Original Article

Cytotoxic and Genotoxic Effects of the Cold Aqueous Extract of Glycyrrhiza Glabra Roots on MCF-7 Breast Cancer Cells and REF Normal Cells in Vitro

Muslim Mohammed Kadhim and Aseel Raheem Mardan

ABSTRACT

Objective: To evaluate the cytotoxic and genotoxic effects of the cold aqueous extract of Glycyrrhiza glabra roots on MCF-7 breast cancer cells and REF normal cells in vitro.
Study Design: Experimental study
Place and Duration of Study: This study was conducted at the Al-Nahrain University Biotechnology Research Centre from 22nd December 2024 to 20th March 2025,
Methods: Glycyrrhiza glabra extract was administered to MCF-7 breast cancer cells at different concentrations (70, 140, 210, 280, and 350 µg/ml) for 24, 48, and 72 hours. The embryonic fibroblast cells were treated 72 hours. Cell viability, IC_{50} values, and deoxyribonucleic acid damage was assessed using the comet assay.
Results: This extracts reduced MCF-7 cell proliferation time- and dose-dependently at 350 µg/ml inhibition rates was significantly ($p<0.05$): 77.778% (24 h), 79.349% (48 h), and 87.262% (72 h). The IC_{50} of embryonic fibroblast cells was 5.59 mg/ml after 72 hours, demonstrating selective susceptibility. In the comet test, MCF-7 cells treated with increasing extract concentrations demonstrated moderate and severe DNA damage after 72 hours.
Conclusion: Gglycyrrhiza glabra root cold aqueous extract is dose and time-dependently cytotoxic and genotoxic to MCF-7 breast cancer cells but not normal fibroblast cells.

Key Words: Glycyrrhiza glabra, Cytotoxicity; Comet assay; MCF-7 cells

Citation of article: Kadhim MM, Mardan AR. Cytotoxic and Genotoxic Effects of the Cold Aqueous Extract of Glycyrrhiza glabra Roots on MCF-7 Breast Cancer Cells and REF Normal Cells in Vitro. Med Forum 2025;36(9):43-48. doi:10.60110/medforum.360908.

INTRODUCTION

Medical plant bioactive substances are investigated globally for therapeutic and pharmacological uses. To cure cancer, plants help. Traditional medicine prevents sickness using herbs. Modern medicine uses plants and drugs. Principal herbal drug is plant secondary metabolites, alkaloids, glycosides, chlorophyll, carotenoids, protein, minerals, vitamins.[1] Plant extracts treat diabetes, heart, and cancer, Glycyrrhiza glabra L., bushy perennial licorice with lateral blooms and semi-creeping branches. Licorice grows 50–125cm^2, depending on blossom color. Fabaceae has licorice. The bioactive components glycyrrhizin, 18-β glycyrrhetinic acid, and isoflavones have medicinal promise.[3]

Department of Biology, College of Education, University of Al-Qadisiyah, Diwaniyah, Iraq.

Correspondence: Muslim Mohammed Kadhim, Department of Biology, College of Education, University of Al-Qadisiyah, Diwaniyah, Iraq.
Contact No: +964 7809345528
Email: edu.bio.posta124@qu.edu.iq

Received: April, 2025
Reviewed: May, 2025
Accepted: June, 2025

Immunomodulatory, anti-inflammatory, antiviral, antioxidant, and antidiabetic, this plant kills bacteria. Cancer is uncontrolled cell development that tumors. A tumor might be malignant or benign cancer may spread.[4] DNA alterations that disrupt cell cycle may cause cancer.[5] Bone, brain, lungs, blood cells, etc. may spread cancer. Tissue origin classification of malignant cancers diseases may spread from blood or lymphatic vessels, complicating therapy.[6]

METHODS

This experiment study was conduct at Al-Nahrain University Biotechnology Research Centre from 22nd December 2024 to 20th March 2025. Glycyrrhiza glabra roots were available in Baghdad. After washing off dust and dirt, the roots were air-dried at room temperature and crushed into powder. 30 g powder and 300 ml distilled water were put to a clean flask. The mixture rested 24 hours at room temp. The flask extracted surface-active components best when power-swirled. The extract was filtered through sterile gauze after 24 hours to separate liquid and solid residues. The filtered extract was lyophilized at 4°C before use.

Cell Lines and Culture Conditions: Al-Nahrain University's Biotechnology and Genetic Engineering Research Center offered MCF-7 breast cancer cells and

normal rat embryonic fibroblasts for investigation. The cell lines were grown in DMEM with 10% FBS and 1% penicillin-streptomycin, cells grown in a sterile CO_2 incubator (5% CO_2, 37°C).

Cytotoxicity Assay (MTT Assay): MTT was used to investigate Glycyrrhiza glabra root cold aqueous extract cytotoxicity. The first MTT experiment tested MCF-7 cells with cold aqueous extract at various concentrations (70, 140, 210, 280, 350 µg/ml) for 24 hours, 48 hours, and 72 hours. For 72 hours, REF cells received the same cold aqueous extract. MCF-7 and REF-exposed cells received MTT after 4, 24, 48, and 72 h. An ELISA reader measured 620 nm absorbance after dissolving formazan crystals in 0.04 ml DMSO.

Genotoxicity Assay (Comet Assay): The extract was administered to MCF-7 cells for 72 hours and tested for genotoxicity using the alkaline comet assay. Cells were prepared and stained with Green SYBR dye as normal. Image analysis program estimated genotoxicity from comet tail DNA after staining (a greater ratio indicates more genetic damage). The data was entered and analyzed through SPSS-25.

RESULTS

Cold aqueous Glycyrrhiza glabra root extract was evaluated for cytotoxicity on normal REF. The table demonstrates extract concentration lowered cell viability. Cytotoxicity was concentration-dependent with an IC_{50} of 5.59 mg/ml. Cytotoxicity was shown by REF cell density decrease, rounding, and detachment after 72 hours with the cold aqueous G. glabra extract (Table 1). These morphological changes were more obvious at increasing dosages, but untreated control cells did not change (Figs. 1-2)

After 24 hours, the cold aqueous extract showed considerable cytotoxicity ($p<0.05$) compared to the control group (Table 2). Cancer cell proliferation decreased by 4.483% at 70 µg/ml. After 24 hours, cytotoxic activity peaked at 77.778% at 350 µg/ml as concentration increased. After 48 hours, inhibition increased at all levels, with 70 µg/ml reaching 5.881% and 350 µg/ml reaching 79.349%. The percentage inhibition increased significantly after 72 hours, reaching 13.887% at 70 µg/ml and 87.262% at 350 µg/ml.

Table No.1: The impact of cold aqueous extract of Glycyrrhiza glabra root at different concentrations on the viability of normal cells (REF) after 72 hours of exposures

Concentrations (mg/ml)	Mean±SD
1	92.12±1.72
2	79.04±5.66
4	52.31±6.25
6	39.81±3.82
8	30.56±1.40
10	15.94±2.87
12	12.50±1.29

Table No.2: The effect of Cold Aqueous Extract of Glycyrrhiza glabra roots on MCF-7 Cells at different concentrations after 24, 48, and 72hrs

Time/Conc. (µg/ml)	IR (%) 24 hrs	IR (%) 48 hrs	IR (%) 72 hrs	LSD
0µg/ml	E, b 3.520±1.678	E, b 4.773±1.694	F, a 6.656±1.046	1.293615
70µg/ml	E, b 4.483±2.566	E, b 5.881±1.624	E, a 13.887±3.010	2.125452
140µg/ml	D, b 24.505±5.220	D, b 26.518±5.330	D, a 38.551±5.080	4.482866
210µg/ml	C, b 35.809±6.500	C, b 37.979±6.640	C, a 49.930±5.860	5.455869
280µg/ml	B, b 60.630±3.480	B, b 62.254±3.930	B, a 73.529±2.150	2.818712
350µg/ml	A, b 77.778±2.794	A, b 79.349±3.010	A, a 87.262±2.980	2.520545
P-value	0.00012	0.00046	0.00068	
LSD	3.416072	3.479814	3.145972	

Table No.3: The impact of cold aqueous extracts of Glycyrrhiza glabra roots on DNA damage and the Comet assay in MCF-7 breast cancer cells at various concentrations

Parameter/Conc. (µg/ml)	No Damage	Low Damage	Medium Damage	High Damage
0µg/ml	A: 42.563±1.756	AB: 42.218±1.707	D: 7.045±0.428	D: 8.174±0.755
70µg/ml	B: 39.123±4.030	A: 42.439±3.482	C: 8.867±0.393	D: 9.571±0.516
140µg/ml	BC: 37.468±2.130	C: 38.981±1.823	B: 11.471±1.751	C: 12.080±1.636
210µg/ml	CD: 35.762±3.176	BC: 39.820±1.885	B: 12.133±1.373	C: 12.285±1.406
280µg/ml	CD: 34.859±1.229	D: 35.335±0.999	A: 14.684±0.757	B: 15.122±0.921
350µg/ml	D: 32.981±0.879	D: 34.413±1.060	A: 15.649±0.818	A: 16.957±1.094
P-value	0.0014	0.0007	0.00033	0.00056
LSD	2.643509	2.153496	1.122144	1.205672

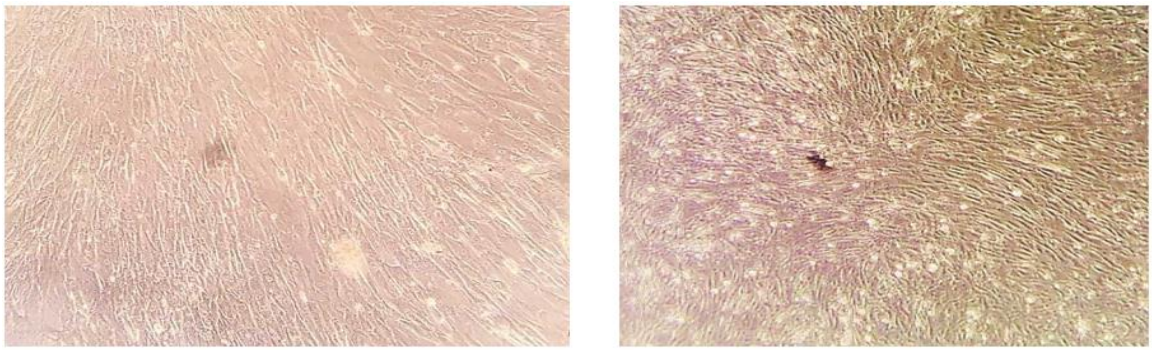

Figure No. 1: Glycyrrhiza glabra root old aqueous extract treatment alters normal cell morphology (REF). REF cells treated with extract for 72 hours, 40X magnification. Control (untreated REF cells)

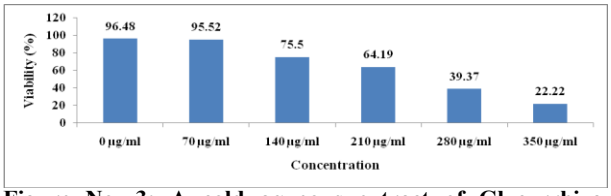

Figure No. 3: A cold aqueous extract of Glycyrrhiza glabra roots and its effects on the viability of MCF-7 breast cancer cells, after 24 hours of exposure to different concentrations

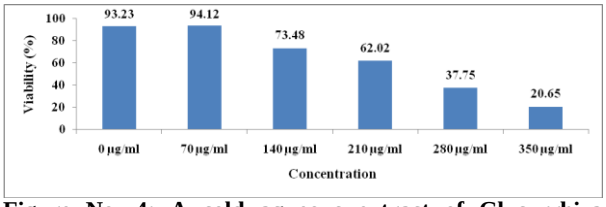

Figure No. 4: A cold aqueous extract of Glycyrrhiza glabra roots and its effects on the viability of MCF-7 breast cancer cells, after 48 hours of exposure to different concentrations

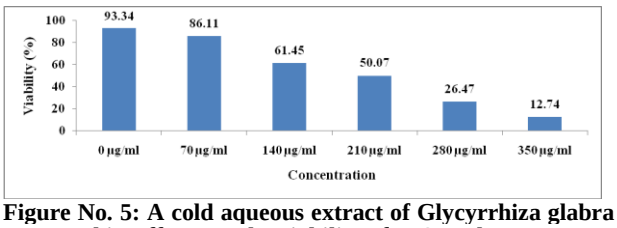

Figure No. 5: A cold aqueous extract of Glycyrrhiza glabra roots and its effects on the viability of MCF-7 breast cancer cells, after 72 hours of exposure to different concentrations

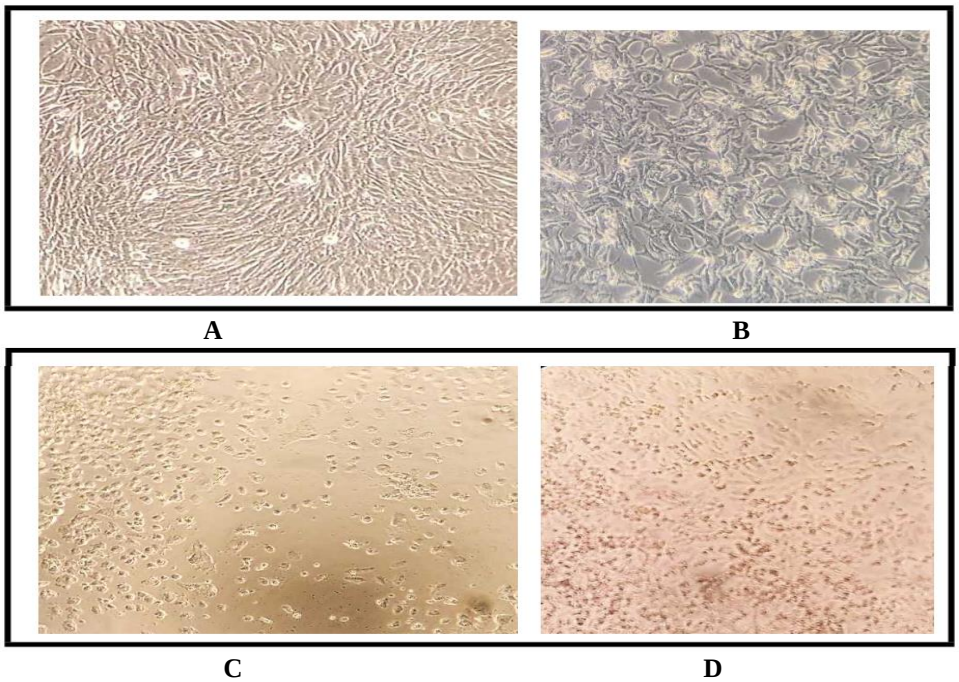

Figure No. 6: Morphological alterations in MCF-7 breast cancer cells treated with the aqueous extract of Glycyrrhiza glabra for different exposure periods

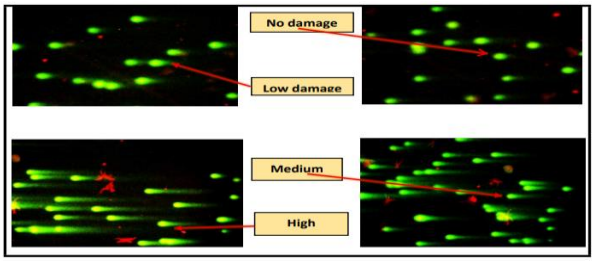

Figure No. 7: DNA damage levels in MCF=7 cells, evaluated using the Comet assay

After 24 hours, cancer cell viability ranged from 22.22% at 350 µg/ml to 95.52% at 70 µg/ml. Time reduced same-concentration cell survival. At 48 hours, viability was 20.65% at 350 µg/ml and 94.12% at 70 µg/ml. Cancer cell viability decreased to 12.74 percent at 350µg/ml after 72 hours. Figures 3–7 show the decay system's Cimabue oty cell cutoff from start to viability.

Table 3 showed the cold aqueous extract of Glycyrrhiza glabra roots substantially affected the comet test on MCF-7 breast cancer cells at low, medium, and high damage levels. At low damage levels, the comet rate was highest at 70 µg/ml (39.123) and decreased to 32.981 at 350 µg/ml ($p \leq 0.005$) after 72 hours. At medium and severe damage levels, the maximum comet rate was from 350 µg/ml concentration, while the lowest comet rate was from 70 µg/ml concentration. The medium damage value was 15.649 at 350 µg/ml, compared to 8.867 at 70 µg/ml while the high damage value was 16.957 at 350 µg/ml, compared to 9.571 at 70 µg/ml, during the same exposure duration and significance level.

DISCUSSION

Aqueous Glycyrrhiza glabra root extract very cytotoxic in embryonic fibroblast normal cells (IC_{50} = ~5.59 mg/ml). Low extract doses injure normal cells less. The extract concentration decreased cell viability from 92.12% at 1 mg/ml to 12.50% at 12 mg/ml. Toxin dose-dependent extraction 72-hour in vitro cytotoxic. Assess cell viability early and late after exposure. Ganguly and Breen[7] reported no oxidative damage or cellular signaling pathways from short dosages. Medical or environmental impacts of the extract may be best examined after 72 hours.

Some bioactive plant compounds may damage normal cells.[7] A significant indicator of toxicity and therapeutic safety is the IC_{50} value. Glycyrrhizin and liquiritigenin in the extract do this. Wang asserts Xuhui[8] reported that oxidative stress and ROS production may destroy these compounds at large concentrations. Yang and Zhang[9] demonstrated that magnesium isoglycyrrhizinate reduces ROS in normal fibroblasts via p38MAPK/Akt/Nox4 signaling and suggest these medicines may affect cell oxidative balance. According to Muhamad Plengsuriyakarn[10], certain phytochemicals attack cancer cells without affecting normal cells. Purity, extract chemical composition, and exposure time effect selectivity. High therapeutic extract concentrations restrict cell viability. Pérez-Soto et al[11] validates the cell growth and antioxidants may be compromised by overdosing.

Cold water Glycyrrhiza glabra root extract inhibits MCF-7 breast cancer cells. High extract concentration and exposure time increased inhibition. Extract bioactives may collectively inhibit cancer cell survival. Husain et al[12] reported that early exposure to lower dosages showed little inhibition (24 h), whereas 350 µg/ml showed considerable inhibition, indicating that dosage concentration considerably affects cytotoxicity. This validates Sohel et al[13] conclusion that active ingredient concentration boost plant extracts' early impacts.

Wang et al[14] found Glycyrrhiza glabra root extract suppresses MCF-7 breast cancer cells concentration-dependently. Ahmad et al[15] also found that MTT and ELISA doses and exposure times killed cells. Cytotoxic inhibition ratios rose considerably across all dosages after 48 hours, demonstrating that bioactive chemicals are cumulative and cellular growth pathway regulation persists. Plant extracts may slow cell development, claim Jain et al.[16] Flavonoids and terpenes may alter gene expression and cause apoptosis, according to Tuli et al.[17]

After 72 hours, cell death and cytotoxic inhibition peaked, allowing the extract to concentrate and activate complicated cancer cell-killing signaling pathways. Nizam et al[18] found that Glycyrrhiza glabra extract inhibited MCF-7 cell growth and promoted apoptosis via the PI3K/Akt and MAPK pathways. Its cytotoxicity and effect on cancer cells may make it an anticancer drug, especially in combination.

Cold aqueous glycyrrhiza glabra root extract damaged MCF-7 breast cancer cells' DNA at various dosages in the comet test. High DNA damage levels reached 70 µg/ml, whereas moderate damage was 350 µg/ml.[19] Too many active chemicals in the extract may protect or reduce cell responsiveness at higher doses. Hormesis occurs when a little amount of chemical boosts biodefenses yet poisons, cytotoxicizes, or inhibits function. Hormetic cellular toxicity is well-known. Al-Naqeb et al[20] used plant extract to preserve and destroy cell DNA. Like cytotoxicology's hormesis.

Rising extract concentration significantly correlates with medium-level DNA damage, from 8.867% at 70 µg/ml to 15.649% at 350 µg/ml. Bioactive chemicals in the extract damaged MCF-7 breast cancer cells' DNA dose-dependently, indicating genotoxicity. Glycyrrhizin and liquiritigenin enhance intracellular ROS, DNA damage, and oxidative stress, according to Sharifi-Rad et al[21], these components may induce apoptosis and double-strand DNA breaking. Zhang et al[22] found that isoliquiritigenin, another licorice component, may increase ROS-mediated DNA damage

in cancer cells and inhibit autophagy, which cleans damaged organelles and proteins and maintains homeostas Study's Comet test confirms this.

Significant DNA damage increased cell damage from 9.571% at 70 µg/ml to 16.957% at 350 µg/ml. Its high genotoxicity hinders DNA repair. DNA damage may damage cell cycle checkpoints, causing apoptosis or preventing it to maintain genomic integrity. Lagunas-Rangel and Bermúdez-Cruz[23] discovered natural chemicals that may hinder cancer cell DNA repair, causing genetic damage or enhancing antitumor effects. The data validate this study's Comet assay.

No harm percentages reduced from 42.563% in the control group to 32.981% at 40% extract. In prescriptive pharmacology and toxicology, the dose-dependent genotoxic theory states that higher dosages injure cells more aligns with Vrbovac-Madunić et al.[24] A statistical study found substantial damage levels at different doses ($P \leq 0.005$), supporting these conclusions. Cordelli et al[25] found that the comet assay, a sensitive DNA damage detection instrument, strongly suggests that the extract affects MCF-7 breast cancer cells' genetic material.

CONCLUSION

In a dose- and time-dependent study, Glycyrrhiza glabra root cold aqueous extract inhibited normal fibroblast proliferation and killed MCF-7 breast cancer cells. A comet experiment reveals cold water Glycyrrhiza glabra root extract destroys breast cancer DNA. The study studies Glycyrrhiza glabra root anticancer properties. In vivo and molecular research is required to understand Glycyrrhizin's breast cancer treatment properties.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Muslim Mohammed Kadhim, Aseel Raheem Mardan
Drafting or Revising Critically:	Muslim Mohammed Kadhim, Aseel Raheem Mardan
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

Conflict of Interest: The study has no conflict of interest to declare by any author.

Source of Funding: None

Ethical Approval: No.388 Dated 29.10.2024

REFERENCES

1. Gezici S, Şekeroğlu N. Current perspectives in the application of medicinal plants against cancer: novel therapeutic agents. Anti-Cancer Agents Med Chem 2019;19(1):101-11.
2. Srivastava AK. Significance of medicinal plants in human life. Synthesis of medicinal agents from plants. Elsevier; 2018.p.1-24.
3. Li F, Liu B, Li T, Wu Q, Xu Z, Gu Y, et al. Review of constituents and biological activities of triterpene saponins from Glycyrrhizae Radix et Rhizoma and its solubilization characteristics. Molecules 2020; 25(17): 3904.
4. Riaz M, Khalid R, Afzal M, Anjum F, Fatima H, Zia S, et al. Phytobioactive compounds as therapeutic agents for human diseases: A review. Food Sci Nutr 2023;11(6):2500-29.
5. Al Bayati AASY. A Morphological and Anatomical Study of the Glycyrrhiza glabra Plant in the Zab District-Hawija District-Kirkuk Governorate. South Asian Res J Agri Fish 2025;7(1):19-24.
6. Batiha GE-S, Beshbishy AM, El-Mleeh A, Abdel-Daim MM, Devkota HP. Traditional uses, bioactive chemical constituents, and pharmacological and toxicological activities of Glycyrrhiza glabra L. (Fabaceae). Biomolecules 2020;10(3):352.
7. Nguyen ST, Nguyen HT-L, Truong KD. Comparative cytotoxic effects of methanol, ethanol and DMSO on human cancer cell lines. Biomed Res Therapy 2020;7(7):3855-9.
8. Wang H, Xuhui G, Huiheng Q, Ning W, Jiawen Z, Wenjing X, et al. Glycyrrhizic acid inhibits proliferation of gastric cancer cells by inducing cell cycle arrest and apoptosis. Cancer Management Res 2020;12(null):2853-61.
9. Yang Q, Zhang P, Liu T, Zhang X, Pan X, Cen Y, et al. Magnesium isoglycyrrhizinate ameliorates radiation-induced pulmonary fibrosis by inhibiting fibroblast differentiation via the p38MAPK/Akt/Nox4 pathway. Biomed Pharmacotherapy 2019;115:108955.
10. Muhamad N, Plengsuriyakarn T, Na-Bangchang K. Application of active targeting nanoparticle delivery system for chemotherapeutic drugs and traditional/herbal medicines in cancer therapy: a systematic review. Int J Nanomed 2018:3921-35.
11. Pérez-Soto E, Estanislao-Gómez CC, Pérez-Ishiwara DG, Ramirez-Celis C, del Consuelo Gómez-García M. Cytotoxic effect and mechanisms from some plant-derived compounds in breast cancer. Cytotoxicity-Definition, Identification Cytotoxic Compounds, 2019; 10-15.
12. Husain I, Bala K, Khan IA, Khan SI. A review on phytochemicals, pharmacological activities, drug interactions, and associated toxicities of licorice (Glycyrrhiza sp.). Food Frontiers 2021;2(4): 449-85.
13. Sohel M, Aktar S, Biswas P, Amin MA, Hossain MA, Ahmed N, et al. Exploring the anti-cancer potential of dietary phytochemicals for the patients

with breast cancer: a comprehensive review. Cancer Med 2023;12(13):14556-8.
14. Wang Y, Xia W, Tao M, Fu X. Oncopreventive and oncotherapeutic potential of licorice chalcone compounds: Molecular insights. Mini Rev Med Chem 2023;23(6):662-99.
15. Ahmad R, Alqathama A, Aldholmi M, Riaz M, Mukhtar MH, Aljishi F, et al. Biological Screening of Glycyrrhiza glabra L. from Different Origins for Antidiabetic and Anticancer Activity. Pharmaceuticals 2023;16(1):7.
16. Jain R, Hussein MA, Pierce S, Martens C, Shahagadkar P, Munirathinam G. Oncopreventive and oncotherapeutic potential of licorice triterpenoid compound glycyrrhizin and its derivatives: Molecular insights. Pharmacol Res 2022;178:106138.
17. Tuli HS, Garg VK, Mehta JK, Kaur G, Mohapatra RK, Dhama K, et al. Licorice (Glycyrrhiza glabra L.)-derived phytochemicals target multiple signaling pathways to confer oncopreventive and oncotherapeutic effects. Oncotargets Therapy 2022;15:1419.
18. Nizam NN, Mahmud S, Ark SA, Kamruzzaman M, Hasan MK. Bakuchiol, a natural constituent and its pharmacological benefits. F1000 Res 2023;12:29.
19. Zhang Y, Huang Y, Li Z, Wu H, Zou B, Xu Y. Exploring natural products as radioprotective agents for cancer therapy: Mechanisms, challenges, and opportunities. Cancers 2023;15(14):3585.
20. Al-Naqeb G, Kalmpourtzidou A, Giampieri F, De Giuseppe R, Cena H. Genotoxic and antigenotoxic medicinal plant extracts and their main phytochemicals: a review. Frontiers Pharmacol 2024;15:1448731.
21. Sharifi-Rad J, Quispe C, Herrera-Bravo J, Belén LH, Kaur R, Kregiel D, et al. Glycyrrhiza genus: Enlightening phytochemical components for pharmacological and health-promoting abilities. Oxidative Med Cellular Longevity 2021; 2021(1):7571132.
22. Zhang Z, Yung KK, Ko JK. Therapeutic intervention in cancer by isoliquiritigenin from licorice: a natural antioxidant and redox regulator. Antioxidants 2022;11(7):1349.
23. Lagunas-Rangel FA, Bermúdez-Cruz RM. Natural compounds that target DNA repair pathways and their therapeutic potential to counteract cancer cells. Frontiers Oncol 2020;10:598174.
24. Vrhovac Madunić I, Madunić J, Antunović M, Paradžik M, Garaj-Vrhovac V, Breljak D, et al. Apigenin, a dietary flavonoid, induces apoptosis, DNA damage, and oxidative stress in human breast cancer MCF-7 and MDA MB-231 cells. Naunyn-Schmiedeberg's Arch pharmacol 2018;391:537-50.
25. Cordelli E, Bignami M, Pacchierotti F. Comet assay: a versatile but complex tool in genotoxicity testing. Toxicol Res 2021;10(1):68-78.