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RESEARCH ARTICLE

# Phycocyanin Mitigates Subacute Alcohol-induced Hepatorenal Toxicity and Impaired Body Growth in Rats

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## Abstract

**Background:** Alcohol consumption induces significant oxidative stress and inflammation, leading to damage in vital organs, particularly the liver and kidneys. **Aim:** Here, we investigate the potential therapeutic effects of phycocyanin in mitigating alcohol-induced liver and kidney damage and body growth. **Methods:** Four groups of male rats were included: control, ethanol-exposed (38% w/v, 1 ml/kg), phycocyanin co-treated (50 mg/kg phycocyanin followed by ethanol), and phycocyanin-only treated (50 mg/kg phycocyanin). **Results:** The results revealed that a 14-day exposure of rats to ethanol resulted in a decrease in body weight, with a significant increase in blood aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) activity and elevated levels of urea, creatinine, iron, and C-reactive protein compared to the control group. However, co-treatment with phycocyanin significantly attenuated these effects, whereas phycocyanin alone did not induce significant effects. Additionally, ethanol exposure increased oxidative stress, lipid peroxidation and caused histopathological damage in both the liver and kidney. Co-treatment with phycocyanin significantly improved oxidative stress markers and preserved organ structure. **Conclusion:** These findings suggest that phycocyanin represents a promising natural therapeutic strategy for the prevention and management of alcohol-induced hepatorenal damage, calcium homeostasis alteration and impaired body growth.

**Major Finding:** Phycocyanin exhibits notable antioxidant and anti-inflammatory properties, highlighting potential to improve organ health in cases of alcohol poisoning.

**Keywords:** Ethanol, Growth, Kidney, Liver, Phycocyanin

## 1. Introduction

Alcohol consumption has well-documented detrimental effects on multiple organs, notably the liver and kidneys[1-3]. Chronic alcohol intake is a major contributor to liver disorders, including fatty liver disease, alcoholic hepatitis, and cirrhosis[4-6]. The liver plays a central role in alcohol metabolism. This action involves various enzymes, affecting the rate of alcohol clearance in the body and its conversion into metabolites, especially acetaldehyde, a highly reactive intermediate that generates Reactive Oxygen Species (ROS) and contributes to oxidative stress. This oxidative burden overwhelms the liver's antioxidant defences, leading to lipid peroxidation, inflammation, and cellular apoptosis, ultimately impairing organ functions[7-9].

The major enzymes responsible for ethanol metabolism are alcohol dehydrogenase (ADH),

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aldehyde dehydrogenase (ALDH), and cytochrome P450 2E1 (CYP2E1). ADH catalyses the conversion of ethanol into acetaldehyde, which is subsequently oxidised by ALDH into acetate, a less toxic metabolite. However, during chronic alcohol consumption, CYP2E1 is induced and contributes to the excessive production of ROS, thereby increasing oxidative stress and promoting inflammatory responses[10].

The kidneys, which are essential for the excretion of alcohol and its metabolites, are similarly vulnerable to oxidative damage induced by alcohol. Both acute and chronic ethanol exposure elevate oxidative stress and inflammation in renal tissues, leading to impaired kidney function. This dysfunction is manifested by disruptions in the glomerular filtration, electrolyte imbalance, and damage to cellular integrity, ultimately contributing to the development of chronic kidney disease[11,12].

In response to these challenges, there is growing interest in natural compounds that offer protective effects against alcohol-induced damage. Phycocyanin, a blue pigment extracted from *Spirulina platensis*, has demonstrated notable antioxidant and anti-inflammatory properties in preclinical studies[13,14]. Its mechanism of action involves scavenging free radicals, reducing ROS levels, and modulating inflammatory pathways, which may provide substantial protection to liver and kidney tissues under alcohol-induced stress[15].

This article explores the physiological impact of alcohol on liver and kidney health and evaluates the emerging potential of phycocyanin as a therapeutic agent. Through biochemical analyses, oxidative stress assessments, and histopathological evaluations, we aim to elucidate phycocyanin's efficacy in mitigating ethanol-induced damage to these vital organs.

## 2. Materials and Methods

### 2.1 Experimental Protocol

Phycocyanin was provided by Bio Algues (Ksour Essef Mahdia-Tunisia). Male Wistar rats weighing 250 - 350 g at their reception were obtained from the Pasteur Institute, Tunisia. Animals were housed under controlled conditions of temperature (25°C) and a 12:12 light/dark cycle and were supplied water and food ad libitum. Rats were acclimatised 10 days before the beginning of the experiment and were cared for in compliance with the Institutional Ethics Committee code of practice for the Care and Use of Animals for Scientific Purposes. The experiment protocol was approved by the Ethics Committee of the Faculty of Sciences, Bizerta, Tunisia.

The rats were divided randomly into 4 groups, each comprising five animals. They were treated daily for 14 consecutive days according to the following: Group Control: Rats received 1 ml/kg of distilled water orally. EtOH Group: Rats received 1 ml/kg of distilled water orally, followed by an intraperitoneal injection of 1 ml/kg of 38% v/v ethanol one hour later[16], PE Group: Rats received 50 mg/kg of phycocyanin (BioAlgues Tunisia) orally[13], followed by an intraperitoneal injection of 1 ml/kg of 38% v/v ethanol one hour later[17], PC Group: Rats received 50 mg/kg of phycocyanin orally. Ethanol was administered intraperitoneally to ensure controlled and reproducible systemic exposure, minimising the variability that often arises from differences in gastrointestinal absorption and first-pass metabolism following oral gavage.

### 2.2 Biochemical Analyses

Rats were sacrificed by decapitation under slight ether anaesthesia. Blood was collected in heparinised tubes and centrifuged at 4°C at 4000 rpm for 15 minutes. Plasma was recuperated and stored at -25°C for further biochemical analyses. Aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), C-reactive protein (CRP), urea, creatinine, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), iron, and calcium levels were determined using commercial analysis Kits (BioMaghreb, Tunisia) and performed according to the manufacturer's instructions.

### 2.3 Oxidative Stress Measurement

The liver and kidney tissues were homogenised in ice-cold Tris-Buffered Saline (TBS) at pH 7.4 and then centrifuged at 4°C at 9000 rpm for 10 min. The resulting supernatants were collected for further analysis. Protein levels in the supernatants were determined using the Bradford method[18]. Superoxide dismutase (SOD) activity was measured following the method described by Misra and Fridovich[19]. To assess lipid peroxidation, malondialdehyde (MDA) levels were measured based on the Draper and Hadley method[20]. The level of hydrogen peroxide ($H_2O_2$) was determined according to the method outlined by Jabri *et al.*[21].

### 2.4 Histology

For histological analysis, some portions of liver and kidney tissue were fixed overnight in a 10% paraformaldehyde at room temperature. Samples were then dehydrated with ethanol (50-100 %), cleared with toluene series and embedded overnight in liquid paraffin. Sections of 5µm were mounted on gelatine-coated glass slides, cut and stained with haematoxylin-eosin for light microscopy observation.

### 2.5 Statistics

Statistical analysis of data was performed using a one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. Data were presented as mean ± Standard Deviation (SD). The significance was accepted at $P < 0,05$. Data was analysed using GraphPad Prism 5 software.

## 3. Results

### 3.1 Effect of Alcohol and Phycocyanin on Body Weight and Plasma Biochemical Parameters

Daily body weight remained stable across all groups until day 5 of the experiment, when a significant decrease was observed in the ethanol-exposed group compared to the control group (Figure 1). However, cotreatment with phycocyanin prevented this effect, while exposure to phycocyanin alone had no significant impact on rats' body growth.

There was a significant increase in blood enzyme activity, including ASAT, ALAT, LDH, and ALP, in the ethanol-intoxicated group compared to the control group. Additionally, rats treated with ethanol alone exhibited significantly higher levels of urea, creatinine, and iron compared to the control group. In contrast, the calcium levels in the ethanol-treated group were significantly lower than those in the control group. Furthermore, C-reactive protein, a marker of inflammation, was significantly elevated in the ethanol-treated groups compared to all other experimental groups (Table 1).

Notably, co-treatment with phycocyanin significantly restored the activity of ASAT, ALAT, LDH, and ALP to levels comparable to those of the control group. Additionally, rats co-administered with phycocyanin showed a significant decrease in urea, creatinine, and iron levels compared to the ethanol group. Ethanol ethanol-intoxicated group also displayed a high CRP level in comparison to the control rats. Interestingly, co-administering phycocyanin for 15 days of phycocyanin decreased plasma CRP level reaching the control values.

### 3.2 Effect of Alcohol and Phycocyanin on Redox Status

Our results revealed a significant increase in $H_2O_2$ and lipid peroxidation as indicated by elevated levels of MDA, in the liver of the ethanol-treated group when compared with untreated rats (Figures 2A and 2B). Additionally, SOD activity was significantly higher in the ethanol group relative to the control group. Notably, co-treatment with phycocyanin significantly reduced MDA and $H_2O_2$ levels as well as SOD activity to levels comparable to the control group. Whereas phycocyanin alone had no significant effect on the above oxidative stress biomarkers (Figure 2C).

In the ethanol-treated group, kidney levels of $H_2O_2$ and MDA were significantly higher compared to the control group (Figures 3A and 3B). The overall activity of SOD was also increased in the ethanol-treated rats relative to the other experimental groups. Interestingly, rats co-treated with phycocyanin

**Table 1.** Plasma biochemical parameters

| | AST U/l | ALT U/l | LDH U/l | ALP U/l | Urea g/l | Creatinine mg/l | Iron mg/l | Calcium mmol/l | CRP mg/dl |
|---|---|---|---|---|---|---|---|---|---|
| **Control** | 22.93±1.95 | 48.83±2.04 | 59.90±2.97 | 49.50±2.98 | 0.25±0,01 | 14.29±0.90 | 1.29±0.09 | 2.06±0.10 | 5.84±0.38 |
| **EtOH** | 47.95±3.08* | 78.23±4.22* | 177.3±14.22* | 80.30±4.79* | 0.44±0.01* | 32.57±1.45* | 1.82±0.08* | 1.39±0.06* | 26.59±1.46* |
| **PE** | 33.08±3.73# | 46.20±2.20# | 72.86±4.78# | 58.30±3.52# | 0.30±0.01# | 19.43±0.57# | 1.19±0.06# | 1.88±0.03# | 5.62±0.45# |
| **PC** | 23.98±2.26# | 41.30±4.02# | 59.90±3.70# | 45.65±2.51# | 0.24±0.01# | 13.71±1.06# | 1.29±0.06# | 2.08±0.07# | 4.72±0.81# |

Values are represented as mean ± SEM; EtOH: Ethanol-treated group; PE: Phycocyanin co-treated group at 50 mg/kg; PC: Phycocyanin treated group; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; LDH: Lactate dehydrogenase; ALP: alkaline phosphatase.
* p <0.05 as compared to the control group, # p<0,05 as compared to the ethanol treated group.

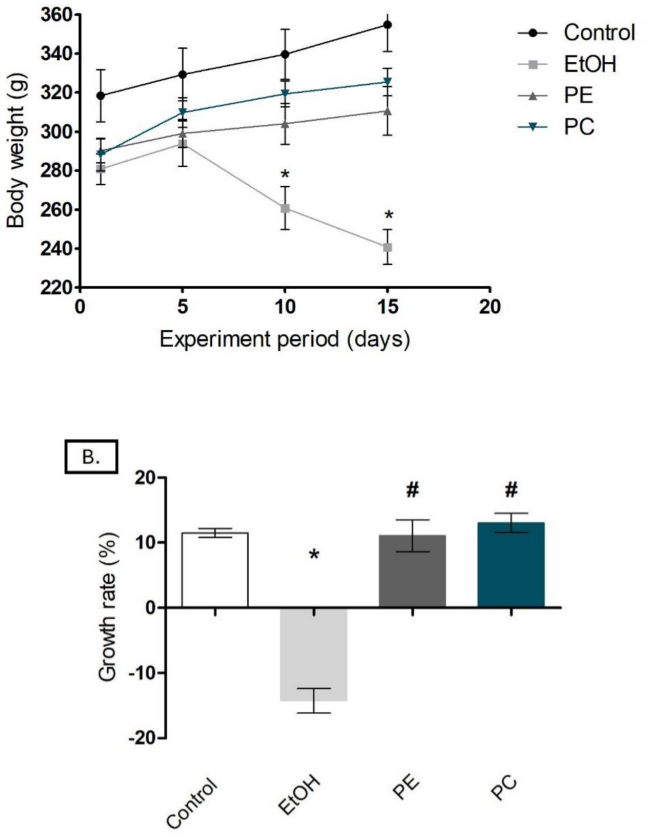



**Figure 1.** Body weight variation (A) and growth rate (B) during the experiment period.

Values are represented as mean ± SEM; EtOH: Ethanol treated group; PE: Phycocyanin co-treated group at 50 mg/kg; PC: Phycocyanin treated group; * p <0.05 as compared to the control group, # p<0,05 as compared to the ethanol treated group

exhibited significantly reduced renal $H_2O_2$ and lipid peroxidation content and SOD activity (Figure 3C). Whereas phycocyanin alone did not exhibit a significant change in these parameters.

## 3.3 Liver and Kidney Histological Examination

The liver sections from the control group (Figure 4A) and the phycocyanin-only treated group (Figure 4B) displayed a typical and well-organised lobular architecture. Hepatocytes were arranged in cords radiating around the central veins, with no significant inflammatory infiltrates or vacuolization detected. In contrast, sections from the ethanol-intoxicated group (Figure 4C) exhibited disrupted hepatocyte organisation, with some hepatocytes displaying fat vacuolization. Additionally, inflammatory cell infiltrations were notably observed, particularly around the portal area.

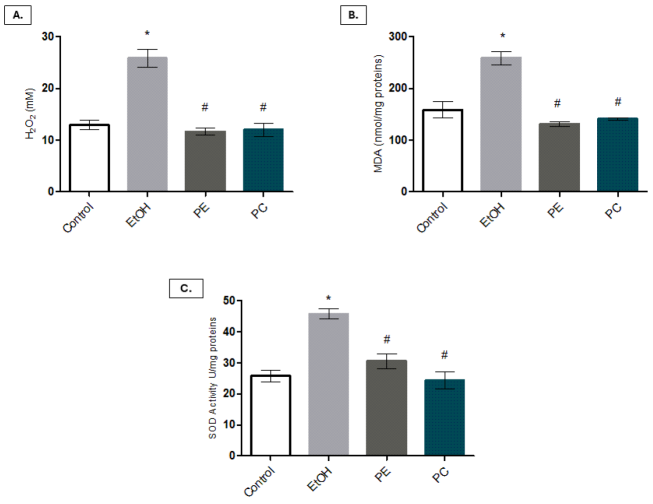



**Figure 2.** Oxidative stress markers in the liver. A: $H_2O_2$; B: MDA, C: SOD.

Values are represented as mean ± SEM; EtOH: Ethanol-treated group; PE: Phycocyanin co-treated group at 50 mg/kg; PC: Phycocyanin-treated group; $H_2O_2$: Hydrogen peroxide; MDA: Malondialdehyde; SOD: Superoxide dismutase. * p <0.05 as compared to the control group, # p<0,05 as compared to the ethanol treated group.

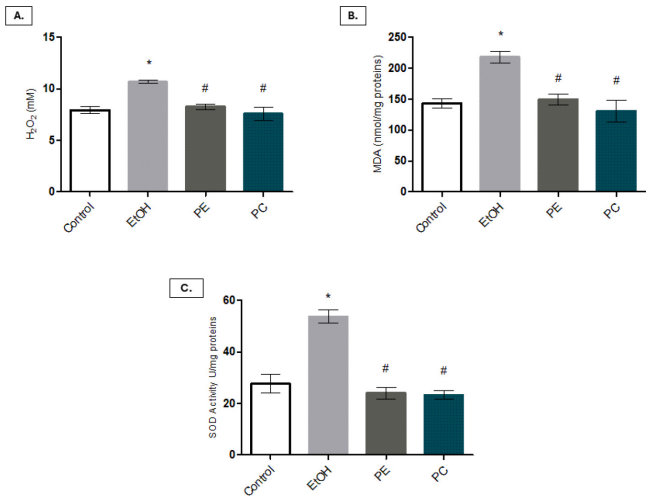



**Figure 3.** Oxidative stress status in the kidney. A: $H_2O_2$, B: MDA, C: SOD.

Values are represented as mean ± SEM; EtOH: Ethanol-treated group; PE: Phycocyanin co-treated group at 50 mg/kg; PC: Phycocyanin treated group; $H_2O_2$: Hydrogen peroxide; MDA: Malondialdehyde; SOD: Superoxide dismutase. * p <0.05 as compared to the control group, # p<0,05 as compared to the ethanol treated group.

Liver sections from rats co-treated with phycocyanin and ethanol showed a partial restoration of hepatocyte structure and arrangement (Figure 4D). However, slight sinusoidal irregularities were still present, suggesting ongoing recovery.

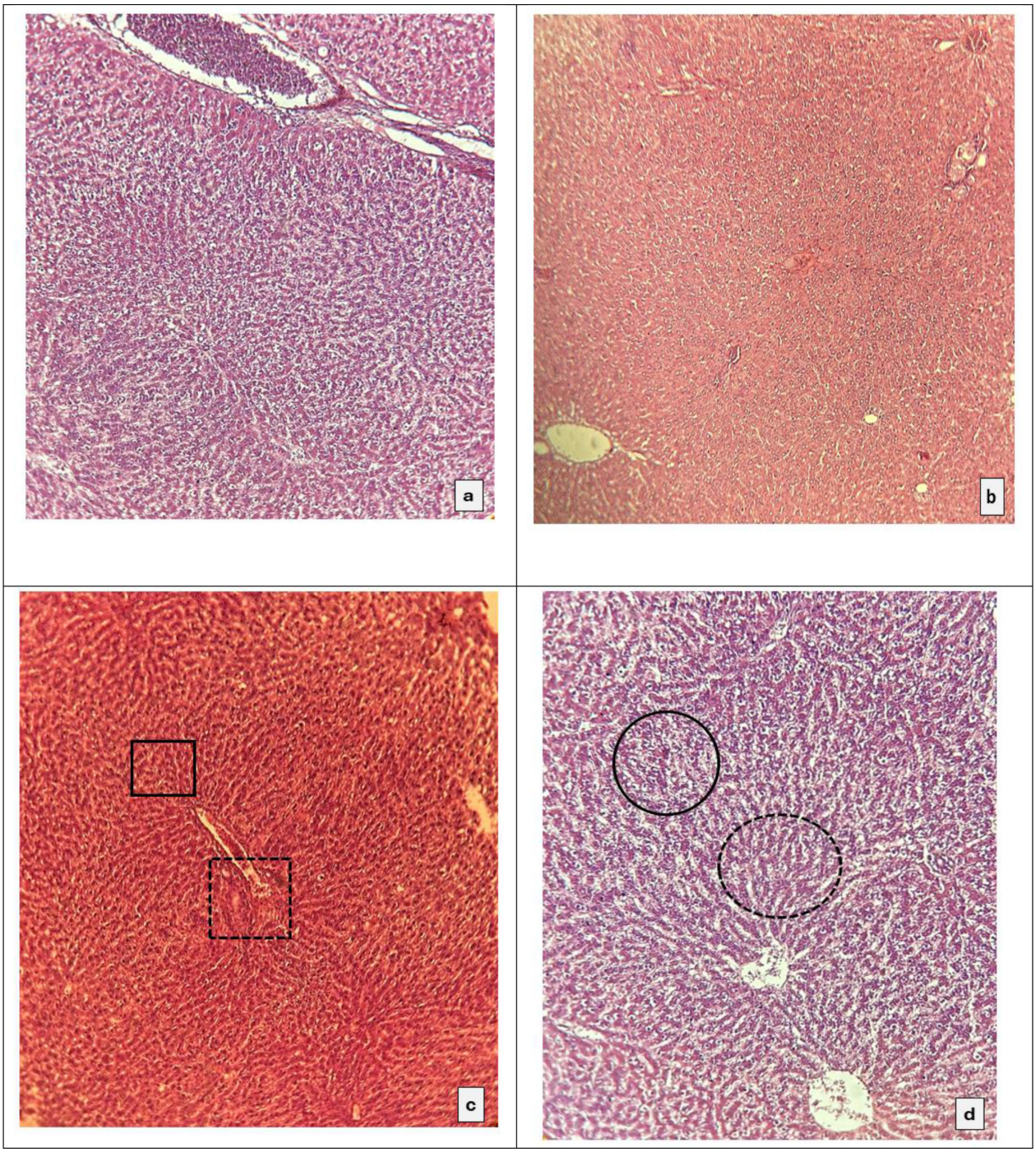



**Figure 4.** Effect of ethanol and phycocyanin on liver histology.

Photomicrographs of hematoxylin-eosin-stained liver sections. (a) Liver from control group; (b) liver from phycocyanin only treated group; (c) sections from ethanol 38% intoxicated group; (d) liver from rats co-exposed to 38% ethanol and phycocyanin. Rectangular: Disrupted hepatocytes organization; Rectangular dashed line: form of inflammatory infiltration; Circle: Normal hepatocytes structure; Circle dashed line: sinusoidal irregularities.

Kidney sections from the control group (Figures 5A and 5B) and the phycocyanin-only treated group (Figure 5C) displayed densely packed renal tubules and glomeruli in the renal cortex, as well as typically elongated collecting ducts within the medulla. All tubular structures appeared intact and well-organised. In contrast, sections from the ethanol-exposed group exhibited pathological features,

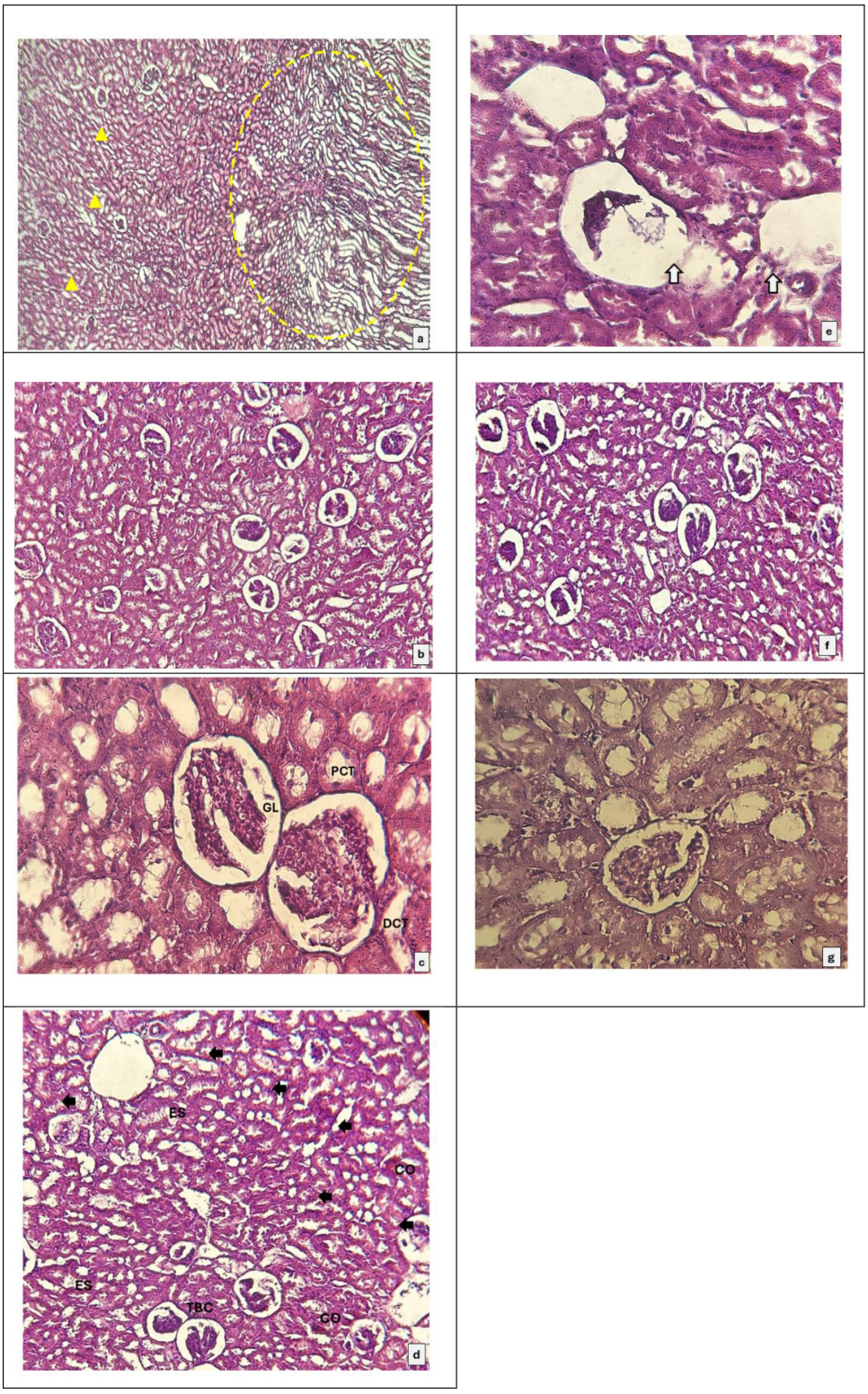



**Figure 5.** Effect of ethanol and phycocyanin on kidney histology.

Photomicrographs of hematoxylin-eosin-stained kidney sections: (a, b) kidneys from control rats; (c) kidneys from rats supplemented with phycocyanin alone; (d, e) kidneys from rats exposed to 38% ethanol; (f, g) kidneys from rats co-exposed to 38% ethanol and phycocyanin. Yellow dashed line = Medullary zone; Yellow arrowheads = Renal cortex; GL = Normal glomeruli; PCT = Proximal convoluted tubule; DCT = Distal convoluted tubule; Black arrows = Highly vascularized stroma; CO = Congestion; ES = Epithelial sloughing; TBC = Thickening of Bowman's capsule; Hollow arrows = Membrane disruption.

notably a highly vascularized stroma with widened interstitial spaces. The tubules appeared disorganised, with some epithelial cells sloughed off. Congestion and vacuolation were also evident (Figure 5D). Additionally, disruption of the glomerular structure was observed, with fragmentation of the capillary tuft (Figure 5E). Kidney sections from the phycocyanin administered group (PE) displayed intact tubular structures and normal glomerular architecture, including visible capillary tufts and Bowman's spaces (Figure 5G). Notably, areas with irregularly shaped tubule lumens were observed, suggesting possible mild tubular dilation (Figure 5F).

## 4. Discussion

Excessive alcohol consumption is a common issue among numerous societies, and it is reported to negatively affect human health with short- and long-term impacts. The World Health Organisation (WHO) reported that alcohol consumption is dangerous with serious health-related consequences, including 200 potential diseases, mainly liver diseases[22-24].

The present study aimed to investigate the protective effects of phycocyanin extract from *Arthrospira Platensis* on alcohol-induced toxicity in adult male rats. Our results showed that a 14-day exposure of rats to 38% v/v ethanol resulted in a 14% decrease in body weight compared to their initial weight, whereas the control group exhibited an 11% increase in body weight during the same period. Our results are in alignment with previous data indicating that in rats, win-drinking for 4 weeks reduces food consumption and weight gain in comparison to control[25]. The decrease in the growth rate might be due to the mechanism by which alcohol depresses the function of the intestine and induces a false feeling of satiation, which reduces food intake[26]. Furthermore, alcohol consumption can lead to malnutrition, triggered by the decreased intake of other food products, impaired digestion and absorption of nutrients[27]. Consequently, nutritional deficiencies result in a decrease of body mass[28], even though alcohol constitutes a source of energy for the body[29].

Remarkably, rats co-exposed to phycocyanin exhibited a growth rate like that of controls. However, body weight gain in the phycocyanin alone-treated group remained unchanged when compared to the control group, suggesting that the impact of this compound may be more pronounced under pathological than physiological states. One possible explanation for phycocyanin's protective effect against alcohol-induced weight loss lies in its ability to improve gastrointestinal tract function by promoting nutrient utilization and absorption. Thus, phycocyanin has been implicated in modulating the gut microbiota, promoting the proliferation of beneficial bacteria, and simultaneously reducing the abundance of harmful bacteria. This modulation of the gut microbiota can modify the intestinal function, promoting a healthier gut environment[30]. Additionally, a recent study examined the effects of phycocyanin supplementation following intense and prolonged exercise in rats. The results showed that phycocyanin supplementation reduced skeletal muscle damage and fatigue by modulating pathways essential for muscle protein synthesis and energy metabolism, suggesting that phycocyanin may improve muscle atrophy and reduce oxidative damage after intense exercise[31]. Our results are consistent with the previous report, indicating that phycocyanin supplementation at 200 mg/kg effectively attenuates streptozotocin-induced weight loss in rats[32]. Additionally, in chickens, phycocyanin supplementation with 1 or 2 g/kg for 35 days enhances antioxidant activities and increases body weight gain[33].

Evaluation of blood markers in rats revealed a significant increase in ASAT, ALAT, ALP, and LDH levels in the ethanol-intoxicated group compared to both the control and the phycocyanin-treated groups. ASAT and ALAT are liver enzymes critical for amino acid metabolism, with elevated levels, particularly ALT, indicating liver damage or inflammation[34]. In this study, C-reactive protein level, an inflammatory marker, was also notably higher in the ethanol-intoxicated rats compared to both the control and the phycocyanin group. These findings suggest a significant inflammatory response due to ethanol consumption and may serve as an early indicator of alcoholic liver disease[35].

Lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) play distinct roles in cellular processes: LDH is involved in energy production by

converting lactate to pyruvate, while ALP is crucial for protein breakdown and bile metabolism. Elevated levels of these enzymes often signal generalised cellular injury. Specifically, increased ALP levels are closely associated with liver damage, particularly in cases involving bile duct obstruction or cholestasis. Interestingly, co-treatment with phycocyanin showed a significantly decreased ASAT, ALAT, CRP, LDH and ALP levels as compared to the ethanol-treated group.

Alcohol exposure may also affect other organs, including the kidneys[36,37]. Accordingly, our study demonstrated that plasma urea and creatinine levels, the main biomarkers of renal injury, were significantly increased in ethanol-treated rats. However, phycocyanin association was found to exert a protective effect against alcohol-induced renal toxicity since the level of these parameters reached those of the control group. Our results are in line with previous data demonstrating that EtOH exposure in rats at a dose of 1.6g/kg for 12 weeks, resulting in an increase of serum urea and creatinine levels, suggesting an impact on renal function [38]. Furthermore, previous data reported that both acute and chronic alcohol consumption influence renal function, either directly or indirectly by products formed through the metabolism process of ethanol, including ROS, acetate and fatty acid ethyl esters[2,3]. These nephroprotective effects could be related to the antioxidant power of phycocyanin[39,40], since a strong link was demonstrated between oxidative stress and renal dysfunction[41]. The increase in plasma levels of urea and creatinine, hallmark indicators of renal dysfunction in ethanol-treated rats, may reflect impaired glomerular filtration and increased retention of nitrogenous waste[42].

Ethanol is well known for causing malnutrition by disrupting nutrient absorption. After ingestion, ethanol travels through the gastrointestinal tract and is initially metabolised mainly by alcohol dehydrogenase enzymes. It is then primarily absorbed in the upper small intestine through passive diffusion, due to its amphiphilic nature. Factors such as ethanol dosage, food intake, gastric emptying rate, ethanol concentration, and intestinal conditions influence the rate of absorption. Once absorbed, ethanol circulates throughout the body, impacting the distal small intestine and colon via the mesenteric vasculature. Ethanol also affects the basolateral membrane of enterocytes, leading to alterations in intestinal villi and crypts, and causing inflammation and haemorrhage in the lamina propria. These disruptions impair the absorption of essential nutrients, including vitamins B, C, and D, as well as minerals like calcium, iron, selenium, and zinc[43].

In this present study, calcium evaluation showed a significantly decreased level in ethanol-treated rats in comparison to the control and phycocyanin-alone treated group. Interestingly, phycocyanin supplementation was able to restore calcium levels. Previous research has elucidated that ethanol impairs calcium signaling by disrupting inositol 1,4,5-trisphosphate (IP3)/phospholipase C (PLC)-linked [$Ca^{2+}$] signaling pathways. This disruption leads to premature cessation of [$Ca^{2+}$] spikes and interferes with both calcium signaling and Protein Kinase C (PKC) pathways in hepatocytes. Ethanol's inhibition of IP3/PLC signaling reduces the effectiveness of calcium signaling, resulting in impaired cellular responses and altered physiological functions[44].

Ethanol's impact on calcium levels may also be partly attributed to its indirect effects on vitamin metabolism, particularly vitamin D, which is crucial for calcium absorption in the intestine. Calcium is absorbed through two pathways: the paracellular pathway, a passive process, and the transcellular pathway, which is actively regulated by the hormone 1,25-dihydroxyvitamin D. Indeed, adequate vitamin D levels are crucial for efficient calcium absorption. Insufficient vitamin D impairs calcium uptake. Therefore, ethanol-induced disturbances in vitamin D metabolism could contribute to reduced calcium absorption and subsequently lower systemic calcium levels[45].

The results of this study also demonstrate that ethanol exposure significantly exacerbates oxidative stress in both the liver and kidney tissues, as evidenced by increased levels of malondialdehyde (MDA) and hydrogen peroxide ($H_2O_2$). These findings align with previous studies showing that ethanol metabolism generates reactive oxygen species (ROS), which overwhelm endogenous antioxidant defences, leading to lipid peroxidation and cellular damage[7]. The increased MDA levels in the ethanol-treated group indicate extensive lipid peroxidation, a hallmark of

oxidative damage to cell membrane injury due to impaired detoxification mechanisms[46].

Interestingly, the activity of superoxide dismutase (SOD), a key antioxidant enzyme, was also elevated in the ethanol-treated group. This increase in SOD activity likely represents a compensatory response to the heightened oxidative stress, as SOD works to convert superoxide radicals into less harmful molecules like $H_2O_2$. However, the persistent elevation of MDA and $H_2O_2$ levels indicates that this compensatory mechanism is insufficient to fully counteract the oxidative damage induced by ethanol. Although decreased SOD activity is often interpreted as impaired antioxidant defence, in the present context, it more likely indicates a reduced need for enzymatic scavenging, owing to the lower generation of superoxide radicals. Thus, it may serve as an indirect marker of alleviated oxidative burden, reflecting a diminished requirement for upregulated antioxidant enzyme activity.

Phycocyanin co-treatment significantly mitigated these effects, reducing MDA and $H_2O_2$ levels to near-normal values in both the liver and kidney. This suggests that phycocyanin's antioxidant properties play a crucial role in scavenging ROS and preventing lipid peroxidation. Phycocyanin's ability to modulate oxidative stress has been well-documented in previous studies, where it has been shown to directly neutralise free radicals and enhance the activity of endogenous antioxidant enzymes[13,37]. The reduction in SOD activity observed in the phycocyanin co-treated group further supports this, as it indicates a decreased need for compensatory antioxidant responses due to the overall reduction in oxidative stress. These findings highlight phycocyanin's potential as a therapeutic agent for mitigating alcohol-induced oxidative damage. By restoring redox balance, phycocyanin not only protects cellular integrity but also prevents the downstream consequences of oxidative stress, such as inflammation and apoptosis. In a recent study in a mouse ethanol intoxication model, Guan *et al.*[47] reported that acute alcohol administration increased liver MDA and decreased SOD and glutathione levels with elevation of serum ALAT and ASAT content, while coadministration of Zanamivir, an antiviral agent, mitigated liver oxidative damage induced by alcohol. Furthermore, in a recent study[3], administration via gavage of ethanol (2mg/kg of 20% alcohol) for eight weeks increased MDA and decreased Total Antioxidant Capacity (TAC), glutathione peroxidase (GPx) and SOD levels in kidney tissue. Additionally, alcohol consumption resulted in elevated levels of proinflammatory cytokines, including TNFα and interleukin-6 (IL-6), and a reduction in anti-inflammatory cytokines, notably interleukin 10 (IL-10). It has been suggested that alcohol metabolism produces ROS, which cause oxidative damage and activate the nuclear factor kappa B (NF-κB) pathway, a key regulator of inflammation, allowing the transcription of proinflammatory cytokines[48,49]. The antioxidant property of phycocyanin has been attributed to hydroxyl, alkyl, and peroxyl radical scavenging activity[50].

It has been suggested that the tetrapyrrole structure of phycobilin in PC is similar to bilirubin, allowing it to reduce inflammation by modulating the expression of antioxidant enzymes such as Heme Oxygenase-1 (HO-1) and NAD(P)H: quinone oxidoreductase 1 (NQO1), both regulated by phosphorylated Nuclear factor erythroid 2–related factor 2 (p-Nrf2), which are typically upregulated during oxidative stress[51]. Moreover, phycocyanin's anti-inflammatory effects have been shown to involve modulation of the nuclear factor kappa-B (NF-κB) and Toll-Like Receptor (TLR) activation pathways as well as a reduction in lipopolysaccharide (LPS)-induced inflammatory signals[52].

The histological analyses provide further evidence of ethanol's detrimental effects on tissue architecture and confirm the protective role of phycocyanin. In the liver, ethanol exposure led to significant structural disruptions, including hepatocyte disorganisation, fat vacuolization, and inflammatory cell infiltration. These changes are consistent with the early stages of alcoholic liver disease, where ethanol-induced oxidative stress and inflammation disrupt normal hepatic function[5]. The presence of inflammatory cells around the portal area further underscores the role of inflammation in ethanol-mediated liver injury.

In the kidney, ethanol exposure caused marked pathological changes, including disorganised tubules, epithelial cell sloughing, and glomerular fragmentation. These alterations are indicative of ethanol-induced nephrotoxicity, which is driven by oxidative stress and

inflammation[11]. The widened interstitial spaces and vascular congestion observed in the ethanol-treated group suggest impaired renal blood flow and filtration, consistent with the elevated levels of urea and creatinine observed in the biochemical analyses.

Phycocyanin co-treatment notably ameliorated these histological changes. In the liver, phycocyanin partially restored hepatocyte organisation and reduced inflammatory infiltration, suggesting a protective effect against ethanol-induced inflammation and cellular damage. Similarly, in the kidney, phycocyanin preserved tubular and glomerular integrity, with only mild tubular dilation observed. These findings align with phycocyanin's known anti-inflammatory and antioxidant properties, which likely contribute to its ability to maintain tissue structure and function under conditions of ethanol-induced stress[13,39]. Phycocyanin acts as a bilirubin analogue by inducing heme oxygenase 1 to suppress inflammation[53].

The combined results of oxidative stress markers and histological analyses provide compelling evidence for phycocyanin's protective effects against alcohol-induced organ damage. By reducing oxidative stress and inflammation, phycocyanin helps preserve cellular and tissue integrity, thereby mitigating the functional impairments associated with ethanol exposure.

Moreover, the ability of phycocyanin to restore calcium levels in ethanol-treated rats further underscores its therapeutic potential. Calcium dysregulation is a known consequence of ethanol exposure, driven by disruptions in vitamin D metabolism and calcium signalling pathways[45]. Phycocyanin's ability to normalise calcium levels suggests that it may also play a role in restoring cellular signalling and metabolic homeostasis, although further research is needed to elucidate the precise mechanisms involved.

While this study provides valuable insights into phycocyanin's protective effects, several limitations should be acknowledged. The study was conducted in a rodent model, and future research is needed to determine whether these findings translate to humans. Additionally, the dose and duration of phycocyanin treatment were fixed; future studies could explore dose-response relationships and long-term effects to optimise therapeutic protocols.

Future research could also investigate the molecular mechanisms underlying phycocyanin's effects, particularly its interactions with key signalling pathways involved in oxidative stress, inflammation, and calcium homeostasis. Exploring the potential synergistic effects of phycocyanin with other antioxidants or anti-inflammatory agents could further enhance its therapeutic efficacy.

## 5. Conclusion

In conclusion, this study demonstrates that phycocyanin effectively mitigates alcohol-induced oxidative stress, inflammation, and tissue damage in the liver and kidney, preserving tissue integrity. Thereby, phycocyanin offers a promising natural therapeutic strategy for preventing and managing alcohol-related organ injury. These findings contribute to the growing body of evidence supporting the use of phycocyanin as a functional food or nutraceutical for promoting liver and kidney health in individuals at risk of alcohol-induced toxicity.

## 6. Author Contribution

Oumayma Boukari: investigation, Latif Hajri : data curation, Thelja Laassili : histological investigation, Wahid Khemissi : resources, Dorsaf Hallegue : methodology, Olfa Tebourbi: supervision, Khemais Ben Rhouma: validation, Mohsen Sakly: writing original draft, Soumaya Ghodhbane: writing -review and editing. All authors have read and agreed to the published version of the manuscript.

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