

EFFECT OF 6-HYDROXYFLAVONE ON CISPLATIN-INDUCED HISTOPATHOLOGICAL AND BIOCHEMICAL CHANGES IN LIVER OF SPRAGUE-DAWLEY RATS

Zia Ud Din¹, Shazia Iftikhar¹, Zainab Rehman¹, Muhammad Harris², Sadaf Ambreen¹, Munila Shabnum Khattak¹, Muhammad Shahid^{2,3}, Rahimullah³, Sobia Haris⁵

¹ Department of Anatomy, Khyber Medical College, Peshawar, Khyber Pakhtunkhwa, Pakistan

² Department of Anatomy, Nowshehra Medical College, Nowshehra, Khyber Pakhtunkhwa, Pakistan

³ Department of Pharmacy, University of Peshawar, Peshawar, Khyber Pakhtunkhwa, Pakistan

⁴ Department of Pharmacy, Sarhad University of Science and Information Technology, Peshawar, Khyber Pakhtunkhwa, Pakistan

⁵ Sarhad Institute of Health Sciences Khyber Medical University, Khyber Pakhtunkhwa, Pakistan

Abstract

Objective: Chemotherapeutic agents like cisplatin benefits cancer patients by decreasing metastasis, relapse and increase the overall survivability. The clinical effectiveness of these beneficial chemotherapeutics is greatly limited by occurrence of side-effects including hepatotoxicity. Flavonoids possess potent hepatoprotective effects and exhibit antioxidant property. In this study, the hepatoprotective effect of the flavonoid, 6-hydroxyflavone was investigated against cisplatin induced liver damage in rats.

Materials & Methods: 6-Hydroxyflavone (25 and 50 mg/kg) and silymarin (100 mg/kg) were administered for 14 days and hepatotoxicity was induced by a single injection of cisplatin (7.5 mg/kg) on the 10th day of experiment. The liver tissues were examined for histopathological and biochemical changes.

Results: Cisplatin produced histopathological changes of sinusoidal dilatation and steatosis and elevated the serum alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase levels. Cisplatin generated oxidative stress in the liver which was observed from significant increase lipid peroxidation and reduction in the level of antioxidant mechanisms of glutathione, catalase and superoxide dismutase. Administration of 6-hydroxyflavone and silymarin protected the liver from cisplatin-induced degenerative changes and reduced the escalated levels of biochemical parameters. These protective doses also significantly inhibited the generation of oxidative stress as significant attenuation of lipid peroxidation and an increase in the antioxidant status were observed in the cisplatin-injected animals.

Conclusion: These findings suggest that 6-hydroxyflavone has prospective hepatoprotective effect mediated by inhibition of oxidative stress in the hepatocytes and thus can be considered when treating cancer patients with chemotherapeutic drugs including cisplatin.

Key Words: Chemotherapy and hepatotoxicity, Flavonoids, Hepatoprotective natural products, Cisplatin and oxidative stress, Antioxidants, Cisplatin-induced hepatotoxicity.

Correspondence:

Dr. Zia Ud Din

Department of Anatomy, Khyber Medical College, Peshawar
25000, Khyber Pakhtunkhwa, Pakistan
Email: ziauddin003@gmail.com
Contact: +923329146044

INTRODUCTION

Conventional chemotherapy benefits patients in the form of decreased metastasis, relapse and a longer survival not only at the early stage of disease,

but also at the late stage, and is currently the main treatment for cancer¹. The current chemotherapeutic drugs are associated with serious adverse effects in addition to their limited effectiveness and their long-term use remain a major source of concern for both patients and clinicians². The platinum-based drugs including cisplatin are effective for the treatment of a variety of cancers. However, their use is greatly limited by severe, dose-limiting side effects³. Thus, there is an urgent need of new approaches to improve tolerance and reduce the sequelae of these effective chemotherapeutic drugs.

Chemotherapy induced hepatotoxicity is a frequent complication of anticancer drugs and occurs frequently from an unpredictable or idiosyncratic reaction. The liver is the major site of drug metabolism and the liver-drug interaction must be kept in account while dosing with chemotherapeutic drugs. Cancer patients must be carefully assessed for liver function both prior to and during cytotoxic chemotherapy⁴⁻⁶.

Flavonoids are naturally occurring polyphenolic compounds and possess disease preventing and health promoting properties. Numerous preclinical and clinical studies revealed that flavonoids are able to limit pathological changes in various disease conditions. Studies have shown that flavonoids have potent hepatoprotective effects^{7,8} and prevent steatosis in the liver⁹. Keeping in view of the intractable adverse effects associated with the use of chemotherapeutic drugs and the myriad biological properties offered by flavonoids along with their strong protective influence on the liver, the present study investigated the hepatoprotective effect of flavonoid, 6-hydroxyflavone in the cisplatin associated hepatotoxicity in rats.

MATERIALS AND METHODS

Chemicals

6-Hydroxyflavone (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in a vehicle comprising of 5% DMSO and 2% Tween80^{10,11}. The positive control, silymarin (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in normal saline prior to administration¹².

Animals

Male Sprague Dawley rats weighing 200-250 g were maintained in a light dark cycle of 12/12 h at 20-24°C. The experiments were approved by the Ethical Committee of Khyber Medical College, Peshawar,

Pakistan and approval for the study was granted vide registration number 42/PG/KMC.

Treatment protocol

The different doses of 6-hydroxyflavone (25 and 50 mg/kg) and silymarin (100 mg/kg)¹³ were administered through intraperitoneal route. The hepatotoxicity was induced by a single intraperitoneal injection of cisplatin (7.5 mg/kg). The animals were divided into the following groups with each group consisted of six animals:

Group A served as negative control and was injected with the vehicle. Group B received cisplatin as a single injection (7.5 mg/kg) on day 10 of the experiment. Group C received daily 6-hydroxyflavone injection at 25 mg/kg for consecutive 15 days along with cisplatin (7.5 mg/kg), which was administered two hours after 6-hydroxyflavone administration on day 10 of the experiment. Group D received daily 6-hydroxyflavone injection at 50 mg/kg for consecutive 15 days along with cisplatin (7.5 mg/kg), which was administered two hours after 6-hydroxyflavone administration on day 10 of the experiment. Group E received daily silymarin injection at 100 mg/kg for consecutive 15 days along with cisplatin (7.5 mg/kg), which was administered two hours after 6-hydroxyflavone administration on day 10 of the experiment. Group F received daily 6-hydroxyflavone injection at 50 mg/kg for consecutive 15 days.

Biochemical and histopathological analysis

At the end of study, the animals were anesthetized by injecting xylazine plus ketamine. The blood was collected and was analyzed for serum alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase using standard diagnostic kits. After blood collection, each animal was euthanized by cervical dislocation and the liver was immediately collected after dissection. A portion of the liver tissue was homogenized in 50 mM phosphate buffer (pH 7.0) and the homogenate was centrifuged at 10,000 rpm for 15 min. The liver tissue was analyzed for malondialdehyde¹⁴, catalase¹⁵, superoxide dismutase¹⁶, and reduced glutathione¹⁷. A portion of the remaining liver was instantaneously transferred to a jar containing 10% neutrally buffered formalin. The tissues were processed and stained for histopathological examination using standard laboratory protocols^{12,18}.

RESULTS

Effect of 6-hydroxyflavone on cisplatin-induced biochemical changes in serum and liver tissue

The single dose of cisplatin at 7.5 mg/kg significantly altered the serum level of liver function enzymes. This was observed in the group of cisplatin injected animals treated with the vehicle as a significant increase ($P < 0.001$) in the serum level of alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase as compared to the respective serum levels of these enzymes in the group of non-cisplatin animals treated with the vehicle. The daily treatment with 6-hydroxyflavone at the tested doses has a protective effect on the cisplatin-induced aberration in the serum levels of liver biochemical enzymes. 6-Hydroxyflavone at 25 mg/kg, significantly reduced ($P < 0.05$, $P < 0.01$) the cisplatin induced pathological elevations of serum alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase, as compared to the cisplatin injected treated only with the vehicle. At the higher dose of 6-hydroxyflavone at 50 mg/kg, a strong reduction in the cisplatin-induced elevated serum enzymes was observed as a significant decrease ($P < 0.01$, $P < 0.001$) in alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase, when compared to vehicle treated cisplatin dosed animals. The positive control, silymarin treated group at a dose of 100 mg/kg, has a robust effect on the liver function enzymes inducing tendency of cisplatin. This occurred as a significant decrement ($P < 0.001$) of serum of alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase ($P < 0.001$) levels in the cisplatin injected animals, as compared to the cisplatin-vehicle animals group. No pathological alterations in the serum biochemical levels were observed after daily treatment with only 6-hydroxyflavone at 50 mg/kg (Table 1).

A marked oxidative stress condition was detected in the tissues of animals treated with the single dose of cisplatin at 7.5 mg/kg. This deleterious condition was prominent in the vehicle treated animals injected with cisplatin as a significant increase ($P < 0.001$) in the content of malondialdehyde, and a significant decrease ($P < 0.001$) in the level of glutathione, and activities of catalase, and superoxide dismutase, as compared to that of the non-cisplatin animals treated

only with vehicle. Treatment with 6-hydroxyflavone and silymarin have a significant main dose effect on the cisplatin-induced oxidative stress in the liver. When the animals were treated with 6-hydroxyflavone at a dose of 25 mg/kg, the cisplatin associated pathological distress occurring in the form of lipid peroxidation and a decline in the antioxidative mechanisms were significantly attenuated as observed from the decrease in malondialdehyde level ($P < 0.05$), and an increase in glutathione content ($P < 0.05$) and activities of catalase ($P < 0.05$) and superoxide dismutase ($P < 0.05$). Similarly, treatment with the higher dose of 6-hydroxyflavone at 50 mg/kg also produced a strong inhibition of cisplatin-induced detrimental changes in the liver, as the reduction in malondialdehyde ($P < 0.001$) along with increment in glutathione ($P < 0.001$), catalase ($P < 0.01$), and superoxide dismutase ($P < 0.01$) was significant underlying the inhibition of oxidative stress in the liver. Treatment with the positive control, silymarin at 100 mg/kg was also effective in counterbalancing the aberrations in the hepatic antioxidant status as the cisplatin-induced elevated malondialdehyde level was significantly declined ($P < 0.001$), and the toxicant-induced reduced glutathione, catalase and superoxide dismutase was significantly elevated ($P < 0.01$, $P < 0.001$) after daily administration. The per se administration of 6-hydroxyflavone at a higher dose of 50 mg/kg was not associated with any deviant changes in the liver tissue antioxidant markers as shown in Figure 1.

Effect of 6-hydroxyflavone on cisplatin-induced histopathological changes in liver

The administration of cisplatin at a single dose of 7.5 mg/kg produced considerable changes in the histoarchitecture of the liver tissue. The histopathological changes were observed as severe necrotic changes around the central vein along with congestion of their lumen with red blood cells and lymphocytes. There was perivenular accumulation of lymphocytes, dilatation of sinusoidal spaces with infiltration of red blood cells and lymphocytes. Extensive microvesicular steatosis and occasional macrovesicular steatosis were observed throughout the lobules of the liver. The hepatocytes also showed glycogen depletion. Hemorrhage was also observed. In contrast, the groups of cisplatin injected animals treated with 6-hydroxyflavone at the tested doses as well as the positive control, silymarin showed almost

normal histological features of the liver tissue, except for occasional microvesicular steatosis and red blood cells accumulation in the sinusoidal spaces and central veins. The vehicle treated animals and the per

administration of 6-hydroxyflavone were devoid of any histopathological changes in the lobules of the liver tissue as shown in Figure 2.

Table 1: Effect of 6-hydroxyflavone on cisplatin-induced serum biochemical changes

Groups	Alanine aminotransferase(U/L)	Aspartate aminotransferase (U/L)	Alkaline phosphatase
Vehicle	34.50± 2.895	41.83± 2.482	74.33± 5.619
Cisplatin	96.67± 10.07###	88.17 ± 4.505###	162.7± 8.188###
Silymarin + Cis	48.17±5.588***	56.00± 5.842***	98.83± 6.145***
6HF-25 + Cis	62.33± 6.391**	67.50± 6.168*	126.5± 11.63*
6HF-50 + Cis	53.67± 6.839***	58.67± 4.318**	108.0± 7.492***
6HF-50	32.17± 4.301	39.33± 2.813	68.67± 3.739

Values expressed as mean ± SEM. ###P < 0.001 as compared to vehicle alone treated group, *P < 0.05, **P < 0.01, ***P < 0.001 as compared to cisplatin un-treated group. One-way ANOVA followed by Tukey’s post hoc test, n = 6 animals per group.

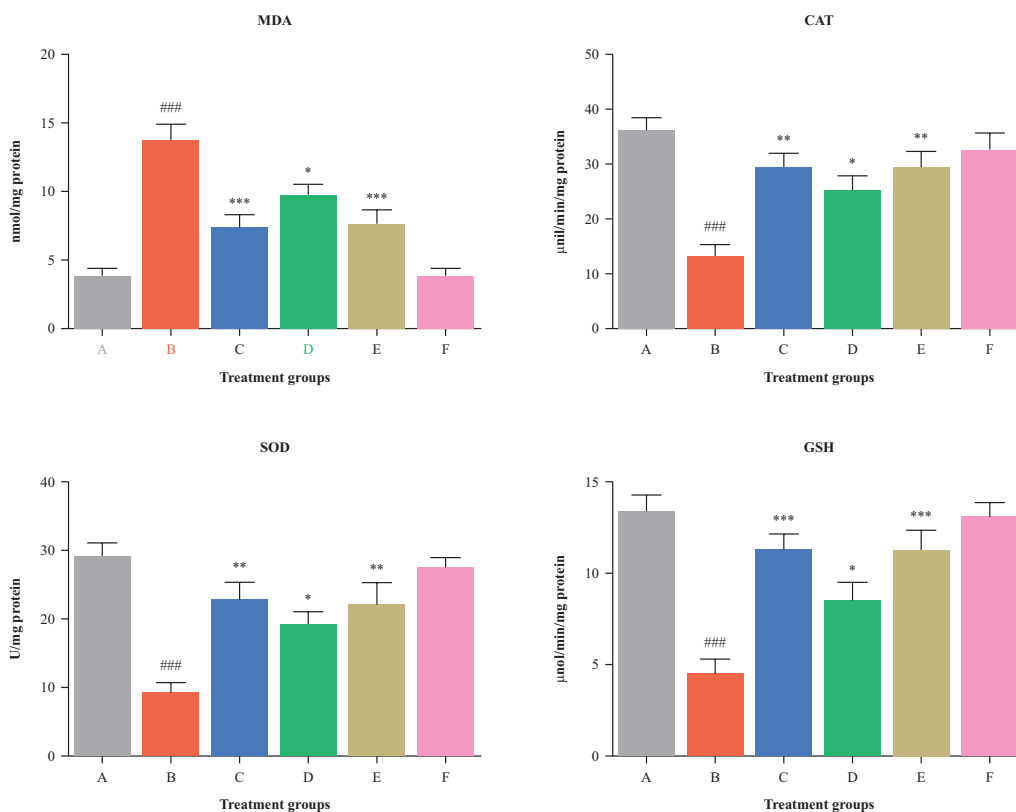


Figure 1: Effect of 6-hydroxyflavone on cisplatin-induced biochemical changes in liver tissue. Each bar represents mean values ± SEM. ###P < 0.001 as compared to vehicle alone treated group, *P < 0.05, **P < 0.01, ***P < 0.001 as compared to cisplatin administered vehicle treated animals group. One-way ANOVA followed by Tukey’s post hoc test, n = 6 animals per group. Group A: vehicle treated control, Group B: cisplatin (7.5 mg/kg) untreated control, Group C: silymarin at 100 mg/kg plus cisplatin, Group D: 6-hydroxyflavone at 25 mg/kg plus cisplatin, Group E: 6-hydroxyflavone at 50 mg/kg plus cisplatin, Group F: 6-hydroxyflavone at 50 mg/kg for 14 days.

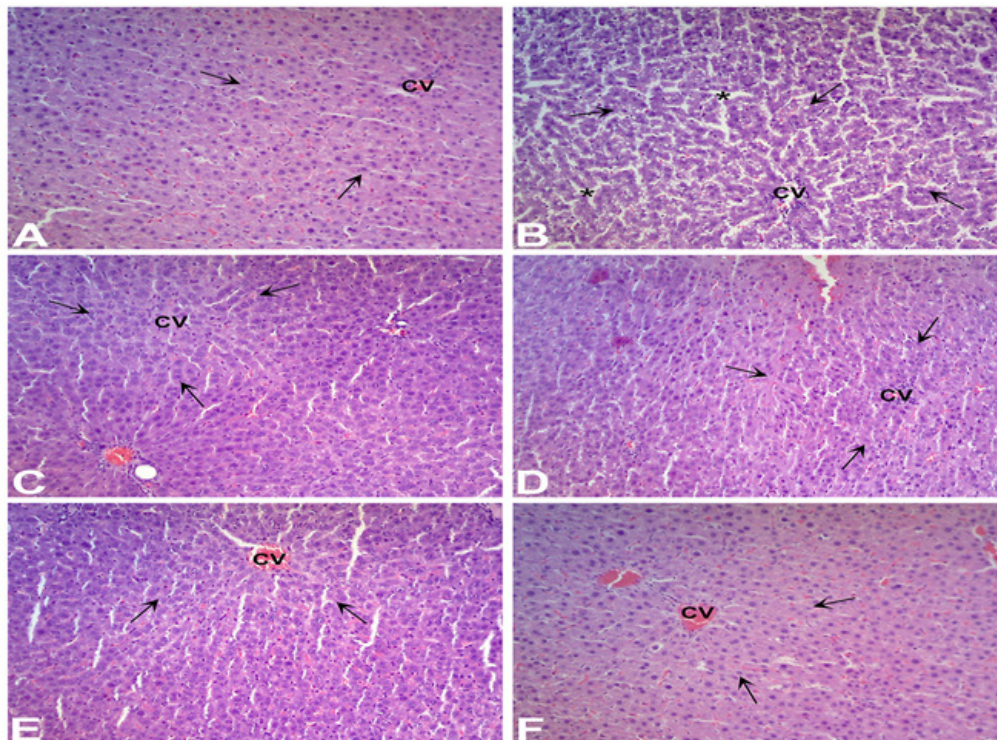


Figure 2: Effect of treatment with 6-hydroxyflavone and silymarin on liver histology in the cisplatin-induced hepatotoxicity (H & E; 100x). (A): Liver section from the vehicle group showing histoarchitecture of central vein (CV) and hepatocytes (arrows). (B): Liver section from cisplatin-vehicle group showing central vein (CV) shrinkage and congestion. The sinusoidal spaces show dilatation and their lumen (asterisks) are infiltrated with red blood cells and lymphocytes. Microvesicular steatosis is visible throughout the hepatocytes (arrows). Normal histological features of the hepatic lobules containing a centrally placed central vein (CV) around which normal appearing plates of hepatocytes (arrows) are visible in groups of cisplatin injected animals treated with silymarin at a dose of 100 mg/kg (C), and 6-hydroxyflavone at 25 mg/kg (D) and 50 mg/kg (E), as well as in the per se treated group of 6-hydroxyflavone at a dose of 50 mg/kg (F).

DISCUSSION

In the present study, the flavonoid, 6-hydroxyflavone was investigated for its therapeutic potential as prospective hepatoprotective agent against cisplatin-induced hepatotoxicity in rats. Liver injury associated with anti-cancer drugs is considered as one of the most serious complication in patients treated for cancer. Chemotherapeutic agents produce diverse histopathological changes in the liver and may occur as severe steatosis, or sinusoidal injury sinusoidal obstruction syndrome⁴. These chemotherapeutic-induced hepatic histopathological changes produce clinical symptoms of hepatotoxicity and may culminate in liver failure and death, but it may also result in a cessation of beneficial anticancer therapy with curative intent¹⁹. Cisplatin associated hepatic toxicity is dose related and produce steatosis and cholestasis with abnormal liver test especially

elevation of alanine aminotransferase, and aspartate aminotransferase⁶. In this study, the nature and degree of histopathological changes caused by cisplatin corroborates the previous studies as reported in the literature^{20,21}.

The mechanism underlying the hepatic injury as occurred with chemotherapeutic drugs is thought to be secondary to the formation of reactive oxygen species, that produce oxidative stress and leads to necrotic and apoptotic changes in the hepatocytes¹⁹. In this study, cisplatin produced severe oxidative stress in the liver as revealed from significant increase in the lipid peroxidation and reduction in the antioxidant mechanism i.e. decrease in the levels of glutathione and antioxidant enzymes of catalase and superoxide dismutase. Oxidant stress has been implicated in a number of liver diseases. Hepatic oxidative and stress can produce hepatocytes injury by enhancing

the generation of mitochondrial superoxide with increase permeability of mitochondria, infiltration of neutrophils and Kupffer cells and generation of hypochlorous acid along with release of cytokines such as TNF- α ²². Cisplatin mediated liver toxicity involves mitochondria and evidence suggests damage to the structural integrity and function of mitochondria, imbalance in the antioxidant defense system, oxidation of proteins and lipids in the mitochondria and induction of apoptosis of hepatocytes²³.

In this study, administration of 6-hydroxyflavone to the cisplatin injected animals produce a significant protective effect demonstrated by preservation of liver histoarchitecture, reduction in the elevated levels of liver function enzymes, and inhibition of hepatic oxidative stress. Natural antioxidants have potent free radicals scavenging abilities and possess strong anti-inflammatory properties, and these beneficial activities are supposed to be responsible for the therapeutic and health benefits potential of medicinal plants²⁴. Flavonoids have demonstrated significant hepatoprotective properties. Isolated flavonoids and medicinal plants rich in flavonoids are able to suppress liver lipid peroxidation, pancreatic lipase activity, tumor necrosis factor, nitric oxide, hepatic mRNA levels for inducible NO synthase and fragmentation of DNA and thus protect liver from various toxicological insults²⁵⁻²⁷.

CONCLUSION

Cisplatin administration produced marked histopathological changes in the liver demonstrated by extensive fatty changes throughout the hepatic lobules with infiltration of lymphocytes and dilatation of sinusoidal spaces. Cisplatin caused severe lipid peroxidation and generates oxidative stress in the hepatocytes. Daily treatment with the flavonoid, 6-hydroxyflavone protected the hepatocytes from the cisplatin-induced degradative changes and inhibited the generation of oxidative stress. Flavonoid ingestion like 6-hydroxyflavone may be effective for the management of cancer patients diagnosed with hepatic problems specifically caused by beneficial chemotherapeutic agents including cisplatin.

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