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The Protective Role of *Camellia Sinensis* Leaves Extract Against Histopathological Changes in Spleen of Rats that Induced by DEN and CCL4

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ABSTRACT

Objective: *The current study was performed in the animal house at the Faculty of Science, University of Kufa, for the period from 2/1/2025 to 2/6/2025.* ***Method:*** *(36) laboratory rats (Rattus rattus) were used. The animals were divided into six groups as follows: The first group(G1): the control group treated only with the feed and water. The second group(G2): The infection group was injected with Diethylnitrosamine (DEN) and given for two weeks, then it was injected with Carbon tetrachloride (CCL4) for six weeks to induce cancer. The third group(G3): treated with alcoholic extract of Camellia sinensis leaves at a concentration of 10 mg/kg for two months. The fourth group(G4): treated with alcoholic extract of C.sinensis leaves at a concentration of 20 mg/kg for two months. The fifth group(G5): treated with alcoholic extract of C.sinensis leaves at a concentration of 10 mg/kg for two weeks, after which the mixture of DEN + CCL4 was given until the end of the experiment. The sixth group(G6): treated with alcoholic extract of C.sinensis leaves at a concentration of 20 mg/kg for two weeks, after which the mixture of DEN + CCL4 was given until the end of the experiment. The treatment continues for two months, after which the animals are dissected, the organs are extracted and preserved in sand for making after completing histological sections, they are studied, the histological changes were read using light microscopy.* ***Results:*** *The histological study in the groups treated with alcoholic extract of C.sinensis leaves showed no histological changes in spleen tissue compared with the second group treated with the substances (DEN and CCL4). The results of the histological study also showed the protective role in the groups were treated with alcoholic extract of C. sinensis leaves were then treated with substances that cause cancer in the spleen, it showed no histological changes or evidence of the presence of cancer cells.* ***Novelty:*** *The results of the current study demonstrate the protective role of alcoholic extract of C. sinensis leaves in preventing histopathological changes in spleen.*

INTRODUCTION

Tea (*Camellia sinensis* (L.) Kuntze) is one of the most important beverages worldwide, is produced in several distinct geographical regions [1]. *C.sinensis* originated in Southwest China, as confirmed by genetic sequencing of 81 tea accessions with diverse origins [2]. According to the Food and Agriculture Organization (FAO) of the United Nations, China is the largest tea producer in the world, followed by India, Kenya, and Sri Lanka [3]. Most tea is produced in humid tropical or humid subtropical climate; however, different varieties appear to be adapted to local climatic conditions [4]. Some researches indicate that drinking green tea is of great beneficial to increase bone density, improve cognitive function and prevent tooth caries and kidney stones [5] Abundant animal and cellular studies have demonstrated that green tea and EGCG may protect against NAFLD initiation and development by alleviating oxidative stress and the related

metabolism dysfunction, inflammation, fibrosis, and tumorigenesis [6]. The different tea cultivars are suitable for different purposes. Higher levels of amino acids and lower levels of flavanols have been observed in green tea cultivars [7] The spleen is a highly compartmentalized organ in humans and compartment has a different structure, cell populations, and function represented by removing microorganisms, proteins, and old or pathologically changed blood cells, this organ also triggers humeral and cellular immune responses [8], [9] and is made up of both red and white pulp, The periarterial lymphatic sheath (PALS) and follicles, two collections of motile T- and B-lymphocytes, are what make up the white pulp. These structures are situated close to the central arteries, which are tiny arteries devoid of corresponding veins. Specialized fibroblasts, known as T-zone reticular cells (fibroblastic reticulum cells) and B-zone reticular cells (follicular dendritic cells, or FDCs), nourish the PALS and the follicles. The red pulp is made up of lymphoid regions that do not filter, the perifollicular zone that divides the red and white pulps, White pulp is surrounded by red pulp, which is mostly made of a particular kind of connective tissue [10], [11]. DEN is toxic and cancerous to the liver, lungs, kidneys, skin, stomach, and blood. In addition to delivering DEN chemicals, CCL4 compounds can operate as tumor promoters, or genotoxic substances raising the risk of genetic errors and driving cells to develop into malignant neoplasms. The combination of DEN with CCl4 induction can cause harm to several organs. In a healthy body, angiogenesis helps with wound healing and the production of new tissue. In addition to being malignant when the body is subjected to toxic substances, angiogenesis contributes to carcinogenesis which is the unrestrained growth of cancer cells [12].

Aim of the study

The purpose of the current study was to use green tea leaves extract to assess its in preventing or reduce histopathological changes in spleen of white male rats.

RESEARCH METHOD

C. sinensis plant leaves were collected at a market in Najaf, Iraq. To prevent the leaves from rotting, they were cleaned with tap water and let too dry at room temperature for 10 days, stirring often. After drying, the leaves were pulverized using a milling machine to preparing alcoholic extract from powdered *C. sinensis* leaves, a cording to method of [13] used 215g of leaves powder using a sensitive balance, and soak it by adding 500ml of Ethanol (C_2H_5OH) as a polar solvent for two days in a dark incubator shaker, it was filtered by filter paper. The resulting filtrate was then collected and dried in an electric oven at 40C^o for a week, The extract was then collected ground with a milling machine, and placed in a clean plastic containe.

In this study, 36 adult rats (*Rattus rattus*), weighting between (220 ± 30) g (8 to 9 weeks old). This experiment was conducted in the University of Kufa in the animal house at the Faculty of Science, Plastic boxes with metal clips covering them held the animals, and holes were drilled in the sides to accommodate the water bottle nozzles. The cages were kept at 26 ± 2 C° and were subjected to a 12 hour light–dark cycle. The animals received water and food in the form of granules, and their cages were cleaned on a regular basis to ensure health conditions 20 To help their adapt, the animals were fed on feed and water for the first 14 days before the trial began.

The animals were separated into six groups, each consisting of six individuals. The first group (G1 negative control group) had a subperitoneal injection of normal saline, whereas the second group (G2 positive control group) received a subperitoneal injection of DEN for two weeks before being injected with CCL4 for six weeks to induce cancer. The third group (G3) received alcoholic extract of *C. sinensis* leaves at a dose of 10 mg/kg for two months. The fourth group(G4) received alcoholic extract of *C. sinensis* leaves at a concentration of 20 mg/kg for two months. The fifth group(G5) was treated with alcoholic extract of *C. sinensis* leaves at a concentration of 10 mg/kg for two weeks, after which the DEN + CCL4 mixture was administered till the end of the study. The sixth group(G6) was treated with alcoholic extract of *C. sinensis* leaves at a dosage of 20 mg/kg for two weeks before receiving a mixture of DEN and CCL4 until completion of experiment. The treatment lasts two months. Animals were anaesthetized with an intramuscular injection (thigh muscle) of a mixture of xylazine (lidocaine) 20mg/ml and ketamine 10mg/ml at the rate of 1/3ml until the righting reflex (LRR) was lost [14]. Following that, the animals are dissected, organs are taken and placed in a 10% formalin solution for the aim of fixing it according to the procedure of [15]. After that, slides prepared the cloth sections according to the method of [16] are studied, the histological changes are read using an optical microscope and notes are taken.

RESULTS AND DISCUSSION

Results

The current study findings revealed that the spleen tissue of the third(G3) and fourth groups(G4), which received treatments of (10, 20) mg/kg from alcoholic extract, respectively. Shows normal structure of splenic sinusoids, red pulp and white pulp as in figures 3 and 4 and no appear histopathological changes but their tissues composition were more similar to that of the negative control groups. To ascertain the protective effect of alcoholic extract against histopathological changes in spleen which induced by DEN and CCL4 at doses of (10, 20) mg/kg respectively, the fifth(G5) and sixth (G6) groups were treated with (10, 20) mg/kg from alcoholic extract shows proliferation of lymphocyte in the both white pulp and red pulp in (G5), dilation in splenic sinusoids and normal red pulp and white pulp in (G6), as in figures 5 and 6.

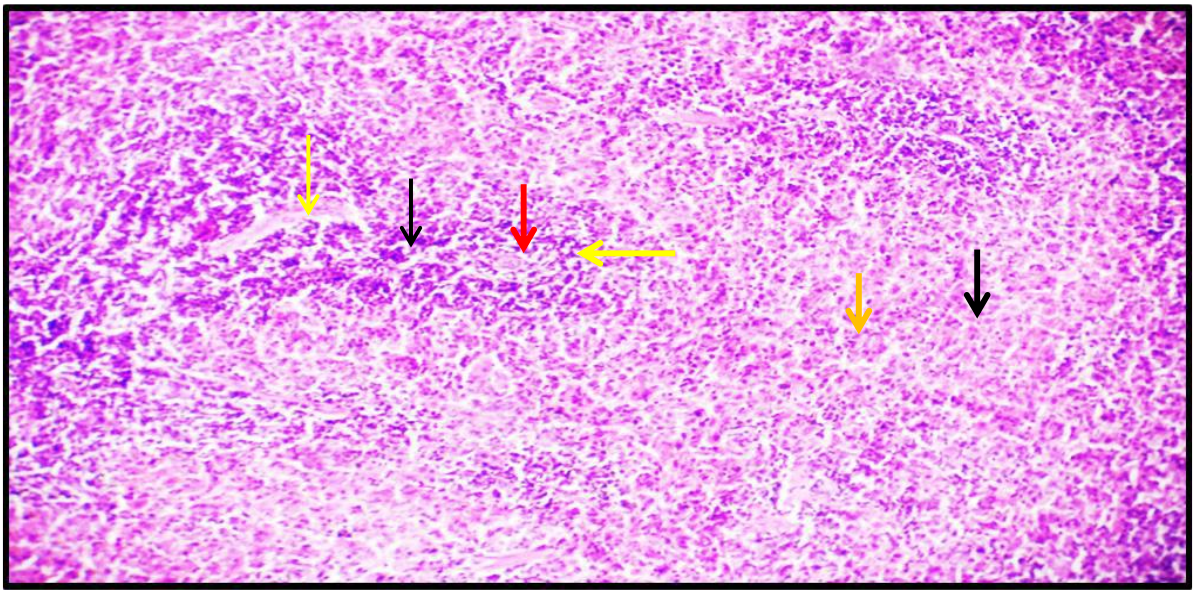

Figure 1. A Cross section of spleen tissue in the negative control group(G1), Shows normal structure of splenic sinusoids (Orange arrow), Red pulp (Thick arrow), White pulp (Thin arrow) Trabeculus(Thin yellow arrow), Germinal center (Thick yellow arrow) and Arteriole (Red arrow) HE X100.

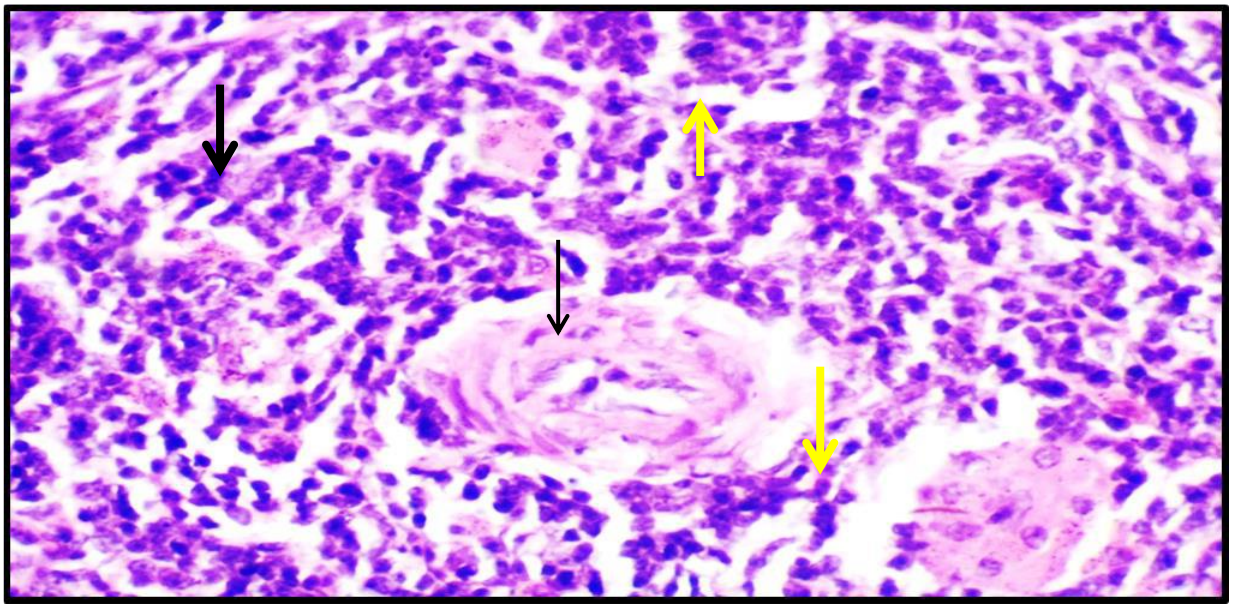

Figure 2. A Cross section of spleen tissue in the positive control group(G2), Shows dilation in the splenic sinusoids (Yellow arrows), Malpighian bodies (Thin arrow), Proliferation of lymphocyte in the white pulp (Thick arrow) HE X400.

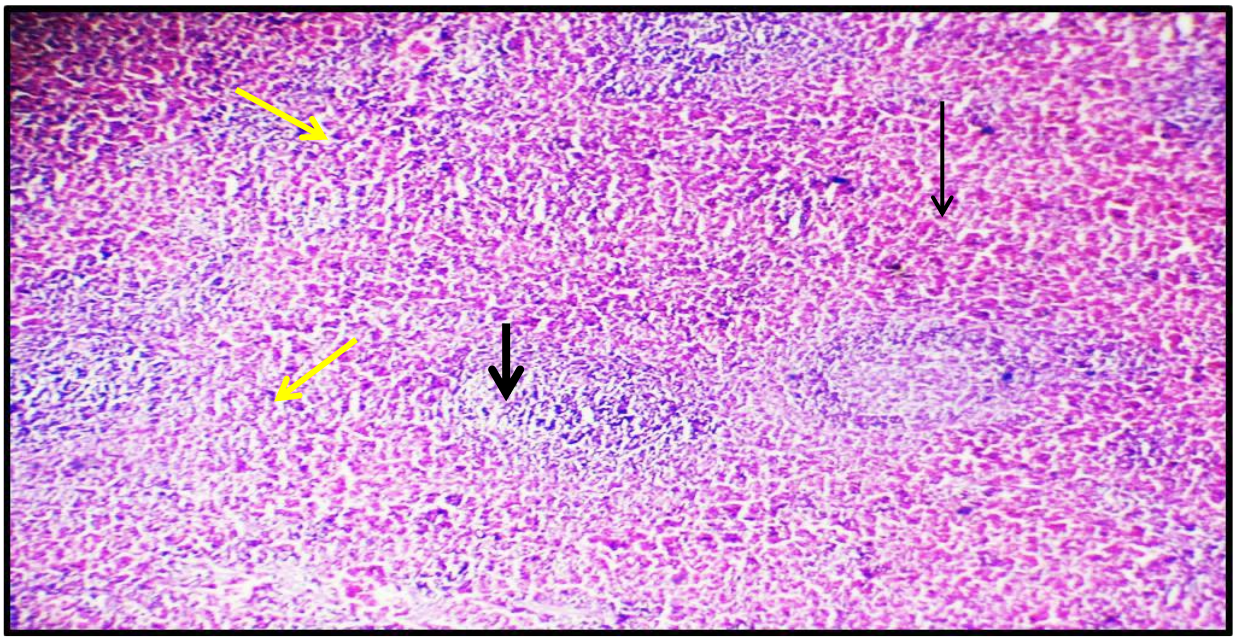

Figure 3. A Cross section of spleen tissue in the third group(G3), Shows normal structure of splenic sinusoids (Yellow arrows), Red Pulp (Thin arrow), and White Pulp (Thick arrow) HE X100.

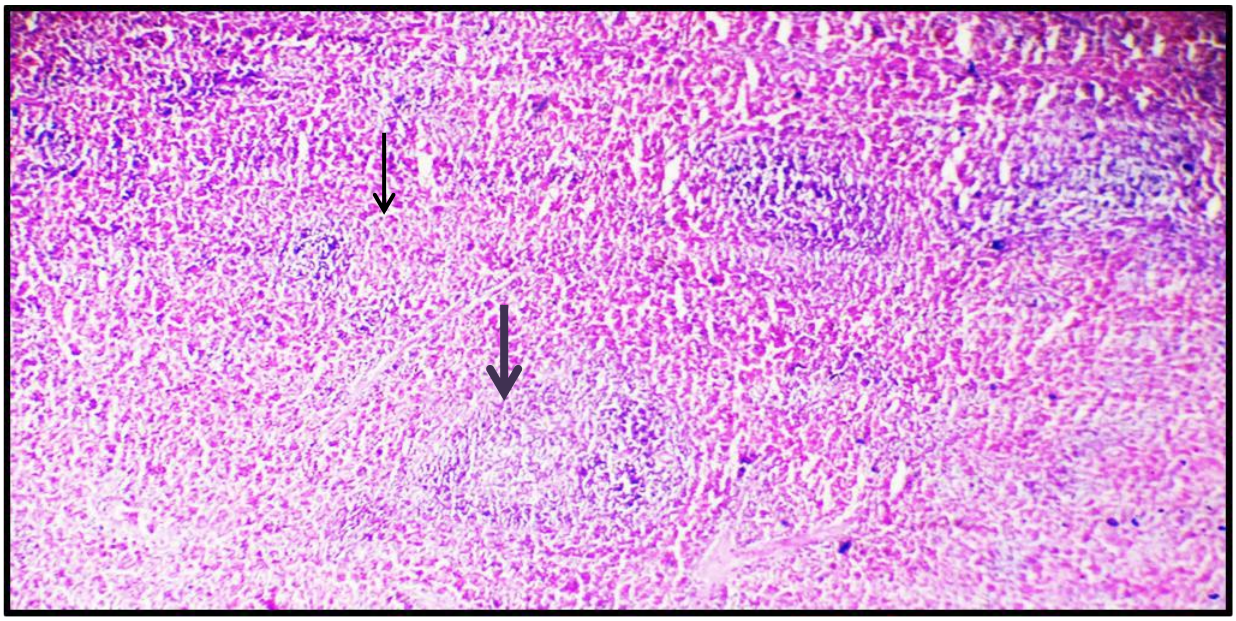

Figure 4. A Cross section of spleen tissue in the third group(G4), Shows the normal of Red Pulp (Thin arrow) and white Pulp (Thick arrow) HE X100.

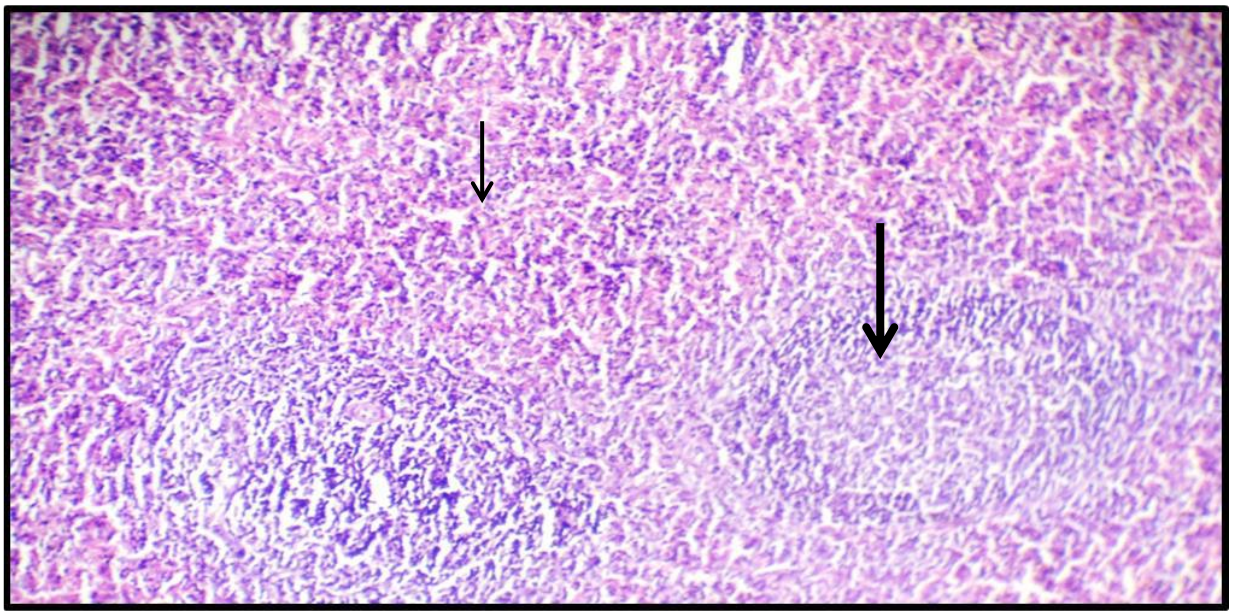

Figure 5. A Cross section of spleen tissue in the fifth group(G5), Shows Proliferation of lymphocyte in the both White pulp (Thick arrow) and Red pulp (Thin arrow) HE X400.

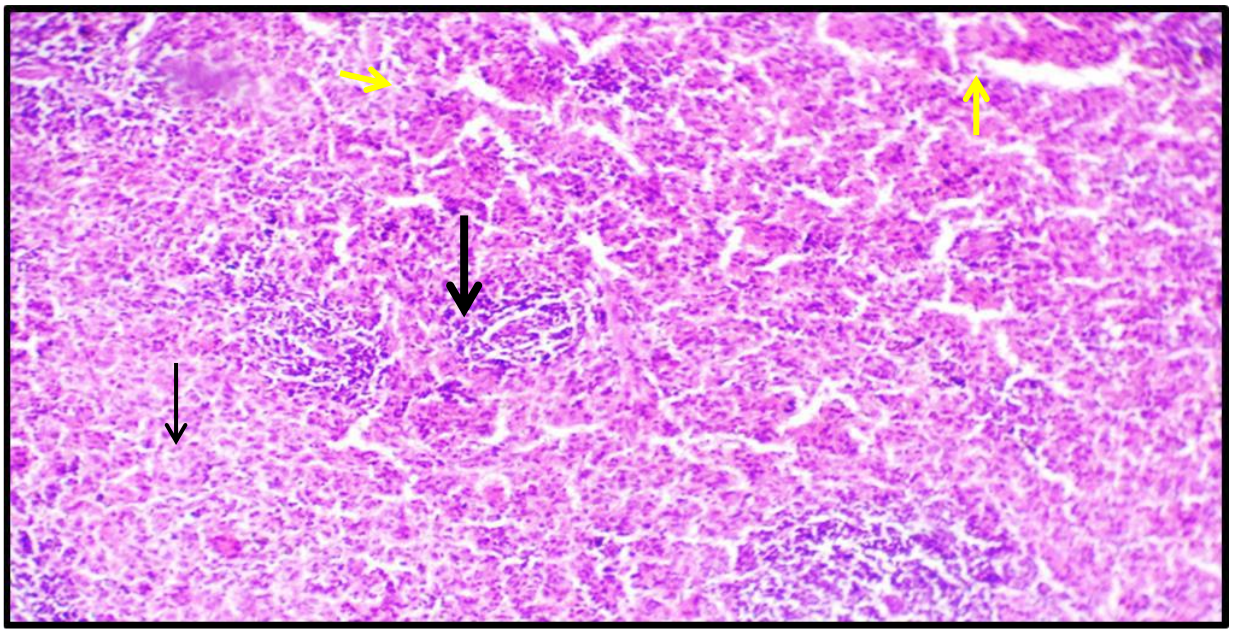

Figure 6. A Cross section of spleen tissue in the sixth group(G6), Shows dilation in splenic sinusoids (Yellow arrows), normal Red pulp (Thin arrow) and White pulp (Thick arrow) HE X400.

Discussion

The results of the histological examination showed the occurrence of pathological tissue changes in the positive control group (G2) treatment with substances (DEN and CCL4) which stimulated these changes, causing dilation in the splenic sinusoids and Proliferation of lymphocyte in the white pulp, the (G3) and (G4) groups the histological structure of spleen is normal, showing no alterations, and Shows normal structure of splenic sinusoids, Red Pulp and White Pulp because the alcoholic extract of *C. Sinensis*

are rich in antioxidants, which worked to suppress free radicals generated by the two substances (DEN and CCL4) and preserve the spleen tissue from damage and thus improve the histological structure, Or may be because the phenolic compounds in *C. Sinensis* which acts as chemopreventive effect of histological structures against the development any change in spleen. the fifth(G5) and sixth (G6) groups were treated with (10, 20)mg/kg from alcoholic extract shows proliferation of lymphocyte in the both white pulp and red pulp in(G5) and dilation in splenic sinusoids, normal red pulp and white pulp in (G6). extract at high concentration 20mg/kg is more effective from concentration 10 mg/kg and have a role in reducing the harmful effects of these two substances (DEN and CCL4). *C. Sinensis* extract significantly improved the histological structure of the spleen in mice with cancer, where the number of helper T cells (CD3+, CD4+) was recovered significantly after giving the extract of *C. Sinensis* leaves, while some previous studies showed that *C. Sinensis* extract induces lymphoblasts to produce lymphocytes and thus helps to improve and raise the level of immunity in the body, or the effect of green tea extract may be due to polyphenols which represent the largest proportion of compounds in it which have anti-inflammatory properties, which are considered as complementary or clinically adjuvant and antitumor during chemotherapy or can be used as natural chemotherapeutic agents and as adjunctive substances in improving histopathological changes for patients with cancer in general [17], [18], [19].

CONCLUSION

Fundamental Finding : The current study findings revealed that the spleen tissue of the third (G3) and fourth (G4) groups, treated with alcoholic extract of C. sinensis at 10 and 20 mg/kg, respectively, showed normal structure of splenic sinusoids, red pulp, and white pulp, with no histopathological changes, resembling the negative control group. In contrast, the positive control group (G2), injected with DEN and CCL4, showed dilation in splenic sinusoids and lymphocyte proliferation. The fifth (G5) and sixth (G6) groups, pre-treated with C. sinensis extract before DEN+CCL4 exposure, showed partial protection, with G6 (20 mg/kg) showing near-normal histology. **Implication :** C. sinensis extract, rich in antioxidants and polyphenols, appears to mitigate DEN and CCL4-induced spleen damage. It preserved splenic architecture and may exert chemopreventive and immunomodulatory effects. The extract may support immune function by influencing lymphocyte production and enhancing histological recovery, making it a potential complementary agent in cancer therapy. **Limitation :** The study was limited to a small sample of rats, focused solely on histological analysis without molecular or immunological assays. Functional immune parameters like CD3+ and CD4+ T cells were discussed but not quantified, and systemic effects were not evaluated. **Future Research :** Future studies should include immunophenotyping, broader systemic toxicity markers, comparisons between extract types, and synergistic effects with conventional drugs. Longer durations and molecular studies on inflammatory pathways could validate C. sinensis as an adjuvant therapy.

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