GSC Biological and Pharmaceutical Sciences

eISSN: 2581-3250 CODEN (USA): GBPSC2

Cross Ref DOI: 10.30574/gscbps

Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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Hepatoprotective effect of aqueous extract of hibiscus leaves in female albino rats treated with Tamoxifen

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GSC Biological and Pharmaceutical Sciences, 2025, 30(01), 186-191

Publication history: Received on 29 November 2024; revised on 11 January 2025; accepted on 13 January 2025

Article DOI: https://doi.org/10.30574/gscbps.2025.30.1.0011

Abstract

Background: Breast cancer is a perilous malignancy and a predominant source of mortality in women. The incidence of breast cancer is steadily rising due to risk factors such as age, menopause, obesity, hormone replacement treatment, familial history, as well as environmental and lifestyle influences

Objective: This study assesses the hepatoprotective efficacy of oral pretreatment with varying dosages of aqueous extract from the leaves of Hibiscus sabdariffa L. against Tamoxifen induced hepatic damage

Material and Methods: It was used 30 female (Sprague Dawley) rats were divided into six groups, each group subjected to five individuals, Group I: The control group was administered normal saline, Group II: The negative control group was administered a single intraperitoneal dosage of Tamoxifen (10 mg/kg body weight) on the eighth day of the experimental period. Groups III and IV : co-treatment groups with 2 doses 250, 500 mg/kg BW of Aqueous extract leaves of Hibiscus sabdariffa L. Groups V and VI : co-treatment groups with 2 doses 250, 500 mg/kg BW of Aqueous extract leaves of Hibiscus sabdariffa L in addition to a single intraperitoneal administration of Tamoxifen. Induction of Hepatoxicity started from day 8 intraperitonelly, up to day 21.

Results: Results showed the beneficial effects of Hibiscus sabdariffa L. leaves on Glutathium and Superoxide desmutase, along with the enhancement of antioxidant capacity in liver tissue, indicate that these leaves will mitigate oxidative stress induced by Tamoxifen and decrease Reactive oxygen species induced lipid peroxidation, as evidenced by the significantly reduced levels of Malondialdehyde in the liver tissue compared to the control group. Lipid peroxidation and mitochondrial dysfunction induced by Tamoxifen will ultimately result in cellular injury and membrane damage, compromising cellular integrity. Consequently, liver cell contents, particularly the enzymes ALT, AST, and ALP, will be released into the bloodstream from the compromised cells.The leaves of Hibiscus sabdariffa L. maintain oxidative equilibrium within liver tissue, so averting hepatic damage, as seen by the considerable and dose-dependent decrease in blood ALT, AST, and ALP levels.

Conclusion: The effectiveness of the aqueous extract of the plant in inhibiting the activity of free radicals and neutralizing the oxidative stress induced by the drug Tamoxifen in the liver tissue and some functional parameters in female white rats.

Keywords: *Hibiscus sabdariffa L*; Hepatopro-tective activity; Hepatotoxicity; Tamoxifen; Liver enzymes

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1. Introduction

Breast cancer is a perilous malignancy and a predominant source of mortality in women. The incidence of breast cancer is steadily rising due to risk factors such as age, menopause, obesity, hormone replacement treatment, familial history, as well as environmental and lifestyle influences [1,2]

Tamoxifen, an oestrogen antagonist, is a well-established medicine utilised as adjuvant endocrine therapy for breast cancer and is regarded as a significant alternative for hormone receptor (HR)-sensitive early breast cancer in both premenopausal and postmenopausal women [3]. Tamoxifen received approval from the United States Food and Drug Administration in 1977 as an adjuvant hormonal therapy for individuals with estrogen-receptor-positive breast cancer.[4] Due to its affordability and favourable tolerance, tamoxifen has emerged as the primary option for adjuvant hormonal therapy. TAM is commonly utilised as an adjuvant therapy for early-stage breast cancer; nonetheless, its hepatotoxicity remains under studied. Despite its good tolerance, numerous side effects, including liver damage or even hepatocarcinoma (described just in rats), are unavoidable [5]. Recent studies have identified the unique mechanism of TAM-induced hepatotoxicity; nevertheless, investigations into TAM-induced morphological alterations in hepatic damage remain to be clarified.

To reduce these toxic effects of the drug, we resorted to medicinal plants because of their great importance. which significantly contribute to the suppression of both free radical generation and oxidative chain reactions inside tissues and membranes [6]. Numerous phytochemicals, particularly polyphenols such as phenolic acids, flavonoids, tannins, and anthocyanins, are recognised for their free radical scavenging and antioxidant properties.

(Hibiscus sabdariffa L.) is an annual and herbaceous medicinal plant and is scientifically called (rosella) It belongs to the (Mavaceae) Family. Many studies have confirmed that the ability of drinking the guava plant or red tea contributes to the treatment of many diseases, the spread of which has increased in our current time, including kidney stones due to its effect on uric acid as well as reducing the level of cholesterol and triglycerides, in addition to its ability to lower blood pressure and protect the liver from oxidizing agents [7]. On the other hand, many studies have indicated the inhibitory effect of guava tea (water extract) on cancerous diseases, including stomach, bladder, colon, liver, breast and mouth cancer [8]. It also contributes to increasing the effectiveness of the kidneys and is a pain reliever for urinary tract infections and is considered a good diuretic in addition to its effectiveness in reducing blood sugar levels and preventing obesity [9,10]. The medical importance of the aqueous extract of the guava plant comes from the fact that this plant contains many active substances that have been discovered through previous studies, as it contains compounds and minerals Ca, niacin, carotene, calcium, C, riboflavin, ascorbic acid, vitamin C, and some important minerals for the body such as potassium. It also contains anthocyanin granules It improves many metabolic functions of the liver, as it has been shown that the anthocyanin pigment present in the aqueous extract has a protective effect against liver toxicity, which is represented by resisting the oxidation of free radicals in the tissues of Hepatotoxicity. It also plays a protective role against hyperlipidemia in the liver [11]. Among the important phenolic compounds is the phenolic compound protocatehenic acid (PCA) [12]. It was found that this extract has a significant effect against the growth of tumors Cancer by fighting free radicals and also works against liver cirrhosis by removing the harmful effect of tamoxifen.

2. Material and Methods

2.1. Collection and preparation of plant extract

leaves of Hibiscus sabdariffa L. were collected from the local markets in the city of kirkuk / Iraq. It was washed with distilled water to To clean it and left to dry for five days at room temperature. After drying, it was ground with a mortar and electric grinder to obtain a coarse powder used in extraction.

2.2. Chemicals and reagents

All materials used in this experiment were of the analytical type. Tamoxifen is sourced from Rosemont, England; diagnostic kits for evaluating serum transaminases (ALT, AST), alkaline phosphatase (ALP) enzymes, and antioxidant enzymes such as catalase and superoxide dismutase are produced by Bio Lab.

2.3. Phytochemical screening

The prepared alcohol extract of Hibiscus sabdariffa L. was used to test various phytochemical components. Different chemical reagents were prepared and specific test for specific phytochemicals was done [13,14].

2.4. Experimental animals

Female Sprague Dawley rats (250-300g body weight) were obtained from the Iraqi Center for Cancer Research, Serum and Vaccine Institute, and the National Centre for Drug Control and Research, Baghdad, Iraq, were maintained under standardised environmental conditions. The subjects were maintained under controlled conditions of temperature, humidity, and photoperiod, with access to conventional rat feed and water ad libitum. Thirty female Sprague Dawley rats were allocated into six groups of five individuals each and subjected to the subsequent treatment regimen.

Group I: The control group was administered normal saline. Group II: The negative control was administered a single intraperitoneal dosage of TAM (10 mg/kg body weight) on the eighth day of the one-month experimental period. Groups III and IV: co-treatment groups,And respectively (250,500 mg/kg b.w) of Aqueous extract leaves of Hibiscus sabdariffa L. Groups V and VI: co-treatment groups,And respectively (250,500 mg/kg b.w) of Aqueous extract leaves of Hibiscus sabdariffa L. contemporaneous with a single intraperitoneal administration of TAM in Group II, which served as the positive control.

Induction of Hepatoxicity started from day 8 intraperitonelly, up to day 21, when all the groups (except I, III and IV), received single intra-peritoneal injections of Tamoxifen at a dose 10 mg /kg of animal body weight.

2.5. Statistical analysis

The values were presented as mean ± SD for five animals per group. The data was statistically evaluated using one-way ANOVA. Duncan's test [15]. identified substantial differences between the groups. Probability levels below 0.05 were deemed significant.

3. Results

Impact on oxidative stress biomarkers (glutathione, superoxide dismutase, catalase, and malondialdehyde) Table 1 Marked consumption of the antioxidant capacity indicated by GSH and SOD was seen in the liver tissue following TAM injection ($p \leq 0.05$) relative to the control group. Conversely, oral pretreatment with aqueous extract of Hibiscus sabdariffa L. leaves at doses of 250 and 500 mg/kg resulted in a significant increase in tissue levels of GSH, SOD, and catalase compared to the TAM (10 mg/kg) group ($p \leq 0.05$) over one month. Additionally, MDA, a by-product of oxidative stress, was significantly elevated ($p \leq 0.05$) following TAM injection at a dose of 10 mg/kg IP compared to the control group. The aqueous extract of Hibiscus sabdariffa L. leaves, administered orally at doses of 250 and 500 mg/kg, significantly and dose-dependently reduced the MDA level, with a P-value of ($p \leq 0.05$) for both groups.

Table 1 Effect of TAM and Aqueous extract of leaves of Hibiscus sabdariffa L. on liver tissue level of oxidative stress biomarker (MDA,GSH,Catalase and SOD)

Mean ± SD				
Group	**MDA nm/l**	**GSH nm/l**	**catalase nm/l**	**SOD nm/l**
CONTROL	1.7221±0.0811 c	0.5112±0.0067 a	1.3212±0.0261 ab	0.5214±0.0231 c
TAM 10Mg/Kg	2.5213±0.0912 a	0.1134±0.261 b	0.791±0.0193 b	0.1941±0.0042 f
Aqueous extract 250mg	1.3291±0.0492 cd	0.5122±0.0213 a	1.3112±0.0799 b	0.7211±0.0211 c
Aqueous extract 500mg	1.5312±0.0139 de	0.3941±0.0192 a	1.4162±0.222 a	0.7312±0.0112 a
TAM+250Mg aqueous extract	1.5333±0.081 cd	0.523±0.0075 a	1.3320±0.0854 ab	0.731±0.0526 b
TAM+500Mg aqueous extract	1.832±0.1921 b	0.426±0.0311a	1.060±0.0623 b	0.496±0.0169 d

Effect on the activity of serum marker enzymes (alanine aminotransferase, Aspartateamino Transeferase and Alkaline Phosphatase) When TAM (10 mg/kg of .w) is injected into the TAM-only group, a significant elevation was observed in the serum (ALT,AST and ALP) [Table 2] level ($p \leq 0.05$) compared to the control group [Table 2]. Pretreatment with Aqueous extract of leaves of Hibiscus sabdariffa L. orally dosed(250,500mg\kg of .w) in both doses will hold down this elevation significantly and dose-dependently when compared to the TAM group ($p \leq 0.05$).

Table 2 Effect of TAM and Aqueous extract of leaves of Hibiscus sabdariffa L. on serum level of enzyme biomarker (AST, ALT and ALP)

Mean ± SD			
Group	**AST U/L**	**ALT U/L**	**ALP U/L**
CONTROL	19.96±5.48 c	18.47±4.11 c	38.23±4.88d
TAM 10Mg/Kg	59.48±8.27 a	83.45±6.21a	99.36±8.65a
Aqueous extract 250mg	20.43±6.16 b	26.25±5.11b	42.42±6.21bc
Aqueous extract 500mg	18.76±4.96 c	20.31±3.98c	40.21±5.32c
TAM+250Mg Aqueous extract	21.35±4.94 b	44.87±5.98 b	47.63±6.53b
TAM+500Mg Aqueous extract	22.47±6.11 c	22.43±3.99c	39.43±4.39d

4. Discussion

The beneficial effects of medicinal herbs include their protective role against oxidative damage and preventing various diseases that can occur due to oxidative stress such as cancer, diabetes and Alzheimer's. This damage, whether in cells or tissues, leads to the emergence of various diseases in humans, as a result of significant damage to the components of the living cell. The medical importance of the aqueous extract of Hibiscus sabdariffa L. comes from the fact that this plant contains many active substances that have been discovered through previous studies, as it contains effective compounds such as phenols, flavonoids and carotenoids, Riboflavin, Vitamin C and Anthocyanins.

This study evaluates the hepatoprotective efficacy of oral pretreatment with different dosages of aqueous extract from Hibiscus sabdariffa L. leaves against TAM-induced liver injury. The results shown that the leaves of Hibiscus sabdariffa L. exhibit a significant and dose-dependent ability to safeguard hepatic tissue from acute damage caused by TAM. The injection of TAM in this study reduces both GSH levels and SOD activity in liver tissue. The direct inhibition of TAM on NADPH, a cofactor for the enzyme GSH reductase in the manufacture of reduced GSH, along with the excessive generation of ROS, will lead to a reduced cellular availability of GSH. [16,17]. SOD is an essential endogenous antioxidant enzyme that converts reactive oxygen species produced by mitochondria, mostly superoxide (O2−), into less detrimental hydrogen peroxide (H2O2) or molecular oxygen (O2).[18] Conversely, MTX causes damage to the mitochondrial membrane, leading to an elevated production of superoxide (O2−) free radicals, which impairs SOD activity [19]. The overproduction of reactive oxygen species (ROS) and the reduction of endogenous antioxidants will disturb oxidative balance, resulting in oxidative stress. Moreover, reactive oxygen species (ROS) will cause mitochondrial dysfunction, resulting in elevated ROS production [20,21]. This leads to lipid peroxidation of cellular lipids and the liberation of malondialdehyde (MDA) [22]. Consequently, in this study, TAM increases MDA levels in hepatic tissue by almost three-fold relative to the control group. Furthermore, lipid peroxidation and mitochondrial dysfunction will ultimately result in cellular injury and membrane impairment. This will jeopardise the cell's integrity, resulting in the leakage of hepatic cell contents, including ALT, AST, and ALP, into the bloodstream. [23,24]. Furthermore, neutrophil activation triggered by reactive oxygen species (ROS) would intensify cellular damage [25]. The leaves of Hibiscus sabdariffa L. contain various compounds, such as riboflavin, vitamin C, and anthocyanins, which possess antioxidant and free radical scavenging capabilities. This activity reduces the surplus reactive oxygen species (ROS) produced by TAM, thereby preserving the levels of both enzymatic and non-enzymatic antioxidants [26]. Furthermore, the leaves of Hibiscus sabdariffa L. promote the translocation of Nrf2 from the cytosol to the nucleus, presumably activating cytoprotective mechanisms and increasing liver levels of GSH and SOD as a result. [27]. The advantageous impact of Hibiscus sabdariffa L. leaves on GSH and SOD, coupled with the augmentation of antioxidant capacity in hepatic tissue, suggests that these leaves will alleviate oxidative stress caused by TAM and diminish ROS-induced lipid peroxidation, as demonstrated by the markedly lower levels of MDA in the liver tissue relative to the control group. Lipid peroxidation and mitochondrial dysfunction caused by TAM will ultimately lead to cellular injury and membrane damage, undermining cellular integrity. As a result, the contents of liver cells, specifically the enzymes ALT, AST, and ALP, will be discharged into the bloodstream from the damaged cells. .[28] The leaves of Hibiscus sabdariffa L. maintain oxidative equilibrium within liver tissue, so averting hepatic damage, as seen by the considerable and dose-dependent decrease in blood ALT, AST, and ALP levels.

5. Conclusion

We conclude from the current study the effectiveness of the aqueous extract of the plant in inhibiting the activity of free radicals and neutralizing the oxidative stress induced by the drug tamoxifen in the liver tissue and some functional parameters in female white rats.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

Ethical approval was obtained.

References

[1] Paliwal SR, Paliwal R, editors. Targeted Nanomedicine for Breast Cancer Therapy. Academic Press; 2022 Aug 6.

[2] Al-Maliki SA, Mussttaf AH, Al-Haidary YA. Comparative Study of Cardiac Radiation Dose With Different Types of Surgery in Breast Cancer Patients. Diyala Journal of Medicine. 2024 Dec 25;27(2):53-64.

[3] NCCN Clinical Practice Guidelines in Oncology: Breast Cancer. Version 1.2006: NCCN, 2006. 5 Howell A, Cuzick J, Baum

[4] Osborne CK. Tamoxifen in the treatment of breast cancer. New England Journal of Medicine. 1998 Nov 26;339(22):1609-18.

[5] Yang G, Nowsheen S, Aziz K, Georgakilas AG. Toxicity and adverse effects of Tamoxifen and other anti-estrogen drugs. Pharmacology & therapeutics. 2013 Sep 1;139(3):392-404.

[6] Rahim SM, Taha EM, Mubark ZM, Aziz SS, Simon KD, Mazlan AG. Protective effect of Cymbopogon citratus on hydrogen peroxide-induced oxidative stress in the reproductive system of male rats. Systems biology in reproductive medicine. 2013 Dec 1;59(6):329-36.

[7] Prasongwatana V, Woottisin S, Sriboonlue P, Kukongviriyapan V. Uricosuric effect of Roselle (Hibiscus sabdariffa) in normal and renal-stone former subjects. Journal of Ethnopharmacology. 2008 May 22;117(3):491-5.

[8] Tsai, P.J., McIntosh, J., Pearce, P., Camden, B. and Jordan, B.R., 2002. Anthocyanin and antioxidant capacity in Roselle (Hibiscus sabdariffa L.) extract. Food research international, 35(4), :351-356..

[9] Rosemary R, Rosidah R, Haro G. Antidiabetic effect of roselle calyces extract (Hibiscus sabdariffa L.) in streptozotocin induced mice.

[10] Alarcon-Aguilar FJ, Zamilpa A, Perez-Garcia MD, Almanza-Perez JC, Romero-Nuñez E, Campos-Sepulveda EA, Vazquez-Carrillo LI, Roman-Ramos R. Effect of Hibiscus sabdariffa on obesity in MSG mice. Journal of ethnopharmacology. 2007 Oct 8;114(1):66-71.

[11] Akindahunsi AA, Olaleye MT. Toxicological investigation of aqueous-methanolic extract of the calyces of Hibiscus sabdariffa L. Journal of ethnopharmacology. 2003 Nov 1;89(1):161-4.

[12] Lin TL, Lin HH, Chen CC, Lin MC, Chou MC, Wang CJ. Hibiscus sabdariffa extract reduces serum cholesterol in men and women. Nutrition research. 2007 Mar 1;27(3):140-5.

[13] Abayomi S. Medicinal plants and traditional medicine in Africa. J Altern Complement Med. 1993;13:195-238.

[14] Harborne JB. Phytochemical Methods.(1973). Chapman and hall Ltd.,London: U.K., 49-188.

[15] Ali, Younis. Principles of Biostatistics. Journal of the Royal Statistical Society, (2023). doi: 10.1093/jrsssa/qnad038

[16] Lodish HF. Molecular cell biology. Macmillan; 2008.

[17] Nagahara Y, Shiina I, Nakata K, Sasaki A, Miyamoto T, Ikekita M. Induction of mitochondria-involved apoptosis in estrogen receptor-negative cells by a novel tamoxifen derivative, ridaifen-B. Cancer science. 2008 Mar;99(3):608-14.

[18] Schieber M, Chandel NS. ROS function in redox signaling and oxidative stress. Current biology. 2014 May 19;24(10):R453-62.

[19] Famurewa AC, Ekeleme-Egedigwe CA, David EE, Eleazu CO, Folawiyo AM, Obasi NA. Zinc abrogates anticancer drug tamoxifen-induced hepatotoxicity by suppressing redox imbalance, NO/iNOS/NF-κB signaling, and caspase-3-dependent apoptosis in female rats. Toxicology mechanisms and methods. 2020 Feb 12;30(2):115-23.

[20] Qays, Assi, Ahmed., Bayan, Mohammed, Mahdi, Almubarak., Amjed, Ạbbawe, Salih. The effect of oxidative stress on the kidneys. GSC biological and pharmaceutical sciences, (2024).;28(2):215-219. doi: 10.30574/gscbps.2024.28.2.0305

[21] Qays Assi A, Dlawer Abdulhammed Ahmad A, Khalid Bahram A. DOXORUBICIN'S ROLE IN CANCER TREATMENT AND ITS SIDE EFFECTS ON HUMAN HEALTH. Academicia Globe: Inderscience Research. 2022;3(11):165-83doi: 10.17605/OSF.IO/EC5AS.

[22] Nazarewicz RR, Zenebe WJ, Parihar A, Larson SK, Alidema E, Choi J, Ghafourifar P. Tamoxifen induces oxidative stress and mitochondrial apoptosis via stimulating mitochondrial nitric oxide synthase. Cancer research. 2007 Feb 1;67(3):1282-90.

[23] Albukhari AA, Gashlan HM, El-Beshbishy HA, Nagy AA, Abdel-Naim AB. Caffeic acid phenethyl ester protects against tamoxifen-induced hepatotoxicity in rats. Food and Chemical Toxicology. 2009 Jul 1;47(7):1689-95.

[24] A'laa HJ, Hussein AL. Correlation Between Lipid Profile and Liver Function in Patients With Non-Alcoholic Fatty Liver. Diyala Journal of Medicine. 2024 Dec 25;27(2):29-39.

[25] Abid-Essefi S, Ouanes Z, Hassen W, Baudrimont I, Creppy E, Bacha H. Cytotoxicity, inhibition of DNA and protein syntheses and oxidative damage in cultured cells exposed to zearalenone. Toxicology in vitro. 2004 Aug 1;18(4):467-74.

[26] Adeyemi DO, Ukwenya VO, Obuotor EM, Adewole SO. Anti-hepatotoxic activities of Hibiscus sabdariffa L. in animal model of streptozotocin diabetes-induced liver damage. BMC complementary and alternative medicine. 2014 Dec;14:1-1..

[27] Liu LC, Wang CJ, Lee CC, Su SC, Chen HL, Hsu JD, Lee HJ. Aqueous extract of Hibiscus sabdariffa L. decelerates acetaminophen-induced acute liver damage by reducing cell death and oxidative stress in mouse experimental models. Journal of the Science of Food and Agriculture. 2010 Jan 30;90(2):329-37.

[28] Li W, Shi X, Xu Y, Wan J, Wei S, Zhu R. Tamoxifen promotes apoptosis and inhibits invasion in estrogen‐positive breast cancer MCF‐7 cells. Molecular medicine reports. 2017 Jul 1;16(1):478-84.