RESEARCH ARTICLE

Editorial Process: Submission:04/29/2024 Acceptance:08/11/2024

*In vivo E*evaluation of Antioxidant Activity of Chamomile Extract against Procyclidine-Induced Oxidative Stress: Potential Application in Cancer Prevention

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Abstract

The study aimed to investigate the effect of the aqueous extract of the chamomile plant on oxidative stress induced by procyclidine in rats. 30 rats were randomly divided into five groups, with 6 rats in each group. The first group was given distilled water only, while the second group was administered procyclidine (1 mg/kg body weight) in three doses daily for a period of 60 days. The third group was given procyclidine in the same doses as the second group for 30 days. Afterward, they were administered an aqueous extract of chamomile (300 mg/kg) for another 30 days. The fourth group was administered the aqueous extract (300 mg/kg) for 30 days. Subsequently, they were given procyclidine in the same doses as the second group for another 30 days. On the other hand, the fifth group was administered the aqueous extract of chamomile (300 mg/kg) for a period of 60 days to investigate the potential effects of the extract. Afterward, blood samples were drawn to measure various biological parameters, including Total Oxidant Status (TOS), Malondialdehyde (MDA), Aspartate Aminotransferase (AST), Alanine Transaminase (ALT), and Acetylcholinesterase (AChE) activity. Finally, an anatomical study was conducted on the kidneys, brain, and liver to enhance the research. The results displayed a significant increase in the levels of TOS, MDA, AST, ALT enzymes, and Ach-E activity in the second group compared to the first group. Groups 3 and 4 significantly decreased compared to the second group based on the same standards. In regard to Group 5, there are no significant moral differences between it and Group 1. Finally, this study demonstrated the importance of using chamomile extract as an antioxidant and its potential in cancer prevention against the oxidative stress induced by excessive doses of procyclidine. ($p \leq 0.005$).

Keywords: Chamomile- cancer- anti-oxidant- oxidative stress- procyclidine

Asian Pac J Cancer Prev, **25 (8)**, 2919-2928

Introduction

Medicinal plants have been critical sources of therapeutically active chemicals for many years. The medicinal and pharmacological benefits of plant-derived compounds are supported by evidence-based research, and there is a growing interest in the identification and characterization of bioactive molecules obtained from natural sources [1]. Chamomile is one of the most significant and well-known medicinal plants [2, 3]. The forename of this plant comes from the Greek words CHAMOS (‘ground’) and MELOS (‘apple’). These words refer to the slow growth and apple-like odor of new chamomile flowers [4]. Traditional uses of M. chamomile include the treatment of a variety of disorders, including gastrointestinal disorders, widespread colds, liver abnormalities, psychological and neurological problems, and respiratory problems. Chamomile is also usually used to treat aches, infections, and eye, skin, and mouth illnesses (Figure 1) [5].

Cancer cells exhibit an elevated production of ROS and a modified redox environment in comparison to normal cells. Additionally, redox regulation and signaling play a crucial role in tumorigenesis and cancer treatment response [6, 7]. The dual role of ROS in tumorigenesis has significant implications for the development of potential anticancer therapies that target cellular redox levels. ROS are implicated in both the initiation and progression of tumors by promoting DNA mutations, genomic instability, and abnormal pro-tumorigenic signaling. Conversely, heightened ROS levels can also be cytotoxic to cancer cells and potentially induce cell death [8]. To counteract oxidative stress, cancer cells enhance their antioxidant capacity, suggesting that elevated ROS levels can potentially impede tumorigenesis [9, 10].

The prevention of oxidative stress-related diseases is primarily attributed to the use of natural antioxidants, as synthetic antioxidants are often associated with carcinogenic or mutagenic effects and high toxicity [11, 12]. Consequently, there is a growing global interest in replacing artificial antioxidants with safe and potent natural antioxidant compounds that act through various

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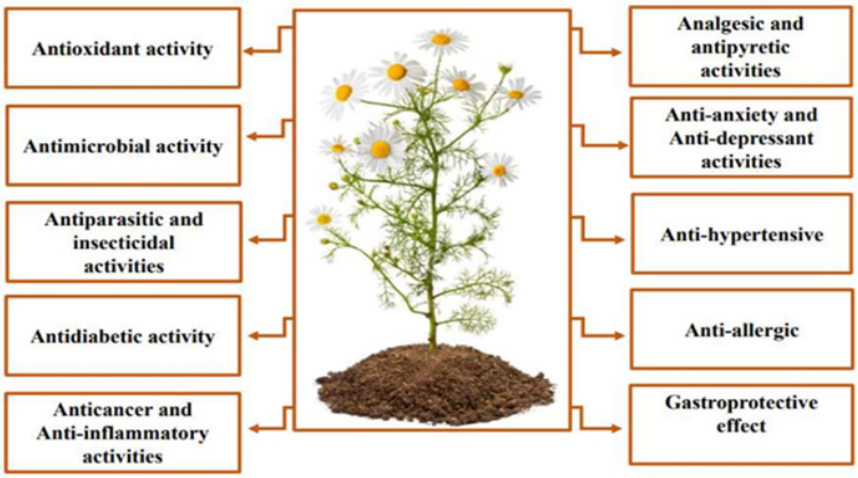

Figure 1. Biological Characteristic of Matricaria Chamomile

mechanisms, including preventing hydrogen abstraction and radical scavenging, chelating transition metal ion catalysts, and inhibiting chain initiation [13].

Procyclidine hydrochloride is an antispasmodic medication utilized in the treatment of parkinsonism and extrapyramidal functional disorders. An overdose of procyclidine can lead to anticholinergic effects, including confusion, restlessness, insomnia, dilated pupils, and hallucinations. Procyclidine overdose can lead to drug-like addiction. Addictive drugs can penetrate the blood-brain barrier, causing oxidative stress and damage to the brain and peripheral nervous system [14]. Free radicals play a role in various health issues, such as inflammation, cataracts, cancer, heart disease, and aging. Oxidative stress occurs when there is an imbalance between the formation of reactive oxygen species and the antioxidant capacity of the system [15]. Oxidative stress promotes peroxidation of membrane lipids, leading to alterations in the biological properties of the membrane and weakening normal cellular function. Lipid peroxidation produces a variety of relatively stable end products, mainly α and β-unsaturated reactive aldehydes, such as malondialdehyde (MDA) and isoprostanes (IsoPs) [16, 10]. Lipid peroxidation is a process involving the abstraction of a proton from a side chain of a fatty acid to produce a carbon-centered radical. This radical can then react with the fatty acid, leading to the formation of a lipid peroxidation product and another carbon-centered radical. This new radical can further react, resulting in a chain reaction [17].

AST is present in the cytoplasm and mitochondria of every cell, with higher concentrations in the liver, heart, and skeletal muscles than in blood serum. The presence of damaged or dead hepatocytes indicates protein breakdown in the body and the existence of destroyed cells, serving as a reliable predictor of chronic liver disease [18, 19]. The coenzyme pyridoxal phosphate is required in this reaction. Hepatic cell ALT activity is approximately 3000 times higher than serum ALT activity. When the liver is injured, alanine aminotransferase (ALT) is released from the damaged cells, leading to a significant rise in serum ALT activity. ALT can also be found in muscles, adipose tissues, the intestines, and the brain. However, the concentration of ALT in these organs is much lower than in the liver [20].

Acetylcholinesterase (AChE 3.1.1.7) is a carboxylesterase enzyme that is secreted from muscle and is located largely at postsynaptic neuromuscular junctions, especially in muscles and nerves. It rapidly hydrolyzes the neurotransmitter acetylcholine (ACh), degrading it into acetic acid and choline. The primary function of AChE is to restrict the dispersal of Ach and the activation of adjacent receptors, thereby halting neuronal transmission and synaptic communication. It is also involved in brain development. Inhibiting AChE reduces the breakdown of ACh, leading to the accumulation of ACh. This accumulating ACh stimulates muscarinic and nicotinic receptors, offering therapeutic relief for memory problems in brain disorders [21]. AChE inhibition potentially increases levels of ACh, a neurotransmitter released by cholinergic neurons, and has a positive effect on cognition, learning, memory, and the suppression of inflammation [22]. In this study, we aimed to investigate the effect of the aqueous extract of the chamomile plant on oxidative stress induced by procyclidine in rats.

Materials and Methods

Experimental Design

This study was conducted at the animal facility of the Department of Biological Sciences, College of Science, University of Al-Qadisiyah. Thirty mature male Sprague-Dawley rats, weighing between 160 g and (8-9) weeks of age, were randomly divided into five groups. Every group consists of 6 male rats. Animals in all groups were administered as follows:

Group 1 (control, negative): The group received normal feeding and was orally administered distilled water.

Group 2 (control positive): It's administrated orally with distilled water containing procyclidine at a dose of 1mg per kg of body weight three times a day for 60 days.

Group 3 was administered orally with distilled water containing (1 mg/kg B.W) of procyclidine three times a day, using an oral drencher for 30 days. Subsequently, they received (300 mg/kg) of chamomile aqueous extract.

Group 4 received 300mg/kg of chamomile aqueous

extract for 30 days, followed by oral administration of procyclidine at a dose of 1mg/kg body weight three times a day, using an oral drencher for 30 days.

Group 5 received only 300 mg/kg of chamomile aqueous extract for 60 days.

Preparation of chamomile extract

Dried chamomile powder was dissolved in boiling water to produce a chamomile aqueous extract (20 g/200 ml). Afterward, a rotary evaporator was used to concentrate the solution by heating it to 40°C under vacuum conditions, and the prepared extract was stored in a freezer [23].

Preparation of samples for biochemical analysis

After the processing was completed, the rats were fasted for 12 hours. Subsequently, the animals were sacrificed, and blood samples were collected from their hearts. The serum was separated by centrifugation at 3000 rpm for 10 minutes at 4 °C and then stored at -70 °C until tests were conducted. The sera were used to measure total antioxidant capacity (TOS), malondialdehyde (MDA), liver enzymes such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT), and the brain enzyme acetylcholinesterase activity (Ach-E). A part of the liver, kidney, and brain tissue was taken for histopathology studies.

Determination of TOS, MDA, ALT, AST, and AChE levels in the blood of rats

Total oxidant status was measured spectrophotometrically at 560 nm using the colorimetric method developed by Erel (2005) [24]. The most frequently used MDA analytical procedures are based on its interaction with thiobarbituric acid (TBA), which results in the formation of a pink-colored MDA-TBA complex. The intensity of its absorption is measured at a wavelength of 540 nm [25]. Furthermore, ALT, AST, and AChE levels in the blood of rats were evaluated using the Agappe and Elabsience spectrophotometric kits.

Histological Study

Liver, kidney, and brain tissues were sliced, preserved in formalin, dehydrated in alcohol, and encased in paraffin wax. Thin sections were cut from each paraffin block, stained with eosin and hematoxylin, and examined under a light microscope for histopathological analysis [26].

Statistical analysis

The results were statistically analyzed using Graph Pad Prism 8.4 software. Data are presented as mean ± standard deviation of three independent experiments. Statistical comparison between groups was done using the ANOVA test with a significance level of $p<0.05$.

Results

Effect of aqueous chamomile extract on total oxidant level

From Figure 2, it is clear that the level of total oxidants (TOS) in the serum of Group 2 (control positive) was significantly increased compared to Group 1 (control negative) ($p \leq 0.05$). Additionally, in Group 3 and 4 (treated with extraction), the TOS level was significantly decreased compared to Group 2 ($p \leq 0.05$). Moreover, the TOS level in the serum of rats treated with the extract only in Group 5 was significantly decreased compared to Group 1.

Effect of aqueous chamomile extract on serum MDA level

Excessive doses of procyclidine induced a significant ($p \leq 0.05$) increase in the concentration of MDA in group 2 compared to group 1 (Figure 3). The therapeutic and preventive effects of the aqueous extract of the chamomile plant are clearly evident in groups 3 and 4. It is observed that there is a significant decrease in the concentration of MDA in these two groups ($p \leq 0.05$) compared to group 2.

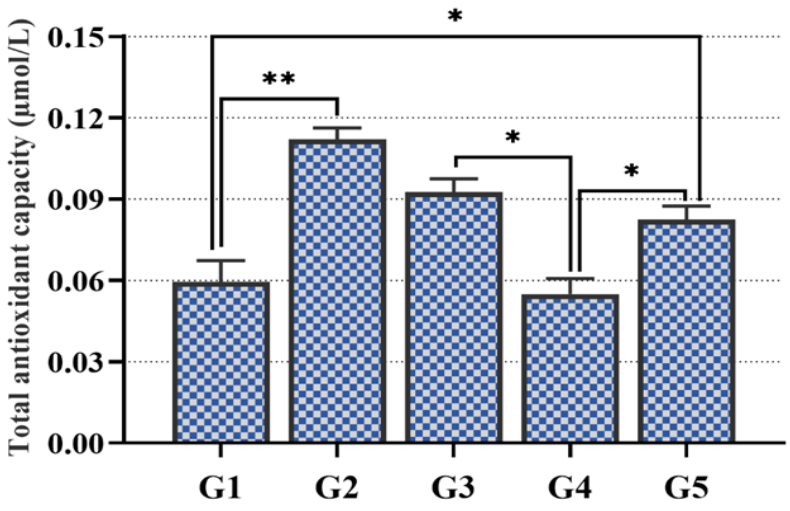

Figure 2. The Effect of Aqueous Chamomile Extract on Total Oxidants Levels. The concentration of total oxidant (TOS) in the serum of group 2 (positive control) showed a significant increase compared to group 1 (negative control). In addition, in groups 3 and 4 (prescribed with the extract), there was a significant decrease in TOS levels compared to group 2. Notably, the TOS concentration in the serum of rats treated only with group 5 extraction showed a significant decrease compared to group 1. ($p \leq 0.05$)

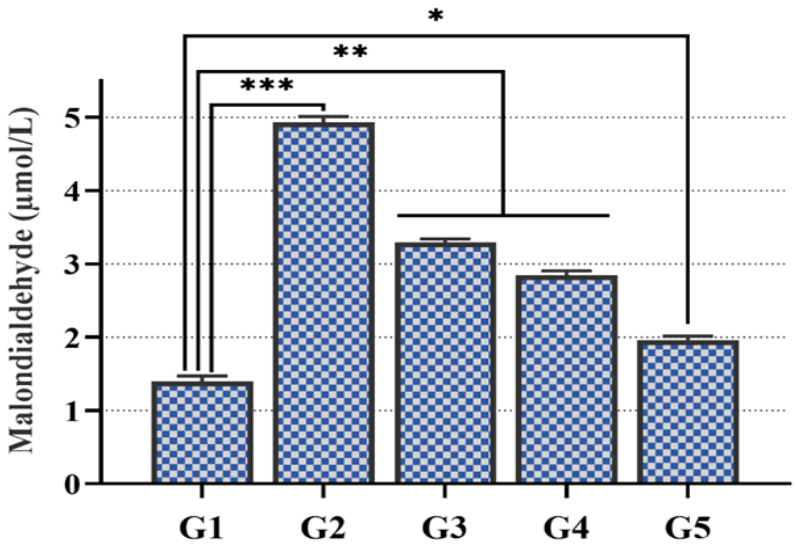

Figure 3. The Effect of Aqueous Chamomile Extract on Serum MDA Level. Elevated levels of procyclidine resulted in a notable ($p \leq 0.05$) rise in the MDA concentration in group 2 when compared to group 1. The therapeutic and prophylactic benefits of the aqueous extract derived from the chamomile plant are notably observable in groups 3 and 4.

In the case of the last group (Group 5) there is a slight yet significant difference between it and group 1.

Effect of aqueous extract of chamomile on the levels of AST and ALT enzymes in the serum of male rats

The current study results showed that oral administration of a higher dose of procyclidine resulted in a significant increase in the levels of liver enzymes in the second group ($p \leq 0.05$) compared to the first group. In the third and fourth groups, there was a noticeable and significant decrease in the levels of these two enzymes compared to the second group. It is also noted that there was no significant difference between the fifth group and the first group (Figure 4).

Effect of aqueous extract of the chamomile on the Acetyl Cholinesterase Activity in the serum of rats

It is clear from Figure 5 that there is a significant increase ($p \leq 0.05$) in the activity of the enzyme acetylcholinesterase in the second group, which was treated with the drug procyclidine, compared to the control group 1. It is also noted that there is a significant decrease ($p \leq 0.05$) in the effectiveness of this enzyme in the third and fourth groups that were dosed with chamomile extract. It becomes clear that the extraction inhibited the activity of the enzyme acetylcholinesterase when compared to

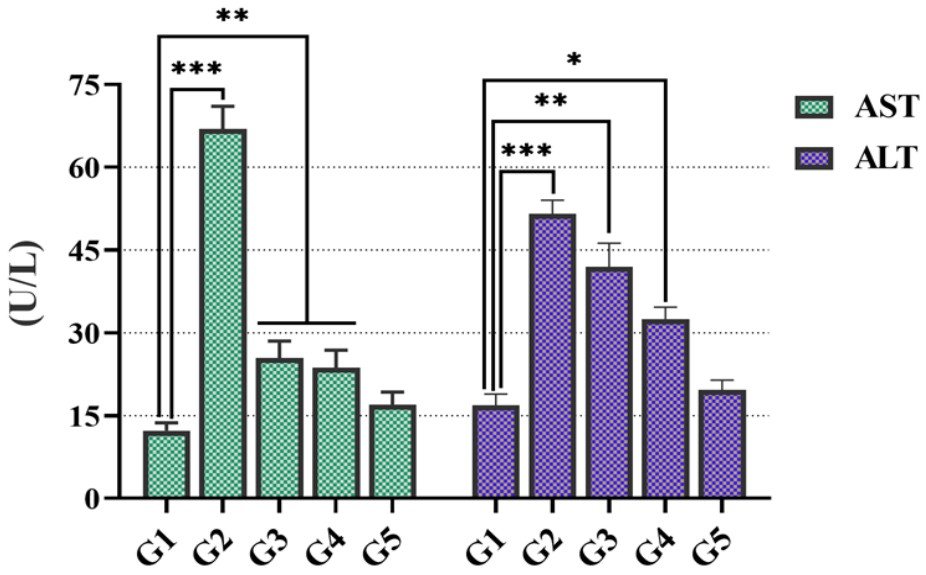

Figure 4. The Effect of Aqueous Extract of Chamomile on AST and ALT Levels of the Serum. The higher dosage of procyclidine led to a notable elevation in liver enzyme levels in the group 2 in comparison to the group 1. Conversely, the groups 4 and 5 exhibited a significant reduction in the levels of these enzymes when compared to the group 2. Furthermore, there was no significant difference between the group 5 and the group 1.

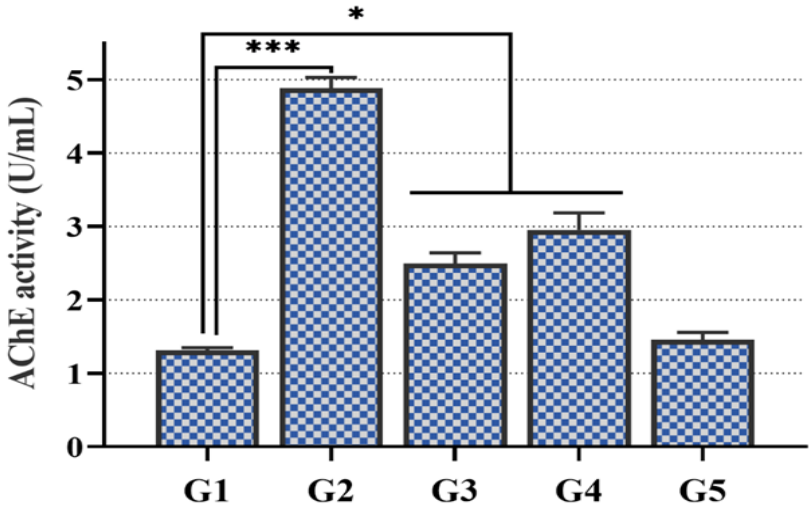

Figure 5. The Effect of Aqueous Extract of Chamomile on AChE Activity. The extract demonstrated a suppressive effect on the enzymatic activity of acetylcholinesterase in comparison to the group 2. Conversely, no substantial differences were observed between the group 5 and the group 1.

the second group. As for the fifth group, no significant differences were noted between it and the first group.

Histological finding

The histological results of the kidney tissue from the control group and the group treated with procyclidine (three doses (1 mg/kg body weight) daily for a period of 60 days) are presented in Figure 6. We found the presence of dilation in Bowman's capsule, glomerular atrophy, and the accumulation of inflammatory cells in the procyclidine-treated group. We also noticed the presence of bleeding and blood congestion (black arrow) also causes expansion

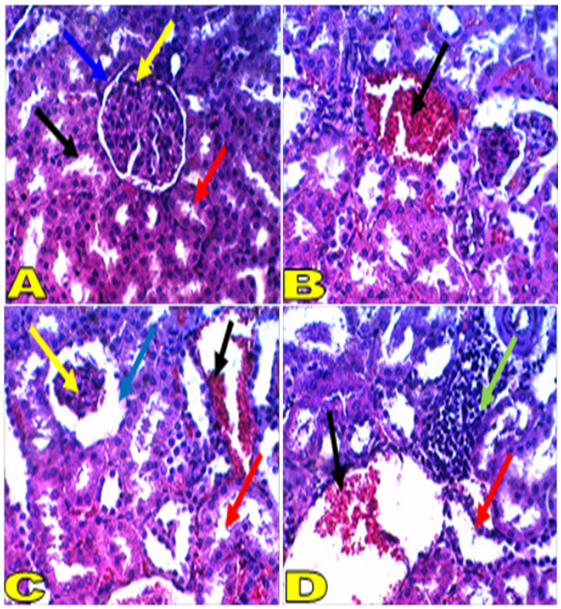

Figure 6. Picture (A) shows a cross-section of the kidney tissue of the control group, highlighting the proximal urinary tubules (red arrow), the distal urinary tubules (black arrow), the glomerulus (yellow arrow), and Bowman's capsule (blue arrow). As for the image (B, C and D), a transverse section of the kidney soft tissue of an individual from the group treated with procyclidine, we noticed the presence of dilation in Bowman's capsule (blue arrow), atrophy in the glomerulus (yellow arrow), and the accumulation of inflammatory cells (green arrow).

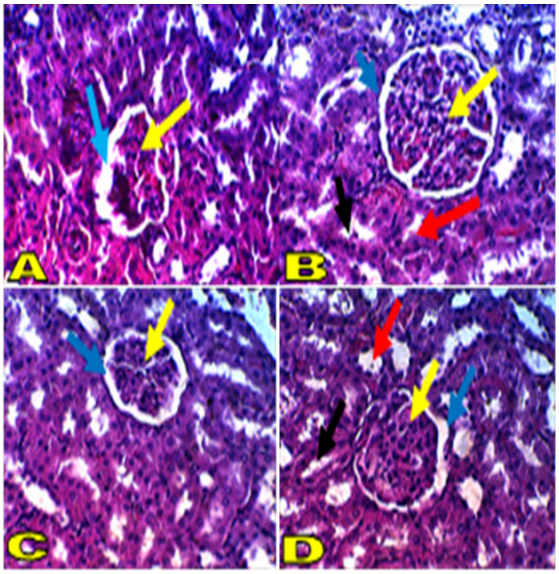

Figure 7. (A) A cross-section of the kidney tissue from the group treated with procyclidine. We noted the presence of atrophy in the glomerulus (yellow arrow) and dilatation of Bowman's capsule (blue arrow). (B) A cross-section of the kidney tissue from the group treated with chamomile extract, in which we noticed the normal proximal urinary tubules (red arrow), distal urinary tubules (black arrow), and the glomerulus (yellow arrow), and Bowman's capsule (blue arrow). (C) A cross-segment of kidney tissue from a group treated with chamomile extract and then with procyclidine, we noticed a slight dilatation of Bowman's capsule (blue arrow) and slight atrophy in the glomerulus (yellow arrow). As for the picture (D), a cross-section of kidney tissue from the group treated with procyclidine and then with chamomile plant extract, we noticed the proximal urinary tubules (red arrow) and the urinary tubules. The distal (black arrow) and glomerulus (yellow arrow), and Bowman's capsule (blue arrow).

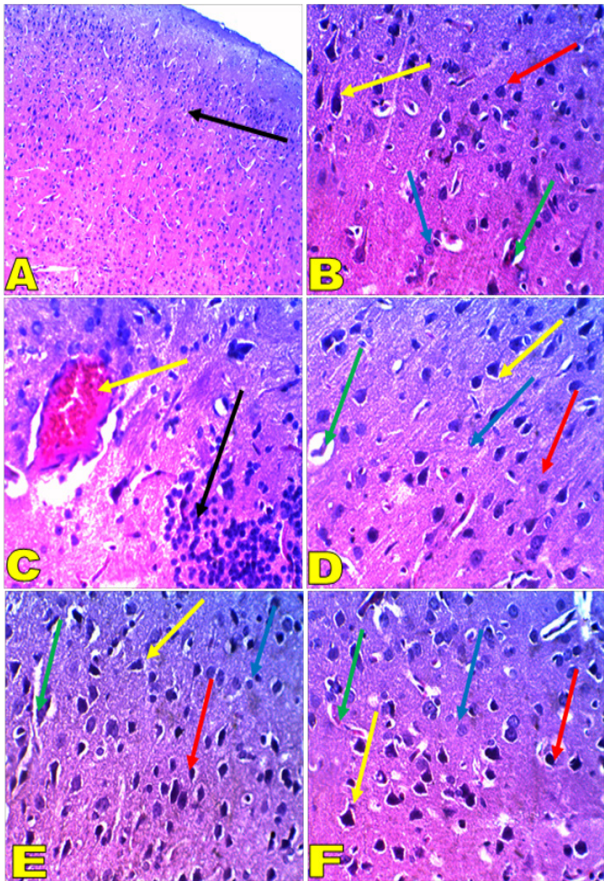

Figure 8. Images A and B show a cross-segment of brain tissue from the control group, we observed the cerebral cortex (black arrow), a blood vessel (green arrow), large pyramidal neurons (yellow arrow), astroglial cells (blue arrow), neurons, and small pyramids (red arrow). Image (C) shows a cross-section of brain tissue from the treated group with the procyclidine, we noticed blood congestion (yellow arrow) and the accumulation of inflammatory cells (black arrow). While the image (D) shows the cross-section of brain tissue from the group treated with chamomile extract, we observed large pyramidal neurons (yellow arrow), astroglial cells (blue arrow), small pyramidal neurons (red arrow), and a blood vessel (green arrow). Image (E) shows a cross-segment of the brain tissue from the group treated with chamomile extract and then with procyclodin. We found that the brain tissue is normal, with large pyramidal neurons (yellow arrow), astroglial cells (blue arrow), small pyramidal neurons (red arrow), and a blood vessel (green arrow). In image (F), the cross-section of the brain tissue from the group treated with procyclidine and chamomile extract shows large pyramidal neurons (yellow arrow), astroglial cells (blue arrow), small pyramidal neurons (red arrow), and a vessel hematoma (green arrow) (H&E 10X, 40X).

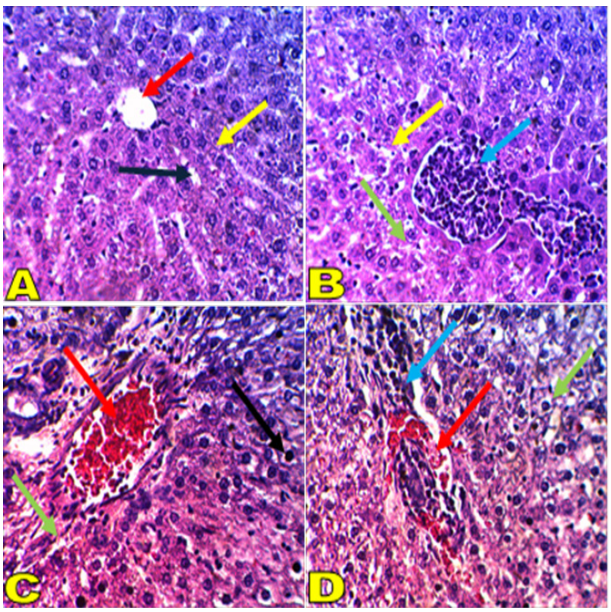

Figure 9. (A) shows a cross-section of the liver soft tissue stripped from the control group, we noticed the central vein (red arrow), the blood sinusoids (black arrow), and the hexagonal-shaped hepatocytes are connected in bands around the central vein (yellow arrow). As for the images (B, C, and D), the cross-section of liver tissue from the treated group with procyclidine showed the presence of hepatocyte necrosis (green arrow) and the accumulation of inflammatory cells (blue arrow). We also noticed the presence of bleeding and blood congestion (red arrow), as well as the thickening of some nuclei cells (black arrow).

and collapse of some urinary tubules (red arrow).

Figure 7 indicates the effect of chamomile extract on the kidney tissues which were treated with procyclidine. In the group treated with procyclidine, we observed atrophy in the glomerulus and dilatation of Bowman's capsule. However, in the group treated with chamomile, we found normal proximal and distal urinary tubules, as well as a normal glomerulus and Bowman's capsule. In the group treated with chamomile extract followed by procyclidine, we observed a slight dilatation of Bowman's capsule and slight atrophy in the glomerulus. As for the group treated with procyclidine followed by chamomile plant extract, we observed the proximal urinary tubules, distal urinary tubules, glomerulus, and Bowman's capsule.

The histological results of the cross-segment of the brain tissue from the control, procyclidine treated, chamomile extract, chamomile extract and then with procyclidine, and procyclidine followed by chamomile groups are illustrated in Figure 8. We observed a potential protective effect of chamomile extract against procyclidine in the treated groups.

As shown in Figure 9, the historical results of the liver tissue of the procyclidine-treated group indicated necrosis of hepatocytes, accumulation of inflammatory cells, and bleeding with blood congestion.

Figure 10 illustrates the impact of chamomile extract on the liver tissue of the group treated with procyclidine. In the group treated with procyclidine, we observed necrosis in hepatocytes and a disruption of the cellular organization of the liver tissue. In the group treated with chamomile, we observed a normal central vein, blood sinusoids, and hexagonal-shaped hepatic cells connected in bands around the central vein. Also, in the group treated with chamomile extract followed by procyclidine, we observed slight irregularity in the central vein and a slight expansion of the sinusoids. In the group treated with procyclidine followed by chamomile plant extract, we observed the central vein, the blood sinusoids, and the hexagonal-shaped hepatocytes connected in the form of bands around

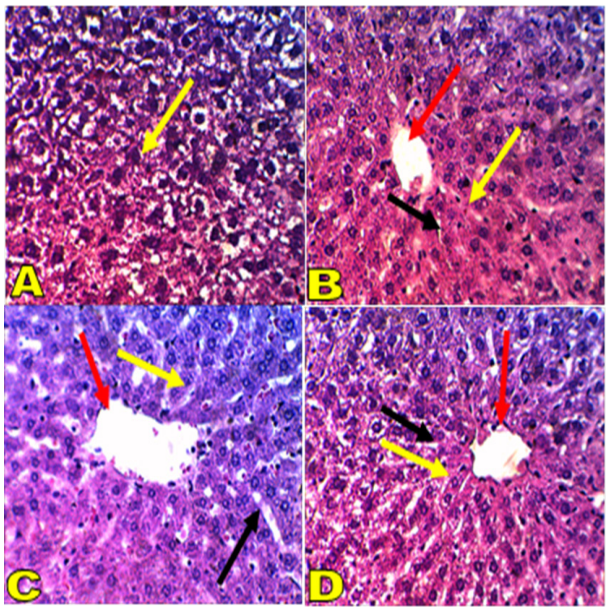

Figure 10. (A) shows a cross-section of liver tissue stripped from the group treated with procyclodin. We observed necrosis in hepatocytes and a disruption of the cellular organization of the liver tissue (black arrow). (B) shows a cross-section of liver tissue stripped from the group treated with chamomile plant extract, we noticed the central vein (red arrow), the blood sinusoids (black arrow), and the hexagonal-shaped hepatic cells connected in the form of bands around the central vein (yellow arrow). (C) shows a cross-section of liver tissue stripped from the group treated first with chamomile plant extract and then with procyclidine. We observed expansion, slight irregularity in the central vein (red arrow), slight expansion of the sinusoids (black arrow), and hexagonal-shaped hepatocytes connected in the form of bands around the central vein (yellow arrow). (D) shows a cross-section of liver tissue stripped from the group treated with procyclidine followed by the chamomile plant extract, we noticed the central vein (red arrow), the blood sinusoids (black arrow), and the hexagonal-shaped hepatocytes connected in the form of bands around the central vein (yellow arrow).

the central vein. This indicates the potential antioxidant effect of chamomile in protecting against procyclidine.

Discussion

In this study, we prepared aqueous chamomile extract via the water solvent. The current study, along with most previous studies, has shown this extraction process's low cost and high selectivity. It also has the advantage of being a safe, gentle, and non-harmful method for the environment. An appropriate amount of the product can be obtained, as the weight of the product was 5.3 grams, resulting in a yield of 26%.

The generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) by phagocytes in response to pathogen activity is a crucial aspect of many beneficial immune responses. These species include superoxide, nitric oxide, and peroxynitrite, which can cause harm to infected cells. There is convincing evidence linking drug-induced oxidative stress to toxicity in many organs, as well as its correlation with disease states. ROS affects important biological targets, such as proteins, lipids, and DNA macromolecules. ROS have the potential to cause molecular damage to these essential biological components. As a result, ROS can impact cell viability through kinase cascades. These elements might be crucial in the process of triggering cell death in response to oxidative damage [27, 28].

Most of the antioxidant effects of various plants are due to their polyphenolic properties. Phenolic compounds, which make them restorative agents, are considered among the most powerful flavonoids and phenolic acids [8]. Flavonoids are oxidized by free radicals to form flavonoid radicals that are more stable but less effective. This is due to its interaction with free radicals because of the presence of hydroxyl groups that donate an electron to the free radical to become more stable, as shown in the following reaction [29].

Flavonoid (OH) + R. → Flavonoid (O.) + RH

Oxidative stress ends with the phenoxyl radical bonding with another free radical

•R through the following reaction

Flavonoid (O.) + R. →FlavonoidO-RH

Flavonoid (O.) + Flavonoid (O.) →FlavonoidO – FlavonoidO

The enzyme xanthine oxidase is involved in causing oxidative damage at the cellular tissue level by producing high quantities of O2* ions. Flavonoids such as Silibin and Quercetin inhibit this enzyme, thus reducing its damage [30]. It was also found that luteolin is one of the most potent inhibitors of this enzyme. Rutin and Epicatechin are also considered to be among the most potent flavonoids in inhibiting the enzyme Xanthine oxidase. Many studies show that flavonoids can inhibit various enzymes that produce free radicals, such as lipoxygenase and cyclooxygenase [31].

Drugs can induce toxicity and oxidative stress in different organs of the body, such as the brain, heart, liver, and kidneys, particularly affecting the central nervous system (CNS). This impact is recognized as a key factor in the development of drug addiction, which is often viewed as a neurological and psychological disorder [32]. Increased levels of oxidants compared to antioxidant defense systems lead to the oxidation of proteins, phospholipids, and nucleic acids, resulting in dysfunction that ultimately leads to cell death [33]. MDA is a crucial indicator of the rise in free radicals and the initiation of the lipid peroxidation process, resulting in enhanced fluidity of the cell membrane. The hydroxyl radical is one of the radicals that contribute to the breakdown of cell membranes [34].

The decrease in the level of MDA in the groups treated with the aqueous extract of the chamomile plant may be due to the presence of flavonoids, particularly the

compound quercetin, which belongs to the flavonol group. Its antioxidant function depends on its chemical structure. The presence of the hydroxyl group in the OH-3 position of the C ring gives flavonoids a great ability to inhibit oxidation (46). The carbonyl group at the C position and the presence of the double bond between carbon 2 and 3 in the C ring play an important role in the antioxidant activity. The catechol group in the B ring also plays an important role in free radical scavenger activity. The hydroxyl radical scavenger activity also increases with an increase in the number of hydroxyl groups in the B ring [35, 36].

The current study results showed that oral administration of a higher dose of procyclidine resulted in a significant increase in the levels of liver enzymes in the second group ($p \leq 0.05$) compared to the first group. When procyclidine is metabolized, free radicals begin to form. These radicals are formed during the action of the oxygen molecule in cytochrome P450 of the endoplasmic reticulum. These free radicals are transported along with several essential biological substances such as fatty acids, lipids, nucleic acids, proteins, and amino acids. The balance between the production of active oxygen species and the antioxidant defense system is disrupted by oxidative stress. This disruption can impair cellular functions and, in some instances, lead to liver damage and necrosis [37]. The interaction of these free radicals alters the integrity and permeability of the cell membrane by oxidizing polyunsaturated fatty acids within the membranes. This causes the leakage of liver enzymes AST and ALT into the bloodstream, leading to an increase in their concentration in the blood [38].

The recent findings indicate that aqueous chamomile extract significantly decreased liver injury by lowering ALT and AST levels. This outcome aligns with a study by Mannaa et al. [39], which illustrated the hepatoprotective properties of chamomile flower extract against liver damage induced by azathioprine. Similar results were also observed in other studies [40].

The activity of chamomile tea has been proven to modulate hepatic cytochrome P450 activity. The therapeutic and preventive activities of chamomile extract can be explained by its content of biologically active compounds, such as phenolic compounds like flavonoids, coumarins, lignans, quinones, and tannins. These secondary compounds are known for their liver-protective, anti-tumor, and anti-inflammatory properties. Additionally, the presence of apigenin contributes to the extract's therapeutic and preventive effects against various types of liver cancers by inhibiting signals and inducing programmed cell death. Other phytochemicals, such as quercetin, rosmarinic acid, caffeic acid, and gallic acid, have been extensively researched in the medical literature for their protective effects on the liver against hepatotoxicity induced by chemicals in rats [41-43].

Procyclidine is considered one of the treatments used for diseases of the nervous system and Alzheimer's disease. It is also used to reduce the effects of oxidative stress. It is one of the acetylcholinesterase inhibitors. The harmful effects of these inhibitors have been reported, such as liver toxicity, digestive system disorders, and nausea [44], in addition to habituation and addiction. These adverse effects were observed in the second group. Those who were treated with this specific treatment showed an increase in oxidative stress and inflammation in the cells of the brain and liver (further explanation will be provided through histological study), along with elevated levels of the enzyme acetylcholinesterase. On the other hand, plants have been traditionally used to improve cognitive function and alleviate other symptoms associated with Alzheimer's disease nowadays [45]. Acetylcholinesterase inhibition is an important drug treatment strategy. Recently, there has been considerable interest in discovering naturally occurring acetylcholinesterase inhibitors to replace synthetic drugs, which may have some adverse effects [46]. For this purpose, our data revealed that chamomile could serve as an inhibitor against the cholinesterase enzyme family. The inhibitory action of chamomile extract against acetylcholinesterase activity in groups 3 and 4 may be related to their content of polyphenols, flavonoids, sterols, and alkaloids. Recently, it was reported that these compounds have been shown to possess anti-acetylcholinesterase properties [47-50].

Author Contribution Statement

Meaad Nasser Hussein: Methodology, Investigation, Data curation, Original draft preparation. Ali NooryFajer: Supervision, Conceptualization, Writing- Reviewing and Editing.

Acknowledgements

Availability of data and materials

The data and materials that support the findings of this study are available from the corresponding author, upon reasonable request.

Ethical Approval

This research protocol was evaluated and approved by Researches Ethics Committee of AL-Qadisiyah University, Iraq.

Conflict of interest

The authors declare that they have no conflict of interests.

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