

CATECHIN AMELIORATES MARKERS OF HEPATIC DAMAGE AND MODULATES IMMUNE RESPONSES IN CARBON TETRACHLORIDE-INTOXICATED RATS

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Abstract

The pathogenesis of drug- or toxin-induced hepatic damage usually involves toxic metabolites that either elicit immune responses or directly impact cellular metabolism. This study evaluates the hepatoprotective, antioxidant and anti-inflammatory activities of (+)-catechin in carbon tetrachloride (CCl₄)-intoxicated Wistar rats. Male Wistar rats randomized into five groups (n = 7) were used for this study. Liver injury was caused using a single intraperitoneal (*ip*) shot of CCl₄ laced in olive oil, while treatments with silymarin and (+)-catechin were orally administered, once daily, for 7 days. Blood samples were collected via cardiac puncture from fasted anesthetized rats for biochemical analyses, and the liver excised for histology and molecular studies. Malondialdehyde-MDA, serum alanine/aspartate aminotransferase-ALT/AST, alkaline phosphatase-ALP, and gamma glutamyl transferase-GGT activities, as well as pro-inflammatory markers; cyclooxygenase-COX-2, interleukin-IL-1 β , interleukin-IL-6 and tumor necrosis factor-TNF- α were significantly ($p < 0.05$) increased, while albumin, reduced glutathione-GSH, superoxide dismutase-SOD and catalase-CAT activities were decreased, with their genes (SOD and CAT) downregulated in Group II rats compared to the control. However, (+)-catechin in a dose dependent manner, significantly ($p < 0.05$) improved albumin levels, lowered hepatic enzymes activities, suppressed inflammatory markers and enhanced the antioxidant status of treated rats. Therefore, these findings support the hepatoprotective, antioxidant and immunomodulatory potentials of (+)-catechin. The results contribute to the existing literature on evidence of catechin as a pharmacological molecule of interest in drug discovery, and highlights the need for its exploits as an adjuvant, supplement or a fortification in therapeutic preparations.

Keywords: antioxidants, carbon tetrachloride, (+)-catechin, hepatic damage, inflammation.

Introduction

The liver is one of the largest organs of vertebrates and the major site of metabolism (Judge and Dodd 2020). Essentially it is linked to almost all biochemical pathways relating to growth, nutrients and energy supply, immunity, reproduction, etc. The liver in addition to its physiological functions protects against hazardous drugs and chemicals via detoxification of xenobiotics. It is estimated that approximately two million people worldwide die yearly from liver related diseases, out of which one million are as a result of complications from cirrhosis, and the other one million are due to hepatocellular carcinoma and viral hepatitis (Asrani et al. 2019, Sung et al. 2020). These diseases of the liver have been associated with toxicants and

certain drugs which are of prime toxicological concerns requiring public health interventions (Asrani et al. 2019, Vornoli et al. 2022).

An example of such chemicals is carbon tetrachloride (CCl_4), a non-naturally occurring aliphatic hydrocarbon. It is a well-established toxin that is used in experimental models, where it targets the liver and kidney (Oriakhi and Orumwensodia 2021, Alejolowo et al. 2025, Mrwad et al. 2025). In hepatocytes, CCl_4 is converted by cytochrome P450 oxygenase to a highly reactive trichloromethyl radical (CCl_3^*) that mediates the transfer of a single electron to the C-Cl bond, giving a radical anion as a transient intermediate that eliminates chlorine to form the carbon centered radical, trichloromethyl (CCl_3^*) radical and chloride. These activated radicals bind covalently with macromolecules and induce peroxidative degradation of the polyunsaturated fatty acid-rich endoplasmic reticulum, leading to formation of lipid peroxides. This in turn gives products like malondialdehyde (MDA), which is an indicator of membrane damage, as well as a chain of other debilitating consequences (Hameed et al. 2022, Singh et al. 2024, Amadikwa et al. 2025). In the liver, these processes are followed by the generation of inflammatory markers from activated hepatic macrophages, thought to initiate the CCl_4 -induced liver damage (Qiang et al. 2020, Oriakhi and Orumwensodia 2021, Hameed et al. 2022, Algefare et al. 2024).

Meanwhile, there is a growing need to complement orthodox medications with less expensive alternatives that elicit fewer side effects and are readily available. Consequently, assaults on the liver are believed to be relieved by plant interventions containing polyphenols such as catechin. Catechins belong to the flavonoid class of polyphenols and are present in high concentrations in a variety of fruits, vegetables and beverages (Dias et al. 2021, Chen et al. 2022, Yao et al. 2025). They are characterized by the presence of a benzopyran structure bearing at least one aromatic ring (Figure 1) and are famed for a host of bioactivity including their role as potent antioxidant molecules (Hidalgo et al. 2022, Yao et al. 2025). Esterification and dimerization of catechin with compounds like itself, gallic acid and other phenolics result in the formation of polyphenolics such as procyanidins, gallocatechin, epicatechin gallate, epigallocatechin, etc.

Previous studies on the hepatoprotective properties of catechin either focused on catechin-rich green tea extract, which also contains several other compounds (Hodges et al. 2020), or combining catechin with other molecules like gallic acid (Oriakhi and Orumwensodia 2021), hydrated catechin (Jing et al. 2018), etc. Nevertheless, there is scarce knowledge on a single-molecule study of catechin amelioration of CCl_4 -induced liver toxicity in rats, conducted under standardized experimental conditions. Thus, it is possible that treatment with catechin alone may suppress oxidative damage like that mediated by CCl_4 and ameliorate its toxic effects. This gap creates a challenge in accurately determining its hepatoprotective potential, which is essential for pharmacological interventions and drug discovery initiatives.

Therefore, this study was designed to address this specific knowledge gap. We aimed to provide further insight into the hepatoprotective effect of catechin, as a single molecule, in CCl_4 intoxicated rats. A multi-parametric approach, including antioxidant, anti-inflammatory, molecular and histopathological analysis was used. We provide a comprehensive and robust assessment of its healing potential, contributing crucial data for therapeutic interventions. This study is expected to advance research translation of the hepatic benefits of catechin to help manage liver-associated morbidity.

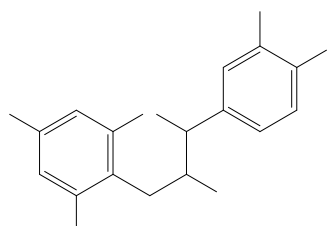


Figure 1: (+)-catechin

Materials and Methods

Drugs and Chemicals

Enzyme assay kits and those for total protein, albumin and bilirubin were obtained from Randox Laboratories Ltd. (Admore, Crumlin, Co-Antrim, UK). Other drugs/chemicals including (+)-catechin were purchased from Sigma-Aldrich (St. Louis, MO, USA) and were of analytical grade with > 99% purity.

Preparation of (+)-catechin

A gram of (+)-catechin was placed into a clean beaker and mixed with 10.0 mL of distilled water under continuous stirring to obtain a homogeneous stock solution. This test compound was prepared fresh daily.

Animals and Experimental Design

Seven weeks old male albino rats (Wistar strain) weighing between 150.0-170.0 g and bred in the animal house of the Department of Biochemistry, University of Benin were used for this study. They were kept in clean galvanized cages, acclimatized for a week and maintained under a 12 h light/dark cycle in accordance with the guidelines for laboratory animal care (ILAR 2011). All animals were placed on a meal of distilled water and rat chow, *ad libitum*, throughout the experiment. The ARRIVE guideline 2.0 on reporting animal studies was followed in conducting this study. Ethical clearance was obtained from the Ethical Review Committee of the Faculty of Life Sciences, University of Benin (Ref No. LS20014).

Following a 7-day acclimatization period, rats (n = 7 per group) were assigned to one of five groups. Carbon tetrachloride (1.25 mL/kg b. wt.) was administered, *ip*, as a single dose in olive oil suspension (1:1) before the administration of treatments, while treatments were orally given through a gastric tube, once daily, for 7 days as shown below.

Group I: received distilled water only and a single dose of olive oil

Group II: received CCl₄ only

Group III: received CCl₄ then silymarin (50 mg/kg b. wt. of rat) 24 h post intoxication

Group IV: received CCl₄ then (+)-catechin (50 mg/kg b. wt. of rat) 24 h post intoxication

Group V: received CCl₄ then (+)-catechin (100 mg/kg b. wt. of rats) 24 h post intoxication

Justification for dose and administration route

The selected dose of CCl₄ (1.25 mL/kg b. wt.) was based on previous studies that demonstrated its sufficiency in inducing hepatocyte degeneration (Ojeaburu and Oriakhi 2021, Oriakhi and Orumwensodia 2021). Olive oil served as an adjuvant to douse the toxicant for effective delivery and assimilation. Also, the dose of catechin (50 and 100 mg/kg b. wt.) were arrived at based on our preliminary acute toxicity and dose-response studies (Data in Press). The intraperitoneal route was chosen because it is a clinically relevant route for both vaccine adjuvants, toxicant and certain nanotherapeutics, allowing for controlled systemic exposure (Hawk et al. 2005, Al Shoyaib et al. 2019, Silva et al. 2021, Yao et al. 2025).

Sample Collection and Preparation

Blood samples were obtained for biochemical analyses via cardiac puncture from overnight-fasted anesthetized (Ketamine/Xylazine: 1.0 mL/kg b. wt.) rats, after the 8-day study. The liver was immediately excised and portions were homogenized with a homogenizer (ART Prozess and Labortechnik, Germany) in ice-cold phosphate-buffered saline to get the homogenates. These were centrifuged for 10 min at 5000 rpm to obtain the supernatants which were preserved at -20°C until needed for the estimation of superoxide dismutase (SOD) and catalase (CAT) activities, as well as MDA concentration, according to the methods (Asru 1972: Misra and Fridovich 1972: Buege 1978), respectively. Liver samples for histology were immediately preserved in 10% formalin-buffered saline. They were then dehydrated in graded alcohol, deparaffinized, hydrated and stained using haematoxylin-eosin staining (Drury 1980).

Thereafter, slides of tissue sections were examined for anatomical features under a binocular light microscope.

Biochemical Analysis

The following biochemical assays were conducted according to standard procedures; alkaline phosphatase- ALP (Kochmar and Moss 1976); γ -glutamyl trans peptidase- GGT (Teitz 1987); aspartate transaminase- AST, alanine transaminase- ALT (Reitman and Frankel 1957); reduced glutathione- GSH (Beutler et al. 1963), albumin- ALB, total and direct bilirubin (Jendrassik and Grof 1938), total protein- TP (Tietz 1995).

Molecular Studies

Quantitative Gene Expression by Real-time-PCR

The IL-1 β , IL-6, TNF- α , and COX-2 gene expressions were quantified using quantitative real-time polymerase chain reaction (qRT-PCR). Ribonucleic acid (RNA) estimation was carried out in triplicate determinations on samples of the liver, which were put in RNAlater contained in Eppendorf tubes. After 3 h, RNA was extracted from harvested cells with Trizol (Ambion, TX, USA), according to the manufacturer's guide. Quantification of samples was done with Nano Drop 2000 spectrophotometer (Thermo Scientific, DE, USA). The reverse transcription of 2.0 μ g of RNA was performed using oligo deoxy-thymidine (dT) primers (Promega, WI, USA). The real-time quantitative polymerase chain reaction (PCR) amplification and detection were performed with 20.0 ng of cDNA on optical-grade 48-well plates in an Eco Real-Time PCR System (Illumina, CA, USA), the KAPA SYBR FAST qPCR Master Mix (Kapa Biosystems, Inc., MA, USA) and specific primers at their annealing temperature. The expression of glyceraldehyde 3-phosphate dehydrogenase (GAPDH), which served as the house keeping gene was estimated for comparative reference and normalization of messenger ribonucleic acid (mRNA) expression, while the relative quantitation of mRNA (fold change) was estimated using the expression; $2^{(-\Delta\Delta C(T))}$ (Livak and Schmittgen 2001). The gene and primer sequences used for the real-time RT-PCR analysis are as follows (were F – forward sequence and R - reverse); IL-1 β primers (F: 5'-CCTTCCAGGATGAGGACATGA -3', R: 5'-TGAGTCACAGAGGATGGGCTC -3'), IL-6 primers (F: 5'-GAGGATACTCACTCCCAACAGACC -3', R: 5'-AAGTGCATCATCGTTGTTTCATACA -3'), COX-2 primers (F: 5'-CAGACAACATAAACTGCGCCTT -3', R: 5'-GATACACCTCTCCACCAATGACC -3'), TNF- α primers (F: 5'-TCAGCCGATTTGCTATCTCATA-3', R: 5'-AGTACTTGGGCAGATTGACCTC-3'), GAPDH primers (F: 5'-CCCATCACCATCTTCCAGGAGC-3'), R: 5'-CCAGTGAGCTTCCCGTTCAGC-3').

Data analysis

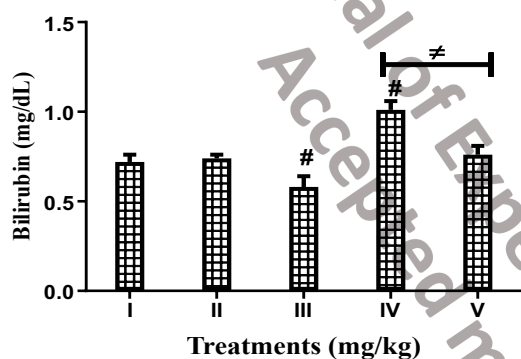
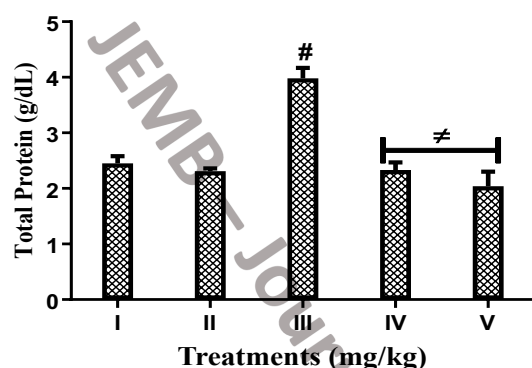
The values presented represent Mean $\pm$ Standard Error of Mean (SEM) and were analyzed for statistical difference using the IBM SPSS software (version 21.0). One-way analysis of variance (ANOVA) followed by posthoc Duncan's test for multiple comparisons were used to determine statistical difference. Differences were considered statistically significant at $p < 0.05$ according to (Levesque 2007). Statistical variations in comparison with negative control, positive control or reference drug groups were represented by *, # and $\neq$, respectively.

Results

Hepatic markers findings

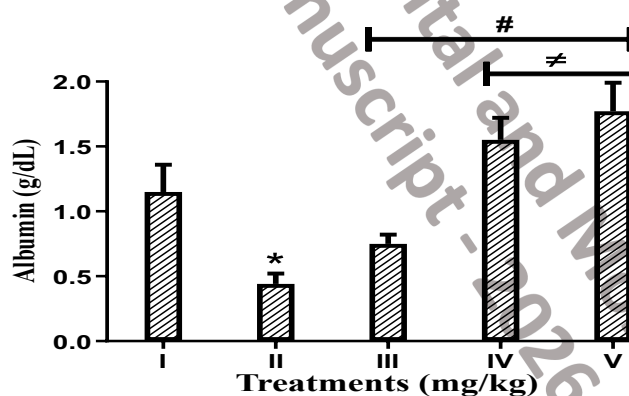
Figure 2, shows the effect of (+)-catechin on total protein, bilirubin and albumin in CCl₄-intoxicated Wistar rats. The result indicated a non-significant reduction in total protein, significant reduction in albumin and a non-significant elevation in bilirubin levels in rats given CCl₄ only (Group II), compared to the control rats (Group I). Administration of (+)-catechin at doses of 50 and 100 mg/kg resulted in a non-significant elevation of total protein concentrations

compared with Group II rats. While both doses of (+)-catechin significantly increased the concentrations of albumin in treated rats, only (+)-catechin (100 mg/kg) lowered bilirubin levels. Meanwhile, silymarin decreased serum bilirubin levels and significantly increased serum protein and albumin concentrations.



(2A)

(2B)



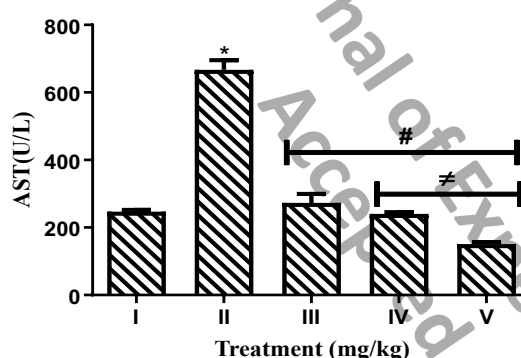
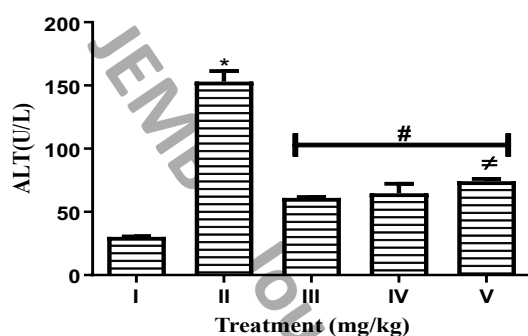
(2C)

Figure 2: Effect of Catechin on Total protein, Bilirubin and Albumin in CCl₄-Intoxicated Wistar rats. Data represent Mean $\pm$ SEM (n=7). 2A = Effect of catechin on total protein conc., 2B = Effect of catechin on bilirubin conc., 2C = Effect of catechin on albumin conc. I = Control, II = CCl₄, III = CCl₄ + Silymarin, IV = CCl₄ + Cat (50 mg/kg), V = CCl₄ + Cat (100 mg/kg). *The mean is significant ($p < 0.05$) when compared with the control; # The mean is significant ($p < 0.05$) when compared with CCl₄; $\neq$ The mean is significant ($p < 0.05$) when compared with CCl₄ + silymarin.

Serum biochemical findings

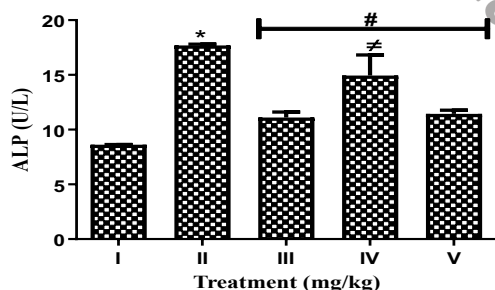
Figure 3 shows the effect of (+)-catechin on serum hepatic enzyme (ALT, AST, ALP and GGT) activities in CCl₄-intoxicated Wistar rats. The results indicated significant elevation in serum levels of hepatic enzymes in rats induced with CCl₄ only as against rats in the control group.

However, treatment with 50 and 100 mg/kg (+)-catechin, significantly lowered serum enzymes (ALT, AST, ALP and GGT) activity when compared with the CCl₄ administered group. Similarly, treatment with silymarin significantly reduced serum hepatic enzyme activities compared to CCl₄ administered group.

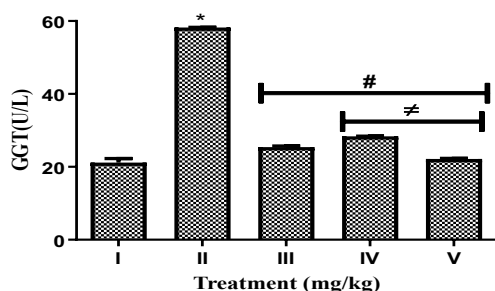


(3A)

(3B)



(3C)



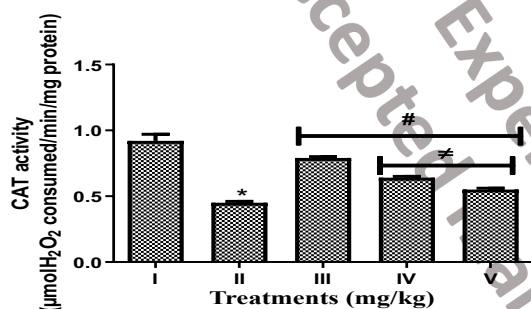
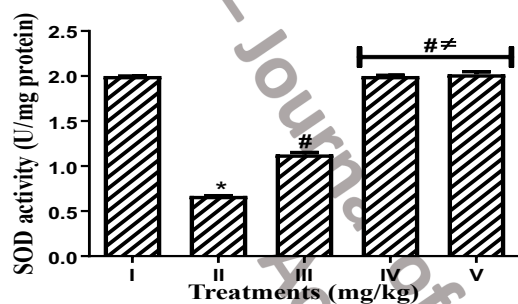
(3D)

Figure 3: Effect of Catechin on Serum Hepatic Enzymes activities in CCl₄-Intoxicated Wistar rats. Data represent Mean $\pm$ SEM (n=7). 3A = Effect of catechin on ALT activity, 3B = Effect of catechin on AST activity, 3C = Effect of catechin on ALP activity, 3D = Effect of catechin on GGT activity. I = Control, II = CCl₄, III = CCl₄ + Silymarin, IV = CCl₄ + Cat (50 mg/kg), V = CCl₄ + Cat (100 mg/kg). *The mean is significant ($p < 0.05$) when

compared with the control; # The mean is significant ($p < 0.05$) when compared with CCl₄; ≠ The mean is significant ($p < 0.05$) when compared with CCl₄ + silymarin.

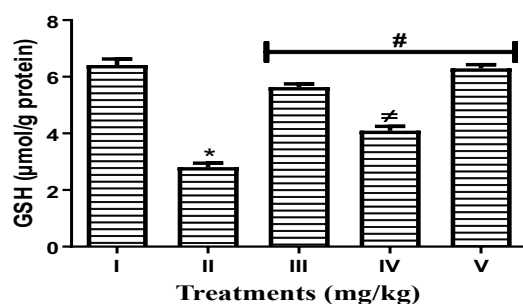
Oxidative stress and antioxidant biomarkers findings

Figure 4, represents the effect of (+)-catechin on the activities of antioxidant enzymes and lipid peroxidation status in CCl₄-intoxicated Wistar rats. The assault from CCl₄ led to a significant decrease in activities of antioxidant enzymes (SOD and CAT), and GSH levels. Similarly, there was increased peroxidation as indicated by elevated MDA levels. However, treatment with (+)-catechin (50 and 100 mg/kg) and silymarin significantly reduced the levels of lipid peroxidation, and increased activities of antioxidant enzymes compared to the untreated CCl₄ exposed group.

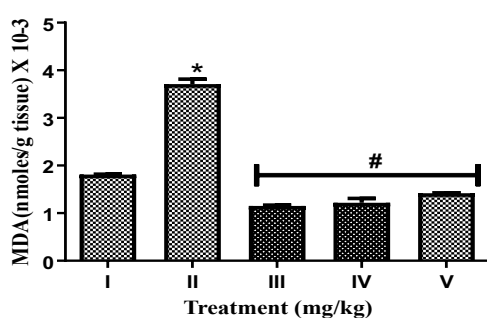


(4A)

(4B)



(4C)



(4D)

Figure 4: Effect of Catechin on Antioxidant enzymes and Lipid Peroxidation in CCl₄-Intoxicated Wistar rats. Data represent Mean ± SEM (n=7). 4A = Effect of catechin on SOD

activity, 4B = Effect of catechin on CAT activity, 4C = Effect of catechin on GSH conc., 4D = Effect of catechin on MDA conc. I = Control, II = CCl₄, III = CCl₄ + Silymarin, IV = CCl₄ + Cat (50 mg/kg), V = CCl₄ + Cat (100 mg/kg). *The mean is significant ($p < 0.05$) when compared with the control; # The mean is significant ($p < 0.05$) when compared with CCl₄; ≠The mean is significant ($p < 0.05$) when compared with CCl₄ + silymarin.

ene expression analysis on antioxidant markers

Figure 5, represents the effect of (+)-catechin on expression of SOD and CAT genes in CCl₄-intoxicated Wistar rats. The assault from CCl₄ led to downregulation of antioxidant genes with a significant repression of the SOD genes. However, treatment with (+)-catechin (50 and 100mg/kg) and silymarin led to a significant upregulation of these antioxidant genes especially those for SOD when compared with groups 1 and 2.

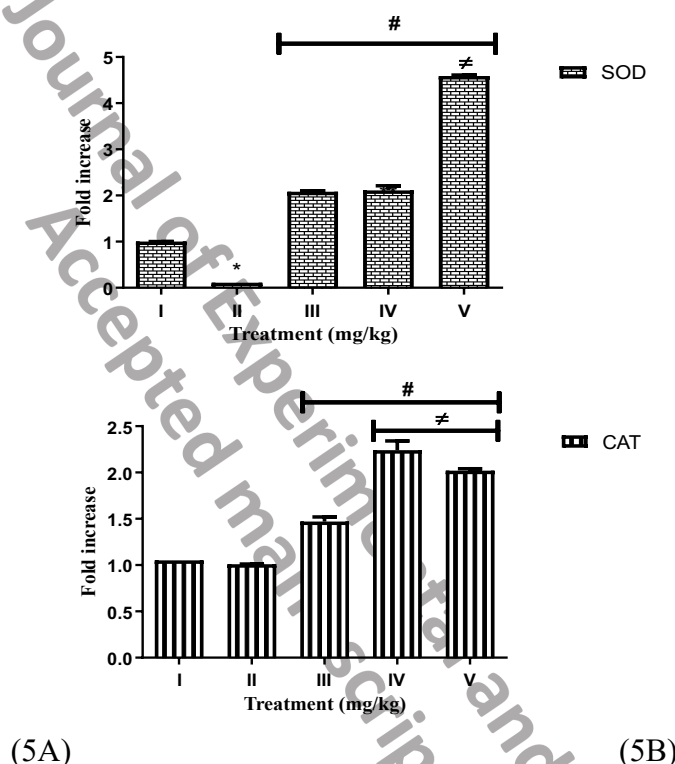


Figure 5: Effect of Catechin on Expression of SOD and CAT Genes in CCl₄-Intoxicated Wistar rats. Data represent Mean $\pm$ SEM (n=7). 5A = Effect of catechin on expression of SOD gene, 5B = Effect of catechin on expression of CAT gene. I = Control, II = CCl₄, III = CCl₄ + Silymarin, IV = CCl₄ + Cat (50 mg/kg), V = CCl₄ + Cat (100 mg/kg). *The mean is significant ($p < 0.05$) when compared with the control; # The mean is significant ($p < 0.05$) when compared with CCl₄; ≠The mean is significant ($p < 0.05$) when compared with CCl₄ + silymarin.

Gene expression analysis on Inflammatory Markers

Figure 6, shows the effect of (+)-catechin on the expression of COX-2, TNF- α , IL-1 β and IL 6 in CCl₄-intoxicated Wistar rats. Results show significant upregulation of genes of COX-2, IL-1 β , IL 6 and TNF- α in rats administered CCl₄ compared to those of the control group. But treatment with (+)-catechin significantly repressed the expression of COX-2, IL-1 β and IL-6 inflammatory markers. Also, silymarin repressed the genes of these markers including TNF- α , which was significantly expressed in rats treated with both doses of (+)-catechin as compared to those administered CCl₄ only.

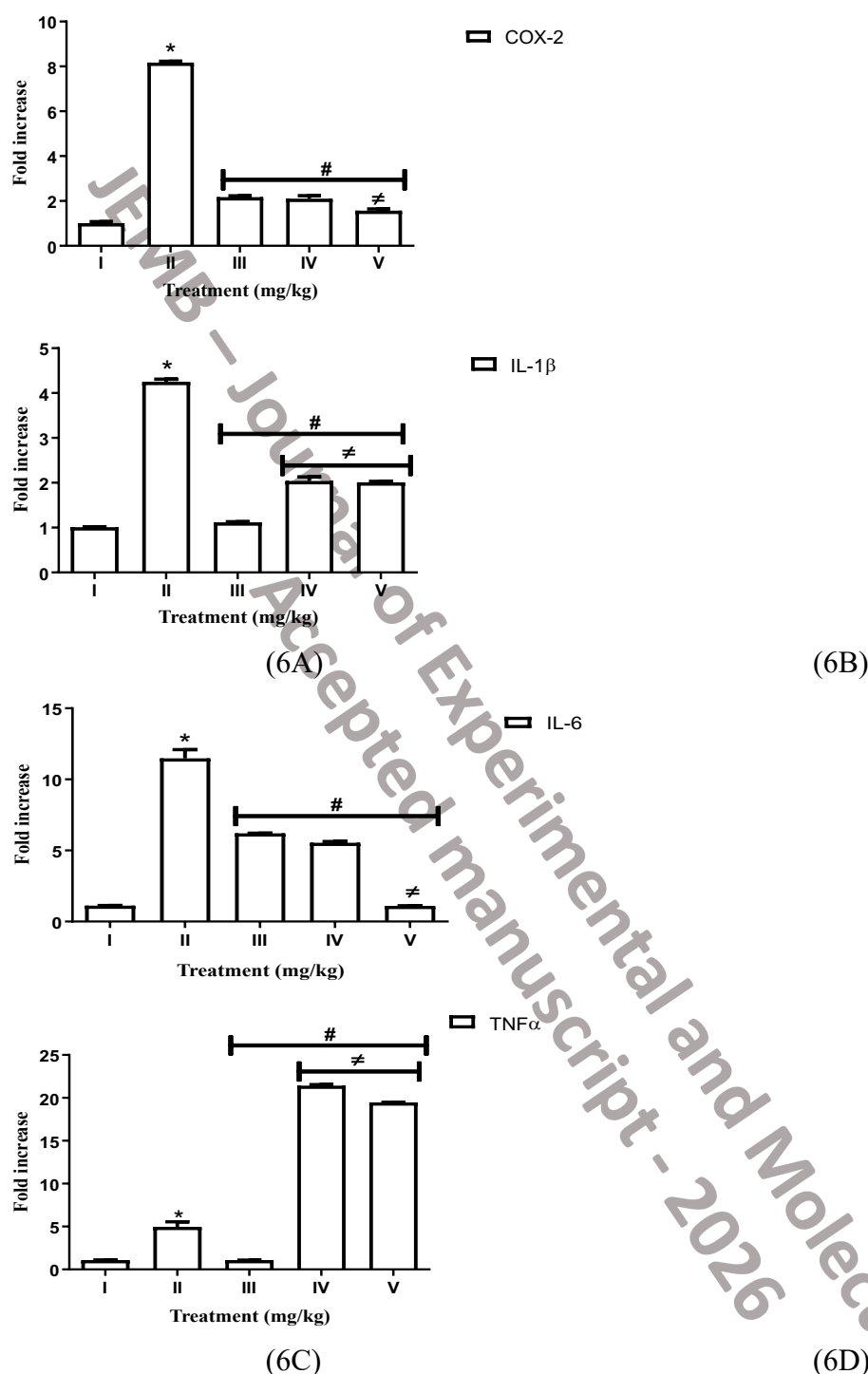


Figure 6: Effects of Catechin on Expression of Inflammatory Markers in CCl₄-Intoxicated Wistar rats. Data represent Mean $\pm$ SEM (n=7). 6A = Effect of catechin on expression of COX-2 gene, 6B = Effect of catechin on the expression of IL-1 β gene, 6C = Effect of catechin on expression of IL-6 gene, 6D = Effect of catechin on expression of TNF- α gene I = Control, II = CCl₄, III = CCl₄ + Silymarin, IV = CCl₄ + Cat (50 mg/kg), V = CCl₄ + Cat (100 mg/kg). *The mean is significant ($p < 0.05$) when compared with the control; # The mean is significant ($p < 0.05$) when compared with CCl₄; ≠The mean is significant ($p < 0.05$) when compared with CCl₄ + silymarin.

Histopathological findings

Figure 7, shows the photomicrograph of the effect of (+)-catechin on liver morphology of Wistar rats intoxicated with CCl₄. A cross-sectional observation revealed pronounced

intracytoplasmic fat vacuoles (macrovesicular steatosis), heavy portal congestion and heavy periportal inflammatory infiltrates in rats administered CCl₄ only. However, treatment with silymarin and (+)-catechin demonstrated restoration, which was comparable to control rats and marked by healthy hepatocytes, slight portal congestion, slight periportal inflammatory infiltrates and reduced intracytoplasmic fat vacuoles (microvesicular steatosis), and activation of Kupffer cell.

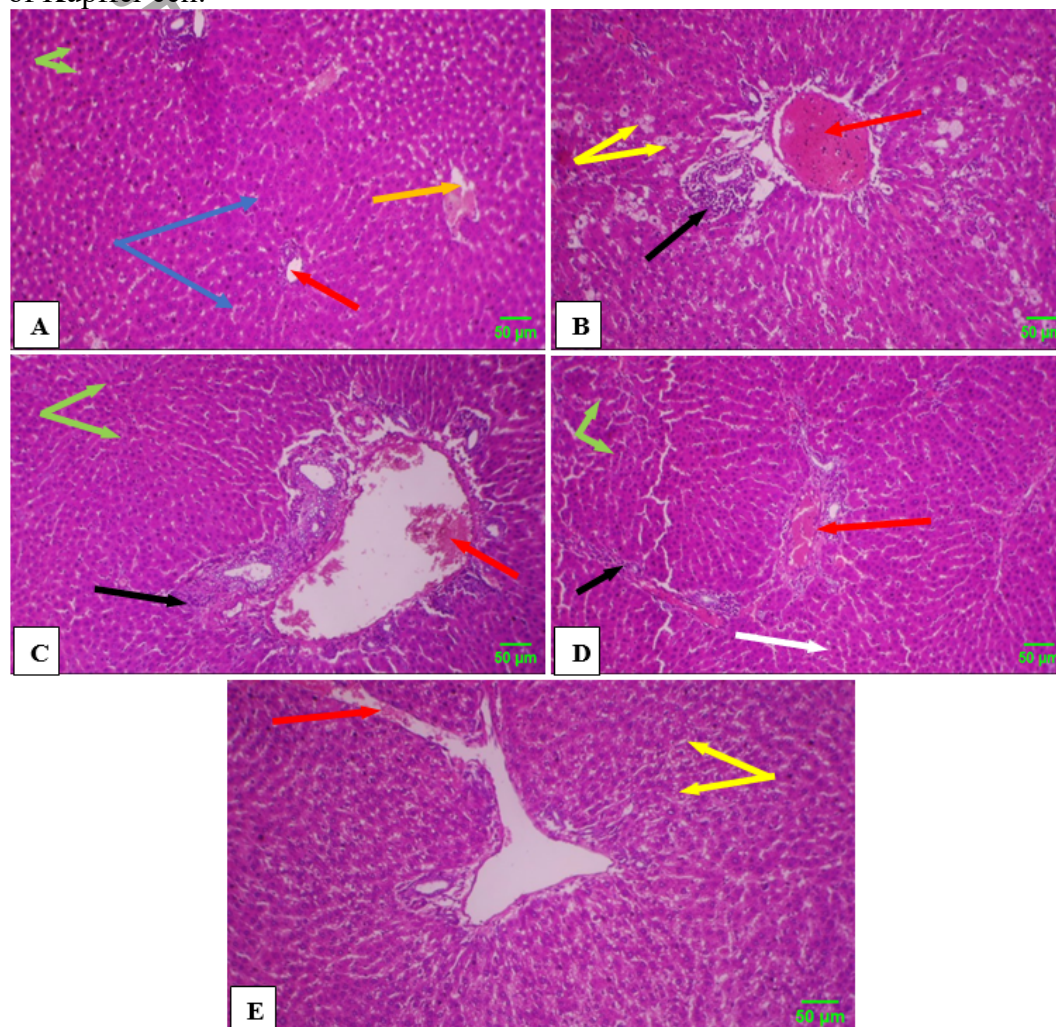


Figure 7: Photomicrograph (x400) of the Effect of Catechin on Liver Histology of Wistar Rats Intoxicated with CCl₄. A (Group I, normal control), green arrows: normal hepatocytes, blue arrows: sinusoids, orange arrow: central vein and red arrow: portal vein; B (Group II, CCl₄ control), yellow arrows: large intracytoplasmic fat vacuoles (macrovesicular Steatosis), red arrows: heavy portal congestion and black arrow: heavy periportal inflammatory infiltrates. C (Group III, CCl₄ + Silymarin), green arrows: normal hepatocytes, red arrow: mild portal congestion and black arrow: mild periportal inflammatory infiltrates. D (Group IV, CCl₄ + Cat 50 mg/kg), green arrows: normal hepatocytes, red arrow: mild portal congestion and black arrow: mild periportal inflammatory infiltrates and white arrow: Kupffer cell activation. E (Group V, CCl₄ + Cat 100 mg/kg), blue arrows: reduced intracytoplasmic fat vacuoles (microvesicular steatosis) and red arrow: mild portal congestion.

Discussion

The pathogenesis of drug- or toxin-induced hepatic damage often involves toxic metabolites that either trigger immune responses or directly impact cellular metabolism. This study

examined the ameliorative effect of (+)-catechin in liver injury caused by CCl₄ toxicity in murine model, and the possible targets employed in cellular recovery. This model is well characterized for the evaluation of the hepato-protective potential of herbal/natural products; given that CCl₄ is an established hepato-toxin (Hameed et al. 2022, Alejolowo et al. 2025, Amadikwa et al. 2025). Results from Group II (intoxicated, but untreated rats) compared with the control (naive) rats (Group I), revealed mild reduction in total protein and a significant decrease in albumin, although bilirubin was not affected. Perturbations in the normal levels of either or all of these molecules, whose metabolisms are linked to the liver, could signify a potential liver challenge. Also, there were elevated serum activities of liver enzymes (ALT, AST, ALP and GGT) post-intoxication, further confirming hepatic membrane compromise. Increased serum activities of hepatic enzymes are potent markers of hepatic injury (Panda et al. 2016, Fu et al. 2020, Algefare et al. 2024) and in this case, could be credited to the heightened oxidative stress triggered by CCl₄. But, (+)-catechin was able to significantly improve serum levels of albumin, while fairly maintaining the levels of total protein and bilirubin in favour of recovery when compared with groups 1 and 2 rats. However, catechin at a dose of 50 mg/kg b. wt. did not resolve the derangement associated with serum bilirubin levels. Nonetheless, there was a significant dose-dependent reduction in serum enzyme activities of AST, ALT, ALP and GGT in treated rats.

The high level of oxidative stress post-CCl₄ administration was further confirmed following increased lipid peroxidation, marked by elevated MDA levels, reduced SOD and CAT activities, and a cellular decline in GSH concentration. Similarly, exposure to CCl₄ led to the downregulation of survival genes coding for these antioxidant enzymes (SOD and CAT), with significance recorded for SOD genes. Aside cellular processes that generate free radicals leading to oxidative stress, the aggressive trichloromethyl radical (CCl₃^{*}) released during the breakdown of CCl₄ can significantly weigh down the endogenous antioxidant defense mechanism leading to a reduction in antioxidant enzymes activities, decreased concentration of antioxidant molecules such as GSH, and repression of survival genes (Bernatoniene and Kopustinskiene 2018, Oriakhi and Orumwensodia 2021, Hameed et al. 2022, Algefare et al. 2024). While, the dismutation of superoxide radicals by SOD into hydrogen peroxide with subsequent conversion into oxygen and water by CAT is a very efficient system of elimination of potentially toxic materials, GSH on the other hand, complements these processes by replenishing oxidized or spent glutathione-related enzymes in a series of reactions involving γ -glutamyl transaminase (GGT), thus drawing a nexus between increased GGT activity during oxidative stress and GSH depletion observed in this study. Hence, a decline in the potency of these vital, yet, naturally occurring antioxidant defense mechanisms has been linked to the etiology or pathology of numerous clinical disorders (Liguori et al. 2018, Forman and Zhang 2021, Hidalgo et al. 2022, Cecerska-Heryć et al. 2025, Nguyen et al. 2025). Meanwhile, treatment with both doses of (+)-catechin alleviated the stress condition to near normal by significantly improving cellular levels of SOD, CAT and GSH. This corroborates the previous findings of Oriakhi and Orumwensodia (2021) and Chen et al (2022), who both reported improved activities of SOD, CAT, and cellular levels of GSH post-administration of catechin. There was also significant expression of SOD genes, suggestive of recovery post-treatment. These results compared favourably with those obtained for the reference drug, silymarin.

Furthermore, increased oxidative stress has been linked to elevation of inflammatory markers. Studies conducted by (Shapouri-Moghaddam et al 2018, Zuo et al. 2019, Rowaiye et al. 2021, Hunt et al. 2025, Liu et al 2025, Nigam et al. 2025), reported that reactive oxygen species (ROS) can function as second messengers activating nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B), which orchestrates the expression of an array of genes involved in inflammatory responses through pathways such as JAK/STAT, PI3K and p38-MAPK, and a non-traditional inflammatory pathway, the Hippo pathway. The unregulated generation of these

inflammatory markers is implicated in chronic inflammation and tissue damage (Chiurchiù et al. 2018, Abdel-Azeem et al. 2019, Choi et al. 2024, Hunt et al. 2025, Liu et al. 2025). This perhaps explains our findings, where a significant fold-increase was noticed in the expression of genes coding for the inflammatory markers; COX-2, IL-1 β , IL 6 and TNF- α , in rats assaulted with CCl₄. But (+)-catechin administration at both doses markedly repressed pro-inflammatory genes coding for COX-2, IL-1 β and 6, although TNF- α remained unusually high after treatment, which is contrary to the usual trend reported in previous studies (Fan et al. 2017, Bernatoniene and Kopustinskiene, 2018). Tumor necrosis factor-TNF presents a remarkable functional duality, in that it is strongly involved in both tissue destruction (by triggering the processes leading to cell death through caspase 8) and regeneration/expansion (by activating NF- κ B, thus, leading to cell protection through the transcriptional upregulation of survival genes) (Wajant et al. 2003, Jing et al. 2018). However, its net action *in vivo* depends on the timing and duration of TNF action, precise cellular context and TNF-R composition, as well as the tissue type (Ağan et al., 2025). In view of this dual call of TNF upon activation, the latter route (i.e., regeneration/expansion) could possibly explain the unusually high amount of TNF- α obtained in this study post-treatment with (+)-catechin. Therefore, allowing the build-up of TNF- α in order to trigger the transcriptional upregulation of survival genes like SOD and CAT could be one of several mechanisms employed by (+)-catechin to instigate hepatic regeneration and recovery as observed in this study.

Results from histopathology demonstrated that treatment with (+)-catechin (both doses) and silymarin restored liver architecture of intoxicated rats to levels similar to those of the naïve rats. Anatomical features such as mild periportal inflammatory infiltrates, mild portal congestion, microvesicular steatosis and activated Kupffer cell, were noticed post-treatment; as against macrovesicular steatosis, heavy periportal inflammatory infiltrates and heavy portal congestion observed in the untreated intoxicated rats. This suggests that (+)-catechin possessed the potential to arrest or prevent the untoward cellular events associated with excess ROS generation. These findings resonate those of (Hodges et al. 2020, Oriakhi and Orumwensodia 2021). While Oriakhi and Orumwensodia (2021) reported improvement in liver architecture of rats administered doses of a combination of gallic acid and (+)-catechin post-intoxication with CCl₄, Hodges et al. (2020) averred that the liver recovered from injuries in clinical and preclinical observations after the administration of green tea (a potent repository for catechins), thereby attenuating intracellular redox distress and pro-inflammatory signaling from extracellular receptors that otherwise activate NF κ B.

Thus, aside functioning as ROS scavenger and metal ion chelator, catechin also plays a role as an indirect antioxidant by the induction of antioxidant enzymes or survival genes (e.g., SOD, CAT and GPx) (Jing et al. 2018, Chen et al. 2022, Hidalgo et al. 2022, Ağan et al. 2025), inhibition of pro-oxidant enzymes (e.g., NADPH oxidase, COX-2, inducible nitric oxide synthase (iNOS), Xanthine oxidase, Lipoxxygenase, etc.) (Fan et al. 2017), modulation of the interaction of signaling molecules with receptors, (Fan et al. 2017), suppression of many oxidative stress-related pathways linked to inflammatory processes (Fan et al. 2017) and the modulation of activities of the redox-sensitive transcription factors such as activator protein-1 (AP-1) and NF- κ B, which are necessary for vital responses during oxidative stress (Hameed et al. 2022). Therefore, the ameliorative potentials exhibited by (+)-catechin in this study could be ascribed to its multifunctional roles including those of mediation of inflammation and oxidative stress.

Conclusion

Current findings reveal that green tea catechin or catechin in combination with molecules like gallic acid alleviate markers of CCl₄ damage by modulating biochemical parameters and

antioxidant functions. Our findings give a wider and more precise perspective on the molecular processes underpinning the ameliorative actions of catechin, only, in CCl₄-induced liver injury. From this study, it can be inferred that (+)-catechin was able to obliterate the assault from CCl₄ on the liver through series of coordinated mechanisms including the antioxidant and inflammatory systems; by upregulating antioxidant genes, lowering oxidative stress, reducing lipid peroxidation and attenuating pro-inflammatory markers. Thus, illuminating its hepatoprotective potentials and priming it as an alternate natural remedy for the prevention or treatment of inflammatory diseases, especially that associated with the liver.

Limitations

Notwithstanding these promising outcomes, several limitations should be noted. This research utilized only a single animal model, which may not fully replicate human physiological conditions, potentially affecting the ramifications of the outcomes. Again, only two dose levels were tested. The molecular mechanisms responsible for the elevated TNF- α levels post-catechin treatment remains explicitly explored, and future research should validate catechin's role in liver regeneration (e.g., measuring NF- κ B or caspase pathway activity). Finally, the study did not assess the long-term effects of catechin on liver regeneration or its efficacy in other liver damage models (e.g., alcohol- or drug-induced models). These limitations necessitate further work to ascertain more pathways involved in catechins recovery actions and a possible translation to clinical studies, including equivalent human doses and potential challenges in practical application.

Abbreviations

ALT: Alanine transaminase, **ALP:** Alkaline phosphatase, **AST:** Aspartate transaminase, **b. wt.:** body weight, **CAT:** Catalase, **CCl₄:** Carbon tetrachloride, **COX-2:** Cyclooxygenase-2, **GGT:** gamma-glutamyl transaminase, **IL-1 β :** Interleukin-1-beta, **IL-6:** Interleukin-6, **INF- γ :** Interferon-gamma, **iNOS:** Inducible nitric oxide synthase, **JAK/STAT:** Janus kinase/signal transducer and activator of transcription, **p38-MAPK:** Mitogen-activated protein kinase, **MDA:** Malondialdehyde, **NF- κ B:** Nuclear factor kappa-light-chain-enhancer of activated B cells, **NADPH:** Nicotinamide adenine dinucleotide phosphate, **PI3K:** Phosphoinositide 3-kinase, **TNF- α :** Tumor necrosis factor-alpha, **TNF-R:** Tumor necrosis factor-receptor, **SOD:** Superoxide dismutase.

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Author Contributions

Orumwensodia KO conceptualized the study, optimized the protocols, carried out the experiments, performed statistical analysis, wrote, reviewed and edited the manuscript. **Ojeaburu SI** conceptualized the study, performed the experiment, reviewed and edited the manuscript. Both authors accept responsibility for the work, ensuring integrity and accuracy of data supplied.

Ethics approval

The experiment was approved by the Ethical Review Committee of the Faculty of Life Sciences, University of Benin, Benin City, Nigeria (Ref No. LS20014).

Institutional Review Board statement

The study was conducted based on the recommended ILAR Guide for the care and use of laboratory animals by the Faculty of Life Sciences Ethics Committee, University of Benin, Benin City, Nigeria. All precautions were followed to diminish animal suffering during the experiment.

Consent to participate

Not applicable

Competing interests

The authors declare no competing interests.

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