How to Cite:
Nikzad, M., & Shokrzadeh, M. (2022). Evaluation of the protective effect of simvastatin on genotoxic effects of doxorubicin via comet assay. *International Journal of Health Sciences*, *6*(S7), 1536–1551.
https://doi.org/10.53730/ijhs.v6nS7.11567

Evaluation of the protective effect of simvastatin on genotoxic effects of doxorubicin via comet assay

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***Abstract*---**Background and purpose: Doxorubicin (Adriamycin) a chemotherapeutic agent belonging to Anthracycline family, is frequently used in the treatment of cancers. Despite its effectiveness, a vast variety of adverse effects have been attributed to this drug with the suggested mechanisms of inducing ROS and mitochondrial damage causing hepatic and cardiac toxicity. In addition to cancer cells, doxorubicin also interferes with the normal cell genome and consequently induce secondary malignancy. Simvastatin, Simvastatin is a fat-lowering drug that has cytoprotective and antioxidant effects. This study attempts to examine the protective effect of simvastatin against doxorubicin induced genotoxicity in the culture medium using the Comte method. Materials and Methods: For this purpose, we measured the DNA damage level with comet assay in HepG2 and HGF cells treated with doxorubicin and simvastatin in pre-treatment condition. Results were reported based on mean ± SEM. $p<0.05$ considered as significant deference. The mean values were compared using SPSS and ANOVA test followed by tukey posttest. Results: In this study, doxorubicin caused significant genotoxicity in both cell lines ($P <0.0001$). Statistical analysis of the data showed the protective effect of simvastatin on doxorubicin-induced genotoxicity by reducing various parameters of comet technique in HepG2 cell line only at 50 and 100µM concentrations and in HGF cell line in all three concentrations studied ($p <0.001$). Discussion and conclusion: According to the results, 1 µM doxorubicin is the lowest genotoxic concentration in both cell lines. Also, simvastatin exerted more protective effects in the normal cell line than in the normal cell line. These effects can be attributed to the antioxidant properties of simvastatin and the inhibition of doxorubicin-induced free radicals.

International Journal of Health Sciences ISSN 2550-6978 E-ISSN 2550-696X © 2022.
Manuscript submitted: 9 April 2022, Manuscript revised: 18 June 2022, Accepted for publication: 27 July 2022

Keywords--- *Doxorubicin, Genotoxicity, Comet, Simvastatin*

Introduction

Cancer is among the leading causes of mortality around the world [1-2]. It is caused by uncontrollable cell division due to various environmental contributors and genetic disorders. Four key genes controlling cancer cells include oncogenes, tumor suppressor genes (TSGs), DNA repair genes, and antiapoptotic genes. Healthy cells become cancerous as a result of a genetic mutation. Accordingly, cancer treatment remains of paramount importance [3].

Ideal anti-cancer drugs (ACDs) should kill cancer cells with no harsh damage to the body's natural tissues. Depending on how they function, ACDs are classified into two main categories: 1) cell cycle-specific drugs (CCS drugs) such as antimetabolites, podophyllin alkaloids, and herbal alkaloids, and 2) cell cycles no specific drugs (CCNS drugs) such as alkylating antineoplastic agent, antibiotics, cisplatin, and dacarbazine (DTIC) [4].

A popular ACD in clinics is an anthracycline antibiotic, doxorubicin (Dox), broadly used to treat various cancers such as leukemia, lymphoma, pediatric cancers (e.g., Wilms' tumor), and many hard-to-treat tumors in adults. However, there are three main tumor-toxicity activities attributed to these anthracyclines, including 1) attachment to DNA with strong binding affinity through becoming integrated to DNA and RNA and thus inhibiting their formation, as well as decomposition of DNA strand by influencing type II topoisomerases, 2) attachment to the membranes to alter the status of liquids and transfer ions, and 3) formation of Quinone (a poisonous free radical) and other free radicals of oxygen through the enzyme-dependent reduction reaction [5].

The lack of selective cytotoxicity usually results in severe outcomes such as cardiac toxicity, bone marrow scarring, and nephrotoxicity, restricting the clinical use of these drugs [6]. The most potential contributors include damage to various organs, especially heart damage (congestive heart failure), free oxygen radical production during the metabolism of this drug in mitochondria walls, and insufficient enzymes neutralizing free radicals in damaged tissues [7]. Mutagenic agents and clastogens also contribute to carcinogenicity as promoters. These substances initiate carcinogenicity and contribute to the numerous genetic modifications that result in cancer progression. Moreover, the genetic toxicity of drugs could result in secondary tumors other than the initial tumors. Thus, research at various levels and exploring proper solutions to avoid or mitigate genetic toxicity seem necessary [8].

Simvastatin is a fat-lowering drug and a strong antioxidant compound that exerts its antioxidant properties in different ways [9]. In one pathway, simvastatin acts via intracellular antioxidant mechanisms such as the Heme oxygenase-1 (HO-1) pathway and glutathione peroxidase (GPx) enzyme and thus elevated glutathione contents. The other pathway is simvastatin's indirect antioxidant function by inhibiting iron-dependent oxidative damage. According to studies, simvastatin prevents the toxic effects of various pharmaceuticals via antiapoptotic functions

and antioxidant pathways [10-12]. Thus, concerning simvastatin's antioxidant properties, this study investigates simvastatin's protective effect against the damage caused by Dox-induced oxidative stress.

Materials and methods

This study investigated simvastatin's protective effects against Dox-induced genetic toxicity by employing the Comet assay. After 24 h of incubation, HEPG2 and HGF cells were treated with varying concentrations of Dox (0.01, 1, 5, and 10 μM) to evaluate the genetic toxicity of the Dox. Before treating cells with Dox, they were adjacent to varying concentrations of simvastatin (25, 50, and 100 μM) to investigate the protective effects of simvastatin and then underwent the Comet assay.

The migration of ethidium bromide (EtBr)-stained DNA was observed under a fluorescent microscope. In this study, 100 cells were randomly analyzed in each sample [13]. The percentage of DNA in the tail is proportional to the percentage of DNA damage. The relevant parameters, including the percentage of DNA in the tail, tail moment, and tail length, are then measured and analyzed in the Comet Score software. This study utilized these parameters, where tail length and tail moment may be computed differently in other visual analysis systems. The percentage of DNA in the tail seems to be the proper parameter for comparing DNA damage between laboratories [14].

There are numerous statistical methods to analyze data obtained from the Comet assay. In addition, numerous regular tests are required to perform appropriate statistical analysis for in vitro tests. For instance, it is crucial to determine the mean rate of DNA migration per 100 cells in each sample and the mean data of two or three samples.

A proper analysis requires utilizing the prepared slides containing adequate high-quality cells to allow appropriate evaluation of the rate of DNA damage. Three slides were prepared for each sample in each experiment. The number of Comet assays required for each sample was determined. Tail length, the percent of DNA in the tail, and tail moment were parameters intended for analyses. The range of each cell was measured in the Comet Score software.

The results and data obtained were reported as the mean of two experiments a day, i.e., three slides from each sample and three specific tests on three different days. The data's standard deviation (SD) was determined in the relevant tables. The results were analyzed as SEM ± Mean. Comet assay data were analyzed, and the diagram of the parameters was drawn in Prism 3 graphic software. One-way ANOVA and posthoc Tukey's multiple comparison test were employed at the 0.05 significance level [14].

Results

Dox's influential genetic toxicity concentration in the liver cancer cell line (HepG2) was determined using the Comet assay. Then simvastatin's varying concentrations were then tested to determine the degree of conservation. In all

experiments, negative controls were cells incubated with a complete cell culture medium having no additional material. Conversely, positive controls were cells incubated with the intended Dox concentrations, where the results were compared with those reported in DNA conservation studies.

The alkaline comet assay was employed to evaluate the genetic toxicity of Dox.

Comet assay results were analyzed in terms of tail length, the percentage of DNA in the tail, and tail moment and were compared with negative control results, i.e., cells incubated with other cells without adding any supplementary substances. This study used cells treated with the genotoxic agent H2O2 (200 μM) as positive controls. The accuracy of tests was verified using negative control and positive control slides with DOX-treated cells. HepG2 cells were adjacent to varying concentrations of Dox (0.01, 1, 5, and 10 μM) for an hour to evaluate the Dox's genotoxic effect and determine its minimum genotoxic concentration required.

Figures 1,2, and 3 demonstrate DNA damage concerning tail length, DNA % in the tail, and tail moment, respectively, compared to the control group. One-way ANOVA results were significant for all three parameters. A significant increase was observed at all concentrations for tail length, DNA % in the tail, and tail moment compared to the control group. A concentration of 1 μM was the lowest Dox's genotoxic concentration in the HepG2 cell line. This concentration was chosen for the next step as it did not increase the number of cells during incubation.

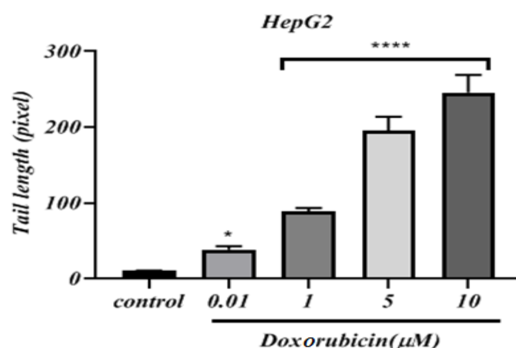

Figure 1 *Comparison of tail length obtained from Comet assay at Dox's varying concentrations in HepG2 cell line.*

(*) (p <0.05) compared to the control group

(****) (p <0.0001)) compared to the control group

The diagram in Figure 1 depicts the tail length at Dox's varying concentrations compared to the control group. The results are shown as Mean ± SEM from three replications. The control group refers to cells incubated in a complete culture medium with no supplementary substances for an hour.

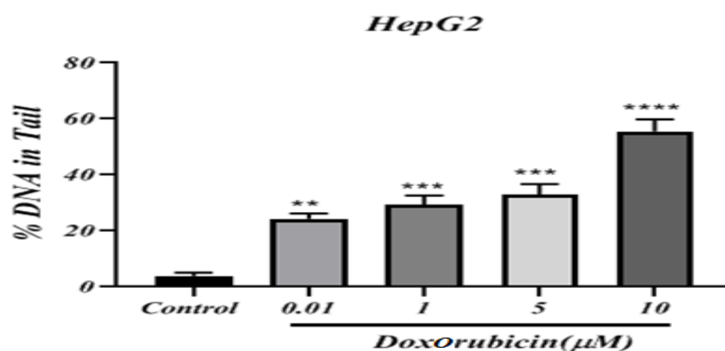

Figure 2 *Comparison of the DNA % in the tail obtained from Comet assay at Dox's varying concentrations in HepG2 cell line.*

(*) (p <0.05) compared to the control group

(**) (p <0.001) compared to the control group

(****) (p <0.0001)) compared to the control group

The diagram in Figure 2 illustrates the migration of DNA at Dox's varying concentrations compared to the control group. The results are given as Mean ± SEM from three replications. The control group refers to cells incubated in a complete culture medium with no additional substances for an hour.

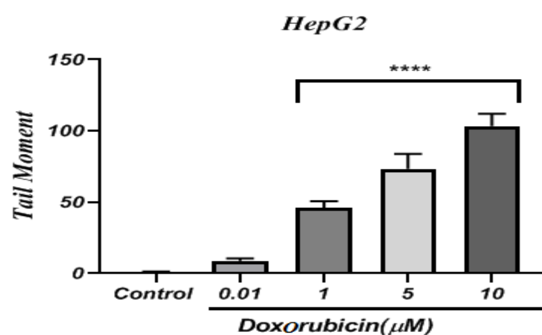

Figure 3 *Comparison of tail moment obtained from Comet assay at Dox's varying concentrations in HepG2 cell line.*

(****) (p <0.0001)) Compared to the control group

The diagram in Figure 3 depicts the tail moment at Dox's varying concentrations compared to the control group. The results are shown as Mean ± SEM from three replications. The control group refers to cells incubated in a complete culture medium with no supplementary substances for an hour. Using the alkaline comet

assay, this study investigated simvastatin's protective effect on Dox-induced genetic damage on the HepG2 liver cancer cell line.

The comet assay results were analyzed in terms of tail length, DNA % in the tail, and tail moment and were compared with the results of the positive control group. HepG2 cells were incubated for 24 hours with varying simvastatin concentrations to determine the protective effects of simvastatin on Dox-induced genotoxicity. Then, after washing, cells were treated with Dox (1 μM) for an hour.

The results obtained from varying concentrations (25, 50, and 100 μM) were compared with the control group in all three factors to evaluate simvastatin's genotoxicity. There were no significant differences with the control group. Consequently, the concentration of 100 μM was used as a control group in the statistical analysis.

Figures 4,5 and 6 demonstrate a reduction in DNA damage in HepG2 cells compared to a positive control (1 μM Dox) in terms of tail length, DNA % in the tail, and tail moment.

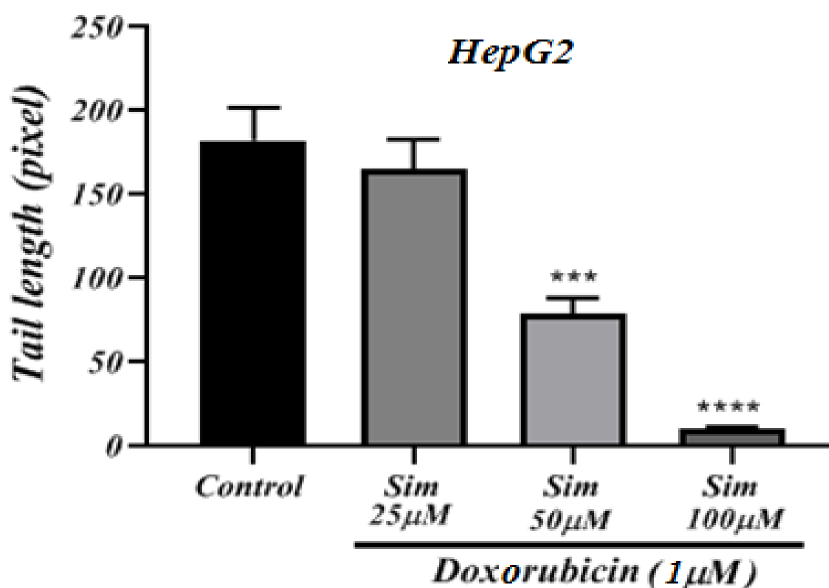

Figure 4 Comparison of the simvastatin's effect on Dox (1 μM)-induced in HepG2 cells using the comet assay in terms of tail length.

(***) ($p < 0.001$) compared to the control group

(****) ($p < 0.0001$)) compared to the control group

In the tail length graph, varying pre-treatment concentrations of simvastatin and Dox (1 μM) are given compared to the control group. The control group refers to cells incubated only with Dox (1 μM). The results are given as Mean ± SEM from

three replications. As illustrated in Figure 7.4, only 50 and 100 µM simvastatin concentrations reduced the Dox-induced genotoxicity in the HepG2 cell line.

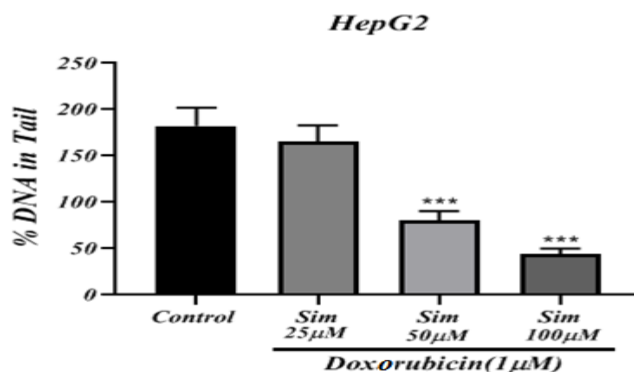

Figure 5 *Comparison of the effect of simvastatin on Dox (1 µM)-induced genotoxicity in HepG2 cells using the comet assay in terms of DNA % in the tail.*

(***) (p <0.001) compared to the control group

In the graph shown for DNA % in the tail, varying pre-treatment concentrations of simvastatin and Dox (1 µM) are compared to the control group. The control group refers to cells incubated only with Dox (1 µM). The results are given as Mean ± SEM from three replications. As illustrated in Figure 5, only 50 and 100 µM simvastatin concentrations reduced the Dox-induced genotoxicity in the HepG2 cell line.

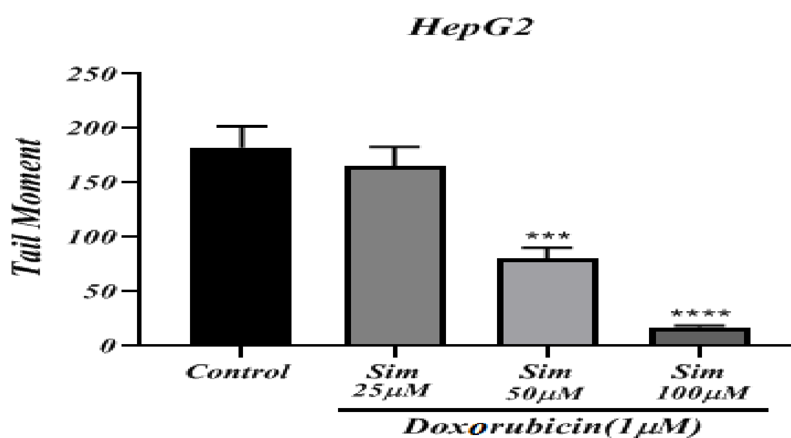

Figure 6 *Comparison of the effect of simvastatin on Dox (1 µM)-induced genotoxicity in HepG2 cells using the comet assay in terms of the tail moment.*

(***) (p <0.001) compared to the control group

*(**** (p <0.0001 relative to the control group*

In the tail moment graph, various pre-treatment concentrations of simvastatin and Dox (1 µM) are compared to the control group. The control group refers to cells incubated only with Dox (1 µM). The results are given as Mean ± SEM from three replications. As illustrated in Figure 7.6, only 50 and 100 µM simvastatin concentrations reduced the Dox-induced genotoxicity in the HepG2 cell line.

The alkaline comet assay was employed to evaluate the genetic toxicity of Dox. Comet assay results were analyzed in terms of tail length, the percentage of DNA in the tail, and tail moment and were compared with negative control results, i.e., cells incubated with other cells without adding any supplementary substances. This study used cells treated with the genotoxic agent H2O2 (200 µM) as positive controls.

The accuracy of tests was verified using negative control and positive control slides with DOX-treated cells.

7.1.2.6. Results of varying Dox concentrations in HFG cell line

HepG2 cells were adjacent to varying concentrations of Dox (0.01, 1, 5, and 10 µM) for an hour to evaluate the Dox's genotoxic effect and determine its minimum genotoxic concentration required. Figures 7,8, and 9 demonstrate DNA damage concerning tail length, DNA % in the tail, and tail moment, respectively, compared to the control group.

One-way ANOVA results were significant for all three parameters. A significant increase was observed at all concentrations for tail length, DNA % in the tail, and tail moment compared to the control group.

According to the results, a concentration of 1 µM was the lowest Dox's genotoxic concentration in the HepG2 cell line. This concentration was selected for the next phase of the study as it did not reduce the number of cells during incubation.

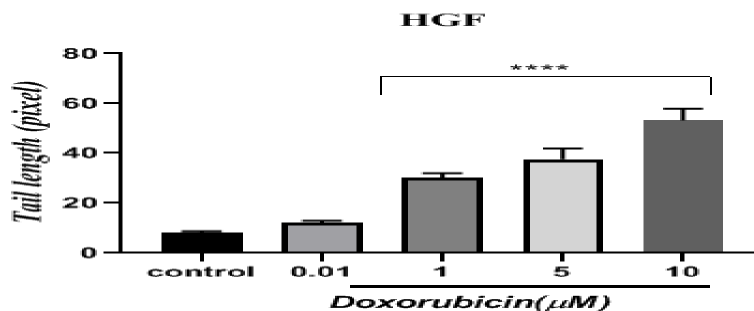

Figure 7 Comparison of tail length obtained from comet assay of varying Dox concentrations in the HGF cell line.

(****) ($p < 0.0001$)) compared to the control group

This graph compares the tail length with the control group at varying Dox concentrations.

The results are given as Mean ± SEM from three replications.
The control group refers to cells incubated only with Dox (1 µM).

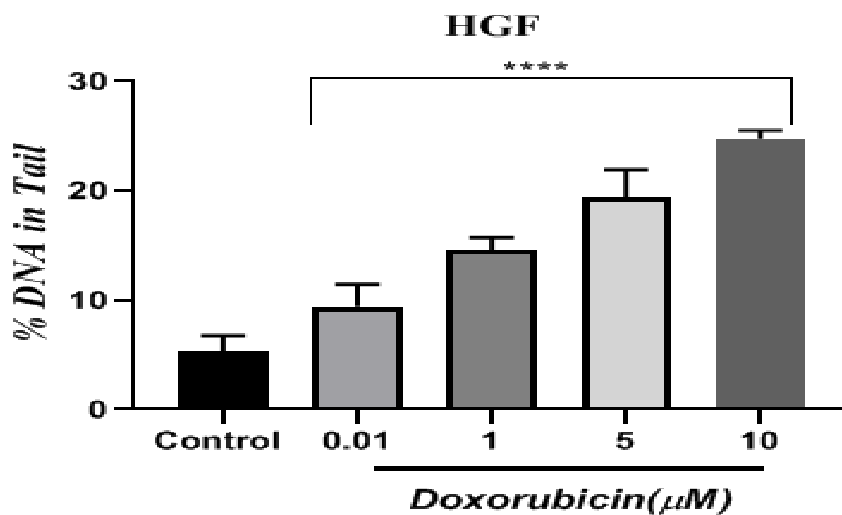

Figure 8 *Comparison of DNA % in the tail obtained from comet assay of varying Dox concentrations in the HGF cell line.*

(****) (p <0.0001)) compared to the control group
This diagram illustrates the rate of DNA migration at varying Dox concentrations compared to the control group. The results are given as Mean ± SEM from three replications. The control group refers to cells incubated only with Dox (1 µM).

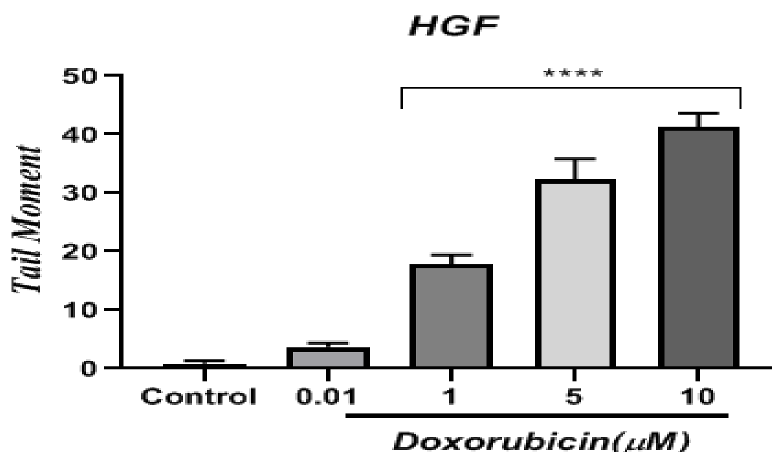

Figure 9 *Comparison of the tail moment obtained from comet assay of varying Dox concentrations in the HGF cell line.*

(****) (P <0.0001) compared to the control group.

In the tail moment graph, varying Dox concentrations are compared with the control group.

The results are given as Mean ± SEM from three replications. The control group refers to cells incubated only with Dox (1 µM).

7.2.1.7. Evaluation of the simvastatin's protective effect against Dox-induced genotoxicity in normal human gingival fibroblasts (HGnFs)

This study used the alkaline comet assay to investigate simvastatin's protective effect against Dox-induced genetic damage in normal HGnFs.

The comet assay results were measured in terms of tail length, DNA % in the tail, and tail moment and were compared with positive controls.

HepG2 cells were incubated for 24 hours with varying simvastatin concentrations to determine the protective effects of simvastatin on Dox-induced genotoxicity. Then, after washing, cells were treated with Dox (1 µM) for an hour.

The results obtained from varying concentrations (25, 50, and 100 µM) were compared with the control group in all three factors to evaluate simvastatin's genotoxicity. According to the results, there were no significant differences with the control group. Consequently, the concentration of 100 µM was used as a control group in the statistical analysis.

Figures 10, 11, and 12 demonstrate a reduction in DNA damage in HepG2 cells compared to a positive control (1 µM Dox) in terms of tail length, DNA % in the tail, and tail moment.

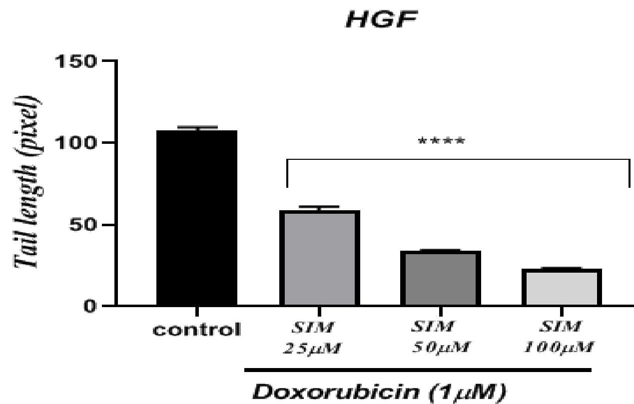

Figure 10. *Comparison of the effect of simvastatin on Dox (1 µM)-induced genotoxicity in HGF cells using the comet assay in terms of the tail length.*

(****) (p <0.0001) compared to the control group

The tail length graph shows the varying pre-treatment concentrations of simvastatin and Dox (1 µM) compared to the control group. The control group refers to cells incubated only with Dox (1 µM). The results are given as Mean ± SEM from three replications. As Figure 10 illustrates, all concentrations of simvastatin reduce Dox-induced genotoxicity in the HGF cell line.

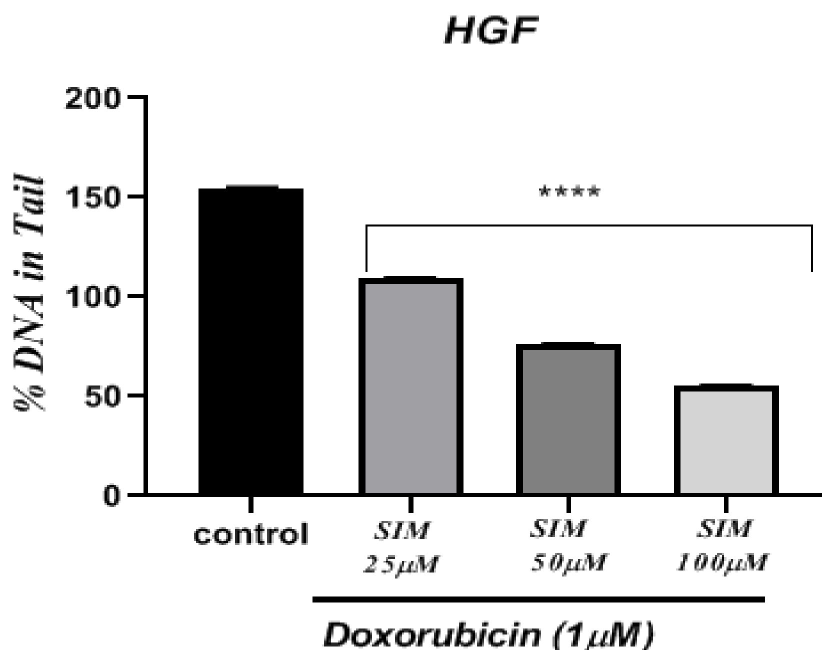

Figure 11. *Comparison of the effect of simvastatin on Dox (1 μM)-induced genotoxicity in HGF cells using the comet assay in terms of DNA % in the tail.*

(****) (p <0.0001) compared to the control group

This graph compares the varying pre-treatment concentrations of simvastatin and Dox (1 μM) with the control group. The control group refers to cells incubated only with Dox (1 μM).

The results are given as Mean ± SEM from three replications. As shown in Figure 11, all concentrations of simvastatin have diminished Dox-induced genotoxicity in the HGF cell line.

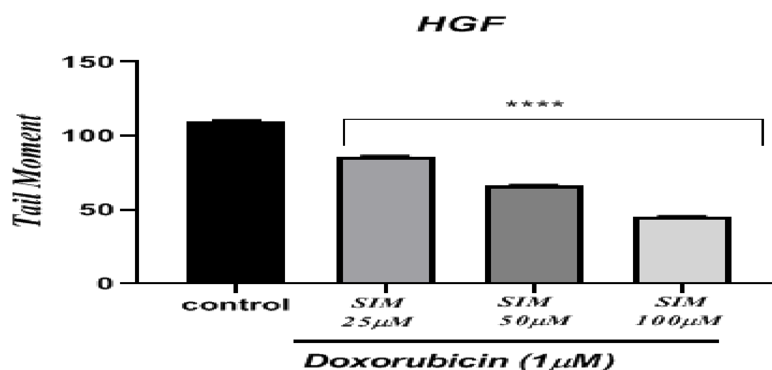

Figure 12. *Comparison of the effect of simvastatin on Dox (1 µM)-induced genotoxicity in HGF cells using the comet assay in terms of the tail moment.*

(****) (p <0.0001) compared to the control group

The tail moment graph shows the varying pre-treatment concentrations of simvastatin and Dox (1 µM) compared to the control group. The control group refers to cells incubated only with Dox (1 µM). The results are given as Mean ± SEM from three replications. As Figure 12 depicts, all simvastatin concentrations have mitigated Dox-induced genotoxicity in the HGF cell line.

Discussion

This study investigated the protective effect of simvastatin and its role in preventing Dox-induced genotoxicity. Here, the results obtained are compared with those reported in other studies. Using cell culture techniques provides detailed knowledge about the effects of various pharmaceuticals on normal and cancer cells. Research on the impact of multiple compounds such as Dox and simvastatin on cells in a controlled and detectable cell culture space identifies the underlying mechanisms and biological effects and interactions with different intracellular factors. These technologies enable us to efficiently identify intracellular processes and interactions during cancer therapy, culminating in enhanced treatments.

This study evaluated the protective effect of simvastatin on Dox-induced genotoxicity in liver cancer cell lines and HGnFs. According to the above tables, graphs, and results, the rate of Dox-induced DNA damage and the protective effect of varying concentrations of simvastatin on this drug in the studied cell lines was significant.

Dox was applied at varying concentrations on normal and cancer cell lines. According to the results concerning the tail moment parameter, the genotoxicity of Dox at its optimum concentration (1 µM) results in more severe DNA damage in HepG2 cell lines (52 ± 2.6) than in normal HGF cell lines (19 ± 1.4). Accordingly, though Dox's genotoxicity is higher in cancer cell lines, it also causes significant

damage in normal cells than in negative controls (2 ± 0.4). Hence, exploring techniques to diminish Dox's genotoxicity during chemotherapy courses is crucial. Numerous studies have researched Dox's cytotoxicity and genotoxicity effects and the underlying mechanisms.

In 2015, Wacharee Limpanasithikul and Darinee Dangkong investigated Dox's cytotoxicity and apoptotic effects using Citral, an essential oil extracted from lemon grass. This compound possesses potential helpful effects in patients with B-cell lymphoma undergoing chemotherapy. Citral (10, 20, and 40 µM) was utilized in Dox-treated patients. Cytotoxic and apoptotic studies were carried out 18 and 24 hours after incubation. Citral concentrations applied increased the cytotoxic effects of Dox and reduced its IC50 value [15]. In their study, Dox concentrations similar to ours were used, and remarkable cytotoxic effects were observed at concentrations above 1 µM. Collectively, Dox's genotoxicity can lead to apoptosis and cell death.
In a 2011 study by Thorn CF et al. to explain the mechanism of Dox toxicity in normal cells (e.g., cardiac cells), it was found that Dox acts by producing doxorubicinol (DOXol) and consequently iron-dependent free radicals through the reaction between DOXol with iron employing ACO1, calcium regulators (e.g., ATP2A2 and RYR2) and the mitochondrial F0F1 proton pump. In addition, Dox causes damage to and death of normal cells via disrupting mitochondria and inducing apoptosis following cytochrome C release [16]. In our study, Dox also induced considerable genetic damage in normal HGnFs. Accordingly, concerning the results of these two studies, Dox affects both cancerous and normal cells and causes cellular and genetic damage by various mechanisms, resulting in an increased risk for secondary cancers.

Mahalaxmi Mohan et al. (2010) investigated the protective effect of *Solanum torvum* (the turkey berry) on Dox-induced toxicity. They explored the protective effect of *Solanum torvum* on Dox-induced nephrotoxicity in rats employing biochemical and histopathological approaches. Oxidative stress is a primary cause of DOX-induced nephrotoxicity. In their study, nephrotoxicity was examined by measuring creatinine and BUN contents. They also measured natural antioxidants, including superoxide dismutase (SOD) and catalase (CAT), in kidney tissues at the end of the treatment plan. Treatment with 100 and 300 mg/kg concentrations remarkably reduced creatinine and BUN levels ($P < 0.05$). In addition, SOD and CAT levels were significantly increased ($P < 0.05$). Histopathological changes revealed that Dox leads to considerable damage such as tubular necrosis, renal lesions, and glomerular obstruction [17]. The results of studying the protective effect of *Solanum torvum* against Dox-induced toxicity are similar to our results. Dox resulted in renal toxicity in both studies by producing reactive oxygen species (ROS) and oxidative stress. Dox-induced genotoxicity can be attributed to oxidative stress.

Helena Kaiserova et al. (2007) studied the protective effect of coumarin and its antioxidant activity against Dox. Using Dox to treat solid tumors is restricted due to oxidative stress caused by carbon toxicity. Accordingly, the antioxidant activity of a set of 80 synthesized coumarin derivatives was investigated. The antioxidant potential of these derivatives was assessed using the ferric reducing ability of plasma (FRAP) assay in MCF-7 human adenocarcinoma cells. According to the

results, Dox caused cytotoxicity by producing ROS, while coumarin derivatives improved these effects [18]. Dox's toxicity effects in this study are consistent with our results, where Dox caused cytotoxicity in cancer cell lines at concentrations above 1 µM. This study used simvastatin to reduce Dox-induced genotoxicity. According to the results, simvastatin (50 and 100 µM) has significantly diminished the Dox-induced genotoxicity in the liver cancer cell line in all three comet assay parameters ($p < 0.001$).

Moreover, in normal HGnFs, simvastatin (25, 50, and 100 µM) has significantly diminished Dox-induced genotoxicity by reducing DNA fragmentation and comet duration by examining all three comet assay parameters ($p < 0.0001$). In the normal cell line, simvastatin has caused more protective effects than in the cancer cell line. However, at a concentration of 25 µM, simvastatin has shown a protective impact only in the HGF cell line. This is a positive factor when using this substance in chemotherapy protocols.

Conclusion

Based on the conducted study, doxorubicin, which is used widely as an anticancer drug in the market, shows significant genetic toxicity effects in both normal and cancer cells. Based on the results of the Comet assay, doxorubicin at concentrations of 10 and 5, 1 µM increased all three parameters of tail length, % DNA in tail and tail moment. Several mechanisms can be involved in the genetic toxicity of doxorubicin, including reducing the body's antioxidant defenses by reducing intracellular glutathione, increasing the amount of active oxygen species, and ultimately causing oxidative stress .Simvastatin, as a prodrug and a potent cytoprotective agent, could reduce doxorubicin-induced genetic toxicity. Based on the obtained results, simvastatin as a free radical scavenger can reduce the amount of doxorubicin-induced reactive oxygen species significantly and increase intracellular glutathione levels and consequently reduce doxorubicin-induced genetic toxicity. Thus, based on the data obtained from the present study, simvastatin can be considered as an important protective agent against genetic toxicity caused by anti-cancer drugs such as doxorubicin.

References

1. *Ahmed LA, Darwish HA, Abdelsalam RM, Amin HA. Role of Rho kinase inhibition in the protective effect of fasudil and simvastatin against 3-nitropropionic acid-induced striatal neurodegeneration and mitochondrial dysfunction in rats. Molecular neurobiology. 2016;53(6):3927-38.*
2. *Almehmadi M, Alzahrani K, M.Salih M, Alsharif A, Alsiwiehri N, Shafie A, and et al. Assessment of thyroid gland function by evaluating of TSH, FT3 and FT4 hormones in cancer patients. J Adv Pharm Edu Res 2020;10(4):37-42.*
3. *Avendaño C, Menendez JC. Medicinal chemistry of anticancer drugs: Elsevier; 2015.*
4. *Ewer MS, Ewer SM. Cardiotoxicity of anticancer treatments. Nature Reviews Cardiology. 2015;12(9):547.*
5. *Jaouen G, Vessières A, Top S. Ferrocifen type anti cancer drugs. Chemical Society Reviews. 2015;44(24):8802-17.*
6. *Kaiserová H, Šimůnek T, Van Der Vijgh WJ, Bast A, Kvasničková E. Flavonoids as protectors against doxorubicin cardiotoxicity: role of iron chelation,*

antioxidant activity and inhibition of carbonyl reductase. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 2007;1772(9):1065-74.

7. *Kemp JA, Shim MS, Heo CY, Kwon YJ. "Combo" nanomedicine: co-delivery of multi-modal therapeutics for efficient, targeted, and safe cancer therapy. Advanced drug delivery reviews. 2016;98:3-18.*
8. *Khaled H Alhomayani F, Hameed M, Althumali A, Abdullah Abed A A, Hamad Hamed Alhamyani A, Ahmed Hussain Alsufyani A, Abdulaziz Burayd Alharthi N, Abdulmohsen Safar Almalki M, Awareness of hemodialysis patients regarding symptoms of cancer at king Abdul-Aziz specialized hospital, Taif city. Arch Pharm Pract 2020; 11(2):69-80.*
9. *Kubásek J, Vojtěch D, Jablonská E, Pospíšilová I, Lipov J, Ruml T. Structure, mechanical characteristics and in vitro degradation, cytotoxicity, genotoxicity and mutagenicity of novel biodegradable Zn–Mg alloys. Materials Science and Engineering: C. 2016;58:24-35.*
10. *Luo D, Cheng SC-S, Xie H, Xie Y. Effects of Bcl-2 and Bcl-XL protein levels on chemoresistance of hepatoblastoma HepG2 cell line. Biochemistry and Cell Biology. 2000;78(2):119-26.*
11. *Ma H, Marti-Gutierrez N, Park S-W, Wu J, Lee Y, Suzuki K, et al. Correction of a pathogenic gene mutation in human embryos. Nature. 2017;548(7668):413.*
12. *Maheshwari RA, Balaraman R, Sailor GU, Sen DB. Protective effect of simvastatin and rosuvastatin on trinitrobenzene sulfonic acid-induced colitis in rats. Indian journal of pharmacology. 2015;47(1):17.*
13. *Mohan M, Kamble S, Gadhi P, Kasture S. Protective effect of Solanum torvum on doxorubicin-induced nephrotoxicity in rats. Food and chemical toxicology. 2010;48(1):436-40.*
14. *Nishihira M, Taira S, Tamayose S, Adachi T, Terukina S, Kikumura T, et al. SP550 EFFECT OF COMET SCORE ON SURVIVAL AMONG CHRONIC HEMODIALYSIS PATIENTS IN OKINAWA, JAPAN. Nephrology Dialysis Transplantation. 2018;33(suppl_1):i534-i.*
15. *Ostrowski SM, Johnson K, Siefert M, Shank S, Sironi L, Wolozin B, et al. Simvastatin inhibits protein isoprenylation in the brain. Neuroscience. 2016;329:264-74.*
16. *Quasnichka H, Budd E, Tarlton J, Roberts S, Jackson C, Caterson B, et al. The effect of simvastatin on novel exploratory biomarkers of cartilage degradation. Osteoarthritis and Cartilage. 2018;26:S192.*
17. *Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA: a cancer journal for clinicians. 2016;66(1):7-30.*
18. Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Health and treatment of diabetes mellitus. *International Journal of Health Sciences*, *5*(1), i-v. https://doi.org/10.53730/ijhs.v5n1.2864
19. *Thorn CF, Oshiro C, Marsh S, Hernandez-Boussard T, McLeod H, Klein TE, et al. Doxorubicin pathways: pharmacodynamics and adverse effects. Pharmacogenetics and genomics. 2011;21(7):440.*