



RESEARCH ARTICLE

Study of Role Flavonoid Compound Isolated from *Camellia Sinensis* (Black Tea) Extract against Methotrexate Drug Induced Liver Toxicity in Male Rate

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ARTICLE INFO	ABSTRACT
Received: May 22, 2023	<p>The objective of this study was to determine the antioxidant and protective effects of a flavonoid compound derived from <i>Camellia sinensis</i> (Black tea) extract against methotrexate drug-induced liver damage caused by lipid peroxidation (LPO), malondialdehyde (MDA), and MDA in male rats. This experiment was conducted in the postgraduate lab at the University of Kerbala from August to January 1, 2023. On August 11th, 2023, the <i>Camellia sinensis</i> (Black tea) plant was taken from Karbala, and methanol alcohol was used to extract the flavonoid component. leaves for black tea, Eight groups of rats were present. G1-the control group has only received saline solution (0,85%). 100 mg/kg B.W. of flavonoid and 15 mg/kg of MTX were injected into the G2. 15 mg/kg of MTX and 150 mg/kg B.W. of flavonoid are injected into G3. G4: MTX 500 mg/kg and flavonoid injection 200 mg/kg B.W. The G5 injection contains 15 mg/kg of MTXl and 100 mg/kg B.W. of black tea extract. G6-injection with 150 mg/kg B.W. black tea extract and 15 mg/kg MTX G7: 200 mg/kg B.W. of black tea extract and 15 mg/kg of MTX were provided intravenously; G8: 15 mg/kg of MTX was given intravenously. By measuring the effectiveness of the liver enzymes MDA, LPO, and GPX in the blood serum of male rats treated with flavonoids and Black tea leaf and comparing the result with rats treated with methotrexate (MTX), it was found that the concentration of MDA and LPO was significantly higher in G8 (injection with 15 mg/kg of MTX) than in G1 control. Hence, administering MTX with drinking water causes oxidative stress and an increase in ROS by raising MDA and LPO levels in liver tissue and lowering GPX.</p>
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INTRODUCTION

The plants known as medicinal plants are those that are used to treat illnesses and discomfort because they are rich in certain compounds. High active ingredients determine physiological efficacy and tanning, excluding higher-containing plants. Pilot oils, which are added to medicinal fibers to increase their strength and flavor, include peppermint, linseed, cumin, and lemongrass oils. [1]

Camellia sinensis Black tea and peppermint were used for centuries as a traditional drinking tea in the worldwide. Currently, biologically active compounds from peppermint and black tea sources

always have been a great interest for scientists working on infectious diseases [2] flavonoids present in *Camellia sinensis*. *Camellia sinensis* as it includes portions of "flavonoids" which is a powerful anti-oxidants. Divided flavonoids into several types depending on their chemical composition which include Anthocyanides responsible for the pigments of red and blue fruits and flowers, Catechines which is concentrated in the subject of tea, Flavonone, and Glycoside flavonones also found in lemon, orange and honey [3].

Oxidative stress causes damage to cellular components and biological and physiological processes within cells through its effect on major biomolecules such as proteins, fats and carbohydrates, and slowing down the action of enzymes, affecting the properties of membranes, and then cell death [4]. Antioxidant : any substance that reduces or reduces the damage caused by Reactive oxygen species (ROS), which are a number of molecules that are derived from molecular oxygen, such as O_2^- , H_2O_2 , OH^- [5] Antioxidants are classified into antioxidants Super Enzymatic Antioxidants include enzyme oxidation and other enzymes that trigger CAT, catalase and SOD, oxide dismutase into non-interacting molecules while ROS plays an important role in converting The second type of antioxidant is a non-enzymatic antioxidant A, C, E and Menia Vitamins Antioxidants Non-Enzymatic albumin, chmotathione, zinc, copper, and other antigens Which plays an important role in addressing the damage of free radicals inside the body The research aims to identify the critical changes in the levels of Some non-enzymatic antioxidants in the serum of infected women Breast cancer compared to women with toxic disease [6]

Methotrexate (MTX) , the rival of folic acid, is one of the remedies for tumor treatment. It is worn out in handling some evil-minded diseases of the immune system. Noxious properties are imperative on loads of organs like the bone marrow, liver, and kidney. 90% of MTX or extra than it is vacant through the urinary system. Hence, the hurt to the kidney caused by MTX holdup itis ending. Ensuing persistent and grand plasma expression results in a manifest augmentation of other MTX's toxicities [7]

MATERIAL AND METHOD

1- Plant collection :*Black tea* leave from Karbalaa city in hindia /Iraq in 11/11/2022, powdered grinded by using mechanical grinder [8].

2- *Black tea* extraction: For 24 hours, 80 grams of plant material were added to a soxhlet apparatus in a thimble with 200 milliliters of (75%) methanol in a 1000-milliliter flask. The extract was then evaporated at 45°C using a rotary evaporation device. [9,10]

3 - Separation flavonoid compounds: Twenty gram from dried methanol extract was placed in a reflex apparatus for 8 hr. using 400 ml of 2M HCL . The filtrate extract was cooled and transferred to a separator funnel , the glycol moiety was extracted by 100 ml ethyl acetate . The collected ethyl acetate layers were washed with distilled water to eliminate the excess acid then evaporated to dryness by a rotary evaporator at 40°C for 20 minute [11]

4- Phytochemical reagent study

4-1: Phenols

Test for lead acetate, the production of precipitate was seen after adding 0.5 ml of 1% lead acetate solution to 5 mg of menthe extract..

4-2 : Glycosides

After dissolving 0.5 mg of menthe extract in 1 ml of creation of the color yellow. Water, aqueous NaOH solution was added

4-3 :Tannins

Added 5 ml of distilled water (D.W) to 5 ml of extract , the mixture heated at 80-100 °C for 10 minutes in water bath apparatus , after being filtered , added 5 drops of 1 % ferric chloride , the dark green color.

4-4 : Alkaloids:

The gathered filtrate was mixed with Wagner reagent, which is a solution of potassium iodide and iodine. reddish-brown precipitate formation.

4-5: Flavonoids:

4mL of extracts were combined with 1.5ml of 50% methanol. After mixing, 5–6 drops of concentrated HCl were added, and the mixture was heated with magnesium metal till red color was created. Flavonoids are present when a color is red [11].

5-Experiment Design: 40 adult male rats, weight (180-290) gm, age (7-10) weeks, were used in this study and isolated in a relatively controlled environment at temperature 25°C in an animal house , in the University of Karbala . They were given food. Rats were divided into eight groups.

G1-the control group has given normal saline (0,85%) only.

G2- injection 100mg/ kg B.W of flavonoid with 15 mg/kg of MTX.

G3- injection 150mg/ kg B.W of flavonoid with 15 mg/kg of MTX.

G4: injection 200 mg/ kg B.W of flavonoid with 500 mg/kg of MTX.

G5- injection 100 mg/kg B.W of black tea extract with 15 mg/kg of MTXl .

G6- injection 150 mg/kg B.W black tea extract with 15 mg/kg of MTXl.

G7 : injection 200 mg/kg B.W black tea extract with15 mg/kg of MTX

G8: injection with 15 mg/kg of MTX.

6: Oxidative and antioxidant assay

6:1: Malondialdehyde (MDA)

Malondialdehyde (MDA) , Glutathione peroxidase (GPX) and Lipid Peroxidation (LPO) analysis by using kit from chin Bioassay Technology Laboratory (BT LAB)

7- Statistical Analysis:

Mean was used to express the data. One-way analysis of variances was used to assess the statistical significance of differences between the control and other groups (ANOVA). The SPSS for Windows version was used for statistical analysis, and P values of 0.05 or less were considered significant (SPSS, Inc., Chicago, Illinois).

RESULTS

Black tea leave extract contains a wide active components are natural product and formation and accumulation in plants, The results of table (1) showed that the black tea leave extract and flavonoid yield percentage gave (4% and 2%) respectively .

Table 1: Percentage of volatile oil yield that was extracted

Extract	Percentage yield of extracted
black tea leave	$4/100g * 100 = 4\%$

flavonoid	$2/100g * 100 = 2\%$
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2- Secondary metabolism screen

The Secondary Metabolic Screen Study was conducted to determine the black tea leave active components. in table (2) Flavonoid , Alkaloid, Phenol, Tannins and Glycoside showed positive results on the phytochemical screen.

Table 2: Shows black tea secondary metabolism.

Bioactive Compounds	Results
Flavonoid	+
Phenol	+
Tannins	+
Alkaloid	+
Glycoside	+

Table 3: Male Rat Groups Treated with the Coefficients Level MDA , LPO and GPX Concentration mol/L:

Groups	MDA	LPO	GPX
G 1	0.685±0.018	6.98±0.24	66.98±2.7
G2	0.587±0.020	5.89±0.33	55.45±3.87
G3	0.543±0.024	5.34±0.34	57.98±3.98
G4	0.522±0.027	5.22±0.34	59.98±3.99
G 5	0.677±0.28	6.46±0.35	50.76±3.42
G6	0.653±0.29	6.23±0.36	51.98±3.43
G7	0.622±0.029	6.01±0.37	52.98±3.34
G8	0.765±0.022	7.87±0.25	45.87±4.87
L.S.D	0.089	0.56	6.25

In the present study table (3) , the MDA level of groups G8, rats showed a significant increase (**0.765**µmol/ L) compared with control **6.85**µmol while G2,G3,G4 G5,G6 and G7 showed a significant decrease (**0.587 0.543, 0.522 , 0.677, 0.653 and 0.622**µmol/ L) respectively compared with G8 **0.765**µmol/ L.

the LPO level of groups G8 showed a significant increase (**7.87**µmol/ L) compared with control **6.98** µmol while G2,G3,G4 G5,G6 and G7 showed a significant decrease (**5.89 , 0.534, 0.522 , 6.46, 6.23and 6.01**µmol/ L) respectively compared with G8 **0. 7.87**µmol/ L.

the GPX level of groups G8 showed a significant decrease (45.87µmol/ L) compared with control 66.98µmol while G2,G3,G4 G5,G6 and G7 showed a significant increase (55.45, 57.98, 59.98, 50.76, 51.98 and 52.98µmol/ L) respectively compared with G8 45.87µmol/ L.

DISCUSSION

The current study sought to determine how black tea extract and flavonoid compounds affected liver enzymes' resistance to toxicity and ROS brought on by MTX. The results reported in table 3 showed that, when compared to G1 control, the MDA and LPO concentrations in G8 (injection with 15 mg/kg of MTX) were significantly higher. Accordingly, administering MTX with drinking water causes oxidative stress and an increase in ROS by raising MDA and LPO levels in liver tissue and lowering GPX levels. These outcomes were in line with prior research. [12, 13] The resulting ROS may cause cellular damage through the peroxidation of membrane lipids, inactivation of the sulfhydryl enzyme, protein cross-linking, and DNA synthesis. [14] It has been established that MTX therapy decreases the efficiency of antioxidant defense mechanisms and lowers glutathione levels in cells. 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. [15] It has been established that MTX therapy decreases the efficiency of antioxidant defense mechanisms and lowers glutathione levels in cells. 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 [16]

Lipid peroxidation and mitochondrial dysfunction can both harm cells over time and harm their membranes. As a result, the cell will get damaged and lose its integrity, allowing the contents of the liver cell—particularly ALT and LDH—to leak out and enter the bloodstream. [17] Moreover, ROS-induced neutrophil activity will intensify the cell damage.[18]

About the medication (G4: injection of 500 mg/kg of MTX and 200 mg/kg B.W. of flavonoid extracts. Furthermore, when compared to G8 (injection with 15 mg/kg of MTX), (G7 injection 200 mg/kg B.W tea extract with 15 mg/kg of MTX) it led to a significant decrease in the concentration of MDA, LPO, and a significant increase in GPX the blood serum rats because flavonoids have the capacity to scavenge electrons of free radicals for this reason is considered antioxidant. [19] have an anti-inflammatory action at intracellular or extracellular antioxidants, inhibiting xanthine oxidase activity that converts xanthine oxidase product to xanthine dehydrogenase [20] The quercetin prevents lipid peroxidation, lowers levels of cardiac markers like aspartate aminotransferase (AST), creatine kinase (CK), and lactate dehydrogenase, and increases the production of glutathione - peroxidase (GSH-PX), superoxide dismutase (SOD), glutathione reductase (GR), and catalase (CAT) (LDH) [21] . Increased resistance or the activation of the enzyme glutamyl cysteine synthesis (GCS) could be the causes of the flavonoid's large increase in GSH levels. This substance or glutathione production may activate glutamyl trans peptidase. [22] Hydrogen peroxide eventually turns into H₂O under the action of GSH [23] Because the antioxidant enzyme GSH (glutathione peroxidase) removes super oxygen free radicals, super oxide, and hydrogen peroxide and can also lessen or prevent the generation of free radicals, [24] Compare with study [25] show Malondialdehyde (MDA) levels in tired animals are reduced by oral rutin (15.30,60 mg/ kg) B.W. for 7 days, which also boosts SOD and GPx activity. It has been discovered that plant extracts of various kinds can remove hazardous chemical substances and shield

liver cells from harm caused by their treatment. [26] demonstrated by decreasing the activity of the liver enzymes GOT and GPT in serum, the flavonoids derived from the plant licorice had a protective effect against the toxic effects and destructive to liver cells generated by a chemical molecule carbon tetrachloride (CCl₄). As noted [27] that care for mice exposed to a real estate development's effects. When compared to mice who were not treated, the levels of the enzymes GOT, GPT, and ALP in the liver cells of heterogeneous mice treated with methotrexate (MTX) and cyclophosphamide (CP) separately in various concentrations of extracts of the seeds of *Nigella sativa* water and alcohol were maintained. Treatment of male rats with parsley extract lowers the levels of (MDA and LPO) and increases Glutathione [28]

CONCLUSION

- 1- Flavonoid compound have high antioxidant
- 2- Methotrexate (MTX) overdose leads to hepatotoxicity in male rats.

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