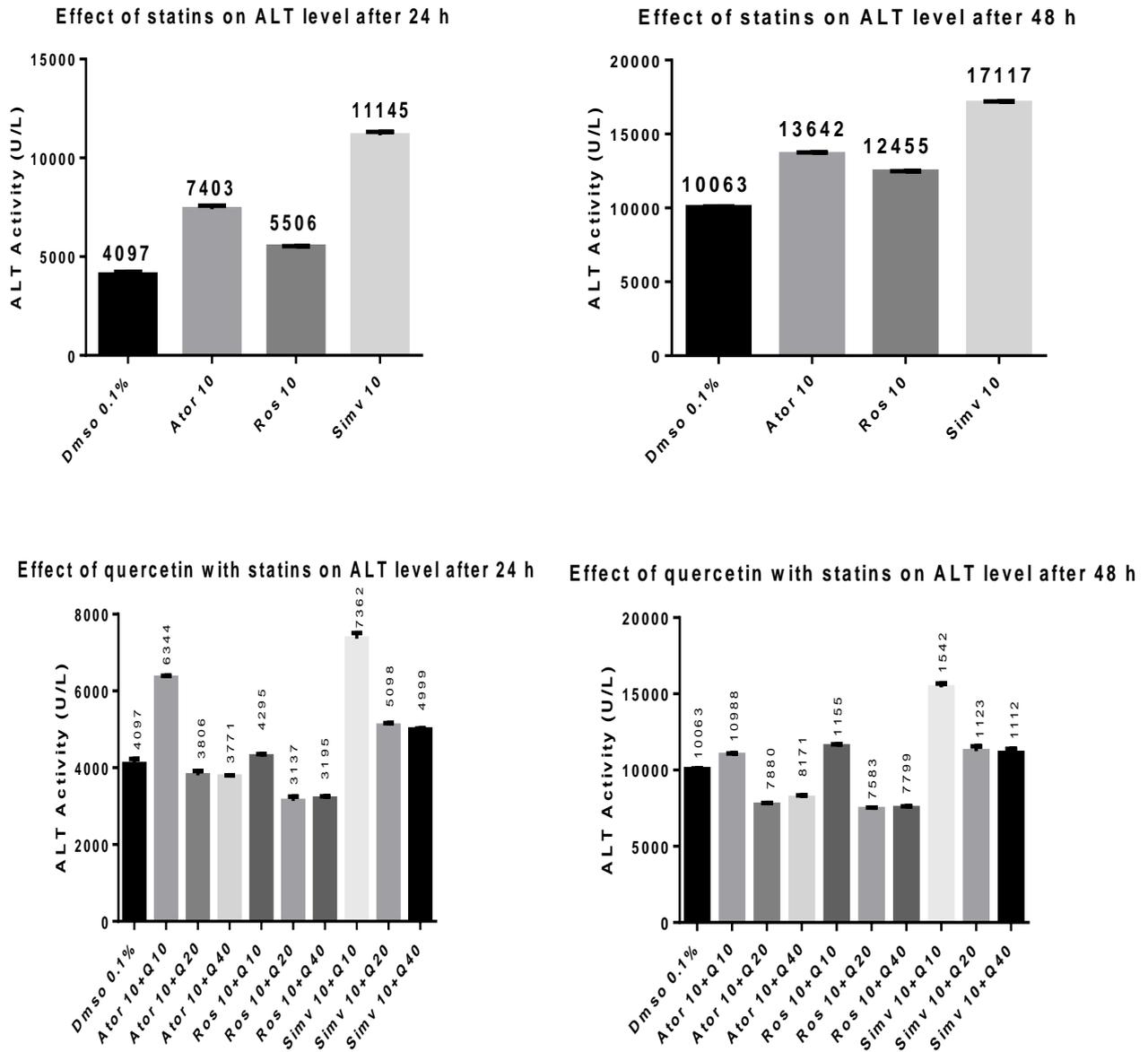
Figure 2: Effect of statins on ALT level after incubation for 24 and 48 hours and after the addition of 10, 20 and 40μM quercetin; Ator: Atorvastatin, Ros: Rosvastatin, Simv: Simvastatin, Q: Quercetin.
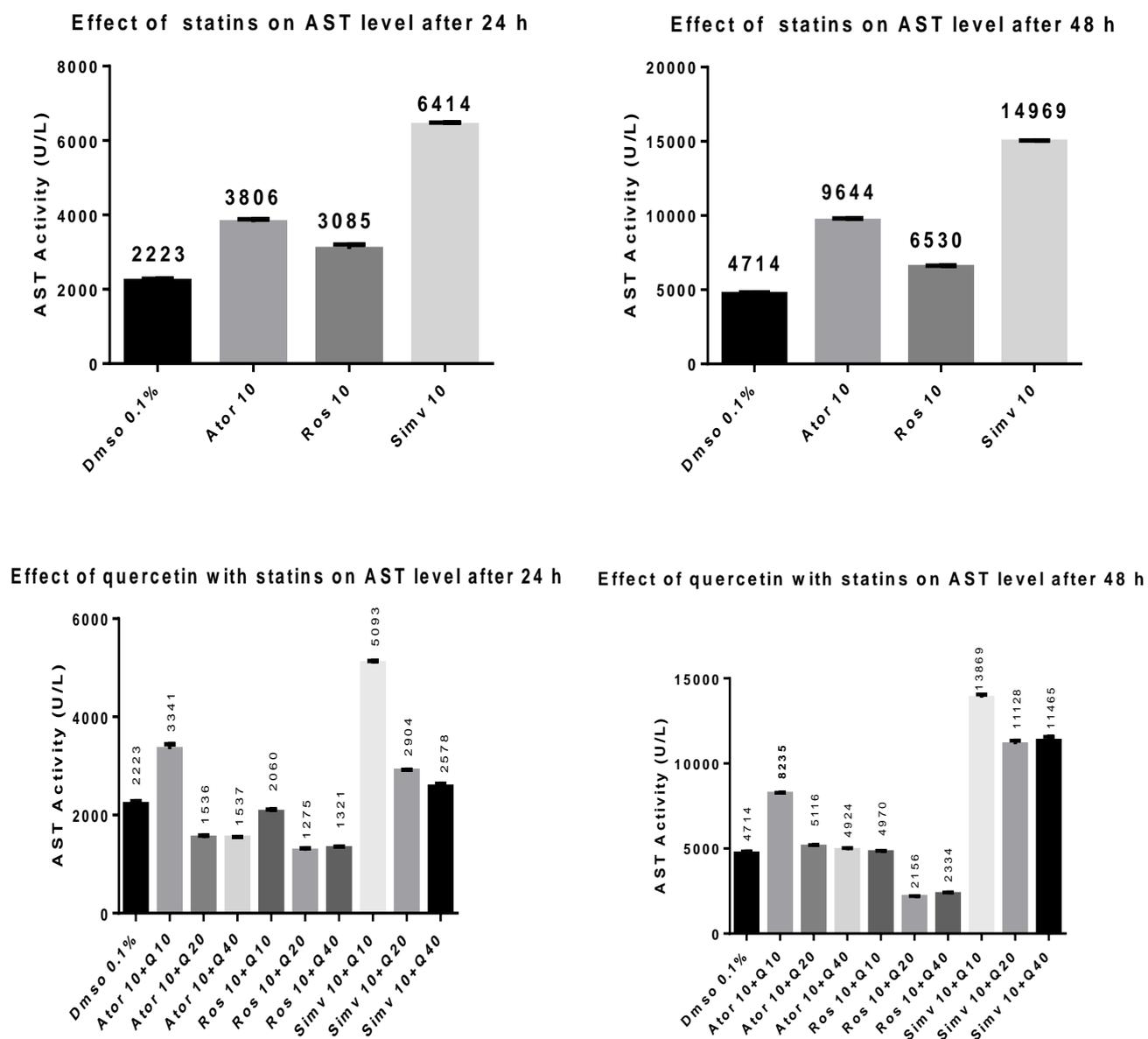
Figure 3: Effect of statins on AST level after 24 and 48 hours, and with 10, 20 and 40μM quercetin; Ator: Atorvastatin, Ros: Rosvastatin, Simv: Simvastatin, Q: Quercetin.

Malondialdehyde assay (MDA): Cytoplasmic concentrations of MDA were measured in HepG2 cells treated with statins after incubation for 24 and 48 hours, which evoked a significant increase of MDA level. Pretreatment of the cells with 10, 20 and 40μM quercetin 4 hours before the addition of statins decreased MDA concentrations, this indicates reduction in lipid peroxidation in response to treatment with quercetin which acts as a protective agent as shown in figure 4.
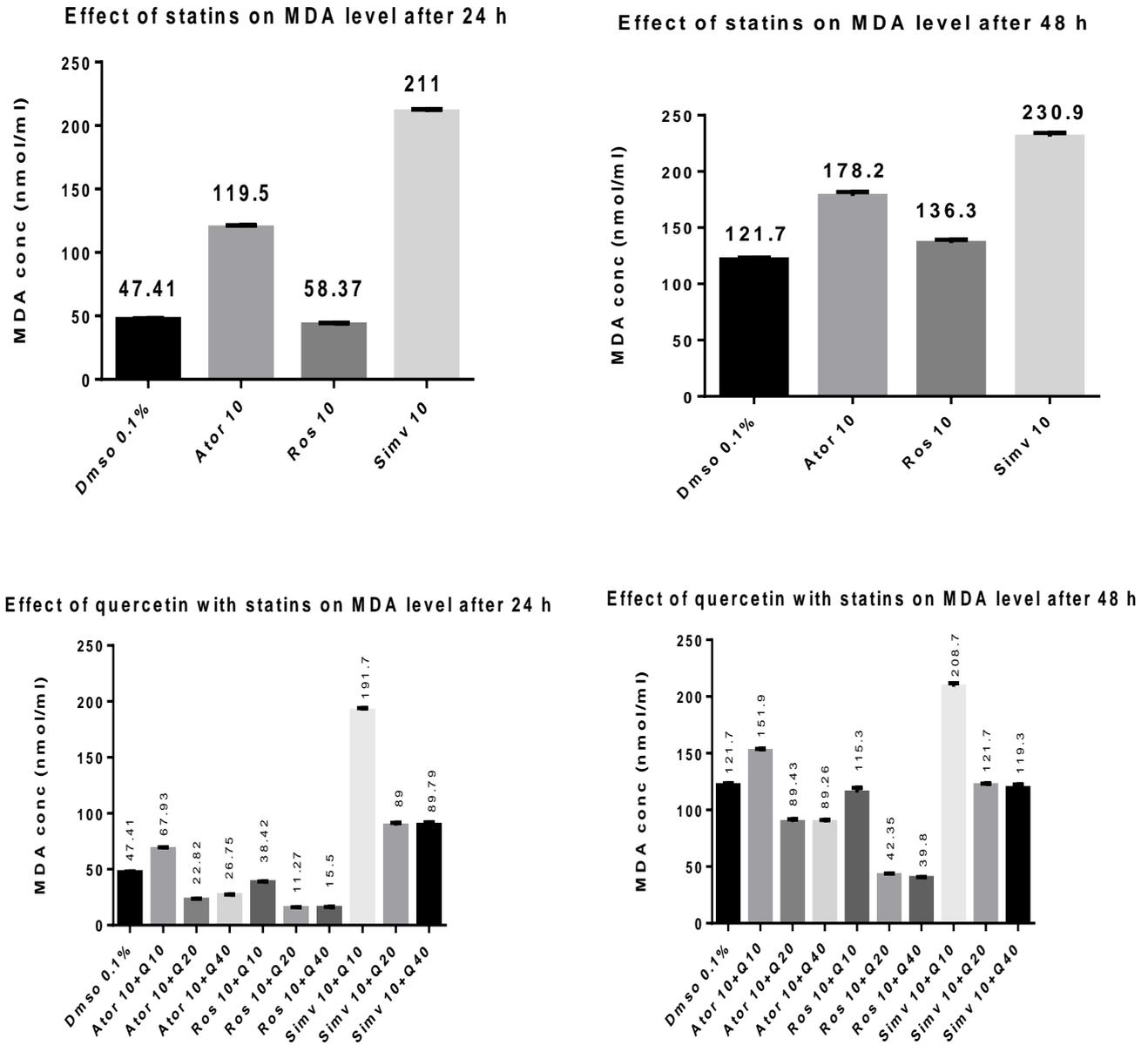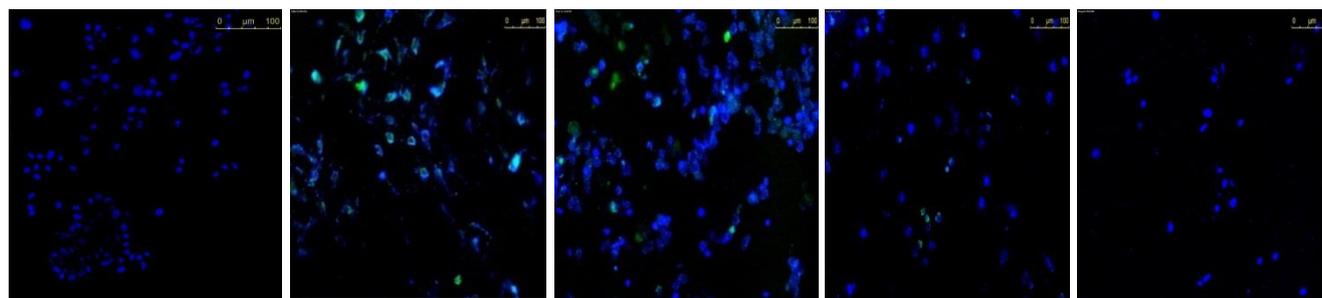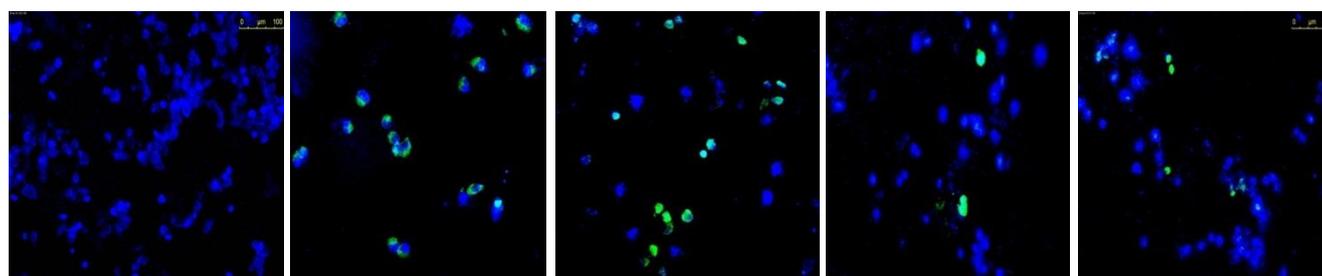
Effect of statins on MDA level after 24 h

Effect of statins on MDA level after 48 h

Effect of quercetin with statins on MDA level after 24 h

Effect of quercetin with statins on MDA level after 48 h

Figure 4: Effect of statins on MDA level after incubation for 24 and 48 hours and after the addition of 10, 20 and 40µM quercetin; Ator: Atorvastatin, Ros: Rosvastatin, Simv: Simvastatin, Q: Quercetin.

Immunofluorescence assay: Fluorescence intensity was measured in the cells treated with statins after 24 and 48 hours incubation which evoked a significant increase in fluorescence intensity, which means elevation of reactive oxygen species (ROS). Pretreatment of the cells with (10, 20 and 40µM) quercetin 4 hours before the addition of statins decreased fluorescence intensity that indicates reduction in ROS levels in response to the addition of quercetin in a dose-dependent manner.
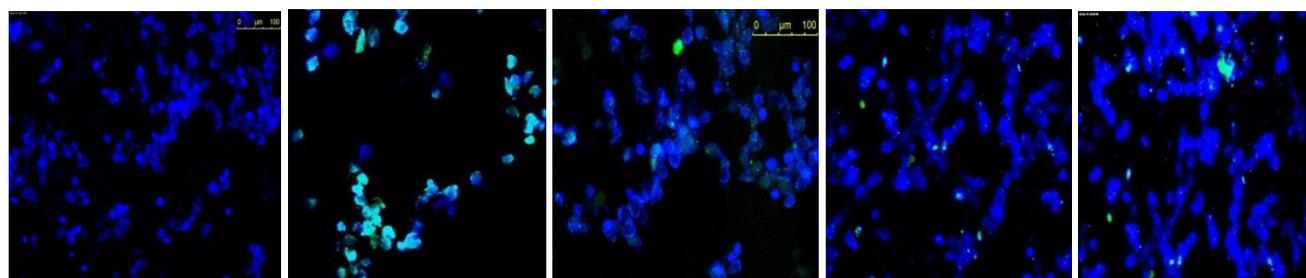
After 24 hours incubation:

Control (Dmso0.1%)   A 10μM   A 10μM+Q 10μM   A 10μM+Q 20μM   A 10μM+Q 40μM

Control (Dmso0.1%)   R 10μM   R 10μM+Q 10μM   R 10μM+Q 20μM   R 10μM+Q 40μM

Control (Dmso0.1%)   S 10μM   S 10μM+Q 10μM   S 10μM+Q 20μM   S 10μM+Q 40μM
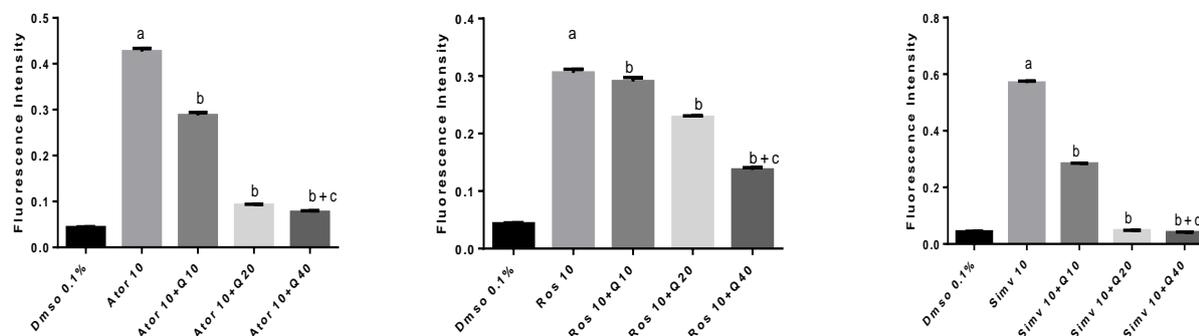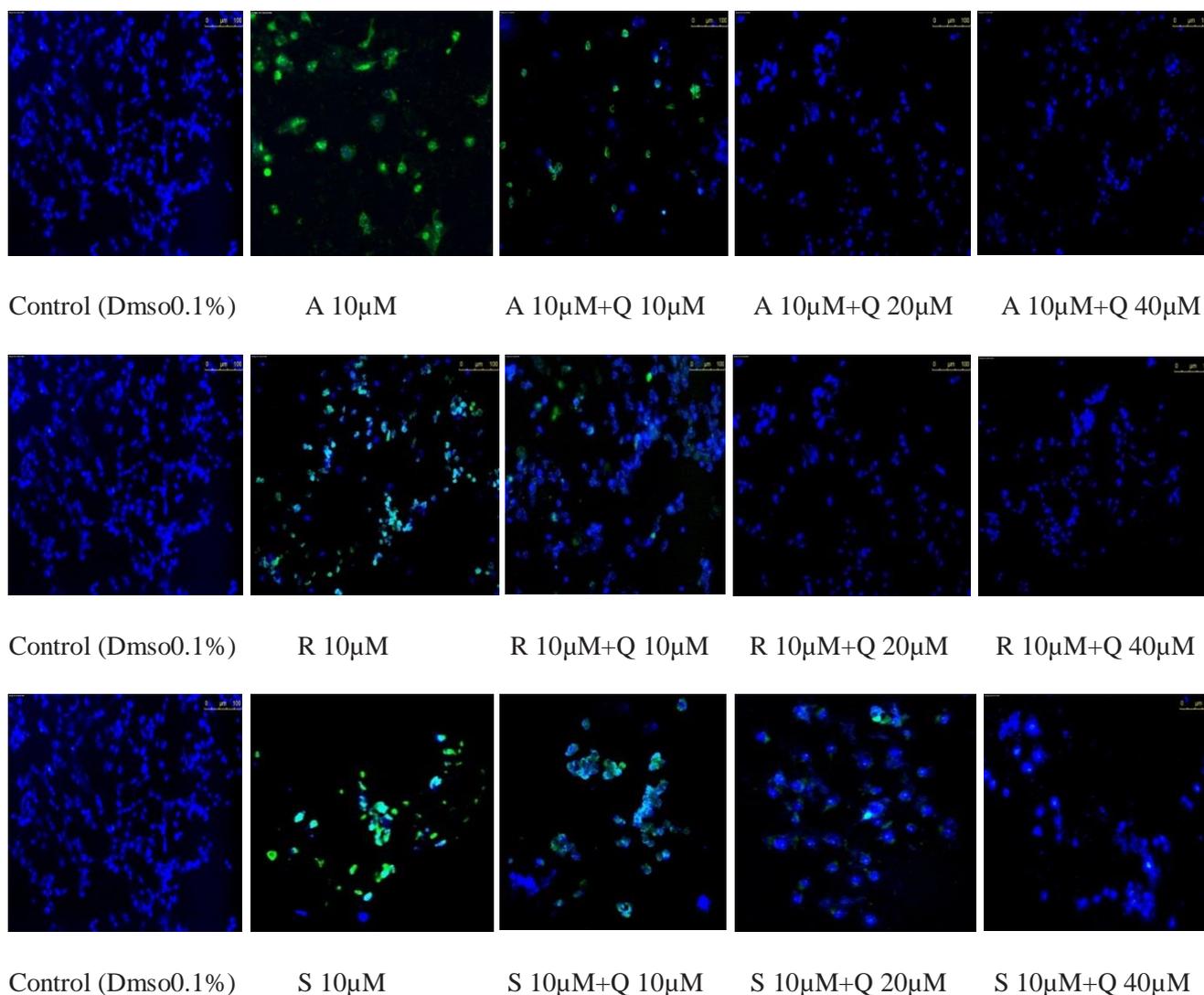
Figure 5: Expression of ROS by fluorescence intensity in HepG2 cells after treatment with atorvastatin, rosuvstatin and simvastatin with and without pretreatment with 10, 20 and 40μM quercetin 4 hours before the addition of statins *(after 24 hours of incubation)*.

A: Atorvastatin; R: Rosuvastatin; S: Simvastatin; Q: Quercetin; blue (DAPI): nucleus; green (Dichlorofluorescen): ROS. [a]p <0.05: significant difference from the control group; [b]p <0.05: significant difference from the toxic group; [c]p <0.05: significant difference from 20µM quercetin group; Dmso: Dimethyl sulfoxide; DAPI: 4', 6-diamdino-2-phenylindol.

As shown in figure 5, the addition of 10µM atorvastatin, 10µM rosuvastatin and 10µM simvastatin significantly elevated ROS in HepG2 cells, which can be determined by measurement of the fluorescence intensity compared with the control group. Pretreatment of HepG2 cells with quercetin 4 hours before the addition of statins showed significant decrease in ROS in a dose-dependent manner. 40µM querctin is slightly more protective than 20µM quercetin and is extremely more protective than 10µM quercetin. We observed that, simvastatin elevated ROS more than atorvastatin, and atorvastatin elevated ROS more than rosuvastatin.

After 48 hours incubation:

Control (Dmso0.1%)   A 10µM   A 10µM+Q 10µM   A 10µM+Q 20µM   A 10µM+Q 40µM

Control (Dmso0.1%)   R 10µM   R 10µM+Q 10µM   R 10µM+Q 20µM   R 10µM+Q 40µM

Control (Dmso0.1%)   S 10µM   S 10µM+Q 10µM   S 10µM+Q 20µM   S 10µM+Q 40µM

Effect of atorvastatin on ROS in HepG2 cells after 48 h   Effect of rosuvastatin on ROS in HepG2 cells after 48 h   Effect of simvastatin on ROS in HepG2 cells after 48 h
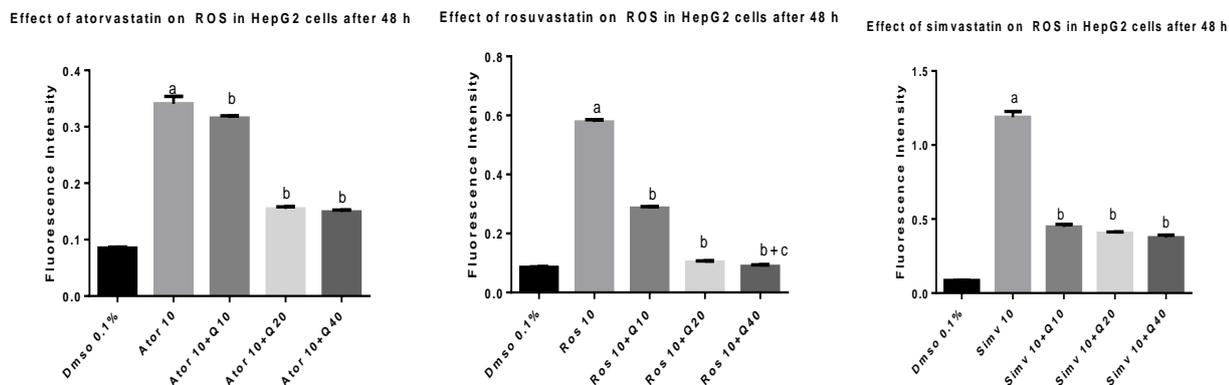

Figure 6: Expression of ROS by fluorescence intensity in HepG2 cells after treatment with atorvastatin, rosuvastatin and simvastatin with and without pretreatment with 10, 20 and 40μM quercetin 4 hours before the addition of statins *(after 48 hours of incubation)*. A: Atorvastatin; R: Rosuvastatin; S: Simvastatin; Q: Quercetin; blue (DAPI): nucleus; green (Dichlorofluorescen): ROS. [a]$p < 0.05$: significant difference from the control group; [b]$p < 0.05$: significant difference from the toxic group; [c]$p < 0.05$: significant difference from 20μM quercetin group; Dmso: Dimethyl sulfoxide; DAPI: 4', 6-diamdino-2-phenylindol.

A shown in figure 6, the addition of 10μM atorvastatin, 10μM rosuvastatin and 10μM simvastatin significantly elevated ROS in HepG2 cells, which can be determined by measurement of the fluorescence intensity compared with the control group. Pretreatment of HepG2 cells with quercetin 4 hours before the addition of statins showed significant decrease in ROS in a dose-dependent manner.

In case of atorvastatin and simvastatin, there was no significant difference between 20μM quercetin and 40μM quercetin but they offered more protection than 10μM quercetin. But in the case of rosuvastatin, 40μM quercetin was slightly more protective than 20μM quercetin and was extremely more protective than 10μM quercetin. We concluded that, simvastatin elevated ROS more than rosuvastatin and rosuvastatin elevated ROS more than atorvastatin.

DISCUSSION

Statins are the most commonly prescribed drugs for treatment of cardiovascular diseases and hyperlipidemia and atorvastatin, rosuvastatin and simvastatin are the most frequently prescribed statins. Hepatotoxicity is one of the most important side effects of statins therapy **(Abdoli et al., 2014)**. However, the mechanism of hepatotoxicity induced by statins is not clearly understood yet and the process of apoptosis is either aberrant or inhibited in cancer cells **(M. Schuler and D.R. Green, 2001)**. However, the effect of statins on cancer cells have been studied before, the molecular mechanism involved in their apoptotic effects remains a major focus of investigation.

In our study, we tried to recognize the toxic effect of statins on hepatic cells in vitro using HepG2 cell line and identify the protective effect of quercetin against statins-induced hepatotoxicity**.**

Our study showed that statins induced hepatocellular toxicity by different ways, by decreasing cell viability% in agreement with **(Janine Kah et al., 2012),** we observed that atorvastatin decreased cell viability% more than rosuvastatin more than simvastatin after 24 and 48 hours when compared with the control group (DMSO 0.1%), and 10, 20 and 40μM quercetin protect the cells by increasing cell viability % when added 4 hours before statins without significant difference in protection between 20 and 40μM quercetin. We observed that statins increased alanine transaminase level (ALT level), simvastatin increased ALT level more than rosuvastatin more than atorvastatin after 24 and 48 hours when compared with the control group, pretreatment with 10, 20 and 40μM quercetin 4 hours before statins could protect the cells by decreasing ALT levels without significant difference in protection between 20 and 40μM. Also we observed that statins increased aspartate transaminase (AST) level, simvastatin increased AST level more than atorvastatin more than rosuvastatin after 24 and 48 hours when compared with the control group, pretreatment of the cells with 10, 20 and 40μM quercetin 4 hours before treatment with statins could protect them by decreasing AST levels without significant difference between 20 and 40μM quercetin. But there is a little significant difference in protection between 20 and 40μM quercetin when added with 10μM simvastatin after 24 hours incubation. We concluded that statins increase ALT level more than AST level.

In order to investigate the mechanism of statins hepatotoxicity, we determined malondialdehyde MDA as a lipid peroxidation parameter **(Giuliana Cighetti et al., 2002)**. We observed that, statins increased MDA concentration after 24 hours incubation which was in accordance with **(Taskeen Fathima et al., 2016),** simvastatin increased MDA level more than atorvastatin more than rosuvastatin after 24 and 48 hours when compared with the control group, pretreatment of the cells with 10, 20 and 40μM quercetin 4 hours before addition of statins could protect the cells by decreasing MDA levels without significant difference between 20 and 40μM, there is no significant difference in protection with 10μM quercetin when added with rosuvastatin, and 10μM rosuvastatin does not affect MDA level when compared with the control group after 24 hours incubation. But after 48 hours incubation, 10μM rosuvastatin significantly increased MDA when compared with the control group.

It is very important to investigate the effect of statins on hepatic cells by determination of reactive oxygen species (ROS) and investigation of the protective effect of quercetin by immunofluorescence staining assay using DAPI. We observed that statins increased ROS in HepG2 cells when added and incubated for 24 and 48 hours, simvastatin increased ROS level more than atorvastatin more than rosuvastatin after 24 hours when compared with the control group which was in accordance with **(Abdoli N et al., 2014)**. But after 48 hours incubation, rosuvastatin toxicity is more than atorvastatin toxicity, and pretreatment of the cells with 10, 20 and 40μM quercetin 4 hours before addition of statins decreased ROS level according in a dose-dependent manner without significant difference in protection between 20 and 40μM quercetin with simvastatin toxicity after 24 hours incubation. And after 48 hours incubation there

is no significant difference in protection between 20 and 40µM quercetin with atorvastatin and rosuvastatin.

CONCLUSION

Atorvastatin, rosuvastatin and simvastatin have a toxic effect on HepG2 cells with a dose of 10 µM. Quercetin could protect the HepG2 cells from statins induced-toxicity with 10, 20 and 40µM doses without significant difference between 20 and 40 µM concentrations.

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دراسة التأثير الوقائي للكيرسيتين ضد سمية الكبد التي تسببها عقاقيرالاستاتينات المخفضة للكوليسترول باستخدام خط خلايا سرطان الكبد البشرية في المختبر

[1]*محمد كمال العناني, [2]محمد محمد عبدالفتاح بدر, [1]أسامة عبد المتعال منصور

[1]قسم الكيمياء الحيوية والبيولوجيا الجزيئية ـ كلية الصيدلة (بنين) جامعة الأزهر

[2]قسم الكيمياء الحيوية والبيولوجيا الجزيئية ـ كلية الصيدلة جامعة المنوفية

*البريد الالكتروني للباحث الرئيسي : *Mohamadkamal.202@azhar.edu.eg*

أجري هذا البحث بغرض دراسة التأثير الوقائي لمادة الكيرسيتين ضد سمية الكبد التي تسببها عقاقير الاستاتينات المخفضة للكوليسترول باستخدام خط خلايا سرطان الكبد البشرية في المختبر عن طريق قياس بعض المعايير الحيوية بعد 24 و48 ساعة من حضانة الخلايا.

وهي كالآتي:

أ. مقايسة جدوى الخلية.
ب. قياس مستوى إنزيم ألانين أمينوترانسفيراز وإنزيم أسبارتات أمينوترانسفيراز في الخلايا.

ج. قياس أكسدة الدهون عن طريق قياس تركيز مستوى المالونديالديهيد في الخلايا.

د. قياس أنواع الاكسجين التفاعلية في الخلية عن طريق مقايسة التألق المناعي باستخدام مادة الديكلوروفلوريسين وصبغ النواة باستخدام مادة دابي.

وقد استخدمت في هذه الدراسة 3 عقاقير من مجموعة الاستاتينات وهي: أتورفاستاتين وروزوفاستاتين وسيمفاستاتين, وقسمت المجموعات كالآتي:

- مجموعة المقارنة: تحتوي علي الخلايا مضافًا إليها مادة ثنائي ميثيل سلفوكسيد.
- مجموعة الخلايا+10 ميكرومولار من الأتورفاستاتين.
- مجموعة الخلايا+10 ميكرومولار من الأتورفاستاتين+10 ميكرومولار من الكيرسيتين.
- مجموعة الخلايا+10 ميكرومولار من الأتورفاستاتين+20 ميكرومولار من الكيرسيتين.
- مجموعة الخلايا+10 ميكرومولار من الأتورفاستاتين+40 ميكرومولار من الكيرسيتين.
- مجموعة الخلايا+10 ميكرومولار من الروزوفاستاتين.
- مجموعة الخلايا+10 ميكرومولار من الروزوفاستاتين+10 ميكرومولار من الكيرسيتين.
- مجموعة الخلايا+10 ميكرومولار من الروزوفاستاتين+20 ميكرومولار من الكيرسيتين.

- مجموعة الخلايا+10 ميكرومولار من الروزوفاستاتين+40 ميكرومولار من الكيرسيتين.
- مجموعة الخلايا+10 ميكرومولار من السيمفاستاتين.
- مجموعة الخلايا+10 ميكرومولار من السيمفاستاتين+10 ميكرومولار من الكيرسيتين.
- مجموعة الخلايا+10 ميكرومولار من السيمفاستاتين+20 ميكرومولار من الكيرسيتين.
- مجموعة الخلايا+10 ميكرومولار من السيمفاستاتين+40 ميكرومولار من الكيرسيتين.

وقد تمت دراسة تأثير عقاقير الاستاتينات على خلايا سرطان الكبد البشرية قبل وبعد إضافة ثلاث تركيزات مختلفة من الكيرسيتين (10 , 20 , 40 ميكرومولار) وتم رسم منحنى الاستجابة للجرعات المختلفة لكل عقار من الاستاتينات على الخلايا قبل وبعد إضافة مادة الكيرسيتين وكذلك تم تحديد تركيز مادة الكيرسيتين الموافق للتثبيط النصفي.

وقد وجد أن:

1-عقار السيمفاستاتين هو العقار الأكثر سمية على الخلايا الكبدية يليه عقار الأتورفاستاتين ثم عقار الروزوفاستاتين.

2- الكيرسيتين يحمي خلايا الكبد من السمية التي تسببها عقاقير الاستاتينات بتركيزات مختلفة من الكيرسيتين, حيث يوفر تركيز 10 ميكرومولار الحماية للخلايا من السمية, أما تركيز 20 ميكرومولار من الكيرسيتين فيوفر حماية أكبر للخلايا مثل تلك التي يوفرها تركيز 40 ميكرومولار من الكيرسيتين دون فرق واضح بين التركيزين.

وبذلك نستخلص من هذه الدراسة أن الكيرسيتين يمكنه حماية الكبد من سمية عقاقير الاستاتينات عن طريق زيادة نسبة بقاء الخلية الكبدية وتقليل نسبة إنزيمات خلايا الكبد المختلفة وتقليل التحلل التأكسدي للدهون الموجودة في الخلية وتقليص نسبة أنواع الأكسجين التفاعلية التي تؤدي إلى موت الخلية.

ويعد الكيرسيتين من العناصر الحيوية الموجودة في العديد من المواد الغذائية, لذا ننصح بإضافته إلى عقاقير الاستاتينات كعامل حماية, حيث إنها تعتبر من أشهر العقاقير التي تستخدم بنسبة كبيرة في تخفيض مستوى الكوليسترول في الدم عالميًا.

الكلمات المفتاحية: الاستاتينات, إصابة الكبد الناتجة من تناول العقاقير, الكيرسيتين, مالونديالديهيد, مقايسة جدوى الخلية, مقايسة التألق المناعي, صبغ النواة باستخدام مادة دابي.