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Antioxidant Traits and Protective Impact of Vorinostat against Cisplatin Induced Hepatoxicity in Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author OMOB did the practical investigation. Authors HSAKE and ASA supervised and reviewed the investigation. Author SSA worked the histology. Authors OMOB, HSAKE, ASA and SSA wrote the manuscript. Authors OMOB, HSAKE, ASA and SSA read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Background and Aim: Cisplatin "cis diamminedichloroplatinum [II] (CDDP) is the most widely used drug in cancer chemotherapy and hepatotoxicity is one of its major side effects. Vorinostat (VST) has been recognized to have an antioxidant and anti-inflammatory effect in low doses. The present study aimed to explore the potential protective effects of low dose VST against CDDP induced-liver toxicity in male Wistar rats.
Methods: The rats were randomly divided into 4 groups (10 rats each); I-control group, II-CDDP group (7.5 mg/kg I.P. single dose 5 days before the end of the experiment) III-, VST group (15 mg/kg/day by gastric gavage for 28 days) and IV-CDDP + VST group (as in group II & III). Blood and livers samples were collected at the day 28th for biochemical and histopathological examinations.

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Results: Administration of CDDP significantly decrease hepatic GSH levels and increase serum alanine transaminase, aspartate transaminase and hepatic MDA, p53, TNF-α, and NF-κB levels compared to control. Pretreatment with VST significantly attenuated all unfavorable changes in these parameters. Histopathological analysis showed that VST significantly decreased liver inflammatory and degenerative changes induced by CDDP. VST also significantly increased Bcl-2 and decreased Caspas-3 immunoexpression in hepatic tissues.
Conclusion: VST alleviates CDDP induced hepatic toxicity in rats by modulating MDA, p53, TNF-α, and NF-κB. It also significantly increased Bcl-2 and decreased Caspase-3.

Keywords: Vorinostat; cisplatin; hepatotoxicity; GSH; malondialdehyde; tumor necrosis factor-alpha; and nuclear factor kappa B.

ABBREVIATIONS

CDDP: Cisplatin "cis diamminedichloroplatinum [II]; MDA: Malondialdehyde; NF-κB: Nuclear factor kappa B; P53: Tumor suppressor protein 53; ROS: Reactive oxygen species; SAHA: Suberoylanilide hydroxamic acid; TNF-α: Tumor-necrosis factor-α; VST: Vorinostat.

1. INTRODUCTION

Many drugs have been implicated in hepatotoxicity which usually results in cell death of hepatocyte [1]. According to world health organization WHO assessments, drug-induced liver injury affects about 500 million persons resulting in the death of one million annually worldwide [2]. Cisplatin "cis-diamminedichloroplatinum [II]; CDDP; Platinol" is an alkylating agent frequently used for the treatment of several solid tumors and other types of neoplasms. It has been reported that CDDP causes hepatic injury by the activation of various pathways: oxidative stress, apoptosis, and inflammatory pathways leading to variances in the structure and function of the liver (destructive changes in liver tissues associated with deterioration of its function) [3].

CDDP efficacy is dose dependent, however, higher doses increase the risk of liver toxicity [4]. CDDP; induced hepatotoxic effects was manifested as an increased level of liver enzymes [5,6]. It can be explained because of suppresses antioxidant defense system by increases the production of reactive oxygen species (ROS) [7]; Previous research showed that there are signs of CDDP induced liver toxicity which indicate and the acceleration of the peroxidative mechanisms in the liver cell [8]. These changes lead to a marked reduction of levels of glutathione (GSH) and increase levels of hepatic malondialdehyde (MDA) [9]. In liver tissues, CDDP leads to an elevation pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and suppression of the anti-inflammatory cytokines such as Interleukin 10 (IL-10) [6].

Many antioxidant or anti-inflammatory compounds have been shown to ameliorate the drug induced oxidative stress and potentially provide protective effects against drug-induced liver toxicity [10]. Histone deacetylase (HDACs) plays a critical role in inflammation by coordinating the communication of intracellular signaling pathways, chromatin remodeling, and transcriptional factors. In experimental animal research, pharmacologically modulators of HDACs have shown strong anti-inflammatory outcomes. Moreover, inhibition of HDACs generally results in apoptosis and growth arrest of a certain cancer cell line [11].

Suberoylanilide hydroxamic acid (SAHA), is an HDAC inhibitor known as vorinostat (VST), was the first HDAC inhibitor approved by the food and drug administration (FDA) to treat certain cutaneous T-cell lymphoma [12]. It has been shown that VST with a low dose can enhance liver function and reduce liver fibrosis [13]. However, the mechanism of action behind that is still not clear. Besides, little work has been done to explore the effect of low dose VST as anti-inflammatory and antioxidant agent.

The present study aimed to investigate the potential protective effects of low dose VST against CDDP induced-liver toxicity in male Wistar rats. In addition to investigating possible molecular mechanisms that explain the ability of VST to reverse or protect the liver against CDDP induced toxicities.

2. MATERIALS AND METHODS

2.1 Drugs and Chemicals

VST was purchased from Advanced Engineering, WI, USA. It was dissolved in Dimethyl sulfoxide

(DMSO) (66 mg/ml) [14]. CDDP ampule 50 mg/100 ml (0.5 mg/ml) was purchased from Manufacturer: Ebewe Pharma, Origin: Austria.

2.2 Experimental Protocol

Forty male Wistar rats (weighing 180-230 g) were purchased from an animal house in King Fahd Center for Medical Research (KFMRC). Rats were housed in an animal room with an adaptation period of one week for vehicle administration was allowed before initiation of the experimental protocol.

2.3 Experimental Design and Study Group

Induction of liver injury: A model of liver injury was induced by intraperitoneal injection of CDDP. A single dose of CDDP 7.5 mg/kg was intraperitoneally injected into rats. This dose was found to cause inflammation and hepatic toxicity after five days [15].

Study groups (10 rats each): Group I: (control) rats received normal saline (IP) single dose and vehicle by gastric gavage daily (DMSO) for 28 days. Group II: (CDDP) Rats received CDDP (7.5 mg/kg I.P. single dose, on day 23). Group III: (VST) Rats received VST (15 mg/kg) by gastric gavage daily for 28 days. Group IV: rats received CDDP as in group II, alongside were given daily VST (15 mg/kg for 28 days) by gastric gavage (Fig. 1). The dose of CDDP was selected because of the reported hepatotoxic dose [15]. The dose of VST was extrapolated from the previous study that examined the anti-inflammatory effect of it [16].

2.4 Biochemical Measurements

On day 28th, rats were anesthetized by a large dose of ether, cardiac blood samples were collected in plain tubes. After that rats were sacrificed, and livers were removed.

Blood samples preparation: Blood was left to clot for 15 minutes at room temperature and then centrifuged at 3500 r.p.m. for 15 min to separate the serum. Serum Separation by Gel Tubes SSGT to obtain high quality serum specimens. The serum kept at -80 until analysis within one month for biochemical assessment.

Liver samples preparation: The right lobe of the liver was removed, cut into thin slices, some of these slices were kept in 10% formalin for histological and immunohistochemical examination. The left lobes of the livers were washed with cold buffer solution and slices were kept at -80 for the assessment of hepatic GSH, MDA, p53, TNF- α, and NF-κB. level in tissue homogenate.

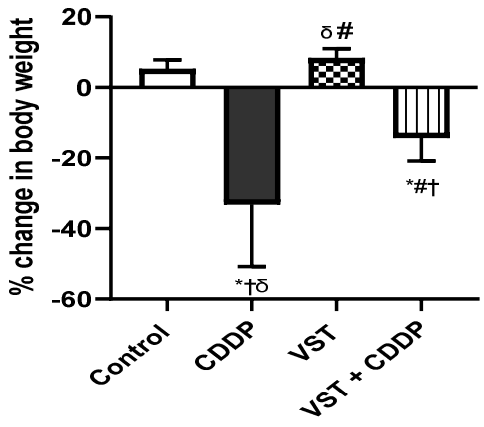

Fig. 1. Effect of pre-treatment with vorinostat (VST) on cisplatin (CDDP) induced % change in the body weight of rats

*Statistical analysis was carried out by using one-way analysis of variance (ANOVA) followed by Tukey multiple comparisons test. *Significant difference from normal control (vehicle) group at p<0.05, #Significant difference from CDDP (7.5 mg/kg) group at p<0.05, † Significant difference from VST (15 mg/kg) group at p<0.05 and δ Significant difference from VST + CDDP group at P <0.05*

2.4.1 Serum alanine aminotransferase and aspartate aminotransferase levels

The serum ALT and AST levels were measured according to the method of [17]. ALT (Alanine Transaminase) ELISA Kit purchased from MyBioSource, USA (Catalog # MBS269614) and aspartate aminotransferase ELISA Kit purchased from MyBioSource, USA (Catalog # MBS264975). Levels of ALT and AST were expressed as IU/L.

2.4.2 Hepatic homogenate assessment

Rinse 100 mg of the liver tissues with cold phosphate buffered saline (PBS) (pH 7.4) to remove any blood before homogenization. Add 10 ml PBS (pH 7.4) to the tissues and homogenize using a polytron homogenizer (PT 3100) (five cycles of 10 s at 3000 rpm). Freeze at -20°C then centrifuge at 3000 rpm for 20 min (using a HERMLE centrifuge, Germany). The supernatant was collected and stored at -80°C till assayed.

2.4.2.1 Assessment of reduced glutathione (GSH)

Levels of hepatic reduced glutathione (GSH) were measured to evaluate the antioxidant state in liver homogenate. Levels of hepatic GSH in rats were determined by using the ELISA kit (Catalog # MBS724319) purchased from MyBioSource, USA. Levels of GSH was expressed as ng/ ml.

2.4.2.2 Assessment of malondialdehyde (MDA)

Levels of hepatic MDA in rats were measured to evaluate the oxidative damage of the liver by using the ELISA kit (Catalog # MBS738685) purchased from MyBioSource, USA. Levels of MDA was expressed as µmol /l.

2.4.2.3 Assessment of p53

Levels of hepatic p53 in rats were determined to evaluate apoptosis of hepatocyte in liver homogenate by using ELISA kit (Catalog # MBS723886) purchased from MyBioSource, USA. Levels of p53 were expressed as µg/l.

2.4.2.4 Assessment of TNF-α

Levels of hepatic TNF-α in rats were determined to evaluate inflammation level in liver homogenate by using ELISA kit (Catalog # MBS355371) purchased from MyBioSource, USA. Levels of TNF-α were expressed as pg/ml.

2.4.2.5 Assessment of NF-κB

Levels of hepatic NF-κB in rats were determined to evaluate inflammation level in liver homogenate by using ELISA kit (Catalog # MBS268833) purchased from MyBioSource, USA. Levels of NF-κB was expressed as µmol/l.

2.5 Hepatic Histopathological and Immunohistochemical Assessment

Following fixation of liver tissues in 10% formalin for one week, liver tissues were processed using standard methods. The tissues of the liver were embedded in paraffin blocks. Serial sections were cut at a thickness of 5 µm. Finally, visualization and photographing of slides were observed by objective lens X100 (low power field) and X400 (high power field) using an Olympus light microscope (model: BX51TF- Japan).

Hepatic sections were subjected to the following techniques:

2.5.1 Hepatic histopathological assessment

The sections were stained with hematoxylin and eosin stain (H&E) for studying general histology [6,18]. The examination of five different non-overlapping fields in three serial sections of 3 different animals in each group was done.

Scoring of hepatic histopathological alterations stained with H&E-stained [6].

a. Hepatocytes degeneration, necrosis or apoptosis:

Early apoptotic changes are characterized by hepatocytes shrinkage, dense acidophilic cytoplasm, and smaller pyknotic nuclei with condensed chromatins at the periphery of the nuclei (Goldsworthy et al. 1996). The results were confirmed by immunohistochemistry for caspase -3 apoptotic markers.

- = no watched changes; + = mild changes; + + = moderate changes and + + + = extreme changes.

b. Van Kupffer cells activation:

- = no watched activation of Van Kupffer cells; + = mild activation of Van Kupffer cells; + + = moderate activation of Van Kupffer cells and + + + = extreme activation of Van Kupffer cells.

c. Congestion and dilatation of central vein and hepatic sinusoids:

- = no watched changes; + = mild changes; + + = moderate changes and + + + = extreme changes.

d. Karyomegaly activation:

- = no watched changes; + = mild changes; + + = moderate changes and + + + = extreme changes.

e. Cytoplasmic vacuolation of hepatocytes:

- = no watched changes; + = mild changes; + + = moderate changes and + + + = extreme changes.

f. Focal area of hepatocyte swelling and necrosis:

- = no watched changes; + = mild changes; + + = moderate changes and + + + = extreme changes.

g. Hydropic degeneration of hepatocytes:

- = no watched changes; + = mild changes; + + = moderate changes and + + + = extreme changes.

2.5.2 Immunohistochemical assessment

Paraffin sections of the liver were cut at 5µm thickness on positively charged slides and stained with Caspase-3 stain for detection of apoptosis [19] and Bcl-2 stain for detection of anti-apoptotic effect [6]. The staining procedures followed the protocol provided by the company for the kit used. Caspase-3 stain (Catalog # PP229AA) purchased from Biocare medical, USA. Bcl-2 stain (Catalog # 94538) purchased from BioGenex, USA.

2.6 Statistical Analysis

A statistical study was conducted out using graph pad prism version 8 (2019), USA. All data were given as means ± standard deviation. For comparison of the different groups, analysis of variance (ANOVA) was calculated with significance set at $P < 0.05$. When the ANOVA test was significant, it followed by the post hoc Tukey test for multiple comparisons between means of groups.

3. RESULTS

3.1 Rat's Body Weight

A single dose of CDDP produced a significant ($P<0.05$) decrease in % change of rat's body weight in CDDP group (60 g, 22% decrease in weight) compared to that in the control group. This weight loss was attenuated in VST + CDDP group (34.17 g, 12% decrease in weight). Regarding VST rats' group, not significantly different in the % change of rat's body weight compared to that in the control group (Table 1 and Fig. 1).

3.2 Biochemical Mesurments

3.2.1 Serum level of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) after 28 days

Administration of CDDP produced a significant ($P<0.05$) increase in the mean serum ALT level (128.33 ± 29.01) compared to that in the control group (15.22 ± 3.40) [about 10-fold increase confirming massive deterioration of liver function induced by CDDP]. The mean ALT level in serum of VST + CDDP group (19.93 ± 2.57) and VST group (14.37 ± 2.42) is not significantly different from that of control. The same pattern was observed with AST in Fig. 2. The serum AST level increased from 18.92 ± 2.75 (control) to 162.32 ± 31.79 IU/L (CDDP) after administration of CDDP [about 8-fold increase in mean level]. Concomitant administration of VST + CDDP prevents the CDDP induced an increase in AST to mean serum level. These data confirm the protective effect of VST against the hepatotoxic effect of CDDP or in other words achievement of normal liver function tests.

3.2.2 Hepatic homogenate studies

The data demonstrate the ability of VST to attenuate the CDDP induced MDA level, reduced GSH level and induced expression of p53, TNF-α and NF-κB levels in liver tissues Fig. 3. A significant ($P<0.05$) decrease in the mean GSH level was observed due to the administration of CDDP (2.32 ± 0.91) compared to the corresponding value of the control (21.77 ± 2.39). In the case of CDDP + VST almost normal values of mean GSH level were observed (18.73 ± 1.73) also in VST alone. The mean MDA level was significantly increased ($P<0.05$) from 0.85 ± 0.09 (control) to 2.80 ± 0.22 µmol/l (CDDP); while that in VST + CDDP is comparable to the control. The mean MDA level in liver tissue VST + CDDP group (0.73 ± 0.21) and VST group (0.69 ± 0.17) was comparable to control. The mean p53 level was significantly ($P<0.05$) increased from 0.53 ± 0.06 µg/l compared to the corresponding normal control group (0.14 ± 0.03). The mean p53 level in the liver tissue of the VST + CDDP group (0.14 ± 0.03) was the same as that of the control group. VST alone produced insignificant differences compared to control. Similarly, CDDP increased the expression of TNF-α in liver tissues and this effect is abolished by VST. The mean TNF-α level was significantly ($P<0.05$) increased from 48.33 ± 4.19 (pg/ml) compared to the corresponding normal control group (21.83 ± 3.12). The mean TNF-α level in the liver tissue of the VST + CDDP group (23.88 ± 2.26) and VST alone insignificantly different compared to the control group. CDDP also increased the expression of NF-κB in renal tissues and this effect is abolished by VST. The mean NF-κB level was significantly ($P<0.05$) increased from 72.90 ± 6.81 (µmol/l) compared to corresponding normal control group 23.07 ± 4.64 µmol/l. The mean NF-κB level in the liver tissue of the VST + CDDP group (24.17 ± 4.29) and VST alone insignificantly different compared to the control group.

Table 1. Effect of pre-treatment with vorinostat (VST) on cisplatin (CDDP) induced change in the body weight of rats

Body weight	Control	CDDP	VST	VST + CDDP
I- Before injection of cisplatin, g	273.33 ± 23.38	271.67 ± 34.45	260.00 ± 18.97	279.17 ± 19.08
II-At the end of experiment, g	288.33 ± 28.58	211.83 ± 20.20	283.33 ± 19.66	245.00 ± 22.58
Difference (II-I)	15 g increase	60 g decrease	23.33 g increase	34.17 g decrease
% change *	5.49 %	-22.03 % $^{*\dagger\delta}$	8.97 % $^{\#\,\delta}$	-12.24 % $^{*\#\dagger}$

% change = (weight after injection - weight before injection) x 100; weight after injection;

Values are expressed as mean ± standard deviation (SD), n = number of rats (6 rates/group); One-way ANOVA followed by Tukey's multiple comparison test; $^{*}P$ <0.05 compared to normal control group; $^{\#}P$ <0.05 compared to CDDP group; $^{\dagger}P$ <0.05 compared to VST group; $^{\delta}P$ <0.05 compared to VST + CDDP group

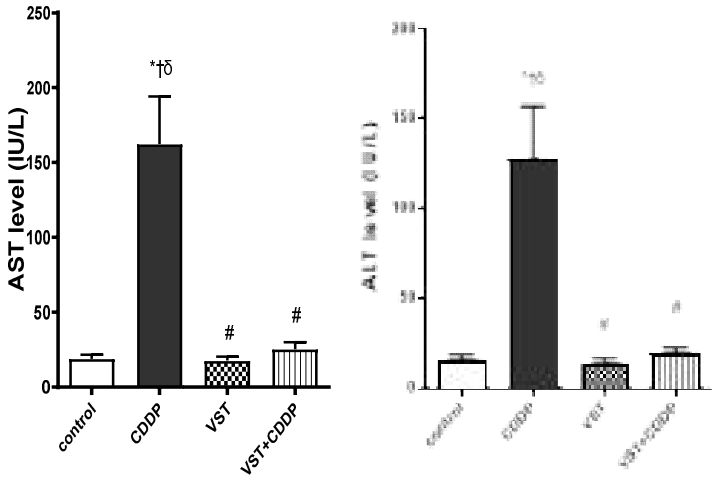

Fig. 2. Effect of pre-treatment with vorinostat (VST) on cisplatin (CDDP) induced increase in rat's serum alanine transaminase serum (ALT) level and serum aspartate transaminase (AST) level

*Statistical analysis was carried out by using one-way analysis of variance (ANOVA) followed by Tukey multiple comparisons test. *Significant difference from normal control (vehicle) group at p<0.05, #Significant difference from CDDP (7.5 mg/kg) group at p<0.05, † Significant difference from VST (15 mg/kg) group at p<0.05 and δ Significant difference from VST + CDDP group at P <0.05*

3.3 Histopathological and Immuno-histochemical Assessment of Hepatic Tissue

3.3.1 Hepatic histopathological assessment

Hepatic tissue of control rat showed normal hepatocytes arranged in cords or plates radiating from the central vein. Portal areas are located at the periphery of the lobule and showed branches of portal blood vessels and bile ducts. Hepatocytes showed normal active nuclear appearance. Blood vessels and hepatic sinusoids between cell cords showed normal no congested appearance. Rats received VST alone showed almost the same pattern.

CDDP showed marked alteration of hepatic tissue in the form of dilatation and congestion of hepatic vessels. In some samples, the central veins are markedly dilated and showed damaged detached endothelial lining. Dilatation and congestion of portal vessels were also observed. Hepatocytes at the perivascular central vein region showed signs of necrosis where cell outlines are ill-defined and cell nuclei showed irregularity or karyolitic features. Some samples showed a peri-ductal inflammatory cell infiltrate.

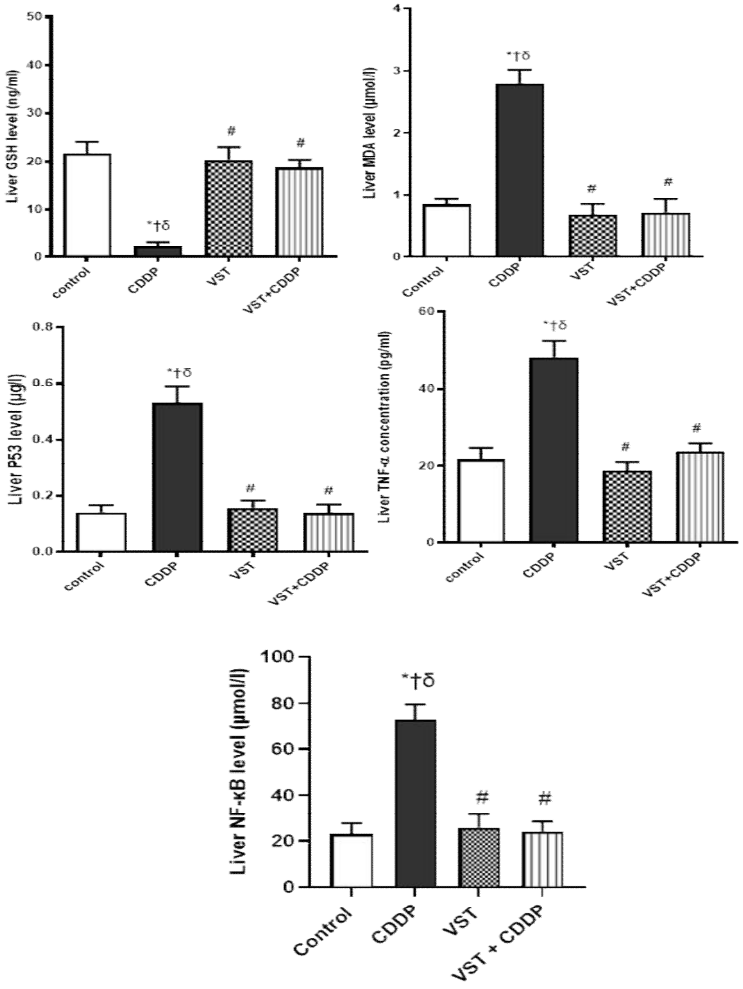

Fig. 3. Effect of pre-treatment with vorinostat (VST) on cisplatin (CDDP) induced change in liver tissue

*Statistical analysis was carried out by using one-way analysis of variance (ANOVA) followed by Tukey multiple comparisons test. *Significant difference from normal control (vehicle) group at p<0.05, #Significant difference from cisplatin (7.5 mg/kg) group at p<0.05, † Significant difference from vorinostat (15 mg/kg) group at p<0.05 and δ Significant difference from vorinostat + cisplatin group at P <0.05*

Administration of VST to rats before receiving CDDP resulted in marked preservation of hepatic tissue with mild individual variation in the extent of response. Hepatic tissue in most rats showed normal hepatocytes with sporadic cells possessing enlarged nuclei (Karyomegaly). Focal few areas of vascular congestion and few hepatocytes swelling and vacuolation (Figs. 4 and 5).

3.3.2 Hepatic histopathological alterations scoring

As showed in Table 2 hepatocytes degeneration and necrosis or apoptosis scoring congestion and dilatation of central vein and hepatic sinusoids, karyomegaly activation, cytoplasmic vacuolation of hepatocytes, focal area of hepatocyte swelling and necrosis, Van Kupffer cells activation: and hydropic degeneration of hepatocytes were significantly higher in CDDP than CDDP and VST combination group.

3.3.3 Hepatic immunohistochemical staining

3.3.3.1 Immunoexpression of Caspase-3 in hepatic tissue (Figs. 6 and 7)

Hepatic tissue of control rat showed occasional Caspase-3 positive apoptotic cells in the

pericentral areas located around the central vein and periportal areas located at the periphery of the lobule around the branches of the portal blood vessels and bile ducts. Administration of a single dose (7.5 mg/kg) of CDDP via I.P. route resulted in a frequently observed Caspase-3 positive apoptotic cells in the pericentral area and periportal area as well as in many endothelial cells lining the blood sinusoids compared to the control group. Liver tissue from rats daily received VST alone for 28 days showed occasional Caspase-3 positive apoptotic cells in the pericentral and periportal areas as was observed in the control group. Administration of VST to rats before receiving CDDP resulted in a reduction in the Caspase-3 positive apoptotic cells compared to the group received CDDP alone.

3.3.3.2 Immunoexpression of Bcl-2 in hepatic tissue (Fig. 8)

CDDP leads to a marked reduction in Bcl-2 immunoexpression in hepatocytes compared to the control group but few cells that still showing a positive reaction. Liver tissue from rats received VST alone for 28 days showed positive Bcl-2 immunoexpression in hepatocytes nearly similar to that observed in the control group. Administration of VST to rats before receiving CDDP resulted in increased Bcl-2 immunoexpression in hepatocytes compared to CDDP group.

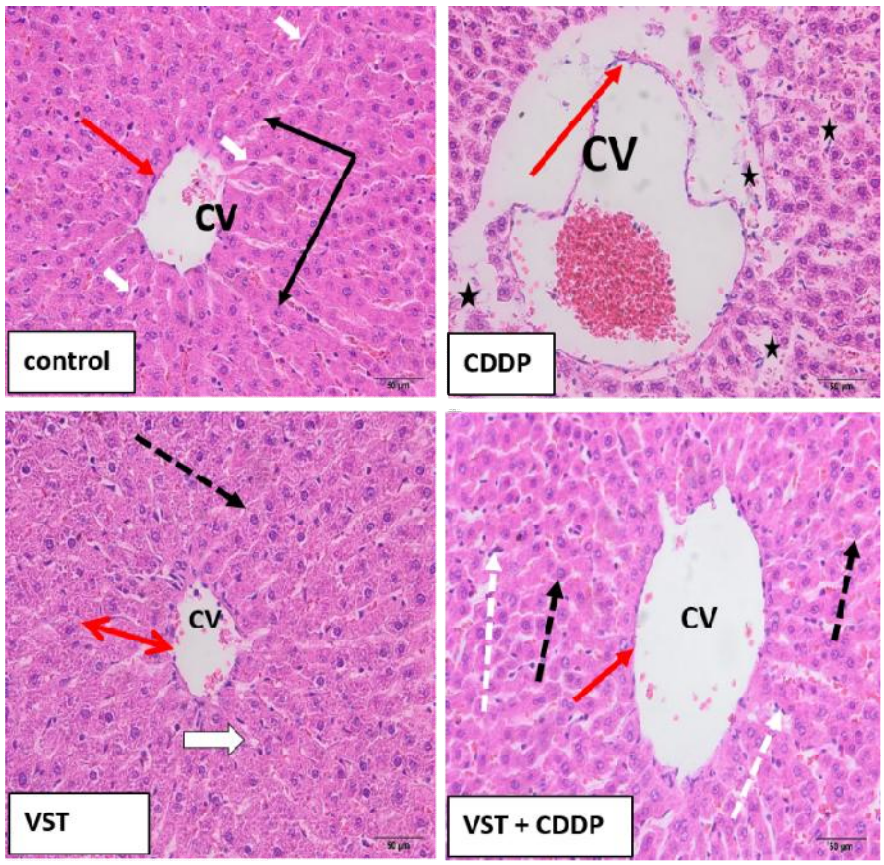

Fig. 4. Photomicrographs of sections of rat's liver central vein region (CV) (H&E stain, X 400)

Control: non-dilated CV with intact endothelial lining (red arrow) and normal hepatocyte cell cords (black arrows) separated by normal hepatic blood sinusoid (white arrows). CDDP: showing the detached endothelial lining (red arrow) of markedly dilated congested CV. Nearby hepatocytes cell necrosis where cells lost their outlines and showed some nuclear-altered staining (black stars). VST: showing non-dilated CV with intact endothelial lining (red arrow). Hepatocytes showed granular cytoplasm with active nuclei similar to control (dotted arrow). No dilation or congestion in hepatic vessels or sinusoid (white arrow). VST + CDDP: showing slight dilated CV with intact endothelial lining (red arrow). Hepatocyte looked normal (black dotted arrows). Van Kupffer cells are prominent (white dotted arrows)

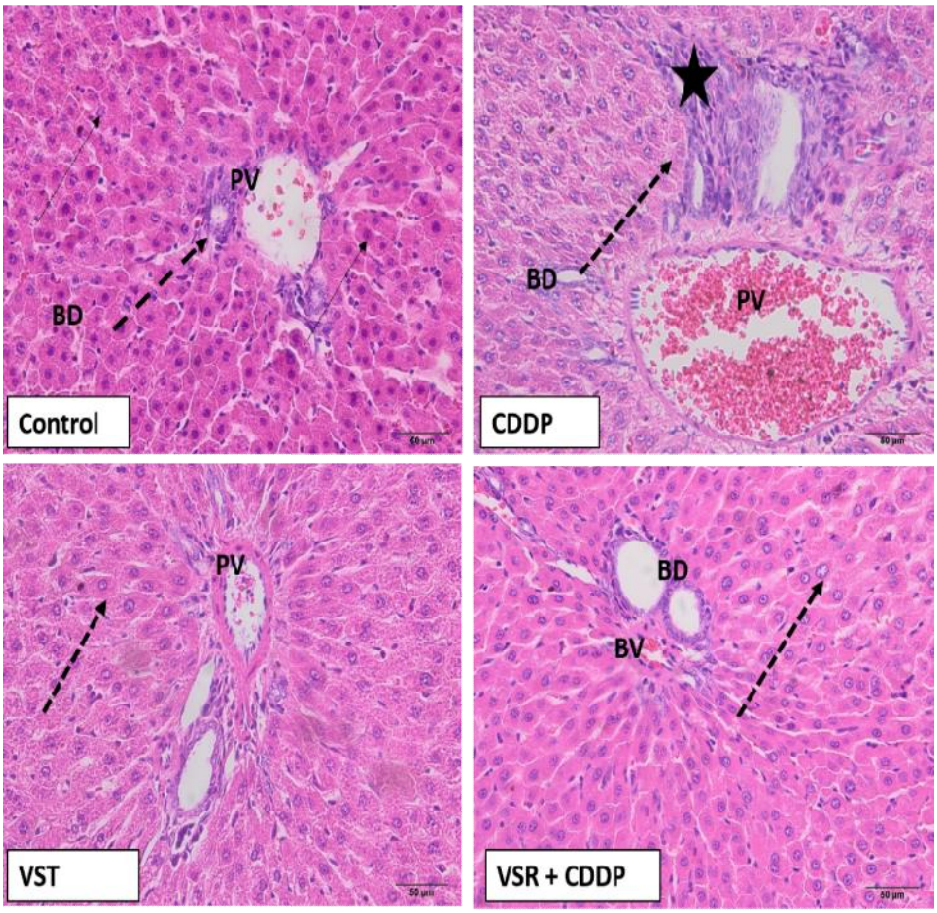

Fig. 5. Photomicrographs of sections of rat's liver portal vein region (PV) (H&E stain, X 400)
Control: showing normal PV and bile ducts (BD) surrounded by normal hepatocytes (dotted arrows). CDDP: showing marked dilatation and congestion of PV, bile ducts (BD) proliferation (dotted arrow) and peri-ductal inflammatory cell infiltrate (black star). VST: showing PV looked normal with no change in cytoplasm or nuclei (dotted arrow). VST + CDDP: showed normal contents of blood vessels (BV) and BD. Hepatocytes are normal. Some showed large nuclei (dotted arrow)

Table 2. Hepatic histopathological alterations scoring

Hepatic histopathological alterations	Control	CDDP	VST	VST + CDDP
a-Hepatocytes degeneration and necrosis or apoptosis	-	+++	-	+
b-Van Kupffer cells activation	-	++	-	+
c-Congestion and dilatation of central vein and hepatic sinusoids	-	+++	-	+
d-Karyomegaly activation	-	+++	-	+
e-Cytoplasmic vacuolation of hepatocytes	-	+++	-	-
f-Focal area of hepatocyte swelling and necrosis	-	++	-	+
g-Hydropic degeneration of hepatocytes	-	++	-	-

- = no watched changes; + = mild changes; ++ = moderate changes; +++ = extreme changes

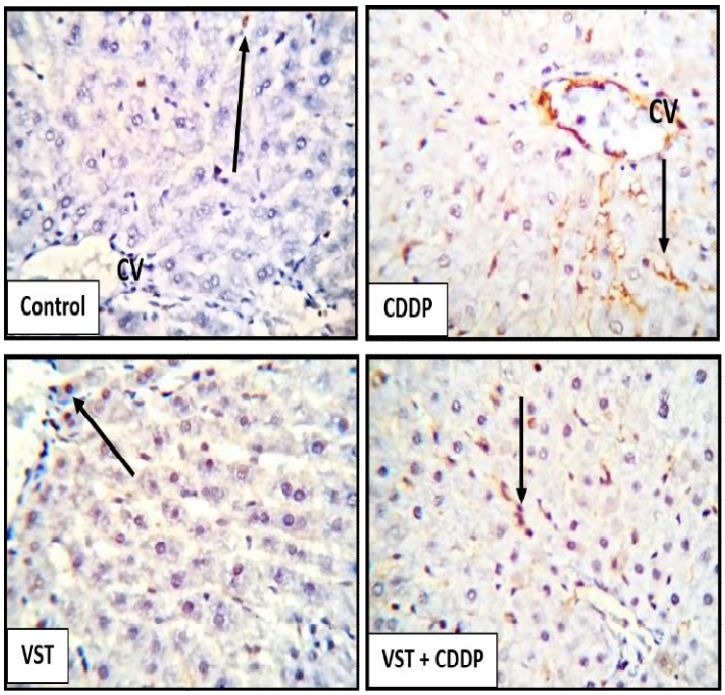

Fig. 6. Photomicrographs of immunohistochemical staining of Caspase-3 in rats' hepatic central vein region (CV) (X 400 magnification)

Control: showing occasional Caspase-3 positive apoptotic cells (black arrow) distinguished by brown cytoplasm in the pericentral areas around the central vein located at the periphery of the lobule around the branches of the portal blood vessels and bile ducts. CDDP: showing frequent Caspase-3 positive apoptotic cells (black arrow) in the pericentral areas compared to the control group. VST: showing occasional Caspase-3 positive apoptotic cells (black arrow) in the pericentral areas as observed in the control group. VST + CDDP: showing less frequent Caspase-3 positive apoptotic cells compared to the CDDP group

3.4 Effect of Pre-treatment with Vorinostat (15 mg/kg) on Cisplatin (7.5 mg/kg) Induced Changes in the Levels of Quantitative Assessment of Caspase-3 and Bcl-2 Immunoexpression in Liver Tissues

Caspase-3 immunoexpression in the liver: When the Caspase-3 immunoexpression was assessed in the liver, it was noticed that it was significantly increased (P<0.05) in CDDP-treated rats compared to the control one, while it was significantly decreased (P<0.05) in VST + CDDP group compared to the CDDP-treated group. There was no significant difference in Caspase-3 immunoexpression in the liver of VST or VST + CDDP compared to the control group (Table 3).

Bcl-2 immunoexpression in the liver: Immunoexpression of Bcl-2 in the liver was significantly reduced (P=0.05) in CDDP-treated animal group compared to the control one, while it was significantly increased (P=0.05) in VST + CDDP group compared to the CDDP-treated group. There were no significant differences in Bcl-2 immunoexpression in the liver of VST group compared to the control group (Table 3).

Table 3. Quantitative assessment of Caspase-3 and Bcl-2 immunoexpression in the liver and kidney

Mean ± SD	Control	CDDP	VST	VST + CDDP
Caspase-3 expression in the liver	0.17 ± 0.08	1.96 ± 0.87$^{*\dagger\delta}$	0.19 ± 0.14$^{\#}$	0.32 ± 0.18$^{\#}$
Bcl-2 expression in the liver	2.04 ± 0.98	0.32 ± 0.21$^{*\dagger\delta}$	2.12 ± 0.34$^{\#}$	1.43 ± 0.62$^{\#}$

Values are expressed as mean ± standard deviation (SD), n = number of rats; One-way ANOVA followed by Tukey's multiple comparison test; *P <0.05 compared to normal control group; $^{\#}$P <0.05 compared to cisplatin group; †P <0.05 compared to vorinostat group; $^{\delta}$P <0.05 compared to vorinostat + cisplatin group

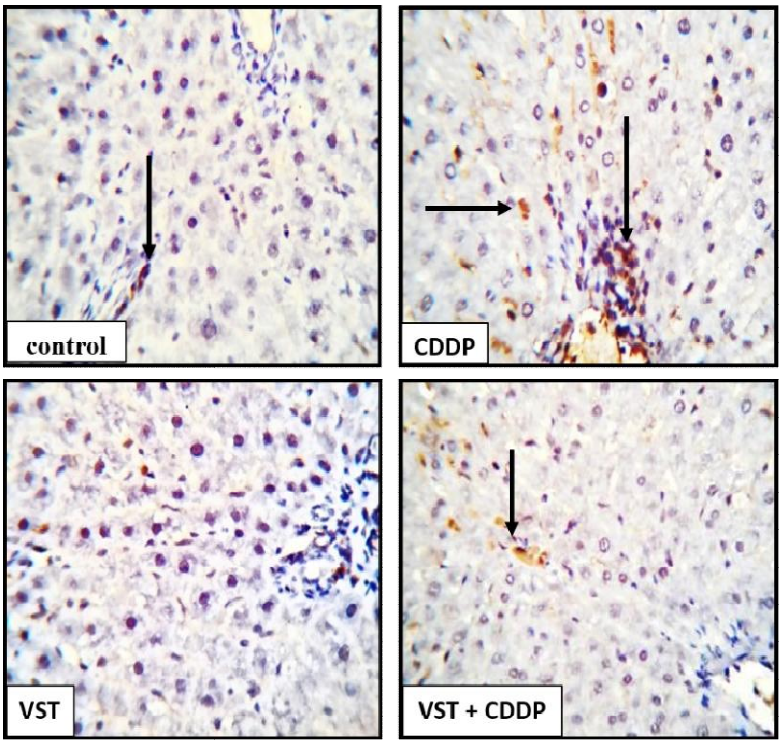

Fig. 7. Photomicrographs of immunohistochemical staining of Caspase-3 in rats' hepatic portal vein region (PV) (X 400 magnification)

Control: showing occasional Caspase-3 positive apoptotic cells (black arrow) distinguished by brown cytoplasm in the periportal areas located at the periphery of the lobule around the branches of the portal blood vessels and bile ducts. CDDP: showing frequent Caspase-3 positive apoptotic cells (black arrows) in the periportal areas compared to the control group. VST: showing occasional Caspase-3 positive apoptotic cells in the periportal areas as observed in the control group. VST + CDDP: showing less frequent Caspase-3 positive apoptotic cells compared to the CDDP group

4. DISCUSSION

CDDP is the most widely used drug in chemotherapy either alone in combination with radiotherapy or with other anticancer agents [20]. It is used for the treatment of several human neoplasms [21-23]. The hepatotoxicity of CDDP is the major side effect of this drug which occurred during high doses treatment [24,25]. The present study sheds light on the possible protective effects of VST on CDDP -induced hepatotoxicity in male Wistar rats. Recently, VST has been recognized to have antioxidant and anti-inflammatory efficacy both in in-vitro and in vivo models [13]. A low dose of VST has a hepatoprotective effect against drug induced liver damage [14].

The cell of liver contains high concentrations of transaminases enzymes (ALT and AST) [9]. The progressive increase in serum level of liver enzymes have been considered as an early indicator for hepatic damage [26]. The present finding demonstrated that CDDP caused damage to liver cells to lead to leakage of cytosol resulted in significant elevation of ALT and AST levels in serum in rats. The results of the present study are in agreement with several studies [3,15,27].

In the present study, VST prevented CDDP induced increase in rat's serum level of transaminase in combination groups confirming its hepatoprotective effect.

Oxidative stress and ROS were noted to be involved in CDDP induced hepatic and renal toxicity which is considered to be one of the main causes for liver injury [27]. GSH, is an antioxidant defense enzyme that plays an important role in cellular defense against ROS and cellular damage [28], MDA, is a harmful product of lipid peroxidation produced by oxidative stress which causes the damage of cellular membrane integrity and leakage of cellular contents to blood [28].

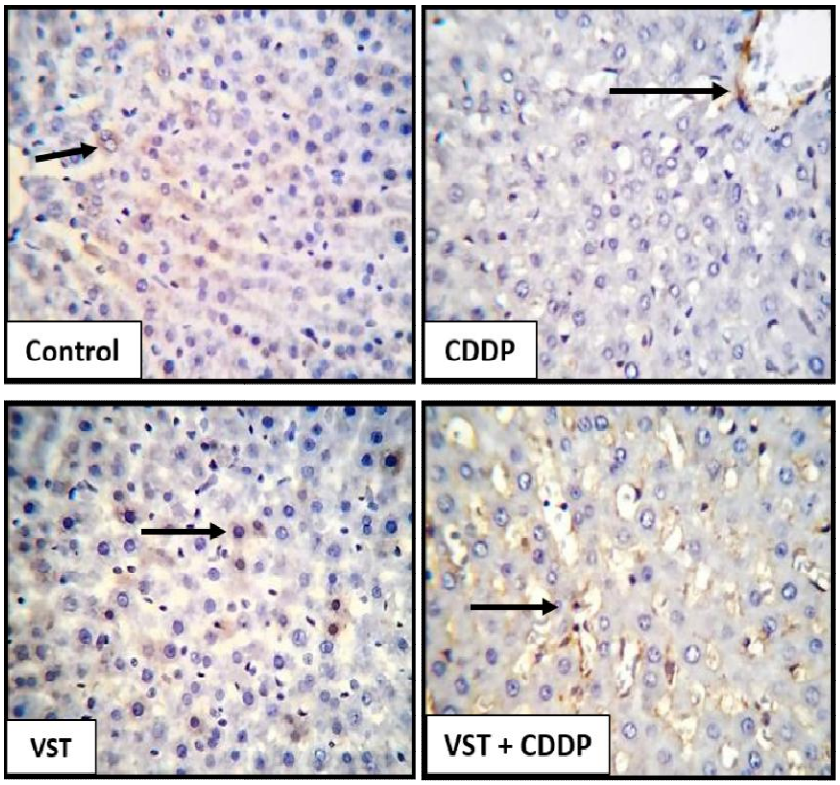

Fig. 8. Photomicrographs of immunohistochemical staining of Bcl-2 in rats' hepatic tissue (X 400 magnification)

Control: Showing positive Bcl-2 immunoexpression in hepatocytes (black arrow). CDDP: showing marked reduction in Bcl-2 immunoexpression in hepatocytes compared to the control group. Few cells still show positive reactions (black arrow). VST: showing positive Bcl-2 immunoexpression in hepatocytes (black arrow) nearly similar to that observed in the control group. VST + CDDP: showing increased Bcl-2 immunoexpression in hepatocytes (black arrow) compared to the CDDP group

CDDP administration was known to induce oxidative stress in renal epithelial cells and liver cells with depletion of the hepatic GSH and triggered the formation of lipid peroxide [9]. These effects were confirmed in the present study by a significant reduction of GSH and an increase of MDA in liver tissues of rats received a single IP dose of CDDP. Several studies documented these conventional pathways of CDDP induced hepatic toxicity.

In agreement with the results of the current study, several studies suggested that the administration of CDDP rapidly reacting with antioxidant defense leading to reduce the activity of the antioxidant enzyme and decreasing concentrations of intracellular GSH lead to dysfunction of mitochondria [18,27,29]. Besides, other studies reported that CDDP produced a change in oxidative stress marker which is a reduction of GSH and elevation of MDA [3,9,27,30].

Pre-treatment with VST (50 mg/kg/day IP) for 7 days in male C57BL/6J mice exhibited increased renal levels of GSH and decreased renal levels of MDA [31]. In the present study, CDDP induced marked elevation of MDA along with the reduction of GSH in renal and hepatic tissues. These CDDP induced changes were prevented by chronic treatment with VST. The protective effect of VST against CDDP induced increase in oxidative stress was explained by VST ability to scavenge intracellular ROS and reduce levels of free radicals as cited before [14].

CDDP can enter the cell which forms DNA adduct and generates ROS leading to activation of p53 [32]. In the present study, CDDP caused a significant elevation in p53 which explained as an early sign of apoptosis in rat hepatic tissue. Activated P53 caused cell cycle arrest and change in the potential of the mitochondrial membrane in cells. These observations lead to the release of cytochrome *c* and activation of

caspase 9 and a caspase 3 and then lead to apoptosis as cited before [32].

Huang et al. (2017) demonstrated that single IP injection of CDDP (8 mg/kg) induced liver and kidney injury in rats which were demonstrated as marked pathological changes of tissues of these affected organs mediated by expression of p53 and caspase 3 in the liver. Furthermore, Sun et al. (2014) utilized Western blot analysis and Immunohistochemical staining to demonstrate an increase in oxidative stress, and apoptosis in the liver tissues mediated by activation of p53 after single dose 10 mg/kg IP CDDP in Kunming mice model.

The current data revealed that VST inhibited acetylation, phosphorylation, and activation of p53 induced by CDDP. These cascades of events explained the protective effect of VST against CDDP induced liver damage is likely to be mediated by its anti-apoptotic effects. These effects were similar to those afforded by the hepatoprotective natural compound, silymarin [33]. The present finding confirms previously reported observation regarding the efficacy of VST to suppress the expression of the p53 level [34]. The toxicity of CDDP was associated with an increased expression of the proinflammatory cytokine TNF-α due to the increased production of ROS and lipid peroxidation. Increased expression of TNF-α induces liver cell damage and accelerates apoptotic changes [35]. Such effect was confirmed in the current study by assay of TNF-α. In the present study, CDDP induces a massive inflammatory response in hepatic tissues to lead to an increase in the production of TNF-α which acts as a mediator in the pathogenesis of the liver inflammation in rats [36].

In agreement with the results of the present study, many studies clearly showed the ability of CDDP to induce inflammation and increase the expression of TNF-α. After a single dose of CDDP similar to that used in our study. Cure et al (2016) reported that a single dose of CDDP (7 mg/kg IP) in male Wistar albino rats and sacrificed 5 days after CDDP treatment produced a significant increase in TNF-α levels. Also, El-Shitany and Eid in (2017) demonstrated that a single (6 mg/kg IP) of CDDP produced a marked elevation in TNF-α levels in adult male Sprague Dawley rats and sacrificed after one day of CDDP treatment. Omar et al. [27] noted that CDDP single dose (7.5 mg/kg IP) induced an increase in TNF-α levels in adult male Wistar rats.

Another study suggested that the administration of CDDP with dose (15 mg/kg IP) in rats induced the production of pro-inflammatory cytokines [37].

Moreover, NF-κB is well known as a nuclear transcription factor found in the cytoplasm of the liver cells and binds to its inhibitory subunit I-κB. It controls several inflammatory genes, including IL-6, IL-1β, and TNF-α [9,38]. In the present study, Oxidative stress induced by CDDP significantly increased the levels of NF-κB. Activated NF-κB transfers to the nucleus of the liver cell and binds to DNA lead to induce expression of the pro-inflammatory cytokines and chemokines which was observed. These observations suggested that hepatotoxicity of CDDP by increased expression of NF-κβ in the liver tissue in rats. Thus obtained results are supported by a previous study of Al-Malki and Sayed [9] who showed that a single dose of CDDP (12 mg/kg IP) in male Wister rats produced a marked activation of NF-κB in the immunoreactivity in the cytoplasm of hepatocytes.

It has been noted that reduction of the NF-κβ expression was related with reduced production of certain cytokines such as IL-1β, IL-6, and TNF-α caused by CDDP and prevention of hepatic injury produced by an excessive inflammatory response [38]. In the current study, reductions in the TNF-α and NF-κB liver contents in the combination groups due to pretreatment with VST were observed.

In this study, it was found that administration of a single dose (7.5 mg/kg) of CDDP resulted in marked histopathological changes in hepatic tissue in the form of dilatation and congestion of hepatic vessels included central veins and portal veins which were markedly dilated and showed damaged detached endothelial lining. Hepatocytes at the perivascular central vein region appeared degenerated with ill-defined cell outlines and irregular, karyolitic cell nuclei. Some samples showed peri-ductal inflammatory cell infiltrate. There finding was in full agreement with those previously reported by Bilgic et al. [39]. They found that the most prominent alteration in the liver of CDDP-treated animals' group was sinusoidal congestion. They added that hepatocytes with eosinophilic cytoplasm and

pyknotic nuclei were detected in some conditions [39].

The administration of CDDP, in a previous study, was found to cause a severe liver injury evidenced by the dilatation and congestion of central vein and hepatic sinusoids with Kupffer cell activation and focal hepatic necrosis. Which also was accompanied by diffuse inflammatory cell infiltration [6]. These histopathological alternations induced by CDDP might be attributed to reactive oxygen species. It was reported that exposure of the liver to oxidative stress, as in CDDP treatment, liver cells encounter large quantities of reactive oxygen species that overwhelm their detoxification capacity. Reactive oxygen species then cause hepatocyte injury by injuring DNA, protein oxidation, lipid peroxidation presented in the form of inflammation, and direct action on signal pathways. When DNA injury could not be repaired, both proliferations are limited and apoptosis is augmented [9,40].

In this study, CDDP resulted in a frequently observed Caspase-3 positive apoptotic cells in the pericentral area and periportal area of the liver compared to the control group. The quantitative assessment of the Caspase-3 immunoexpression in the liver revealed that it was significantly increased in this group compared to the control one. This finding was supported by this of Bilgic et al. [39] who reported that CDDP group showed a significant increase in the caspase-3 expression in the liver [39].

Administration of a single dose of CDDP, in this study, resulted in a marked reduction in Bcl-2 immunoexpression in the hepatic tissue specifically the hepatocytes compared to the control group. Quantitative assessment of Bcl-2 immunoexpression in the liver revealed that there was a significant reduction in its expression in CDDP -treated group compared to the control one. This finding was supported by that of Omar et al. [6]. They mentioned that the administration of CDDP to rats had resulted in an extensive expression of Bax and decreased Bcl-2 in the hepatic tissues [6].

In this study, the administration of VST to rats before receiving CDDP alleviated the histopathological impact of CDDP in the liver. VST significantly increased Bcl-2 immunoexpression while it decreased Caspas-3 immunoexpression in hepatic tissue compared to CDDP-treated group. It is beneficial effect has been reported in previous studies. VST is a drug that was reported to have a beneficial *in vivo* effect over preclinical kidney injury, including acute kidney injury (AKI), chronic kidney disease (CKD), and the AKI-to-CKD transition, through decreasing DNA methylation by increase histone acetylation [41].

These findings result suggested that VST markedly ameliorated the pathological effect of CDDP on the structure of the liver and regained most of the normal structural features.

5. CONCLUSION

These results suggested that LdVST alleviates CDDP induced renal and hepatic toxicity in rats and that might show a novel therapeutic potential for management of CDDP induced renal and hepatic toxicity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The protocol was approved by the research animal ethics committee at King Abdul-Aziz University, faculty of medicine. Reference No 463-18 on Thursday, September 13, 2018.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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