ORIGINAL ARTICLE

Green Tea Extract's Hepatoprotective Properties against Cyclophosphamide-Induced Liver Damage

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Abstract

Background: Consuming green tea has been linked to a decreased risk of cancer, cardiovascular disease, and neurological diseases. For this reason, green tea extract has been added to diets along with other multivitamins and supplements. **Aim of the Work:** investigating the impact of cyclophosphamide (CP) on male albino rat livers and the potential protective function of green tea extract. **Materials and Methods:** Twenty-four adult male albino rats were used in the current study. Three equal groups were formed out of them, each with eight rats. For 9 weeks, Group I (the control group) received I. P. (intraperitoneal) injections of normal saline at a dosage of 0.5 mg/kg body weight twice a week. Group II received 2 weeks of intraperitoneal injections of CP at a dose of 150 mg/kg/day. Group III: For 2 weeks, rats received intraperitoneal injections of 150 mg/kg/day of CP in addition to 3 weeks of oral green tea extracts (50 mg/kg/day). **Results:** The livers of rats given CP exhibited a number of histological and histochemical alterations. Green tea was used to ameliorate these modifications. **Conclusion:** The current study demonstrated the preventative and curative effects of green tea on albino rats' livers following CP exposure.

Key words: Albino rats, cyclophosphamide, green tea, liver

INTRODUCTION

The liver is the organ in the body that is in charge of metabolism and purification. The liver is susceptible to poisons from medications, chemicals, viruses, and free radical damage.[1] When the body's production of free radicals is out of balance with antioxidant activity, it can lead to oxidative stress, which can alter cellular structure.[2]

Nutritious antioxidants are generally considered to be safe substances present in medicinal plants.

They have intriguing benefits in complementary medicine, one of them being the significant reduction of oxidative stress.[3] Green tea in particular has a considerable amount of polyphenols and catechins, which are nutritional antioxidant groups.[4] These nutrient compounds not only have antioxidant properties but also anti-diabetic, anti-atherothrombogenic, antihypertensive, anti-carcinogenic, anti-oxidative, anti-fungal, and anti-inflammatory properties.[5] As a result, drinking green tea is associated with a decreased death rate, especially from heart attacks.[6] The antioxidant qualities of

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green tea's polyphenols and catechins have been suggested to play a preventive role in the prevention of chronic diseases, even though the precise mechanism by which consumption of the tea shields against these conditions is unknown.[7]

With more than 50 years of clinical experience, cyclophosphamide (CP) is a cytotoxic alkylating drug that is useful in treating both non-neoplastic conditions, such as systemic lupus erythematosus and rheumatoid arthritis as well as neoplastic conditions, such as solid tumors and lymphomas.[8] One of the main adverse effects of CP is hepatotoxicity because the drug is mostly metabolized by the hepatic microsomal cytochrome p450 mixed function oxidase system in hepatocytes, producing phosphoramide mustard and acrolein, which are its two active metabolites.[9] Research has indicated a possible link between oxidative stress and its hepatotoxic effects. Acrolein binds to cellular antioxidant nucleophiles, such as glutathione, causing the antioxidant defense system to be depleted and lipid peroxidation to start, which leads to CP toxicity.[10] The purpose of this study was to look into the histological alterations in the liver tissue of rats exposed to CP and whether green tea might have any protective effects.

MATERIALS AND METHODS

The present investigation was conducted on twenty-four mature male albino rats, weighing between 100 and 110 g. They came from Prince Sattam Bin Abdulaziz University's animal house. Before being utilized in the experimental methods, the rats were kept in temperature-controlled rooms (25°C) with a consistent humidity (40–70%) and a 12-h light/dark cycle. Throughout the trial, rats were given a regular pellet meal and had unrestricted access to water. Tecno Med Company in Saudi Arabia produced 300 mg tablets containing green tea extract. After crushing the tablets, the necessary quantity was diluted in distilled water. The rats were kept in standard housing, which included cages, a central ventilation environment with a temperature of 20°C ± 2°C, and a relative humidity of 55%. After the 2-week accommodation phase, the rats were split into three equal groups, each consisting of eight rats. Each experimental rat was weighed every day using a compact scale weighing balance (FEJ-3000 B, 3000 g capacity, China) to track changes in their body weights on a weekly basis.

The three groups were fed a typical chow diet for the duration of the investigation. The experimental animals were split up into the following three groups. Group I (control group): For a total of 9 weeks, the animals in this group served as negative control without any treatment. Group II (CP): For 2 weeks, two daily doses of CP (150 mg/kg/day) were administered intraperitoneally to rats. Group III (Green tea + CP): Rats were given 3 weeks of oral green tea extract (50 mg/kg) through a stomach tube once a day, followed by 2 weeks of concurrent CP administration (150 mg/kg). The livers of the sacrificed animals were processed, stained, and inspected under a microscope.

At the end of our experiment, a halothane overdose resulted in the sacrifice of all three groups of rats. Liver specimens from autopsies were removed, cleaned several times with normal saline, and then immediately fixed in 10% neutral buffered formalin. The specimens were then rinsed in tap water, dehydrated in increasing concentrations of ethyl alcohol, cleared in xylol, and embedded in paraffin to create tissue blocks.

Next, five-micron sections were cut, put on charged glass slides, and stained with hematoxylin and eosin stain (Hx. and Eo.) to look at structural morphology and see changes in histopathology. Mallory's trichrome stain was also used to determine the amount of collagen fibers, and the periodic Acid-Schiff (PAS) reagent method was used to determine how much cytoplasmic polysaccharides there were.[11] The Statistical Packages for the Social Sciences statistical version 22 was used for the statistical analysis. The mean ± standard deviation was used to express all the data. *P*-values were deemed statistically significant if they were <0.05. Using the Paired-Samples "*t*" test, the outcomes of groups 2 and 3 were compared to those of group 1 (control group), and the outcomes of group 2 were compared to those of group 3.

RESULTS

Rats treated with CP showed a considerably lower percent change in body weight (4.98%). Furthermore, it has been observed that administering green tea extract to rats resulted in a 9.92% recovery to normal body weight [Table 1]. Investigations of normal liver sections stained with hematoxyline and eosin in Group 1's untreated negative control showed that each hepatic lobule is made up of a central vein lined with simple squamous epithelium, surrounded by polygonal hepatic cells that are distributed radially in the form of strands with clearly visible blood sinusoids in between. In addition, there is no inflammation inside the lobules [Figure 1].

There were no lymphocytes or signs of liver cell necrosis in the central vein. Hepatocytes often have one or two nuclei. The cytoplasm of the hepatocytes is eosinophilic and has a homogeneous appearance with numerous coarse basophilic granules that are uniformly dispersed. The nuclei of hepatocytes are big, vesicular, and comprise one or more nucleoli. Between the hepatocytes are several spindle-shaped Kuppler's cells with highly pigmented nuclei. The hepatic artery, bile duct, and hepatic portal vein are branches of the hepatic portal tracts. Fine threads of collagen fibers surrounding the major vein were observed in Group 1's examinations of normal liver sections stained with Mallory's trichrome stain. These fibers appeared to be small amounts of collagenous matter surrounding the hepatocytes and hepatic sinusoids. In addition, tiny collagen fiber threads were found

Table 1: Changes in body weight (g) in the control, CP and green tea treated groups. The CP group's parameter values showed a substantial drop (P<0.01) as compared to the control group

Groups	G1 (Control)	G2 (CP)	G3 (CP and green tea)
Body weight at the start of the study	100.1±0.04	100.82±0.36	100.16±0.06
Body weight at the completion of the study	110.02±0.01	105.01±0.02	110.09±0.07
Change in percentage	9.92	4.98	9.92

CP: Cyclophosphamide

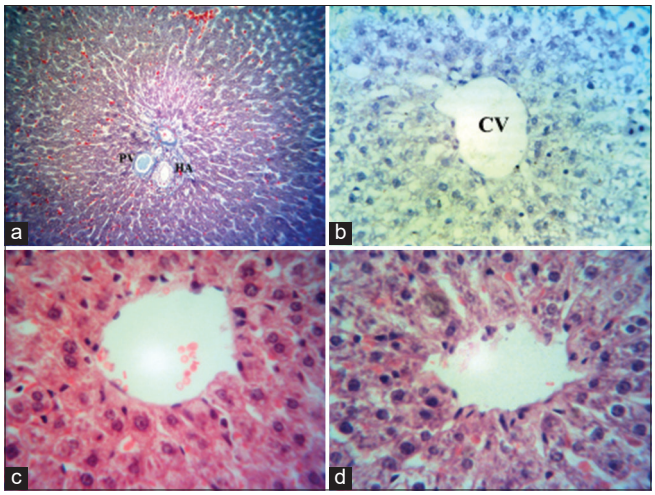

Figure 1: Different images of the control group (a) showing normal lobular pattern normal portal triad consisting of a branch of the portal vein, branch of the hepatic artery (Hx. and E. ×200) (b) displaying a centrilobular vein, irregularly branched hepatocytes, and hepatocyte anastomosing plates with endothelial cell-lined sinusoids in between. The majority of hepatocytes possess vesicular nuclei (T.B. ×400). (c) displaying the dispersion of collagen fibers in tiny threads around the major vein (Mallory's trichrome. ×400). (d) showing an increased amount of dispersed carbohydrates (Hx. and E. ×400)

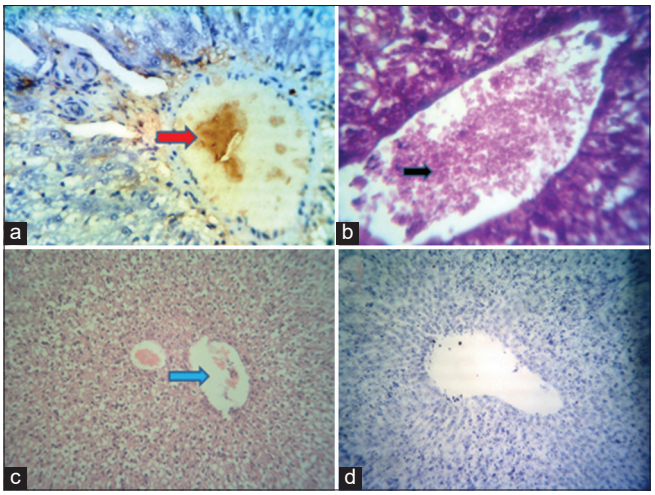

Figure 2: Different images of the cyclophosphamide group (a) showing mild cellular infiltration in the portal triad (red arrow) (T.B. ×400). (b) showing that the central and portal veins were dilated and congested (black arrow) and decreased amount of dispersed carbohydrates (periodic Acid-Schiff. ×400). (c) showing hepatocytes necrotic changes such as marked hepatocytes pyknotic nuclei ballooning, and fatty degeneration (Hx. and E. ×200). (d) showing mild cellular infiltration in the portal triad (T.B. ×42)

in the portal region, which is home to the bile duct and hepatic portal vein. Nevertheless, the distribution of carbohydrates in the cytoplasm of these cells was irregular. The bulk of the hepatocytes included both fine and coarse pink granules that were identified as carbohydrates. The nuclei's affinity for the reaction was negative [Figure 1].

Rat liver slices in the CP group revealed dilated hepatic sinusoids. Hepatocytes affected by the condition lost their nuclei, which contained some small pyknotic nuclei and vacuolated cytoplasm. Hepatic portal veins were dilated and widened, and their walls were thickened and heavily infiltrated by fibroblasts. The veins were seen to be packed with erythrocytes and amyloidal particles. The area around the portal vein also showed a large number of pyknotic nuclei. Rat liver slices from Group 2 revealed that the quantity of liver carbohydrates was considerably lower than that of the control group (poor PAS+ve reaction) and that the carbohydrates were mostly concentrated close to some hepatocytes' basement membranes. In addition, the most damaged hepatocytes had a feeble response [Figure 2].

Also noted were progressively wider hepatic sinusoids and asymmetric central veins. Moreover, a perforated bile duct's cellular wall showed an amazing growth. Hepatocellular necrosis manifested as lobular inflammation accompanied by infiltration of lymphocytes. Rats in Group 2 had liver sections after receiving CP, and compared to the control group, there was a clear and significant increase in the collagenous fiber deposition surrounding the hepatic sinusoids, central vein, and portal tract structures [Figure 2].

Significant progress was noted in Group 3 (Green tea + CP) for the hepatic cellular structure, hepatic sinusoids, and portal tracts [Figure 3].

Group 3 showed a significant decrease in the amount of collagenous fiber deposition surrounding the hepatic sinusoids, central vein, and portal tract structures compared to the CP group.

When compared to the CP group, the majority of the hepatocytes in Group 3 showed a notable improvement in the PAS+ve reaction [Figure 3].

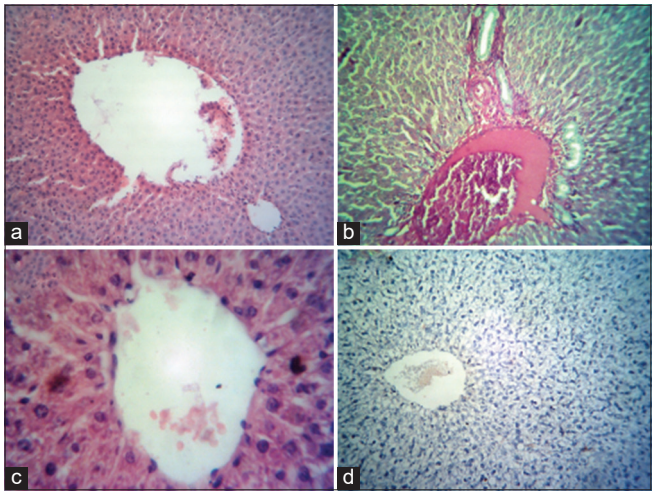

Figure 3: Different images of the green tea treated group (a) Hepatic cords are positioned radially around the major vein in a typical hepatic design similar to that of the control group) (Hx. and E. ×400). (b) distribution of fine threads of collagen fibers around the hepatic portal vein and bile duct (Mallory's trichrome. ×400). (c) displaying the typical distribution of collagen fibers in tiny threads surrounding the major vein (Mallory's trichrome. ×400). (d) showing mild cellular infiltration in the portal triad (T.B. ×200)

DISCUSSION

Due to its therapeutic efficacy against a variety of tumors, CP is one of the most widely utilized anticancer medications.

Our investigation identified many degenerative hepatic morphological and structural alterations in the rats given CP treatment (G2).

Previous research has demonstrated that CP ameliorative doses may result in liver damage.[12] Based on experimental studies, oxidative stress appears to be the cause of CP hepatotoxicity. During its oxidative metabolism, it may produce reactive oxygen species such as superoxide anion, hydroxyl radical, and hydrogen peroxide (H_2O_2), which inhibits the liver's antioxidant defense system.[13] Numerous studies have demonstrated the protective effect of natural compounds with antioxidant activity against CP hepatotoxicity.[14,15]

Liver sections for the group treated with green tea and CP were similar to those of the control. The antioxidant properties of green tea may be the cause of this improvement in the histological image. Thus far, evidence indicating green tea inhibits hepatotoxicity has been consistent with this theory.[16] Green tea's polyphenol (catechin) concentration is primarily responsible for its health-promoting properties. Alpha-tocopherol was not as effective at reducing the generation of peroxides as catechins were.[17]

The liver sections of CP that had been given green tea showed an essentially identical image to the control group. This could be explained by green tea's anti-inflammatory and anti-oxidative properties, which may be able to stop excessive lipid peroxidation due to its polyphenol concentration.

Lipid peroxidation is one of the primary characteristics of CP -induced hepatotoxicity. Lipid peroxidation is caused by free radicals attacking cell membranes, with malondialdehyde (MDA) being the primary byproduct. Consequently, MDA is employed as a biomarker of damage caused by lipid peroxidation. When a hepatoprotective treatment lowers the amount of MDA in liver tissue, it is deemed successful.[2] Further studies are needed to evaluate the levels of hepatic MDA -like antioxidant activity.

Green tea has been shown by Chacko *et al*.[17] to considerably reduce lipid peroxidation, which could provide weight to this theory. Furthermore, it was observed by Shimizu *et al*.[18] that the primary mechanisms of green tea catechins (polyphenols) are their anti-oxidative and anti-inflammatory properties.

Ultimately, group 3 showed a highly significant structural hepatic amelioration and protection against the previously identified severe degenerative alterations shown in group 2 in our current investigation. In addition, a slight dilating of the portal vein, a mild periportal mononuclear cellular infiltration, the formation of very few perivenous and portal collagen fibers, and an ultrastructural electron-dense matrix in some of the liver cell mitochondria were among the mild morphological changes that appeared to improve the liver architecture in that group.

CONCLUSION

The rat liver treated with CP exhibited pathological characteristics that were discovered in this investigation. In addition, it offers proof that green tea may have a hepatoprotective impact against the hepatotoxic effects of CP.

To prevent and treat these negative effects of the medication on the liver, it is therefore recommended that patients receiving CP supplements take green tea on a regular basis.

To identify the specific green tea target components that protect the liver, more research is required. Furthermore, it is advised that human studies be conducted to show whether comparable outcomes may be achieved.

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INFORMED CONSENT

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data are available upon request from the authors.

ETHICS APPROVAL

All series of steps that were implemented in this study that included animal models were in compliance with the Ethics Committee of Prince Sattam bin Abdulaziz University Institutional Review Board (SCBR-300-2023).

REFERENCES

1. Ramos-Tovar E, Muriel P. Free radicals, antioxidants, nuclear factor-E2-related factor-2 and liver damage. J Appl Toxicol 2020;40:151-68.
2. Nguyen AG, Tran HT, Tran CV, Pham BN, Nguyen HN, Nguyen HN, *et al*. Antioxidant and hepatoprotective activities of *Paris polyphylla* Sm. rhizome extract in cyclophosphamide-induced hepatic damaged mice. Trop J Nat Prod Res 2023;7:3652-7.
3. Elzoghby RR, Ahlam FH, Abdel-Fatah A, Farouk M. Protective role of vitamin C and green tea extract on malathion-induced hepatotoxicity and nephrotoxicity in rats. Am J Pharmacol Toxicol 2014;9:177.
4. Higdon JV, Frei B. Tea catechins and polyphenols: Health effects, metabolism, and antioxidant functions. Crit Rev Food Sci Nutr 2003;43:89-143.
5. Al-Attar AM, Abu Zeid IM. Effect of tea (*Camellia sinensis*) and olive (*Olea europaea* L.) leaves extracts on male mice exposed to diazinon. Biomed Res Int 2013;2013:461415.
6. Kuriyama S, Shimazu T, Ohmori K, Kikuchi N, Nakaya N, Nishino Y, *et al*. Green tea consumption and mortality due to cardiovascular disease, cancer, and all causes in Japan: The Ohsaki study. JAMA 2006;296:1255-65.
7. Frei B, Higdon JV. Antioxidant activity of tea polyphenols *in vivo*: Evidence from animal studies. J Nutr 2003;133:3275S-84.
8. Lawson M, Vasilaras A, De Vries A, Mactaggart P, Nicol D. Urological implications of cyclophosphamide and ifosfamide. Scand J Urol Nephrol 2008;42:309-17.
9. Oyagbemi AA, Omobowale OT, Asenuga ER, Akinleye AS, Ogunsanwo RO, Saba AB. Cyclophosphamide-induced hepatotoxicity in wistar rats: The modulatory role of gallic acid as a hepatoprotective and chemopreventive phytochemical. Int J Prev Med 2016;7:51.
10. Adams JD Jr., Klaidman LK. Acrolein-induced oxygen radical formation. Free Radic Biol Med 1993;15:187-93.
11. Bancroft JD, Gamble M. Bancroft's theory and practice of histological techniques. 6th ed. Churchill Livingstone: Elsevier; 2008. p. 178-86, 221-4.
12. Zarei M, Shivanandappa T. Amelioration of cyclophosphamide-induced hepatotoxicity by the root extract of *Decalepis hamiltonii* in mice. Food Chem Toxicol 2013;57:179-84.
13. Bhattacharya A, Lawrence RA, Krishnan A, Zaman K, Sun D, Fernandes G. Effect of dietary n-3 and n-6 oils with and without food restriction on activity of antioxidant enzymes and lipid peroxidation in livers of cyclophosphamide treated autoimmune-prone NZB/W female mice. J Am Coll Nutr 2003;22:388-99.
14. Haque R, Bin-Hafeez B, Parvez S, Pandey S, Sayeed I, Ali M, *et al*. Aqueous extract of walnut (*Juglans regia* L.) protects mice against cyclophosphamide-induced biochemical toxicity. Hum Exp Toxicol 2003;22:473-80.
15. Kumar KB, Kuttan R. Chemoprotective activity of an extract of *Phyllanthus amarus* against cyclophosphamide induced toxicity in mice. Phytomedicine 2005;12:494-500.
16. Crespy V, Williamson G. A review of the health effects of green tea catechins in *in vivo* animal models. J Nutr 2004;134(12 Suppl):3431S-40.
17. Chacko SM, Thambi PT, Kuttan R, Nishigaki I. Beneficial effects of green tea: A literature review. Chin Med 2010;5:13.
18. Shimizu M, Shirakami Y, Sakai H, Kubota M, Kochi T, Ideta T, *et al*. Chemopreventive potential of green tea catechins in hepatocellular carcinoma. Int J Mol Sci 2015;16:6124-39.

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