Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 9, No. 4, 677-683
2025
Publisher: Learning Gate
DOI: 10.55214/25768484.v9i4.6039

Study the antioxidant of phenol compounds isolated from black tea Camellia Sinensis L. in male rats exposed to methotrexate

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Abstract: This study aims to comprehend the function of phenolic compounds and black tea (Camellia sinensis L.) against oxidative stress brought on by methotrexate medication overdose in male rats. The study used 60 adult male rats, and groups of eight rats were formed. T1: 0.85% Normal saline (control). T2: phenol (75 mg/kg body weight) + methotrexate (10 mg/kg). T3: 125 mg/kg phenol with 10 mg/kg methotrexate. T4: 150 mg/kg phenol with 10 mg/kg methotrexate injection. T5: injection: 200 mg/kg black tea + 10 mg/kg methotrexate. T6: Methotrexate 10 mg/kg + 250 mg/kg black tea injection. T7: 300 mg/kg black tea injection + 10 mg/kg. T8: Methotrexate treatment, 10 mg/kg body weight. Blood samples were taken 30 times a day for 14 days after oral dosing. The total protein, blood sugar, hepatic enzyme efficiency, lipid peroxidation, and glutathione peroxidation of male rats were measured. The results indicated that treated black tea and phenol isolated from black tea showed that MDA and LPO concentrations were significantly higher in T8 rats than in T1 rats treated with methotrexate. With drinking water, methotrexate increases ROS and oxidative stress, raising liver tissue MDA and LPO levels and lowering GPX. In male rats, injections of 300 and 125 mg/kg of phenol and phenol extracts prevent and reduce oxidative stress caused by methotrexate by increasing protein and GPX levels and decreasing blood sugar, MDA, and LPO levels in liver tissue.

Keywords: *Camellia sinensis L, LPO, MDA, Methotrexate, Phenol.*

1. Introduction

Methotrexate is used to treat cancer and psoriatic rheumatoid arthritis. Methotrexate was first utilized as a chemotherapeutic agent [1]. It is effective for the treatment of breast, bladder, lung, and blood cells cancers and The dihydrofolate reeducates enzyme (DHFR) is competitively inhibited by methotrexate, and this prevents some proteins, nucleic acids, and folic acid from being synthesized. [2, 3]. Its success in treating refractory inflammatory bowel illness gave Methotrexate a new application. [4]. However severe adverse responses and dangerous side effects frequently limit this medication's usefulness. The cytotoxic action of methotrexate affects healthy tissues that proliferate quickly, such as the bone marrow's hematopoietic cells and the gastrointestinal mucosa's actively dividing cells [5]. In addition to allogeneic bone marrow and organ transplantation, methotrexate has also been used to treat psoriasis, a non-neoplastic skin condition characterized by rapid proliferation of epidermal cells [6]. The liver toxicity brought on by methotrexate may be caused by free radicals. Hepatic fibrosis and chronic liver damage have both been connected to oxidative stress. The development of methotrexate-induced tissue damage has been linked to lipid peroxidation, which is thought to be a significant cause of cell membrane degradation and damage. Lipid peroxidation is mediated by oxygen free radicals.

History: Received: 17 January 2025; Revised: 10 March 2025; Accepted: 14 March 2025; Published: 7 April 2025
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Reactive oxygen species have received attention due to their involvement in mediating the micro vascular disruptions that precede tissue damage brought on by a variety of toxins. Methotrexate induces oxidative stress and melatonin. Free radicals appear to generate an increase in leukocytes in the damaged tissues in addition to their direct tissue-damaging effects. This increase in leukocytes exacerbates tissue damage indirectly by activating neutrophils. Active neutrophils have been shown to emit [7]. Green pharmacy is now very important in the disciplines of pharmacology and medicine. It is projected that 80% of people on the planet will use plant-based medicines to address their medical needs. [8]. The phrase "medicinal plants" describes a broad range of plants that are employed in herbal medicine, have a favorable effect on health, and can be utilized in both conventional and alternative medicine to cure certain ailments. The broad use of medicinal plants in healthcare is supported by a number of factors, including: population growth, a lack of supplies and high medicine prices, adverse effects of many synthetic drugs, and the emergence of infectious germs with multi-drug resistance. The majority of conventional herbs have secondary metabolites [9]. There are three main types of tea, with 78% black, 20% green, and 2% oolong, sold overseas on a global scale. The flavin, 3-gallate aflavin, 3-gallate aflavin, and 3-gallate aflavin are the primary flavons present in black tea. Black tea gets its copper color and astringent flavor from the orange-red aflavins component. In addition to their antioxidant qualities, tea polyphenols are recognized for their antibacterial capabilities [10].

Object from this the study is known important phenol compounds isolated from black tea *Camellia sinensis* L. on antioxidant ,oxidant enzymes protein and blood sugar in male rats exposed to Methotrexate

2. Material and Method

- The black tea leaf was bought at a local Karbala market after being cleaned and having any foreign objects removed. The leaves were washed four times with tap water and once with water distilled water. Every dry component was electrically ground. The powdered ingredients were chilled at 40°C before being used, keeping them in polypropylene tubes [11, 12].
- Soxhlet apparatus: A 500 ml flask containing 100g of powdered black tea leave, 50 milliliters of 75% methanol solvent in a 500 ml flask for extraction through 24 hours, and then separation of alcohol from the extract by using rotary evaporation [13-16].

Phytochemical screening of some secondary metabolic compound in black tea extract (Phenols, Glycosides, Tannins, Alkaloids, Flavonoids).

Separation phenolic compound:

I take an extract of methanol alcohol and acidify it with 2 M HCL (PH 3). Fill a separating funnel with the extract, and then wash it with chloroform (CHCL3). Mixing, creating two layers, and taking the bottom layer. Follow these steps two or three more times. The collection of phenols was dried in an oven at 30 to 20 °C. Before being used, phenol is kept in a refrigerator at 4°C [17].

Research Design: The study used 60 adult male rats weighing 210–310 g and aged 9–11 weeks. Karbala University's animal facility was at 25C°. Met food. Groups of eight rats were formed.T1: 0.85% saline controlling. T2/phenol (75 mg /kg body weight) + methotrexate (10 mg/kg). T3: 125 mg /kg phenol with 10 mg /kg methotrexate. T4: 150 mg /kg phenol with 10 mg /kg methotrexate injection. T5: injection of 200 mg /kg black tea + 10 mg /kg methotrexate. T6: methotrexate 10 mg/kg + 250 mg/kg black tea injection. T7: 300 mg/kg black tea injection+ 10 mg/kg. T8: Methotrexate treatment, 10 mg/kg body weight. Blood spilled after 30 day from oral dosing. The serum protein, blood sugar, hepatic enzyme efficiency, lipid peroxidation, and glutathione peroxide rates of male rats were measured.

2.1. Biochemical Analysis

Blood was drawn using the cardiac puncture technique, The blood serum was spun at 3000 CRF for 10 minutes, and then filtered. After 30 days, blood was collected, and serum was stored at 20 C° for enzyme assays. serum total protein level [18] and blood sugar [19].

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 9, No. 4: 677-683, 2025
DOI: 10.55214/25768484.v9i4.6039

Assay for oxidative and antioxidant enzymes

antioxidant enzymes (Glutathione peroxidase, Oxidative stress parameters such as (Lipid Peroxidation and Malondialdehyde (MDA)) assay with a kit obtained from the Bioassay Technology Laboratory (BT LAB) in Chen [20].

Statistical assay:

The data were expressed as a mean. One-way analysis of variances was used to assess the statistical significance of differences between the control and other groups (ANOVA). When doing statistical analysis using the SPSS program, P≥0.05or less were considered significant (SPSS, Inc., Chicago, Illinois).

3. Results

The active phytochemical components present in black tea extract are listed in the table below (1). Positive results from the screening of chemical compounds for phenol, flavonoid, glycoside, alkaloid, and tannin were obtained..

Table 1.
Phytochemical screening of some secondary metabolic compound in black tea extract.

Compound	Result
Flavonoid	+
Glycoside	+
Phenol	+
Alkaloid	+
Tannin	+

Table 2.
Male Rat Treatment with the Coefficients Level total protein and blood sugar Concentration mg/dl.

Groups	Total protein mg/dl	Blood sugar mg/dl
T 1	0.934±5.87	0.172±0.543
T2	0.382±5.61.	0.197±0.35
T3	0.583±5.81	0.159±0.68
T4	5.92±0.356	0.148±0.49
T 5	6.66±0.393	0.129±0.45
T6	6.88±0.485	0.196±0.43
T 7	7.97±0.276	0.78±0.46
T 8	4.42±0.375	0.297±0.39
L.S.D	0.093	0.67

In the current research table (2) , the total protein concentration level of the group T8, showed a significant decrease 4.42 mg/dl compared to G1 5.87mg/dl while T 2 to T7 showed results a significant increase 5.61 , 5,81, 5.92, 6.66 , 6.88 and 7.97mg/dl consequently compared to T8 4.42mg/dl . the

blood sugar concentration level of groups T8= revealed 0.297mg/dl consequently compared to T1 0.172 mg/dl while T 2 to T7 revealed a notable decline 0.197, 0.159, 0.149, 0.129, 0.196, 0.78 mg/dl consequently compared to T8 0.297 mg/dl.

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ISSN: 2576-8484
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Table 3.
The mean ± SD of the level of MDA, lipid peroxide and GPX in in serum of rats groups under investigation

Groups	MDA µmol/ L	LPO µmol/ L	GPX mol/L
T1	0.611	6.57	68.19
T2	0.664	6.19	39.78
T3	0.640	5.84	41.56
T4	0.634	5.92	60.01
T 5	0.567	4.91	57.94
T6	0.545	4.65	58.45
T7	0.519	4.44	61.86
T8	0.867	8.02	39.96
L.S.D	0.076	0.56	5.48

In the current research table (3), the MDA activity level of groups T8, results showed a significant increase 0.867µmol/ L compared with T1 0.611µmol while T2 to T7 showed a significant decrease 0.664 , 0.640, 0.634, 0.567, 0.545and 0.519µmol/ L consequently compared to T8 0.867µmol/ L., the LPO activity level of groups T8 indicate the results a significant increase 8.02µmol/ L consequently compared to T1 6.57 µmol while T2 to T7 showed a significant decrease 6.19, 5.84, 5.92, 4.91, 4.65and 4.44µmol/ L consequently compared to T8 0.867µmol/ L . the GPX activity level of groups T8showed a significant decrease 39.96µmol/ L compared with control 68.19 µmol/ L while T 2,T 3,T4 T5,T6 and T7 indicate a significant increase 39.78, 41.56, 60.01, 57.94, 61.87and 61.86µmol/ L consequently compared to T8 39.96µmol/ L.

4. Discussion

The goal of the current study was to examine the phenol compound's and *Camellia sinensis* L. extract's antioxidant effects on liver enzymes in response to methotrexate. Table (3) of the results indicates a significant increase in MDA and LPO concentration in the T8. (injection 10 milligram/kg body weight of Methotrexate) when compared to T1 (control) , Therefore, drinking water while taking methotrexate raises the levels of MDA and LPO in the liver enzyme and lowers the levels of GPX, which results in oxidative stress and an increase in ROS. These findings supported earlier studies. Uzar, et al. [21] and Hassan, et al. [22] because proteins, lipids in cell membranes, sulfhydryl enzymes, and DNA synthesis can all be oxidized by ROS, which can cause damage to cells [23]. Because methotrexate medication breaks the link between oxidative phosphorylation and phosphates, which decreases energy when taken in large doses, it has a similar effect on healthy cells [24]. The reduced form of glutathione, which is regarded as a crucial antioxidant for the cell, can become unstable due to the oxidation of unsaturated fatty acids. This results in an increase in MDA and the oxidative phosphorylation of lipids. The sustainability of the reduced form of glutathione is also impacted by the decline in NADPH and malic NADPH [25]. Methotrexate prevents the formation of some amino acids and protein synthesis by acting as a dihydrofolate reductase inhibitor, which affects the generation of folic acid [26]. It has been demonstrated that methotrexate therapy reduces the efficiency of antioxidant defense mechanisms and that methotrexate lowers glutathione peroxide levels in cells [27].

The results of the experiment demonstrated that phenol 200 mg/kg body weight oral enhanced antioxidant enzyme activity and efficiently scavenged free radicals. Phenol is regarded as an antioxidant due to their capacity to scavenge ROS electrons [28]. An antioxidant action occurs either intracellular or extracellular, inhibiting the xanthine oxide enzyme activity that transforms the product xanthine oxide to xanthine dehydrogenase [29]. The activation of the enzyme glut amyl cysteine production or enhanced resistance could be the cause of the notable increase in GPX levels observed in response to phenol. Glutathione production or this material may trigger glutamic trance peptidase to activate [30]. The phenol from *Camellia sinensis* leaves decreases the enzymatic efficacy of methotrexate's damaging impact when this extract and a mutagen overlap [31]. Inhibiting breakdown of hydrogen peroxide into free radicals and neutralizing lipid free radicals are two ways that phenolic compounds exert their

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 9, No. 4: 677-683, 2025
DOI: 10.55214/25768484.v9i4.6039

antioxidant effect [32] injection (10 mg of methotrexate per kilogram of body weight plus 300 mg of black tea) Tea catching have pro-oxidant and antioxidant effects due to the unique ability of tea to undergo oxidation and the resulting increase in ROS [33] and works as a hydrogen donor [34].

This observation is further supported by the apparent structural and chemical similarities between the tea catching and other well-known anti-folic medications, such as methotrexate and trimethoprim. [35, 36]. Previous research has discovered that a variety of antioxidants, including parsley extract [37-39] have protective effects on the liver damage caused by methotrexate in rats. Methotrexate caused substantial liver damage after just one dose, Methotrexate's ability to bind to the enzyme dihydrofolic reductase prevents folic acid from being converted to its active form, folinic acid, which is how it causes hepatotoxicity. This prevents the production of proteins, some amino acids, and nucleic acids, It hinders the ability of hepatic parenchymal cells to function by damaging their organelles and plasma membranes.

5. Conclusions

Phenol compounds have more effect than tea black extract. The phenol compound has an antioxidant role again in oxidant stress induced by the MTX drug in male rats.

Transparency:

The authors confirm that the manuscript is an honest, accurate, and transparent account of the study; that no vital features of the study have been omitted; and that any discrepancies from the study as planned have been explained. This study followed all ethical practices during writing.

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References

[1] P. P. M. Van Lümig, S. P. Menting, J. M. P. A. Van Den Reek, and P. I. Spuls, "An increased risk of non-melanoma skin cancer during TNF-inhibitor treatment in psoriasis patients compared to rheumatoid arthritis patients probably relates to disease-related factors " *Journal of the European Academy of Dermatology and Venereology*, vol. 29, no. 4, pp. 752–760, 2015. https://doi.org/10.1111/jdv.12956

[2] M. Cojoc, K. Mäbert, M. H. Muders, and A. Dubrovska, "A role for cancer stem cells in therapy resistance: Cellular and molecular mechanisms," *Seminars in Cancer Biology*, vol. 31, pp. 16–27, 2015. https://doi.org/10.1016/j.semcancer.2014.06.004

[3] U. E. Martinez-Outschoorn, M. Peiris-Pagés, R. G. Pestell, F. Sotgia, and M. P. Lisanti, "Cancer metabolism: A therapeutic perspective," *Nature Reviews Clinical Oncology*, vol. 14, no. 1, pp. 11–31, 2017. https://doi.org/10.1038/nrclinonc.2016.60

[4] S. T. Helen, T. D. Schiano, S. F. Kuan, S. B. Hanauer, and H. S. Conjeevaram, "Hepatic effects of long-term methotrexate use in the treatment of inflammatory bowel disease," *American Journal of Gastroenterology*, vol. 95, no. 11, pp. 3150-3156, 2000.

[5] V. K. Kolli, A. Premila, and B. Isaac, "Alteration in antioxidant defense mechanisms in the small intestines of methotrexate-treated rats may contribute to its gastrointestinal toxicity," *Cancer Therapy*, vol. 5, pp. 501–510, 2007.

[6] V. K. Kolli, P. Abraham, B. Isaac, and D. Selvakumar, "Neutrophil infiltration and oxidative stress may play a critical role in methotrexate-induced renal damage," *Chemotherapy*, vol. 55, no. 2, pp. 83–90, 2009. https://doi.org/10.1159/000192391

[7] C. L. Coleshowers, O. O. Oguntibeju, M. U. Pong, and E. J. Truter, "Effect of methotrexate on antioxidant enzyme status in a rodent model," *Medical Technology SA*, vol. 24, no. 1, pp. 5–9, 2010.

[8] V. Sureshkumar, G. Sarathchandra, and J. Ramesh, "Biochemical, histopathological, and ultra-structural profile after pulsed water medication of enrofloxacin in broiler chickens," *Veterinary World*, vol. 6, no. 8, pp. 668–673, 2013. https://doi.org/10.14202/vetworld.2013.668-673

[9] E. W. Chan, E. Y. Soh, P. P. Tie, and Y. P. Law, "Antioxidant and antibacterial properties of green, black, and herbal teas of Camellia sinensis," *Pharmacognosy Research*, vol. 3, no. 4, pp. 266–272, 2011. https://doi.org/10.4103/0974-8490.89748

[10] P. Namita and R. Mukesh, "Medicinal plants used as antibacterial agents: A review," *International Research Journal*, vol. 3, no. 1, pp. 32-40, 2012.

[11] L. H. Al-Ghazali, I. H. Al-Masoody, M. H. Ismael, and N. Al-Ibrahemi, "Effect of alcohol extract, volatile oil, and alkaloid isolated from Capsicum frutescens L. fruits on Candida albicans," *IOP Conference Series: Earth and Environmental Science*, vol. 1225, no. 1, p. 012075, 2023. https://doi.org/10.1088/1755-1315/1225/1/012075

[12] D. A. Rabee, G. H. Oleiwi, B. A. H. Musa, N. Al-Ibrahemi, and M. O. Abdulridha, "Study protective role Camellia sinensis L. (black tea) and silver, Zn oxide nanoparticles on antioxidant-oxidant enzymes and biochemical level against paracetamol overdose in adult male rats," *Bionatura*, vol. 5, no. 4, p. 82, 2023. http://dx.doi.org/10.21931/RB/2023.08.04.82

[13] N. AL-Ibrahemi, Z. N. AL-Laith, A. AL-Yassiry, and N. H. AL-Masaoodi, "Phytochemical study of volatile oil for Ocimum basilicum L. and Mentha spicata by gas chromatography technique," *IOP Conference Series: Earth and Environmental Science*, vol. 2031, no. 1, p. 012075, 2022. https://doi.org/10.1088/1755-1315/2031/1/012075

[14] T. A. Salman, A. T. Ahmed, G. Oleiwi, and N. Al-Ibrahemi, "Study of the effect of oil extract of dill (Anethum graveolens L.) plant on oxidative stress parameters of liver enzymes in male rats," *IOP Conference Series: Earth and Environmental Science*, vol. 1215, no. 1, p. 012059, 2023. https://doi.org/10.1088/1755-1315/1215/1/012059

[15] N. M. Hamza, S. M. Yasir, and K. A. M. Hussain, "Biological effects of aqueous extract of Laurus nobilis L. leaves on some histological and immunological parameters in male rat liver affected by aluminum chloride," *Archives of Razi Institute*, vol. 76, no. 6, pp. 1657–1665, 2021. https://doi.org/10.22092/ari.2021.356361.1827

[16] N. Al-Ibrahemi, A. AL-Yassiry, Z. N. AL-Laith, and B. H. Al-Musawi, "Chemical analysis of phytochemicals in Anethum graveolens L. fresh and commercial dry samples using gas chromatography-mass spectrometry," *IOP Conference Series: Earth and Environmental Science*, vol. 1060, no. 1, p. 012089, 2022. https://doi.org/10.1088/1755-1315/1060/1/012089

[17] J. B. Harborne, *A guide to modern techniques of plant analysis*, 2nd ed. London: Chapman and Hall, 1984.

[18] S. M. Lewis, B. J. Bain, and I. Bates, *Dacie and lewis practical haematology*, 10th ed. Germany: Elsevier, 2006.

[19] C. A. Burtis, E. R. Ashwood, and D. E. Bruns, *Tietz textbook of clinical chemistry and molecular diagnostics*, 4th ed. St. Louis, MO, USA: Saunders, an imprint of Elsevier Inc, 2012.

[20] A. M. Aldaamy and N. M. Al-Zubiady, "Study on toxic effect of Tartrazine pigment on oxidative stress in male albino rats," *Journal of Biochemistry and Cell Archives*, vol. 21, no. 1, pp. 1021-1026, 2021.

[21] E. Uzar *et al.*, "The activities of antioxidant enzymes and the level of malondialdehyde in cerebellum of rats subjected to methotrexate: Protective effect of caffeic acid phenethyl ester," *Molecular and Cellular Biochemistry*, vol. 29, no. 1-2, pp. 63–68, 2006. https://doi.org/10.1007/s11010-006-9196-5

[22] A. U. Hassan, S. A. Abeed, and A. K. Obeid, "Histophysiological considerations dealing with the damaging effects of examined doses of heroin on rat kidneys," *Indian Journal of Forensic Medicine & Toxicology*, vol. 14, no. 4, pp. 2357–2360, 2023.

[23] R. M. Babiak, A. P. Campello, E. G. Carnieri, and M. B. Oliveira, "Methotrexate: Pentose cycle and oxidative stress," *Cell Biochemistry and Function*, vol. 16, no. 4, pp. 283–293, 1998. https://doi.org/10.1002/(SICI)1099-0844(1998120)16:4<283::AID-CBF801>3.0.CO;2-E

[24] A. R. Phull, B. Nasir, I. U. Haq, and S. J. Kim, "Oxidative stress, consequences, and ROS-mediated cellular signaling in rheumatoid arthritis," *Chemico-Biological Interactions*, vol. 281, pp. 121–136, 2018. https://doi.org/10.1016/j.cbi.2017.12.024

[25] H. Qiu and V. Schlegel, "Impact of nutrient overload on metabolic homeostasis," *Nutrition Reviews*, vol. 76, no. 9, pp. 693–707, 2018. https://doi.org/10.1093/nutrit/nuy023

[26] D. Rushworth, A. Mathews, and A. Alpert, "Dihydrofolate reductase and thymidylate synthase transgenes resistant to methotrexate interact to permit novel transgene regulation," *Journal of Biological Chemistry*, vol. 290, no. 38, pp. 22970–22976, 2015. https://doi.org/10.1074/jbc.M115.669322

[27] G. Şener, E. Ekşioğlu-Demiralp, M. Çetiner, F. Ercan, and B. C. Yeğen, "β-Glucan ameliorates methotrexate-induced oxidative organ injury via its antioxidant and immunomodulatory effects," *European Journal of Pharmacology*, vol. 542, no. 1-3, pp. 170–178, 2006. https://doi.org/10.1016/j.ejphar.2006.02.056

[28] C. A. Rice-Evans, N. J. Miller, and G. Paganga, "Structure-antioxidant activity relationships of flavonoids and phenolic acids," *Free Radical Biology and Medicine*, vol. 20, no. 7, pp. 933–956, 1996. https://doi.org/10.1016/0891-5849(95)02227-9

[29] C. M. Deaton and D. J. Marlin, "Exercise-associated oxidative stress," *Clinical Techniques in Equine Practice*, vol. 2, no. 3, pp. 278–291, 2003. https://doi.org/10.1053/j.ctep.2003.09.001

[30] Y. Li, O. P. Dhankher, L. Carreira, A. P. Smith, and R. B. Meagher, "The shoot-specific expression of γ-glutamylcysteine synthetase directs the long-distance transport of thiol-peptides to roots conferring tolerance to mercury and arsenic," *Plant Physiology*, vol. 141, no. 1, pp. 288–298, 2006. https://doi.org/10.1104/pp.105.074815

[31] A. S. Jalal and R. A. Mahmood, "Enzymatic effect of flavonoids extracted from the leaves of Camellia sinensis on liver enzymes in mice," *Al-Mustansiriyah Journal of Science*, vol. 34, no. 4, pp. 123-130, 2023.

[32] K. Durgo, L. Vuković, G. Rusak, M. Osmak, and J. Franekić, "Effect of flavonoids on glutathione level, lipid peroxidation, and cytochrome P450 CYP1A1 expression in human laryngeal carcinoma cell lines," *Food Technology and Biotechnology*, vol. 45, no. 1, pp. 69–79, 2007.

[33] L. Elbling *et al.*, "Green tea extract and (-)-epigallocatechin-3-gallate, the major tea catechin, exert oxidant but lack antioxidant activities," *FASEB Journal*, vol. 19, no. 7, pp. 807–809, 2005. https://doi.org/10.1096/fj.04-2915fje

[34] S. Mandel, O. Weinreb, T. Amit, and M. B. Youdim, "Cell signaling pathways in the neuroprotective actions of the green tea polyphenol (−)-epigallocatechin-3-gallate: Implications for neurodegenerative diseases," *Journal of Neurochemistry*, vol. 88, no. 6, pp. 1555–1569, 2004. https://doi.org/10.1046/j.1471-4159.2003.02291.x

[35] L. Sánchez-del-Campo, M. Sáez-Ayala, S. Chazarra, J. Cabezas-Herrera, and J. N. Rodríguez-López, "Binding of natural and synthetic polyphenols to human dihydrofolate reductase," *International Journal of Molecular Sciences*, vol. 10, no. 12, pp. 5398–5410, 2009. https://doi.org/10.3390/ijms10125398

[36] M. A. Kareem and A. K. Obeid, "Evaluation of the effect of aqueous extract of Lepidium sativum seed on the adverse effects of rat liver injury induced by sodium nitrite," *International Journal of Pharmaceutical Research*, vol. 12, no. 4, pp. 4212–4216, 2020.

[37] R. K. Mohammed and N. Al-Ibrahemi, "Phytochemical and protective study for Petroselinum sativum L. (parsley) on oxidative stress and antioxidants in rats," *The Egyptian Journal of Hospital Medicine*, vol. 89, no. 2, pp. 8039–8042, 2022. https://doi.org/10.21608/ejhm.2022.277520

[38] A. S. Abd and L. H. Saqban, "Study of the effect of cytotoxicity of extracts from Origanum majorana leaves on human breast cancer cell line (AMJ13) in vitro," *AIP Conference Proceedings*, vol. 2414, p. 020027, 2023. https://doi.org/10.1063/5.0133924

[39] E. M. Hersh, V. G. Wong, E. S. Henderson, and E. J. Freireich, "Hepatotoxic effects of methotrexate," *Cancer*, vol. 19, no. 4, pp. 600–606, 1966. https://doi.org/10.1002/1097-0142(196604)19:4<600::AID-CNCR2820190420>3.0.CO;2-3