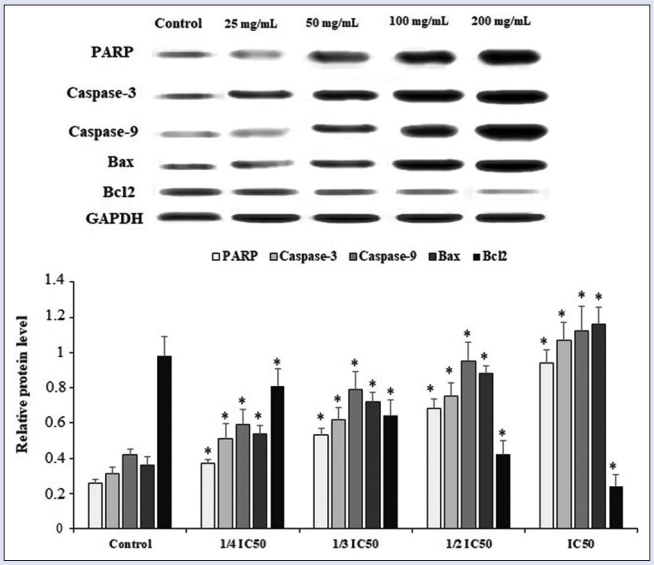

Figure 5: Effects of various concentrations of RJ (0.25, 0.5, 1, and 2 mg/ml) on the protein level of PARP, Caspase-3, Caspase-9, Bcl-2, and Bax in HepG2 cells. Data are expressed as the mean ± SD (n = 3). * $P < 0.001$ difference was significant compared with the control

signaling pathway alteration, suppression of cell cycle actions, apoptosis stimulation, and their anti-metastasis and anti-angiogenic properties. Moreover, Kopustinskiene *et al.*[23] (2020) have revealed that flavonoids exhibit their anti-cancer effects through mechanisms such as autophagy, controlling ROS-scavenging enzyme activities, suppressing the cell cycle, promoting the apoptosis, and inhibition of multiplication of cancer cells.

The incidence and development of cancer are often accompanied with abnormal proliferation and apoptosis resistance of cancer cells. Apoptosis is a gene-regulated process associated to extra-ordinary morphological changes, condensation of chromatin, and DNA damages.[24] Some factors and proteins are related to the mechanism of apoptosis, such as cysteine proteases (also called caspase enzymes and the Bcl-2 family). Among the caspases, Caspase 3 and 9 enzymes play a key role in the apoptotic process and are considered responsible for some mechanisms of apoptosis that cause DNA fragmentation, chromatin condensation, the cleavage of nuclear and cytosolic substrates and apoptotic bodies, and so on.[24,25] Instead, Bcl-2 as an anti-apoptotic prevents the apoptosis process through blocking cytochrome *c* release from mitochondria.[26] miR-34a is recognized as a tumor suppressor biomarker that has been used to assess and modulate cancer cell invasion, drug resistance, metastasis, diagnosis, and prognosis of cancers.[27] miRNA-34a shows different expressions in many cancer types such as colon cancer. Subsequently, down-regulation of miR-34a can disturb various processes such as the cell cycle, apoptosis, and differentiation and growth.[28,29] By real-time PCR, the expression of Bax and the Caspase-3 gene was considerably ($p < 0.001$) increased, ranging from 1.92 to 3.34-fold after treatment with RJ. The expression level of the miR-34a gene was noticeably ($p < 0.05$) elevated 2.11 to 4.45-fold after treatment with RJ. In contrast, the Bcl-2 expression level was considerably ($p < 0.05$) declined in the HepG2 cells exposed with RJ.

By AV assay, HepG2 cell treatment with RJ at the concentration of ½ IC_{50} significantly increased ($p < 0.05$) apoptotic and necrotic cells from 0.96% to 28.3% and 9.3%, respectively. RJ at the concentration of IC_{50} significantly increased ($p < 0.05$) apoptotic and necrotic cells from 0.96% to 39.2% and 14.12%, respectively.

PARP is considered an important enzyme involved in the synthesis of the chromatin structure, replication, transcription, and DNA restoration.[30] Previous studies revealed that up-regulation and hyper-activation of PARP result in cell death through a specific apoptosis pathway described by mitochondrial dysfunction, depletion of NAD+/ATP, imbalance of calcium, and release of the apoptosis-inducing factor.[30,31] Our results exhibited that HepG2 cells treated with various concentrations of RJ (0.25, 0.5, 1, and 2 mg/ml) increased PARP expression, indicating that RJ might induce cell death through a specific apoptosis pathway.

CONCLUSION

Our results showed the promising anti-cancer effects of RJ against HepG2 cells, whereas the induction of apoptosis by various pathways is considered as the main mechanism underlying the cytotoxic effect of RJ against HepG2 cells. The present study's findings propose that RJ can be a candidate agent for treating human HCC.

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Conflicts of interest

There are no conflicts of interest.

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