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# Pachypodol, a Plant Flavonoid, Mitigates Cisplatin-induced Hepatotoxicity Through Anti-Oxidant, Anti-Inflammatory and Anti-Apoptotic Mechanisms

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**Abstract** Cisplatin (CP), a widely used chemotherapeutic drug for cancer treatment is associated with multiple toxicities that limit its clinical application. Pogostemon cablin (Blanco) Benth contains pachypodol, a flavonoid with tremendous bioactive properties. The recent study intended to determine the mitigative role of pachypodol against cisplatin-instigated hepatic damage in albino rats. In this research, 32 albino rats were segregated into four groups (n = 8/group) and treated for 28 consecutive days; 1st group was designed as control, rats of the 2nd group received 10 mg/kg of CP; rats of the 3rd group were co-administered with 10 mg/kg of CP + 20 mg/kg of pachypodol, and 4th group rats received 20 mg/kg of pachypodol. Our results disclosed that CP exposure resulted in a substantial decrease in the activities of antioxidant enzymes i.e., catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD), glutathione reductase (GSR), glutathione (GSH), and glutathione S-transferase (GST) as well as a notable rise in malondialdehyde (MDA) and reactive oxygen species (ROS) levels. CP exposure escalated levels of nuclear factor kappa B (NF-κB), interleukin-1β (IL-1β), tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and cyclooxygenase-2 (COX-2) activity. Conversely, it diminished anti-apoptotic protein (Bcl-2) levels and elevated pro-apoptotic markers (Bax, caspase-9 and caspase-3). Besides, histopathological analysis revealed evident morphological alterations in the rats' livers. However, pachypodol significantly abated all the liver damage prompted by CP in rats. Taken together, our findings suggested that pachypodol alleviated cisplatin-induced hepatic impairment by inhibiting oxidative stress, inflammation and apoptosis.

***Keywords:*** *Pachypodol, Oxidative stress, Hepatotoxicity, Cisplatin*

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## 1. Introduction

Drugs based on platinum are revolutionizing the treatment of solid tumours in organs, including the ovary, head, neck, lung, brain, and testicles [1]. By oxidative stress (OS) and crosslinking with DNA, this compound stops mitosis and prompts apoptosis [2]. The platinum-based anticancer drug cisplatin (Cis-diamine dichloroplatinum II) (CP) is highly effective against different types of cancer. The CP treatment has a potent antineoplastic effect, but it has toxic side effects, like nephrotoxicity [3], neurotoxicity [4], ototoxicity [5], myelosuppression [6], hepatotoxicity and hematotoxicity [7] that limit its clinical applications.

The liver has a crucial function in urea synthesis, drug detoxification, glycogen synthesis, and lipolysis [8]. CP enters cells through passive transport, which is considered to be its primary route of cellular entry [9]. Moreover, OS is responsible for CP-induced hepatotoxicity due to the deleterious effects of ROS [10]. The mechanism of CP toxicity involves the generation of ROS (superoxide anion and hydroxyl radicals) that caused lipid peroxidation (LPO) and cell damage. Both malignant and non-targeted cells undergo cell cycle arrest and apoptosis in response to an elevated ROS level [11]. A key challenge in treating a person suffering from cancer is the mitigation or prevention of adverse side effects. Many approaches are being developed for the attenuation of CP damaging effects, such as adding free radical scavengers in combination with CP [12], but the research is inefficient and still needs to be explored further.

Flavonoids are phytochemicals that are reported in multiple vegetables, fruits as well as drinks. Flavonoids are known to have a wide range of health-improving properties. Besides their antimutagenic, antioxidant, anticarcinogenic, and antiviral activities, flavonoids are a vital component of pharmaceutical, nutritional, medicinal, and cosmetics applications [13]. The flavonoid pachypodol is derived from the plant Pogostemon cablin (Blanco) Benth. [14]. P. cablin has been reported to possess gastro-protective, analgesic [15], anti-inflammatory [16], antioxidant [17], antiviral, and antimicrobial properties [18]. Though pachypodol has favourable pharmacological properties, its protective role against cisplatin-induced hepatotoxicity has not yet been studied. Therefore, the present research was intended to explore the therapeutic efficacy of pachypodol on CP-instigated hepatotoxicity in rats based on its potent anti-inflammatory, anti-apoptotic and antioxidant effects.

# 2. Materials and Methods

## 2.1. Chemicals

Both pachypodol and CP were obtained from Sigma-Aldrich (Germany).

## 2.2. Experimental animals

During the trial, 32 male albino rats weighing 150-200 g were used. The rats were housed at room temperature (25 to 27℃) under standard lab conditions, with adequate moisture at a 12-hour day/night cycle. The animals were fed regularly and given access to tap water.

## 2.3. Experimental design

A total of 32 rats were split into four experimental groups, each containing eight rats; i) Group 1 – control group, given clean tap water and normal food, ii) Group 2 – CP (10mg/kg) was injected intraperitoneally once at the start of the experiment, iii) Group 3 – co-administrated with a 10mg/kg dose of CP via intraperitoneal injection along with a 20mg/kg oral dose of pachypodol for seven days and iv) Group 4 – a dose of 20mg/kg of pachypodol was administered orally (once a day) during the trial. On the last day of the experiment, rats were sedated with diethyl ether, decapitated, and using sterile syringes trunk blood was obtained. For biochemical analysis, serum was isolated from blood and kept at -80℃. The liver was divided into equal halves. One portion was preserved at -20℃ in zipper bags. The 2$^{nd}$ half was placed in a 10% formalin solution for histological evaluation.

## 2.4. Biochemical profile

Estimation of catalase (CAT) activity was carried out based on the method described by Aebi [19]. The approach developed by Kakkar et al. [20] was followed to estimate superoxide dismutase (SOD) activity. Glutathione GSH activity was assessed through a protocol developed by Moron et al. [21]. The glutathione peroxidase (GPx) activity was determined based on a methodology proposed by Rotruck et al. [22]. The activity of glutathione S-transferase (GST) was evaluated based on the protocol reported by Younis et al. [23]. To measure glutathione reductase (GSR) activity, the Carlberg and Mannervik method [24] was employed. Based on the Ohkawa et al. [25] approach, malondialdehyde (MDA) was evaluated. Using a method devised by Hayashi et al. [26], the level of reactive oxygen species (ROS) was assessed.

## 2.5. The Evaluation of Inflammatory Biomarkers

An evaluation of inflammatory markers in hepatic tissues was carried out using kits available in the market. Levels of TNF-α, IL-1β, NF-κB, IL-6, as well as cyclooxygenase (COX)-2 activity, were estimated employing an ELISA kit (Shanghai–YL–Biotech. Co. Ltd., China) for rats.

## 2.6. The evaluation of apoptotic biomarkers

To analyze the levels of apoptotic biomarkers (Caspase-9, Bax, Caspase-3 and Bcl-2) of the rats' hepatic tissues, an ELISA kit was employed (Cusabio Technology Llc, Houston, TX, USA).

## 2.7. Histological analysis

To evaluate the liver's architecture histologically, sections were fixated in 10% formalin, dried in successive grades of alcohol, and finally set in paraffin blocks. Then thin slices of around 5 µm were prepared using microtomes. Hematoxylin/eosin staining was performed on the tissue slices. Lastly, the slides were viewed under the microscope and visuals were captured (Leica DM4000B).

## 2.8. Statistical data analysis

The data was compiled as Mean ± SEM. To analyze variations between control and experimental groups, a one-way ANOVA was performed along with a Tukey's test using Minitab. The significance level was set at $p<0.05$.

# 3. Results

## 3.1. Pachypodol elevates hepatic antioxidant enzyme activities

CP considerably ($p<0.05$) diminished hepatic antioxidant enzymes activity, i.e., CAT, GST, GSR, GPx, GSH, as well as SOD than in the untreated group as demonstrated in Table 1. However, as opposed to the CP group, pachypodol co-treatment significantly ($p<0.05$) raised antioxidant enzymes in liver tissues, preventing CP-induced hepatotoxicity. While the group treated with pachypodol alone showed similar results to the control group.

**Table 1. Ameliorative effects of pachypodol on CP decreased activities of antioxidant enzymes in liver tissues**

| Groups | CAT (U/mg protein) | SOD (U/mg protein) | GPx (U/mg protein) | GSR (nM NADPH oxidized/min/mg tissue) | GST (nM/min/mg protein) | GSH (Mm/g tissue) |
|---|---|---|---|---|---|---|
| Control | 9.88 ± 0.39[a] | 8.41 ± 0.03[a] | 19.42 ± 0.39[a] | 5.68 ± 0.23[a] | 26.16 ± 1.08[a] | 16.73 ± 0.11[a] |
| CP | 3.73 ± 0.09[c] | 2.88 ± 0.10[c] | 4.17 ± 0.21[c] | 1.86 ± 0.03[c] | 11.88 ± 0.33[c] | 5.58 ± 0.13[c] |
| CP + Pachypodol | 7.44 ± 0.07[b] | 5.93 ± 0.15[b] | 13.38 ± 0.77[b] | 4.28 ± 0.08[b] | 21.07 ± 0.78[b] | 13.53 ± 0.42[b] |
| Pachypodol | 9.93 ± 0.20[a] | 8.40 ± 0.07[a] | 19.47 ± 0.41[a] | 5.66 ± 0.25[a] | 26.28 ± 1.18[a] | 16.85 ± 0.14[a] |

There is a significant difference between means that don't share the same letter.

**Table 2. Ameliorative effects of pachypodol on CP elevated ROS and MDA levels in rat's liver**

| Groups | ROS (U/mg tissue) | MDA (nmol/mL) |
|---|---|---|
| Control | 1.43 ± 0.04[c] | 0.64 ± 0.11[c] |
| CP | 8.37 ± 0.13[a] | 4.47 ± 0.13[a] |
| CP + Pachypodol | 2.54 ± 0.11[b] | 1.87 ± 0.05[b] |
| Pachypodol | 1.42 ± 0.04[c] | 0.62 ± 0.11[c] |

There is a significant difference between means that do not share similar letters.

**Table 3. Ameliorative effects of pachypodol on CP increased inflammatory biomarkers in hepatic tissues**

| Groups | NF-κB (ng/g tissue) | TNF-α (ng/g tissue) | IL-1β (ng/g tissue) | IL-6 (ng/g tissue) | COX-2 (ng/g tissue) |
|---|---|---|---|---|---|
| Control | 12.89 ± 0.49[c] | 5.77 ± 0.33[c] | 22.29 ± 1.35[c] | 9.42 ± 0.46[c] | 17.94 ± 0.45[c] |
| CP | 73.59 ± 1.49[a] | 21.06 ± 1.57[a] | 89.14 ± 1.58[a] | 38.74 ± 1.43[a] | 76.73 ± 1.42[a] |
| CP + Pachypodol | 27.69 ± 1.23[b] | 9.69 ± 0.38[b] | 33.37 ± 1.32[b] | 18.65 ± 1.02[b] | 31.51 ± 1.45[b] |
| Pachypodol | 12.88 ± 0.47[c] | 5.76 ± 0.30[c] | 22.18 ± 1.28[c] | 9.41 ± 0.43[c] | 17.92 ± 0.45[c] |

There is a significant difference between means with different letters.

## 3.2. Pachypodol ameliorates Hepatic Oxidative Stress Induced by Cisplatin

CP substantially ($p<0.05$) raised MDA and ROS levels versus the control group (Table 2). Conversely, pachypodol co-treatment remarkably ($p<0.05$) diminished ROS and MDA levels in the hepatic tissue, as opposed to the CP-exposed group. Further, the group treated with only pachypodol displayed comparable results to the control group.

## 3.3. Pachypodol Alleviates Cisplatin-induced Hepatic Inflammation

CP noticeably ($p<0.05$) upregulated hepatic inflammatory biomarkers levels (TNF-α, NF-κB, COX-2, IL-6, and IL-1β) compared to the untreated group (Table 3). Conversely, pachypodol co-administration substantively ($p<0.05$) lessened inflammatory biomarkers levels in liver tissues by protecting against CP-induced hepatotoxicity compared to CP-intoxicated group. The pachypodol alone treated group showed similar results as the control group.

## 3.4. Pachypodol Mitigates Cisplatin-induced Apoptosis in rat's Liver

The treatment with CP revealed a notable ($p<0.05$) elevation in caspase-3, Bax and caspase-9 levels in comparison with control group rats, while a decline in Bcl-2 levels was observed (Table 4). In contrast to CP-intoxicated group, pachypodol co-treatment led to a notable ($p<0.05$) reduction in caspase-9, caspase-3 & Bax levels whereas raising anti-apoptotic protein (Bcl-2) levels in hepatocytes. Moreover, just the pachypodol-treated group didn't differ from the control group.

**Table 4. Ameliorative effects of pachypodol on CP-instigated apoptosis in liver of rats**

| Groups | Bax (pg/mL) | Bcl-2 (ng/mL) | Caspase-3 (pg/mL) | Caspase-9 (pg/mL) |
|---|---|---|---|---|
| Control | 2.81 ± 0.14[c] | 14.36 ± 1.11[a] | 1.54 ± 0.07[c] | 3.93 ± 0.20[c] |
| CP | 10.95 ± 0.59[a] | 4.61 ± 0.76[b] | 9.38 ± 0.16[a] | 17.21 ± 0.95[a] |
| CP + Pachypodol | 5.06 ± 0.41[b] | 11.59 ± 0.62[a] | 4.58 ± 0.12[b] | 6.97 ± 0.31[b] |
| Pachypodol | 2.79 ± 0.13[c] | 14.42 ± 1.16[a] | 1.53 ± 0.07[c] | 3.92 ± 0.20[c] |

There is a significant difference between means that do not share similar letters.

## 3.5. Pachypodol Mitigates Cisplatin-induced Histopathological Liver Damage in Rats

According to the histological study, CP treatment changed the architecture of the liver, resulting in disrupted central venules, and dilated sinusoids, adverse alterations in hepatocyte nuclei and Kupffer cells (Fig. 1). In contrast, pachypodol supplementation significantly alleviated all structural changes in the morphology of liver in CP + pachypodol treated rats matched to CP-instigated rats. Moreover, the pachypodol-only treated group's histological state of the liver tissues was noticeably improved than that of the CP-intoxicated group. Pachypodol alone was treated and the control group displayed no substantial differences.

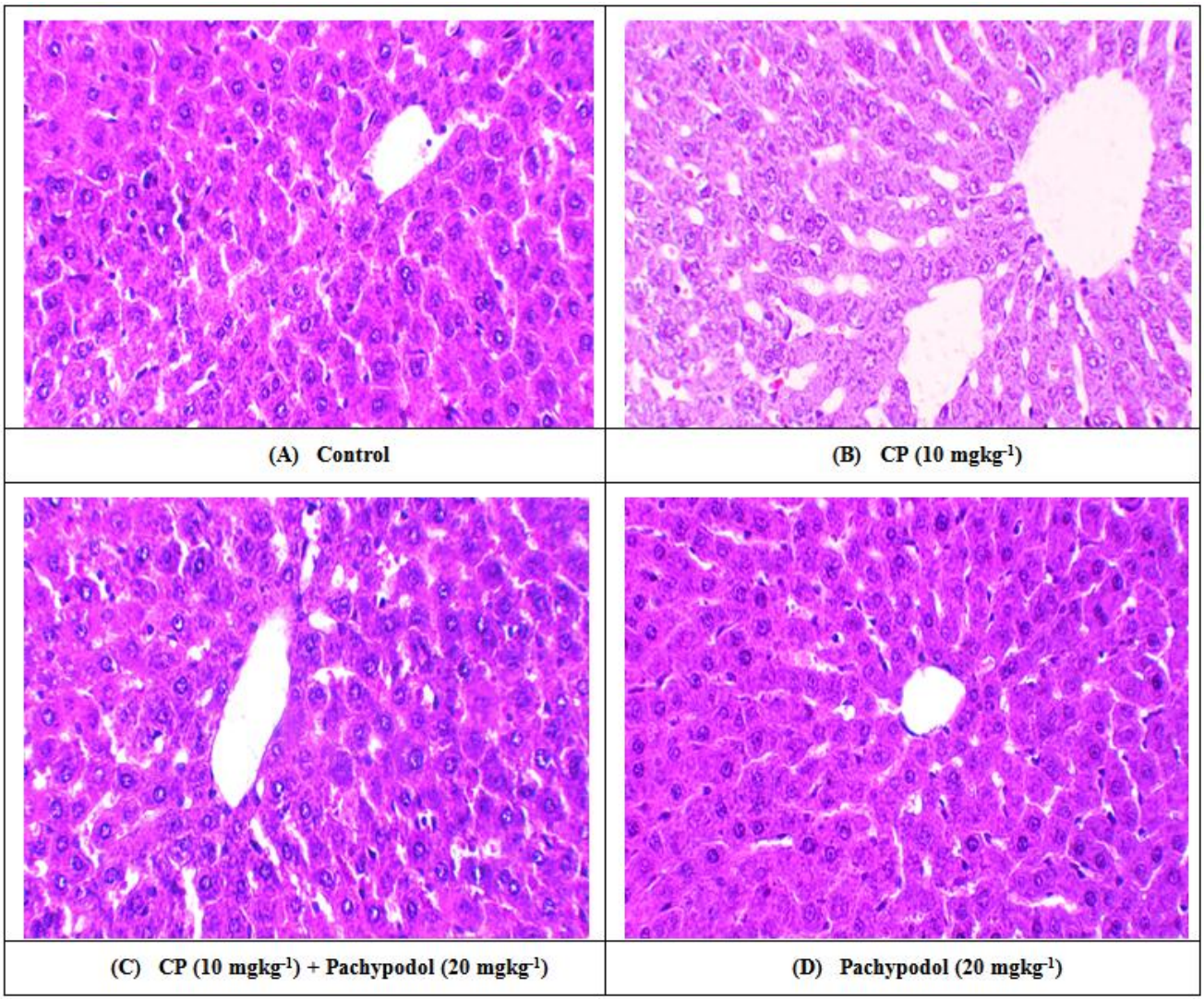


**Figure 1.** Hepato-protective effect of pachypodol on CP-induced alterations in liver histology. A) Control group B) CP (10 mg/kg) group C) CP (10 mg/kg) + pachypodol (20 mg/kg) group D) pachypodol (20 mg/kg) group.

## 4. Discussion

In this study, hepatic toxicity was assessed through the analysis of antioxidant status, oxidative stress markers, inflammation biomarkers, and apoptosis biomarkers. Studies conducted recently have focused on ways to prevent cisplatin-induced hepatotoxicity [27]. Despite this, little is known about flavonoids' effects on cisplatin hepatotoxicity. Flavonoids, polyphenolic compounds, are widely researched as a treatment to alleviate CP-induced hepatotoxicity [28]. Therefore, in the present investigation, the flavonoid pachypodol was used to treat CP-induced hepatic damage in rats.

The findings disclosed that CP supplementation showed a considerable diminution in anti-oxidant enzymes (CAT, GST, SOD, GPx, GSH and GSR) activities. However, MDA and ROS levels were remarkably high. CAT is crucial in the process of converting H2O2 into H2O [29]. SOD plays a key role in the transformation of O-2 into O2 and H2O2 [30]. The cofactor of GPx, GSH, protects hepatocytes from damage by reducing hydrogen peroxide levels and other peroxides, while also being important in xenobiotic detoxification [31]. GST prevents oxidative damage caused by free radicals and removes toxic substances from the body [32]. Based on previous findings, CP causes liver toxicity by raising hydrogen peroxide concentration, which increases ROS production and lipid peroxidation, as indicated by elevated levels of MDA and decreased antioxidant index in the liver [33]. CP toxic metabolites increased the accumulation of ROS as antioxidant enzyme activities declined and ROS production increased, ultimately leading to liver damage. However, pachypodol co-treatment mitigated oxidative damage by removing free oxygen radicals and activating antioxidant enzymes.

Our results revealed that CP exposure escalated the levels of inflammatory indices (TNF-α, IL-1β, NF-κB, IL-6 and COX-2 activity), which are all indicators of elevated inflammatory signalling parameters. NF-κB is considered one of the primary factors responsible for regulating the inflammatory response, by promoting several inflammatory cytokines transcription, which includes IL-1β, TNF-α, IL-6 and COX-2 [34]. The inductive form of the COX family, COX-2, is an important indicator of inflammation that mediates inflammatory reactions in the body [35]. According to this study, rats that had been

exposed to CP had higher COX-2 activity in their hepatic tissues, which is an indication of hepatotoxicity. However, pachypodol showed significant protective effects against CP-induced inflammation by reducing inflammatory markers (IL-6, IL-1β, TNF-α, NF-κB and COX-2) in hepatic tissues. Hence, these results corroborate that pachypodol exhibits an anti-inflammatory effect on hepatic tissues.

Our findings revealed that CP exposure shifted the balance of pro- and anti-apoptotic signals to an apoptotic state [36], as shown by increased Caspase-9, Caspase-3, and Bax levels with concomitantly diminished Bcl-2 levels in hepatic tissue of rats treated with CP. CP intoxication increases Bax expression, a pro-apoptotic effector, and decreases Bcl-2 expression, a pro-apoptotic factor. Moreover, it induces the translocation of Bax from the cytosol to the mitochondria, thereby releasing Cyt-c into the cytosol [37]. As Cyt-c is evicted from mitochondria, it induces the metamorphosis of Apaf1, by binding to the precursor of caspase-9, thereby activating the caspase-9 spontaneously. Caspase-3 is activated by Apaf1, caspase-9, and cytochrome c, creating cascade reactions to initiate apoptosis [38]. Furthermore, the apoptotic effector caspase-3 activates DNA fragmentation factors and cellular proteins that result in discernible alterations [39]. However, pachypodol alleviated apoptosis in the hepatocytes of rats by lowering anti-apoptotic markers and elevating pro-apoptotic markers.

A histomorphological analysis of liver tissues revealed that CP demonstrated adverse effects on hepatic tissues. LPO in hepatocytes was elevated by CP intoxication, which caused morphological abnormalities. Hepatotoxicity due to CP was documented in the treatment group as sinusoid dilation, lobulation, coagulation, blockage, disruption of the central vein, swelling of connective tissues, and multiple necrosis of the biliary duct [40]. By co-treating with pachypodol, these severe abnormalities were reduced. The protective effects of pachypodol might be due to its anti-apoptotic, antioxidant, and anti-inflammatory properties.

## 5. Conclusion

In conclusion, CP-induced hepatotoxicity may be attributed to oxidative stress, inflammation, as well as apoptosis. CP intoxication also caused histoarchitectural changes in liver tissue. However, pachypodol co-treatment extended potent protective effects against CP-induced hepatic damage. This might be because of its anti-inflammatory, anti-apoptotic as well as anti-oxidant effects. Based on our findings, pachypodol may provide a more promising strategy for preventing hepatotoxicity followed by CP-based chemotherapy.

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