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ORIGINAL ARTICLES

HISTOPATHOLOGICAL EVALUATION OF THE HEPATOPROTECTIVE EFFECT OF *CHAENOMELES JAPONICA* FRUIT JUICE IN A RAT MODEL OF PARACETAMOL-INDUCED LIVER DAMAGE

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ABSTRACT

INTRODUCTION: Species of the genus *Chaenomeles* have a long history of use in traditional Eastern medicine due to their health-promoting properties.

AIM: The present study aimed to investigate histopathologically the effect of *Chaenomeles japonica* fruit juice (CJFJ) on the liver in a model of paracetamol (PCM)-induced damage.

MATERIALS AND METHODS: Male Wistar rats (n = 50) were randomly assigned to five groups: Control, PCM, CJFJ2.5+PCM, CJFJ5+PCM, and CJFJ10+PCM. For 12 days, animals were treated with an orogastral tube: the Control and PCM groups received water, while the other three groups received CJFJ at increasing doses of 2.5, 5.0, and 10.0 mL/kg, respectively. On the 11th day, PCM (1.0 g/kg) was intraperitoneally injected to the following groups: PCM, CJFJ2.5+PCM, CJFJ5+PCM, and CJFJ10+PCM. On day 13th, liver samples were taken, fixed in formalin and embedded in paraffin. Tissue slices were stained with hematoxilin and eosin and observed by light microscopy. The severity of necrosis was assessed using a scoring method.

RESULTS: The liver architecture of the Control group was normal. In the PCM group, centrilobular and bridging necrosis was observed. In the CJFJ2.5+PCM group, the zones affected by necrotic and inflammatory changes were reduced. The medium and the high dose of CJFJ succeed in limiting the PCM-induced liver damage to a higher extent without completely preventing it. The necrosis score was significantly reduced by the three juice doses.

CONCLUSION: The current study demonstrated a dose-dependent hepatoprotective effect of CJFJ that is probably attributed to its high polyphenolic content.

Keywords: *Chaenomeles japonica, liver, paracetamol, histopathology, rats*

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INTRODUCTION

The liver is a central organ in the regulation of metabolism and the detoxification of xenobiotics, drugs, and endogenous compounds [1]. Hepatic injury, whether induced by pharmaceuticals, environmental toxins, or oxidative stress, represents a significant global health concern due to its potential to progress to acute liver failure or chronic liver disease [2]. Among pharmaceutical agents, paracetamol (PCM, acetaminophen) overdose is one of the most prevalent causes of drug-induced hepatotoxicity, resulting in oxidative stress, inflammation, and hepatocellular necrosis [3]. Although conventional hepatoprotective drugs such as N-acetylcysteine are clinically effective, their use may be limited by adverse effects and variablility in patient responses, highlighting the need for safe and efficacious natural alternatives [4,5].

Plants with a high content of bioactive compounds, particularly polyphenols, flavonoids, and triterpenoids, have attracted considerable attention for their hepatoprotective properties [6]. *Chaenomeles japonica* (Thunb.) Lindl, also known as Japanese quince, is a species belonging to the *Rosaceae* family. The genus *Chaenomeles* consists of five species: *Chaenomeles speciosa, Chaenomeles thibetica, Chaenomeles cathayensis, Chaenomeles japonica,* and *Chaenomeles sinensis*. *Chaenomeles* fruits are rich in antioxidants and other phytochemicals that might mitigate oxidative and inflammatory liver damage [7].

The *Chaenomeles* genus has gained considerable attention due to its diverse pharmacological potential. Extracts and bioactive constituents from the *Chaenomeles* species have been reported to exhibit a broad range of biological activities, including antioxidant, anti-inflammatory, antimicrobial, antiviral, antitumor, and metabolic regulatory effects [8,9]. These activities are primarily attributed to the high content of phenolic compounds, flavonoids, triterpenoids, and organic acids that contribute to strong free radical scavenging capacity and modulation of inflammatory pathways [8,10].

Among these biological activities, the hepatoprotective potential of the *Chaenomeles* species has been evaluated in both *in vitro* and *in vivo* experiments. *In vitro* studies using hepatocyte models have shown that *Chaenomeles japonica* extracts attenuate oxidative stress, reduce lipid peroxidation, and improve cell viability under chemically induced injury conditions [10]. *In vivo* studies have predominantly employed rodent models of liver injury, including carbon tetrachloride (CCl_4)-induced hepatotoxicity, where administration of *Chaenomeles speciosa* extracts resulted in improved liver histology and reduced serum biomarkers of hepatic damage [11]. Furthermore, metabolic disorder models, such as high-fat diet-induced oxidative stress and liver dysfunction in rodents, have demonstrated that *Chaenomeles japonica* supplementation can improve antioxidant status and liver function parameters [8]. Collectively, these findings support the hepatoprotective activity of *Chaenomeles* species across chemically induced and diet-related models of liver injury.

Overall, evidence from multiple experimental models indicated that the combined antioxidant, anti-inflammatory, lipid-regulatory, and anti-apoptotic mechanisms supported the classification of *Chaenomeles* as a promising multi-target hepatoprotective agent. A study on *Chaenomeles japonica* fruit juice (CJFJ) has been shown to improve the biochemical markers of liver function in healthy rats following a subchronic administration [12].

The chemical composition of CJFJ used in this study was analyzed by Valcheva-Kuzmanova et al. [13]. It proved to be a rich source of bioactive and nutritional compounds. The total phenolic content, determined spectrophotometrically, was 890.00 mg/100 mL (as gallic acid equivalents). High-performance liquid chromatography (HPLC) revealed a high level of procyanidin oligomers (280.52 mg/100 mL as catechin equivalents), along with several phenolic acids and flavonoids. Among the flavonoids, epicatechin (5.59 mg/100 mL), catechin (5.25 mg/100 mL), and quercetin-3-β-glucoside (3.58 mg/100 mL) were the most abundant. The phenolic acids with the highest concentrations were vanillic (14.91 mg/100 mL), caffeic (14.48 mg/100 mL), and chlorogenic (11.00 mg/100 mL), followed by neochlorogenic (2.44 mg/100 mL), p-coumaric (1.52 mg/100 mL), ellagic (1.29 mg/100 mL), ferulic (1.26 mg/100 mL), and 2,4-dihydroxy-benzoic (1.23 mg/100 mL). Six organic acids were also identified—malic (3647.0 mg/100 mL), quinic (1034.0 mg/100 mL), citric (51.00 mg/100 mL), shikimic (30.0 mg/100 mL), ascorbic

(22.0 mg/100 mL), and oxalic (17.0 mg/100 mL). The juice contained several carbohydrates, with glucose (1713 mg/100 mL) and fructose (1237 mg/100 mL) being predominant, along with smaller amounts of sucrose, xylose, rhamnose, and arabinose.

The antioxidant activity of CJFJ was evaluated using two complementary assays. The hydroxyl radical antioxidant capacity (HORAC) assay yielded 18,167.8 ± 938.8 **µmol** gallic acid equivalents per liter, indicating strong potential to prevent hydroxyl radical formation and thereby reduce cellular oxidative stress [13]. The oxygen radical absorbance capacity (ORAC) assay showed 84,401.4 ± 1,934.2 µmol Trolox equivalents per liter reflecting a robust capacity to scavenge oxygen-based free radicals [13].

Despite growing evidence demonstrating the hepatoprotective properties of the *Chaenomeles* species, the effects of CJFJ on PCM-induced liver injury have not been explored. This study aimed to evaluate histopathologically the effect of CJFJ in a rat model of PCM-induced liver damage.

MATERIALS AND METHODS

Expaerimental Substances

Chaenomeles japonica Fruit Juice (CJFJ)

The juice was produced from fully mature fruits harvested in the region of Troyan, Bulgaria. They were manually collected, mechanically crushed, pressed, and filtered. Potassium sorbate (1.0 g/L) was used as a preservative, and the final product was stored at 0°C until experimental use.

The PCM used in the experiment was purchased from Sigma-Aldrich, Germany (CAS number 103-90-2).

Experimental Animals

A total of 50 male Wistar rats (225 ± 25 g) were used in the present study. They were housed in plastic cages in a well-ventilated room, maintained at 22 ± 1 °C and on a 12/12 light-dark cycle (lights on from 07:00 to 19:00). The rats received a standard rodent pelleted diet and water ad libitum except for a 24-hour fasting period prior to PCM administration.

All experimental procedures were approved by the Bulgarian Food Safety Agency (Document № 177/07.07.2017) and were conducted in accordance with national regulations and EU Directive 2010/63/EU on the protection of animals used for scientific purposes.

Experimental Design

The animals were randomly assigned to five experimental groups (n = 10 per group): Control, PCM, CJFJ2.5+PCM, CJFJ5+PCM, and CJFJ10+PCM. A thirteen-day experimental protocol was conducted to evaluate the effects of CJFJ on PCM-induced hepatotoxicity. For 12 days, animals were treated with an orogastral tube in the following way: the Control and PCM groups received distilled water (10 mL/kg), while the other three groups were respectively treated with CJFJ at doses of 2.5, 5.0, and 10.0 mL/kg. The doses of 2.5 and 5.0 mL/kg were diluted with distilled water to a total volume of 10 mL/kg. On the 11th day of the experiment, one hour after the oral treatment, the rats from the following groups: PCM, CJFJ2.5+PCM, CJFJ5+PCM, and CJFJ10+PCM, were injected intraperitoneally with PCM at a dose of 1.0 g/kg in a volume of 4 mL/kg in the form a suspension with 2% Tween 80. Control rats received 4 mL/kg of the vehicle. On the 13th day, 48 hours after PCM administration, animals were sacrificed by cervical dislocation and liver samples were taken for histopathological investigation.

Tissue Collection and Histopathological Investigation

Liver tissues were immediately fixed in 10% neutral-buffered formalin. Fixed tissues were embedded in paraffin, cut into sections, and placed on microscope slides. Slides were stained with hematoxylin and eosin (H&E) for histopathological investigation which was performed by light microscopy.

Evaluation of liver necrosis

The severity of necrosis was assessed by examining 5–6 hepatic lobules per tissue sample and scored as follows [14]:

Score = 0: Normal architectonics of the liver structure;

Score = 1: Mild centrilobular necrosis/apoptosis;

Score = 2: Centrilobular coagulative necrosis involving zone 3 and partially zone 2 of the liver lobe;

Score = 3: Central-to-central (bridging) necrosis;

Score = 4: Massive necrosis erasing liver architecture.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism software, version 5.0 (GraphPad Software, San Diego, CA, USA). Data are presented as means ± standard error of the mean (SEM). Comparisons between the PCM group and CJFJ-treated groups were performed using one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test.

RESULTS

Liver Histopathology

Histopathological examination of the liver of the Control group showed normal liver architectonics. (Fig. 1A).

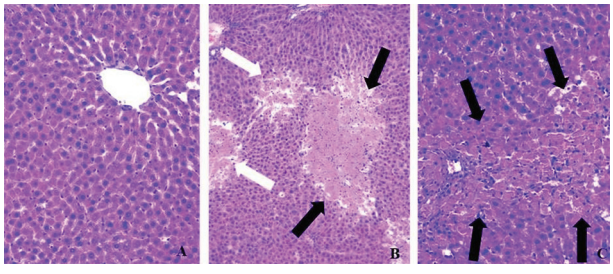


Fig. 1. Control group showed a normal tissue (panel A). Liver tissue from the PCM group exhibited centrilobular coagulative necrosis (white arrows; panel B) and bridging necrosis (black arrows; panels B and C). H&E staining (magnification A x 200; B x100; C x 200).

The histopathological picture of liver tissue from the PCM group revealed centrilobular coagulative necrotic zones in places confluent with each other, thus forming bridging necrosis (Fig. 1 B, C).

The histopathological picture of liver tissue of the CJFJ2.5+PCM group is presented on Fig. 2. In 30% of the cases, the liver architectonics was restored; in 40% of the cases, the necrotic zones were smaller in area compared to the PCM group, single necrotic hepatocytes and many inflammatory cells were observed around the central veins; in 30% of the cases, the histological picture was similar to that of the PCM group.

The histopathological picture of liver tissue of the CJFJ5+PCM group is presented on Fig. 3. In more than half (60%) of the samples from the CJFJ5+PCM group, the structure was normal; in 20% of the cases there were only inflammatory changes and in 20% there were necrotic and inflammatory changes.

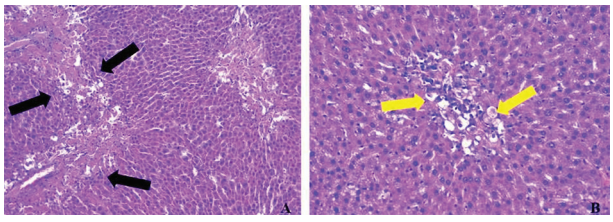


Fig. 2. Liver tissue from the CJFJ2.5+PCM group. Panel A: Bridging necrosis (black arrows). Panel B: Reduction of centrilobular coagulative necrosis; inflammatory cells around the central vein and a few apoptotic hepatocytes (yellow arrows); reduction of the area of damage compared to the PCM group. H&E staining (magnification A x 100; B x 200).

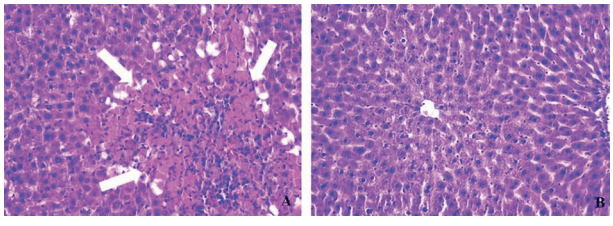


Fig. 3. Liver tissue from the CJFJ5+PCM group. Panel A: Centrilobular coagulative necrosis (white arrows). Panel B: Normal liver tissue. H&E staining (magnification A x 200; B x 200).

The histopathological picture of liver tissue of the CJFJ10+PCM group is presented on Fig. 4. In this group, 70% of the cases were with preserved architectonics, in one sample (10%) there were only inflammatory changes, and in 20% of cases there were some necrotic changes.

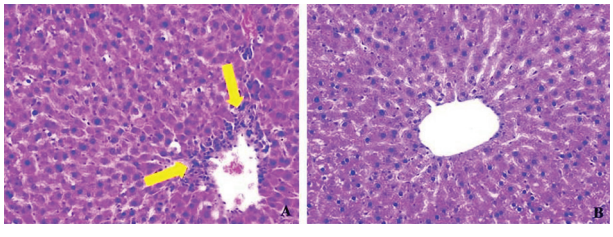


Fig. 4. Liver tissue from the CJFJ10+PCM group. Panel A: Inflammatory cells around the central vein (yellow arrows). Panel B: Normal liver tissue. H&E staining (magnification A x 200; B x 200).

Liver Necrosis Score

The necrosis score of the PCM group was 2.4 ± 0.1, reflecting the extensive hepatocellular damage and disruption of liver tissue integrity. The necrosis scores of the following groups: CJFJ2.5+PCM, CJFJ5+PCM, and CJFJ10+PCM, were respectively 1.4 ± 0.2, 1.1 ± 0.3, and 0.9 ± 0.2, which showed that CJFJ significantly attenuated PCM-induced hepatic injury (Fig. 5).

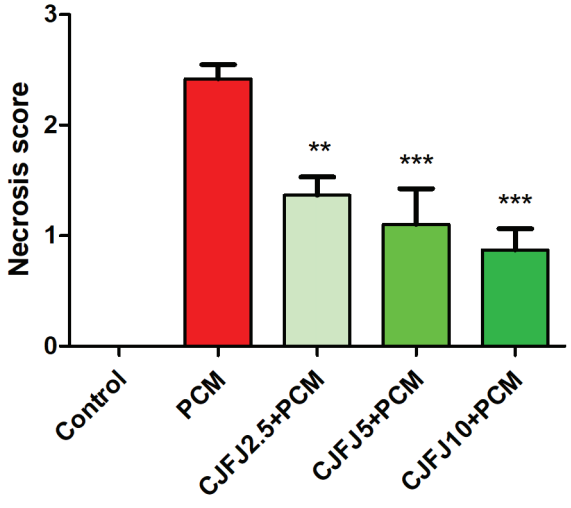


*Fig. 5. Necrosis scores for the severity of necrosis in a rat model of PCM-induced acute liver damage and CJFJ supplementation; **p < 0.01 vs. PCM, ***p < 0.001 vs. PCM.*

DISCUSSION

Paracetamol-induced hepatotoxicity in rats is a highly reproducible model that closely mimics acute liver injury in humans. It is primarily driven by the formation of the reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI), which depletes intracellular reduced glutathione (GSH), causes mitochondrial dysfunction and lipid peroxidation, inflammatory response and hepatocellular necrosis [3,12,15]. The histopathological findings in the current study—centrilobular and bridging necrosis, and inflammatory infiltration—are consistent with the principal mechanism of paracetamol toxicity and confirm the validity of the experimental model.

This experiment demonstrated that CJFJ exerted a dose-dependent hepatoprotective effect in PCM-induced liver damage in rats. The medium and the high dose (5 and 10 mL/kg) succeed in limiting the PCM-induced liver damage to a higher extent without being able to completely prevent it. The observed preservation of hepatic architecture and reduction in centrilobular and bridging necrosis in the treated animals can be attributed, at least in part, to the antioxidant properties of *Chaenomeles japonica,* which were proven by the ORAC and HORAC assays, and to the high polyphenol content of the juice. The CJFJ used in this experiment was a rich source of biologically active compounds, particularly polyphenols and vitamin C, which confer significant antioxidant and anti-inflammatory properties [13]. Polyphenols are capable of scavenging reactive oxygen species (ROS), restoring intracellular GSH levels and modulating endogenous antioxidant systems, thereby limiting oxidative damage [16,17,18]. The bioactive compounds present in *Chaenomeles japonic*a fruits, particularly flavonoids such as quercetin, epicatechin, and catechin, as well as phenolic acids, are known to directly scavenge ROS. This mechanism likely contributed to the liver protection and improved hepatocyte integrity [19]. A study has revealed the protective activity of epicatechin against CCl_4-induced liver damage, which was associated with a direct scavenging effect against free radicals [20]. Treatment with green tea catechins has attenuated iron-induced ferroptosis by reducing the levels of oxidative radicals in hepatocytes [21]. Vanillic acid has also shown promising hepatoprotective actions by enhancing antioxidant potential, reducing oxidative stress, and preserving hepatocyte integrity in drug-induced liver toxicity [22]. In chemical-induced liver injury models (e.g., CCl_4, acetaminophen, and xenobiotics), *Chaenomeles* extracts decreased serum aminotransferases and mitigated oxidative stress by reducing ROS formation and lipid peroxidation while restoring intracellular glutathione levels and enhancing endogenous antioxidant enzyme activity, indicating a central role for modulation of redox homeostasis [7,11,16].

Inflammation is another key contributor to the progression of liver injury. The PCM in this experiment caused inflammatory response in the liver which was reduced by the CJFJ treatment. The observed hepatoprotection by CJFJ might be mediated by suppression of inflammatory signaling pathways. Extracts from *Chaenomeles speciosa* have been

shown to possess anti-inflammatory properties by regulation of inflammatory cytokines [7,23].

Another important mechanism underlying the observed hepatoprotective effect might be the modulation of PCM metabolism. The inhibition of cytochrome P450 2E1 (CYP2E1), the key enzyme responsible for NAPQI formation, reduces the generation of toxic metabolite, thus preventing its covalent binding to mitochondrial proteins. Polyphenols are known to downregulate CYP2E1 activity and expression, thereby limiting NAPQI formation and subsequent protein adduct accumulation, ultimately contributing to the reduction of hepatocellular necrosis [24].

In summary, in the present study, treatment with CJFJ, particularly at medium and high doses, reduced the zones affected by necrotic and inflammatory changes, but the observed effect was not uniform. While the liver damage was limited to a considerable extent, the juice did not completely prevent liver injury. The histopathological improvements observed following administration of CJFJ are supported by multiple mechanistic pathways reported in the literature. These include enhancement of antioxidant defenses, inhibition of toxic metabolite formation, and preservation of mitochondrial function and suppression of inflammatory responses [3,16,18,23,24]. Together, these effects highlight the therapeutic potential of *Chaenomeles japonica* as a natural hepatoprotective agent in paracetamol-induced liver injury.

CONCLUSION

The histopathological findings of this study showed that CJFJ exerted a dose-dependent hepatoprotective effect in PCM-induced liver injury likely due to its polyphenol-rich content. These results support the potential of CJFJ as a natural hepatoprotective agent and suggest further investigations for development of safe, plant-based interventions for the prevention and management of liver disorders.

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