MAHSA International Journal of Health and Medicine
V4, Issue 2; September, 2025
e-ISSN: 2805-5705

Original Article Open Access

IN-VITRO INHIBITORY EFFECT OF CHRYSANTHEMUM HERBAL TEA ON HEPATIC CYP3A4 ACTIVITY IN FEMALE SPONTANEOUS HYPERTENSIVE RATS

Levyaani Manivellu[1]* , Praghya Godavarthy[1] , Chiao Tzi Ng[1] ,N. Athirah[1] , M.Hazim[1] , M.Haanii[1] , M.Fakhri[1] ,Chun Keong Ng[1] , Mayurika Mailvaganam[1] , M. Ariff[1] , Smriti[1] , Siew Keah Lee[2] , Jin Han Chin[1]

[1]Faculty of Medicine, MAHSA University, Bandar Saujana Putra Campus, Jalan SP2, Bandar Saujana Putra, 42610 Jenjarom, Kuala Langat, Selangor, Malaysia.
[2]Department of Pre-clinical Sciences, M. Kandiah Faculty of Medicine and Health Sciences, Universiti Tunku Abdul Rahman, Jalan Sungai Long, Bandar Sungai Long, 43000, Kajang, Selangor, Malaysia.

Corresponding Author(s): levyaanilencie@gmail.com

Published:4th December 2025

Abstract

The increasing use of herbal medicines, such as *Chrysanthemum indicum L.* (*C. indicum L.*) tea, among patients with hypertension raises issues regarding possible interactions with conventional antihypertensive medications. This study seeks to evaluate whether Chrysanthemum tea has any modulation effect on CYP3A4 activity in order to determine the risk of interactions between the herb and drugs in hypertensive patients who are taking medications metabolized by CYP3A4. To address this issue, rat liver microsomes from spontaneous hypertensive female rats were used in an in-vitro experiment. First group served as negative control group treated with distilled water, second group served as positive group treated with 30 µM ketoconazole while four experimental groups were given varying doses of Chrysanthemum tea ranging from 2 µg/ml to 2 mg/ml, respectively. CYP3A4 activity was determined via calorimetric spectrophotometry by measuring the formaldehyde production from aminopyrine. According to the findings, CYP3A4 enzyme activity was significantly (p<0.01) inhibited by 2 µg/ml to 2 mg/ml of *Chrysanthemum indicum* tea compared with the negative control group. This inhibition suggests that Chrysanthemum tea may affect the drug metabolism catalyzed by CYP3A4 which could decrease the effectiveness or raise the toxicity of co-administered treatments like statins, antidiabetics, and antihypertensives. In conclusion, the study highlights the importance of caution when consuming *Chrysanthemum indicum* tea alongside medications metabolized by CYP3A4. Patients and healthcare providers should take note of the possible herb-drug interactions, and further studies are recommended to explore the clinical implications of these findings.

Keywords: Chrysanthemum indicum L., CYP3A4 inhibition, hypertension

1. Introduction

Herbal medicine has been a cornerstone of Eastern traditional healing for centuries, with its roots tracing back to the Zhou dynasty in China (26 B.C.E.). In Malaysia, hypertension remains a major public health challenge, affecting 30% of adults aged 18 and older, as reported in the 2019 Non-Communicable Disease survey. With the growing interest in alternative therapies, medicinal plants like *Chrysanthemum indicum* (Indian Chrysanthemum or Ye Ju Hua) have gained attention for their potential role in managing hypertension. Hypertension, a leading modifiable risk factor for cardiovascular diseases (CVDs), has seen a global surge in prevalence, doubling over the past three decades. In Malaysia, hypertension rates have slightly declined from 2015 to 2019, yet the burden remains significant. Conventional antihypertensive drugs, such as β-blockers and diuretics, effectively lower blood pressure but may cause adverse metabolic effects, potentially worsening conditions like metabolic syndrome. This has fueled interest in plant-based alternatives like *C. indicum*, which offers a promising natural intervention.

A member of the Asteraceae family, *C. indicum* has been referenced in ancient Chinese texts, such as *Sheng Nong's Herbal Classic*, for its therapeutic benefits. The herb is widely cultivated in Malaysia, particularly in the Cameron Highlands, where it contributed to 44.8% of the region's flower production in 2018. Traditionally consumed as herbal tea, *C. indicum* is valued for its antioxidant and antiglycation properties, offering a simple, accessible means of extraction. *Chrysanthemum indicum* has been reported to have more than 191 phytochemicals including 42 flavonoids, 96 terpenoids, 21 phenylpropanoids and phenolic acids, 12 spiro ketones; 20 others and together with their relevant biological activities. Besides, some researchers have asserted that the extracts or monomeric compounds of *C. indicum* contained a wide spectrum of biological properties either in-vivo or in-vitro, such as anti-inflammatory, anti-oxidation, antipathogenic microorganism, anticancer, immune regulation and hepatoprotective effects.[1] One of the effects of this herb include its anti-hypertensive effects, where buddleoside (BUD), a flavonoid, helps in reduction of the number of lipopolysaccharide (LPS) released from cell walls of dead gram- negative bacteria present in the gut. An in-vivo study was done and proven there was a reduction of blood pressure, reduction in liver index and improved lipid deposition in hepatic cells. This shown that the BUD extract of the *C. indicum* L. not only act as antihypertensive, but also exhibit antimicrobial and anti-inflammatory pharmacological activities.

Due to this wide range of effects, there have been questions about the safety assessment of *Chrysanthemum indicum*. One such study conducted in 2018 reported that the aqueous extract of *C. indicum* was found to be safe at 4000 mg/kg body weight with no pathological

changes in male rats over a period of 14 days. However, there has been reported that that *C. indicum* has shown inhibition activity of hepatic CYP3A4 enzyme around 46.18% at 100µg/mL.This information is vital as it can have significant drug interactions between drugs metabolized by the CYP3A4 enzyme; where significant CYP3A4 inhibition may lead to hepatotoxicity due to prolonged drug half-life. [2, 3]

One key area of investigation is the interaction of herbal remedies with the cytochrome P450 (CYP450) enzyme system, which plays a critical role in drug metabolism. CYP450 enzymes, particularly CYP3A4, are responsible for the metabolism of 30-50% of all prescribed drugs. Located primarily in the liver, CYP3A4 facilitates oxidation, reduction, and hydrolysis reactions, transforming lipophilic drug molecules into water-soluble metabolites for excretion. The activity of CYP3A4 can be modulated by inhibitors and inducers, affecting drug efficacy and safety. Potent CYP3A4 inhibitors, such as clarithromycin, erythromycin, diltiazem, ritonavir, verapamil, and grapefruit, reduce the enzyme's activity, potentially leading to toxic drug accumulation in the liver. Conversely, inducers like phenobarbital, phenytoin, rifampicin, and glucocorticoids enhance CYP3A4 activity, accelerating drug metabolism and reducing therapeutic effectiveness. The ability of *C. indicum* to influence CYP450 activity, particularly CYP3A4, presents a critical avenue for research, as it may affect drug interactions and the metabolism of antihypertensive medications. [3, 4]

The aim of this study is to provide preliminary scientific evidence about the safe usage of *Chrysanthemum Indicum* tea with other drugs that are metabolized by the CYP3A4 enzyme in order to keep hypertensive patients safe from potential adverse effects including herb-drug interactions. Therefore, the main objective of the study is to examine the *in vitro* effect *Chrysanthemum Indicum* tea on the rate of *N*-demethylation of aminopyrine *via* CYP3A4 enzyme in hypertensive rat liver microsomes using a spectrophotometric method.

Methodology

Materials and methods

Chemical and solvents

This study investigates the effect of *Chrysanthemum indicum (C. indicum)* flower decoction on CYP3A4 enzyme activity using liver microsomes derived from Spontaneous Hypertensive Rats (SHR). Key apparatus includes a spectrophotometer, filter funnel, water bath, test tubes, cuvette, pipette and dark amber bottle while reagents include aminopyrine, phosphate buffer saline, NADPH, $MgCl_2$, $ZnSO_4$, ammonium acetate, acetone and formaldehyde, all chemicals sourced locally.

Preparation of Rat Liver Microsomes

Five female spontaneous hypertensive (SHR)

rats with 150-170 g body weight were used in this study. The livers of SHR rats were rinsed with ice-cold 0.05 M tris-HCl buffer pH (7.4), sliced into very small pieces, and homogenized with 25 mL of 0.05 M tris-HCl buffer using a lab homogenizer (DYNA-Passion) at the speed of 10,000 rpm. The homogenate was centrifuged at 10,000 × g for 15 min at 4 °C. The resulting supernatant was centrifuged using XL-70 Beckman ultracentrifuge (Beckman, Carlsbad, CA, USA) at 100,000 × g for 60 min at 4 °C to obtain the microsomal pellet. The obtained pellets were resuspended in 500 µL of 0.05 M HEPES buffer (pH 7.4), which contains 20% glycerol and 0.001 mol/L EDTA using a homogenizer at 6000 rpm, and stored at −80 °C until further use. Protein concentration was determined by the Lowry method. Bovine serum albumin (BSA) was used as the standard. Later, the liver microsomes were diluted to 1 mg/ml as initial concentration for CYP3A4 assay. [5]

Plant Materials & Extraction

The decoction preparation follows a modified method from Jiang M.Y *et al*. (2020), using 2 grams of *C. indicum* dry flowers heated in 100 ml of water at 100°C for 10 minutes. [6] For the enzyme activity assay, liver microsomes are pre-treated and then combined with different test groups:

(i) Negative Control (Without *C. indicum* flower decoction)

(ii) Positive Control (With 30 µM ketoconazole)

(iii) Experimental groups (Different dilutions of *C. indicum* flower decoction)

(a) 2 mg/ml (freshly prepared *C. indicum* flower decoction)

(b) 0.2 mg/ml (dilute 10 times)

(c) 0.02 mg/ml (dilute 100 times)

(d) 0.002 mg/ml (dilute 1000 times)

Determination of CYP3A4 activity

Each reaction mixture, incubated at 37°C, undergoes a reaction-termination step followed by centrifugation, after which Nash reagent is added to detect formaldehyde formation, indicated by a yellowish color and measured via spectrophotometer at 405 nm. The CYP3A4 activity is then calculated based on formaldehyde concentration, expressed as nmol formaldehyde/min/mg protein. [6]

Statistical analysis

Data analysis involves ANOVA and post-hoc Dunnett's tests to compare mean values and assess standard deviations, ensuring statistical reliability of the findings.

Results

Standard curve of formaldehyde was plotted to determine the concentration of formaldehyde generated from the N-demethylation of aminopyrine catalysed by CYP3A4 enzyme in rat liver microsomes (Refer to Figure 1). Absorbance reading was increased according to the density of the yellowish color of the formaldehyde after reacting with Nash reagent.

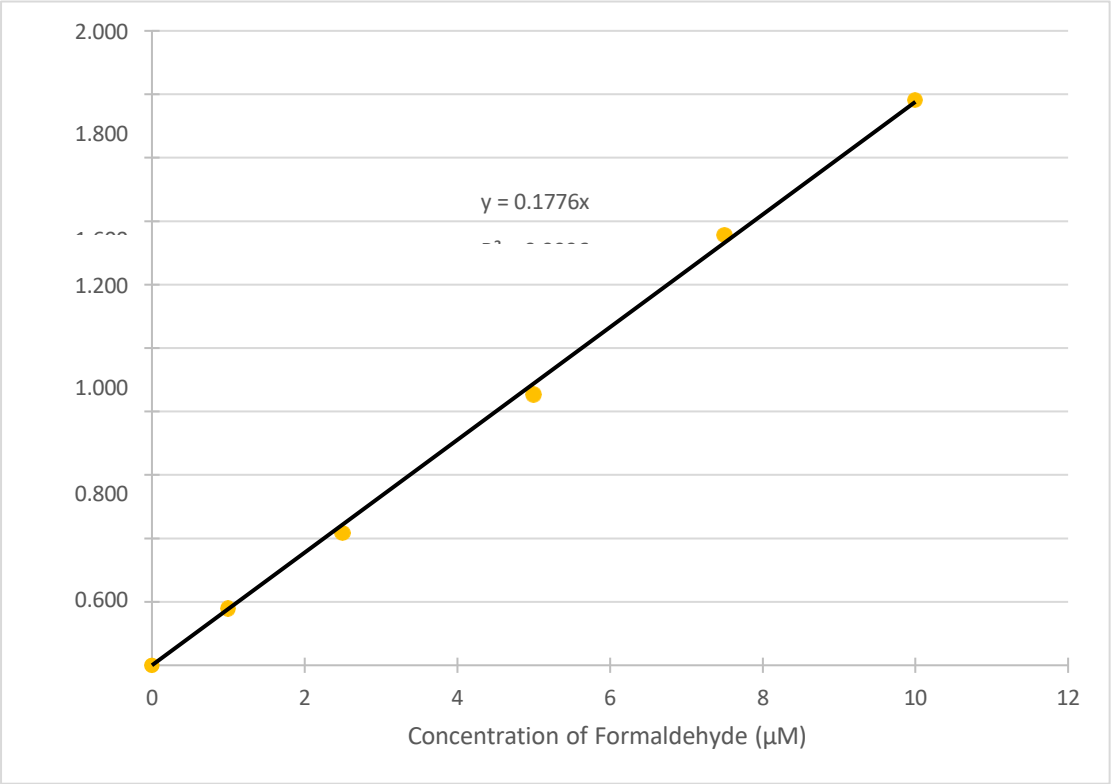
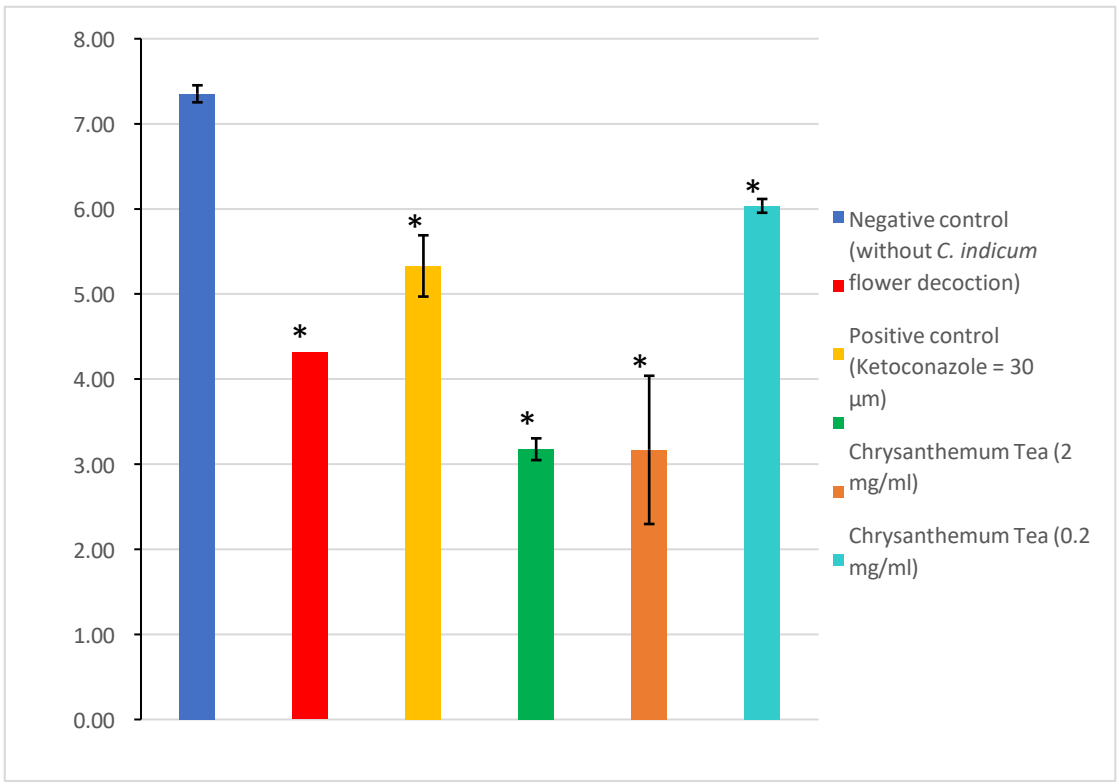


Figure 1: Standard curve of Formaldehyde; (n=3)

The *in vitro* effect of different concentrations of *Chrysanthemum indicum* L. flower herbal tea ranging from 0.002 mg/ml to 2 mg/ml on hepatic CYP3A4 activity was shown in Figure 2. Results obtained from this study demonstrated that *Chrysanthemum indicum* L. flower herbal tea at 0.2 mg/ml produced a significant greatest inhibition effect by 56.92% (p<0.01) compared to the negative control group.



Data = Mean +/- standard deviation; n=3; Analysed by using Dunnett's test.
** $p<0.01$ significant difference compared with the negative control group
Figure 2: Specific CYP3A4 activity (µm/min/mg microsome) against different concentration of *Chrysanthemum indicum* L. flower herbal tea

Discussion

CYP3A4 is the most abundant isoform of the human CYP system accounting for approximately 28% of the whole enzyme system. In addition, the *Chrysanthemum indicum* has been studied to have a secondary metabolite which is a flavonoid that is believed to have a polyphenolic class of compounds that contain several reactive phenolic hydroxyl groups. The presence of phenolic hydroxyl groups causes the formation of hydrogen and ionic bonds at the active sites of an enzyme. [7] This occurs when the charged and polar polyphenols interact with proteins by forming ionic bonds in addition to hydrogen bonds with several amino acids at the active site which might lead to enzyme inhibition and loss of function.

The effects of CYP3A4 are found to be altered by *C. indicum L*. The extracts are known to induce and inhibit CYP3A4 activities and result in changes in blood levels of substrate drugs. The CYP3A4 enzyme is the most important enzyme in drug metabolism, where it is considered to be involved in the metabolism of more than 50% of drugs. [8] CYP3A4 enzymes can be subject to reversible inhibition, where the enzyme is bound by non-covalent bonds, allowing it to be easily removed from the enzyme and return to enzymatic activity. For instance, ketoconazole shows different types of inhibition which are competitive and non-competitive. Significant number of studies highlight these chemical compounds exhibit inhibition of CYP3A4 enzyme.

Our findings were in agreement with studies reported by Jiang Y, Ji X, Duan L, Ye P, Yang J, Zhan R, et al (2023). *C. indicum L.* flower is proven to have inhibition towards CYP450 enzymes because *C. indicum L*. has flavonoids, terpenoids (sesquiterpenes), phenolic acid and other compounds based on several research journals. [9] There are journals suggesting the relationship between flavonoids including acacetin (most prominent 95%), chrysin, apigenin and linarin. Rutin and Isoquercitrin which are flavonoids of *Moringa oleifera* as well as *C. indicum L.* show significant inhibition effect towards CYP3A4 activity as well. [10, 11]

Flavonoid especially taxifolin, apigenin and luteolin inhibit CYP3A4, CYP2E1 and CYP1A2 activity in liver, thus, flavonoid increases the risk of Benzo[a]pyrene toxicity due to decreased metabolism and detoxification process. Flavonoids like Quercetin and Apigenin exhibited inhibitory effects on the metabolism of CYP450 including CYP3A4 which will indirectly inhibit vortioxetine *in vivo* and *in vitro* studies. [12]

According to a journal by Carey (2022). herb drug interactions between cholesterol-lowering drug, Statin and components of *C. indicum.* Chrysanthemum may have the possibility to interact with several statins, thus the author recommended avoiding drinking chrysanthemum tea while using these medications. [13] In an *in vitro* study conducted in 2009, chlorogenic acid has shown to have synergistic effects when given together with antidiabetics 2,4 Thiazolidinedione and Metformin. Due to this, antidiabetic doses may need to be lowered to avoid any adverse effects and toxicity in patients. As for the case of caffeic acid, the effects have not been properly studied yet. Still, it is hypothesized to have a similar effect as chlorogenic acid in a synergistic effect with the two antidiabetics mentioned above. [14, 15] Although we have lack of research findings regarding the herb drug interaction between Chrysanthemum and anticancer drug, based on the research on the biochemical effect of Chrysanthemum, it could enhance anticancer effects, reduce side effects of anticancer chemotherapy and increase the metabolism rate of anticancer drugs due to its inhibition effect towards CYP450 enzymes, antioxidant, anti-inflammatory properties. [16] By referring to in vitro experimental studies, quercetin can increase concentration of doxorubicin by inhibiting CYP enzymes which may pose acute cardiac toxicity due to increased oxidative stress and induction of cardiac myocyte apoptosis [17, 18]

In conclusion, *C.indicum's* influence on CYP3A4 mediated drug metabolism presents both therapeutic opportunities and risks. Its terpenoid and flavonoid content raises the potential for interactions with various drugs, underscoring the need for comprehensive understanding of these interactions, particularly for patients on CYP3A4 metabolized therapies. Further research is essential to ensure the safe use of *C.indicum* as a complementary treatment and to clarify the therapeutic implications of its interactions.

Conclusion

The administration of *Chrysanthemum Indicum* L. herbal tea exhibited significant inhibitory effect on hepatic CYP3A4 activity in female SHR rats. Thus, precautionary steps should be taken to avoid unwanted herb-drug interaction when *CIL.* herbal tea is consumed concurrently with the medications metabolized by CYP3A4 enzyme among hypertensive populations.

Acknowledgement

We are grateful for the facilities and resources provided by MAHSA University, which enabled us to carry out this study successfully.

References

1. Shao Y, Sun Y, Li D, Chen Y. *Chrysanthemum indicum* L.: A Comprehensive Review of its Botany, Phytochemistry and Pharmacology. The American Journal of Chinese Medicine.

2020 Jan;48(04):871–97. DOI: 10.1142/S0192415X20500421

2. Bitwell C, Indra SS, Luke C, Kakoma MK. A review of modern and conventional extraction techniques and their applications for extracting phytochemicals from plants. *Scientific African* [Internet]. 2023 Mar 1;19:e01585. DOI: 10.1016/j.sciaf.2023.e01585

3. Brendler T, Brinckmann JA, Daoust M, He H, Masé G, Steffan K, et al. Suitability of botanical extracts as components of complex mixtures used in herbal tea infusions— challenges and opportunities. *Frontiers in Pharmacology*. 2022 Nov 7;13. DOI: 10.3389/fphar.2022.1013340

4. Carey E. How Chrysanthemum Tea Benefits Your Health [Internet]. Healthline. Healthline Media; 2015. Available from: https://www.healthline.com/health/food-nutrition/how-chrysanthemum-tea-benefits-health

5. Gibson, G. G., & Skett, P. (1994). Introduction to drug metabolism. London, England: Blackie Academic & Professional.

6. Jiang, M., Zhang, W., Zhang, T., Liang, G., Hu, B., Han, P., & Gong, W. (2020). Assessing transfer of pesticide residues from chrysanthemum flowers into tea solution and associated health risks. Ecotoxicology and Environmental Safety, 187, 109859. https://doi.org/10.1016/j.ecoenv.2019.109859

7. Ashour, M. L., Youssef, F. S., Gad, H. A., & Wink, M. (2017). Inhibition of cytochrome P450 (CYP3A4) activity by extracts from 57 plants used in traditional chinese medicine (TCM). Pharmacognosy Magazine, 13(50), 300. https://doi.org/10.4103/0973-1296.204561

8. Kondža M, Brizić I, Jokić S. Flavonoids as CYP3A4 Inhibitors In Vitro. Biomedicines. 2024 Mar 13;12(3):644.

9. Jiang Y, Ji X, Duan L, Ye P, Yang J, Zhan R, et al. Gene mining and identification of a flavone synthase II involved in flavones biosynthesis by transcriptomic analysis and targeted flavonoid profiling in *Chrysanthemum indicum* L. Gene mining and identification of a flavone synthase II involved in flavones biosynthesis by transcriptomic analysis and targeted flavonoid profiling in *Chrysanthemum indicum* L [Internet]. 2019 Aug 1 [cited 2023 May 25];134:244–56. DOI: 10.1016/j.indcrop.2019.04.009

10. Khan, Shabana I, et al. Isolation, Synthesis, and Drug Interaction Potential of Secondary Metabolites Derived from the Leaves of Miracle Tree (Moringa Oleifera) against

CYP3A4 and CYP2D6 Isozymes. Vol. 60, 1 July 2019, pp. 153010–153010, https://doi.org/10.1016/j.phymed.2019.153010

11. Kumar N, Sharma S. Pharmacology, Ethnopharmacology, and Phytochemistry of Medicinally Active Moringa oleifera: A Review. The Natural Products Journal. 2023 Dec 1;13(8):13-41.

12. Lin Y, Wang Y, Ye Z, Gao N, Xu R, Ye L, et al. Effects of dietary flavonoids on the metabolism of vortioxetine and its potential mechanism. Current Medicinal Chemistry. 2023 Jun 7;30.

13. Carey, E. (2022b, March 25). How Chrysanthemum Tea Benefits Your Health. Healthline. https://www.healthline.com/health/food-nutrition/how-chrysanthemum-tea-benefits-health

14. Prabhakar PK, Doble M. Synergistic effect of phytochemicals in combination with hypoglycemic drugs on glucose uptake in myotubes. Phytomedicine. 2009 Dec;16(12):1119–26.

15. Yan Y, Zhou X, Guo K, Zhou F, Yang H. Use of chlorogenic acid against diabetes mellitus and its complications. Journal of Immunology Research. 2020;2020(1):9680508.

16. Sharma N, Kumar M, Kumari N, Puri S, Rais N, Natta S, Dhumal S, Navamaniraj N, Chandran D, Mohankumar P, Muthukumar M. Phytochemicals, therapeutic benefits and applications of chrysanthemum flower: A review. Heliyon. 2023 Oct 1;9(10).

17. Mohos V, Fliszár-Nyúl E, Ungvári O, Kuffa K, Needs PW, Kroon PA, Telbisz Á, Özvegy-Laczka C, Poór M. Inhibitory effects of quercetin and its main methyl, sulfate, and glucuronic acid conjugates on cytochrome P450 enzymes, and on OATP, BCRP and MRP2 transporters. Nutrients. 2020 Jul 31;12(8):2306.

18. Johnson-Arbor, Kelly, and Ramin Dubey. “Doxorubicin.” Nih.gov, StatPearls Publishing, 30 May 2019, www.ncbi.nlm.nih.gov/books/NBK459232/.