CHEMICAL COMPOSITION AND PROTECTIVE EFFECT OF *ANACYCLUS VALENTINUS* AGAINST CISPLATIN-INDUCED HEPATOTOXICITY IN WISTAR RATS

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ABSTRACT

The current study was conducted to evaluate the protective effect of the ethanolic extract of *Anacyclus valentinus* (EEA) against hepatotoxicity resulting from exposure to cisplatin (CP) in male rats. In this study, a total of twenty four adult male Wistar rats were used and divided into four groups. The first group (G1) was the control group, the second group (G2) was exposed to CP (three doses of 7 mg/Kg body weight for 6 days; by intravenous injection), the third group (G3) received 200 mg/Kg EEA orally per day for a period of 28 days and the last group (G4) was exposed to CP and treated with 200 mg/Kg EEA. Thus, the levels of γ-GT, ALP, AST and ALT were significantly increased (p <0.01) due to exposure to CP and these enzymes levels were reversed in the CP group receiving EEA. Further, CP caused a significant decrease (p <0.001) in the activity of antioxidant enzymes (SOD, CAT and GSH-PX) with an increase in the level of MDA and NO. Besides, administration of EEA to rats (G4) restored the impaired activity of the antioxidant system with a decrease in MDA and NO. A significant increase (p <0.001) in markers of inflammation (TNF-α and PGE2) was observed in animals treated with CP; while the effect was diminished by EEA. In addition, histological study of the liver indicated that injection of CP to rats caused vascular congestion, microvacuolar steatosis, inflammatory reaction and chronic hepatitis; these changes were resolved by administration of EEA. In conclusion, EEA possessed a therapeutic effect against CP-induced hepatotoxicity in rats.

Keywords: *Anacyclus valentinus*; HPLC/UV; cisplatin; hepatotoxicity; oxidative stress

INTRODUCTION

Cisplatin (cis-diaminedichloroplatin) is among favored cancer remedies (**Naseem et al., 2015**). It is effective in the treatment of epithelial malignancies such as cancer in lungs, head, neck, ovaries, bladder, testes (**Boulikas and Vougiouka, 2003**), cervix, stomach and some other cancers (**Ghosh, 2019**). Despite its therapeutic efficacy, treatment with cisplatin is associated with various side effects, in particular on the central nervous system and kidneys, as well as ototoxicity (**Ferreira and al., 2018**), hematotoxicity, vomiting (**Hulin and al., 2010**), gastrointestinal toxicity, asthenia (**Boulikas and Vougiouka, 2003**) nausea / vomiting and particularly, hepatotoxicity (**Wang *et al*., 2020**). The hepatotoxic potential is dependent on high doses of cisplatin (**Atasayar *et al*., 2009**).

Cisplatin molecule is composed of one platinum atom, bonded with two amide groups and two reactive chlorides (Xie et al., 2019). Cisplatin primarily targets DNA and induces cytotoxicity (**Liu *et al*., 2013**). Cisplatin chloride ligands are replaced by water molecules, generating a positively charged electrophile $[PtCl(H_2O)(NH_3)_2]^+$ (**Sultana *et al*., 2012**). The aqueous form of cisplatin binds to DNA primarily through the N7 atoms of purine bases with a preference for guanine over adenine (**Madrigal *et al*., 2020**). It efficiently crosslinks adjacent purines and facilitates the formation of DNA folds, thereby preventing DNA replication and transcription (**Liu et al., 2013**) and inducing massive cell death, including by apoptosis (**Rocha et al., 2018**).

Medicinal plants have a great effect on the prevention and treatment of illnesses (**Wang et al., 2020**). Herbal medicines are extracted from plants and are also referred to as secondary metabolites or phytochemicals (**Schuhladena et al., 2019**). Phytochemicals have a high toxicity and yield various molecular responses in prokaryotic and eukaryotic animal cells by modulating several biological processes including cell cycle, metabolism, protein synthesis, stress response, energy metabolism and cell senescence (**Singh et al., 2020**). Phytochemicals, being cheap and available, have limited or lower toxicity as opposed to that in synthetic drugs (**Welcome, 2020**).

Anacyclus which is from the Asteraceae family is a yearly Mediterranean genus (**Torices et al., 2013**). 12 differents species are found in northwest Africa, southern Europe and the Middle East.

Anacyclus valentinus, commonly called "ghertoufa" or valence ancycle, is an annual specie mainly found in different regions of Algeria where it is used as a food condiment (**Houicher et al., 2018**). Anacyclus has various pharmacological activities such as anti-arthritis power and reduction of rheumatoid arthritis (**Side Larbi et al., 2017**), anti-inflammatory and analgesic (**Jawhari et al., 2020**), diabetes and cholesterol (**Tadjeddine et al., 2013**), Antimicrobial and antifungal activities were also observed (**Side Larbi et al., 2016**). Recently, it has also been reported that extract of anacyclus is effective against Human Colorectal Cancer Cell Line (**Mohammadi et al., 2017**). Their medicinal property is due to the presence of flavonoids and terpenoids (**Elazzouzi et al., 2014, Jawhari et al., 2021**).

This study aims to identify the biochemistry of *Anacyclus valentinus* ethanolic extract and evaluate the protective effect it has on cisplatin-induced hepatotoxicity on our lab-subjects.

MATERIAL AND METHODS

Reagents and chemicals

Cisplatin (CP, 25 mg) were procured from Mylan (Saint Priest, France). Ethanol (95% purity), trifluoroacetic acid (TFA), formic acid, acetonitrile, chloroform, tris-HCl, sucrose, EDTA and bovine serum albumin from Sigma - Aldrich Corporation (St. Louis, MO, USA) and formaldehyde from Biochem (Montreal, NJ, Canada). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma glutamyl transpeptidase (γ-GT) were assessed through bioMerieux packs (bioMerieux SA France) The standards (trans-cinnamic acid, gallic acid, benzoic acid, ferulic acid, m-coumaric acid, caffeic acid, rosmarinic acid and ellagic acid, catechin, hesperidin, thymol, galangin, tectochrysin, pinocembrin, acetine, rutin, chrysine, apigenin, kaempferol,

quercétine, ascorbic acid and menthol) were acquired from Sigma-Aldrich as well.

Origin and collection of *Anacyclus valentinus*

Anacyclus valentinus Linnaeus (Asteraceae) used in this study was collected in spring 2018 in El Bayadh located in southwest Algeria, 1313 m above sea level (33° 40' 49.001" N 1° 1' 13.001" E). El-Bayadh has a cold semi-arid climate. We then dried the aerial parts of *Anacyclus valentinus* at room temperature, finely crushed it with an electric grinder and stored away from light and humidity. The plant was identified and authenticated by the botanist Pr Terras Mohamed in Laboratory of Water Resources and Environment from the Saida University.

Plant material and ethanolic extraction of *Anacyclus valentinus*

The extract of *Anacyclus valentinus* was carried out, with some adjustments, through the protocol of **Sujith et al. (2012)**. We cut 500 g of the aerial parts of this plant into small pieces and grind them before extracting the active ingredients in 2 liters of 95% (v/v) ethanol in a cold water bath with ultrasound for 1h30. This extraction operation was repeated 3 times. We then filtered by means of Whatman N° 1 paper before evaporation of the dry solvent under reduced pressure at a temperature of 60°C. These filtrates represented *Anacyclus valentinus* ethanolic extract (EEA).

High performance liquid chromatography/ultraviolet analysis

EEA constituents were identified through high-performance liquid chromatography (HPLC) combined with ultraviolet (UV) detection (Agilent 1100). We performed separation in a HPLC apparatus equipped with an Agilent poroshell 120EC column 100 mm long, 2.1 mm in diameter and 2.7 µm thick. The mobile phase was solvent A (water / TFA / formic acid (99: 0.25: 0.75)) and solvent B (acetonitrile) according to the following gradient (t / min,% B): (0, 0), (1, 10), (2, 12.5), (3, 15), (9, 80), (10, 100), (11, 100), (14, 0) with post 5 min. We set the analytical conditions to maintain the column temperature at 50°C, flow rate of 0.6 mL/min, and directly inject aliquots (10 µL). Absorbance was checked at 270 and 320 nm **(Moulai-Hacene et al., 2020; Boufadi et al., 2021)**.
The sample was treated in the EEA extract with 1: 100 (v/v) diluted in methanol. The constituents of *Anacyclus valentinus* were determined by comparing their retention times and UV spectra with different phenolic standards (trans-cinnamic acid, gallic acid, benzoic acid, ferulic acid, m-coumaric acid, caffeic acid, rosmarinic acid and ellagic acid), flavonoids (catechin, hesperidin, thymol, galangin, tectochrysin, pinocembrin, acetine, rutin, chrysine, apigenin, kaempferol and quercetin) and others (ascorbic acid, menthol).
Stock solutions were obtained by dissolving the standards in methanol (1 mg/mL). The components were quantified in mg through standard curves per 1 g of crude *Anacyclus valentinus*.

Animal study

The experiment was carried out on 24 adult male Wistar rats, with a body weight between 120 and 140 g, from the Pasteur Institute of Algiers. The animals were acclimatized in the animal house of the Department of Biology, Faculty of Sciences, Saida University. All animal experiments were approved by the local ethical committee for animal care of the institution (Saida University) (rat/mouse 20% maintenance, RN-01-20K12; Carfil Quality).
The rats were placed in metabolic cages for a 2 weeks adaptation period at temperatures between 18°C at 25°C and natural environment. The rats are provided with food (kibble; production company, Bouzaréa, Algiers). Two weeks later, the animals were split into four groups. All the rats have access to water and food **(Silici et al., 2011; Chaa et al., 2019)**.

G1: Rats received 1 mL of physiological water for 28 days (control group).
G2: Rats received 1 mL of physiological water for 22 days, followed by three injections of CP (7 mg/Kg) intravenously (*i.v*) (every 48 hours one injection).
G3: Rats received 200 mg/Kg/day of EEA by force-feeding for 28 days.
G4: Rats received 200 mg/Kg/day of EEA by force-feeding for 28 days, with three injections of CP (7 mg/Kg) intravenously (*i.v*) (every 48 hours one injection).

Serum collection and tissue sampling

On day 29 (the end of the treatment period), the animals were abstained from food for 12 hours prior to blood collection. They were as well kept under light chloroform anesthesia to avoid any risk of changing biochemical parameters by general anesthesia, before being sacrificed. EDTA, heparin and dry tubes were used for the collection of blood. The blood was centrifuged at 3000 rpm for 15 minutes. Serum or plasma was collected for biochemical assays.
The liver was retrieved, rinsed in saline solution (NaCl 0.9%) and weighed. A part of the liver was placed in phosphate buffer (PBS) and stored in the freezer at -80°C for the determination of antioxidant status and tumor necrosis factor alpha (TNF-α). In order to make histological sections, another part of the liver was fixed by

formalin (10%). Samples of peritoneal tissue were collected and stored in PBS for the determination of prostaglandin E2 (PGE2).

Biochemical analysis of liver function

Alanine aminotransferase (ALT, EC 2.6.1.2), aspartate aminotransferase (AST, EC 2.6.1.1), alkaline phosphatase (ALP, EC 3.1.3.1) and gamma glutamyl transpeptidase (γ-GT; EC 2.3.2.2) levels were evaluated in serum by the chemistry analyzer (Mindray BS-240, China).

Determination of oxidative stress markers

Frozen liver portions were homogenized in ice buffer (10 mM tris-HCl, pH 7.4, 250 mM sucrose, 0.5 mM EDTA and 0.5% bovine serum albumin). Then centrifuged at 10000 rpm/10 min at 4°C, in order to determine the parameters of the antioxidant status the supernatant was kept at 4°C, in accordance with **Rustin et al. (1994)**.
Thiobarbituric acid was used to determine liver lipid peroxidation. It was quantified in terms of malondialdehyde (MDA) content **(Yagi et al., 1976)**. The protocol of **Elstner et al. (1983)** was used to determine the enzymatic activity of superoxide dismutase (SOD). The method of **Lück (1963)** was used to determine the enzymatic activity of catalase (CAT). The method described by **Rostruck et al. (1073)** was used to measure the activity of hepatic glutathione peroxidase (GSH-Px). The protocol of **Alam et al. (2013)** was used to measure the Nitric oxide (NO) scavenging activity.

Determination of inflammation markers

ELISA kit (Thermo Fisher Scientific, USA) was used to perform the TNF-α assay on liver tissue homogenate, results were expressed in ng/g tissue was quantified using the ELISA kit (Abcam Discover More, UK) was used to quantify PGE2 assay in peritoneal fluid, values were expressed in pg/mL of peritoneal fluid.

Histopathologica studies

The rat liver was fixed by immersion in formaldehyde (10%) and coated in kerosene. A rotating microtome (Leica, Germany) was used to cut 4 µm thick sections which were stained with hematoxylin and eosin in duplicate, observed under an optical microscope with magnification X100 (OPTIKA-B383 PLi, Italy) was used to examine them.

Statistical analysis

SigmaStat software (SPSS, 3.0, SPSS, Inc., Chicago, IL) was used for the analysis. Data was presented as mean±SD and were evaluated by one-way ANOVA, with Dunnett's post hoc test. When appropriate, ANOVA on Rank with Dunn's post hoc test was used.

RESULTS AND DISCUSSION

Determination of the chemical composition of eea by HPLC

Results of the identification of chemical compounds of EEA by HPLC/UV are shown in Table 1 and Figure 1.

Table 1 HPLC/UV Composition of *Anacyclus valentinus* ethanolic extract (EEA) in (mg/g).

Peak number	Components	Retention time per min	EEA (mg/g)
1	Luteolin	0.42	0.45
2	Galangin	0.59	0.11
3	Catechin	2.37	0.89
4	Thymol	2.68	0.12
5	Bis-methylated Quercetin	3.74	1.16
6	Quercetin	4.01	1.36
7	Rutin	4.57	0.26
8	Acacetin	5.25	0.17
9	hesperidin	5.41	0.10
10	Apigenin	5.81	0.02
11	Kaempferol	6.73	0.30
12	Pinocembrin	7.18	0.19
13	Chrysin	7.25	0.95
14	Gallic acid	2.11	0.18
15	Caffeic acid	2.37	1.01
16	ascorbic acid	2.68	0.12
17	Ferulic acid	3.74	1.24
18	m-coumaric	4.01	0.33
19	Rosmarinic acid	4.57	0.41
20	Trans cinnamic	6.73	0.11

Caffeic acid appears at 2.37 min with a level of 1.01 mg/g, chrysine also comes out at 7.25 min, but with a concentration of 0.95 mg/g and luteolin was also present at a level of 0.45 mg/g (Rt=0.42 min). *Anacyclus valentinus* is rich in quercetin (1.36 mg/g at 4.01 min), ferulic acid (1.24 mg/g at 3.74 min) and bis-methylated quercetin (1.16 mg/g at 3.74 min). While it isn't rich in apeginin (0.02 mg/g).
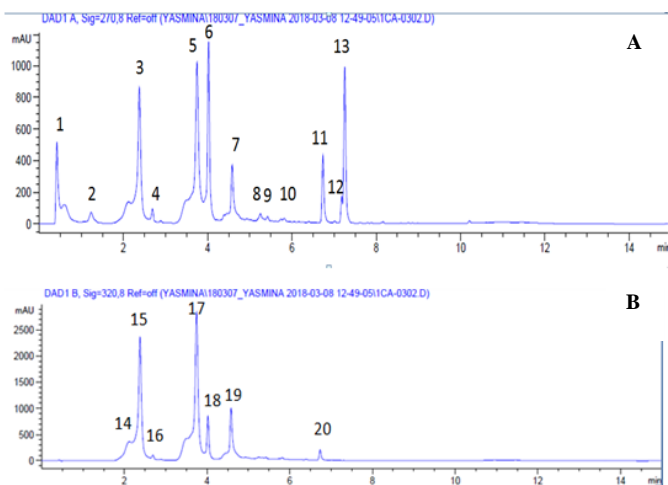

Figure 1 EAA analysis through HPLC/UV Chromatograms , wavelength 270 (A) and 320 nm (B): 1. Luteolin; 2. Galangin; 3. Catechin; 4. Thymol; 5. Bis-methylated Quercetin; 6. Quercetin; 7. Rutin ; 8. Acacetin; 9. Hesperidin; 10. Apigenin; 11. kaempferol; 12. pinocembrin; 13. chrysin; 14. Gallic acid ; 15. Caffeic acid; 16. Ascorbic acid; 17. Ferulic acid; 18. M-coumaric acid; 19. Rosmarinic acid; 20. Trans-cinnamic.

Based on the HPLC results, it can be noted that *Anacyclus valentinus* chemical composition differs from that of **Side Larbi et al. (2017)** who reported the absence of certain compounds in the methanolic extract of *Anacyclus valentinus* such as galangine, rutoside, acetine, hesperidin, pinocembrin, chrysine, caffeic acid, m-coumaric acid, rosmarinic acid gallic acid and trans cinnamic acid, with the presence of myricetin, sinapic acid, malic acid, azelaic acid and lycoperodine. **Canli et al. (2017)** showed that the ethanolic extract of *Anacyclus pyrethrum* (one of the species of the genus *Anacyclus*) consists mainly of palmitic acid, naphthalene, decahydro-1,1-dimethyl, 9,12-octadecadienoic acid (Z,Z) , 7-tetradecenal, gamma-sitosterol, N-isobutyl-tetradeca-2,4-dienamide, (Z) and benzofuran-2-carboxaldehyde. These elements are different from the ethanol extract components of *Anacyclus valentinus*.

Liver function

Table 2 presents the results of the different liver parameter assays (ALT, AST, γ-GT and ALP).

Table 2 Effect of ethanolic extract of *Anacyclus valentinus* (EEA) on the hepatic function parameters on rats dosed by cisplatin.

Parameters	Groups			
	G1	G 2	G 3	G 4
ALT (UI/l)	39.75 ± 2.26	57.26 ± 7.23[a]	45.32 ± 1.49	41 ± 4.40[b]
AST (UI/l)	106.20 ± 3.82	143.50 ± 11.26 [a]	106.04 ± 4.32 [a]	107.45 ± 5.26 [b]
ALP (UI/l)	53.83 ± 1.74	213 ± 9.84 [a]	52 ± 2.88 [a]	79.66 ± 3.38 [b]
γ- GT (UI/l)	29 ± 1.15	174.66 ± 7.51 [a]	34.66 ± 2.02 [b]	53.66 ± 2.60 [b]

Parameters expressed as mean ± SEM (n=6).
[a] Significant difference from the control group (G1) ($p < 0.05$). [b] Significant difference from the G2 group ($p < 0.05$). G1: control ; G2: 7 mg/ Kg of cisplatin; G3: 200 mg / kg of EEA (28 days); G4: 200 mg / kg of EEA (28 days) then 7 mg /kg of cisplatin.

ALP concentration was 75% elevated (P<0.05) in G2 rats (7 mg/Kg cisplatin-injected only) as opposed to G1 (53.83 IU/L). A 63% decrease was noticed in G4 cured by 200 mg/Kg of EEA as to the G opposed to G2. In contrast, G3 which was only dosed EEA for a 21 day period, ALP levels were not significant as opposed to G1.
Based on our results, AST and ALT respectively increased by 26% and 31%, in G2 (which were solely dosed by cisplatin) as opposed to G1 (control). CP exposed rats dosed by EEA (G4) showed a -25 and -28% (p<0.05) decrease AST and ALT activity as opposed to G2.
Our present results show that CP (p<0.05) (G2) significantly increased γ -GT (+145.66 IU/L) concentration as opposed to (G1). In contrast, γ -GT was reduced by -121 IU/L in G4 (dosed by 200 mg/Kg of EEA and CP simultaneously) as opposed to (G1).
Cisplatin is a common platinum metallodrug used in chemotherapy because of its ability to bind to genomic DNA **(Ferreira et al., 2020)**. The cytotoxicity of cisplatin correlates with the formation of DNA adducts that block DNA replication, encourages G2 cell cycle arrest, hinders RNA transcription and ultimately promotes cell death **(Abdel-Daim et al., 2020)**.
The results of our experiment reveal that intravenous injection of cisplatin in male rats produced significant hepatotoxic effects. Significant increase (P<0.05) in the level of liver function parameters (ALT, AST, ALP and γ-GT), hepatic malondialdehyde, NO free radical and inflammation markers (TNF-α, PGE2) were noticeable , together with enzymatic antioxidants (SOD, CAT and hepatic GSH-Px) activity reduction in animals dosed by cisplatin compared to controls. These results were proven by the histopathological alterations observed in the liver. This confirms what has been described by **Hagar et al. (2019)**; **Boraja et al. (2018)**; **Chandel et al. (2018)** and **Kart et al. (2010)**. They reported that exposure to cisplatin decreased antioxidant enzyme activities (SOD, GSH-Px, CAT) and increased lipid peroxidation, NO and serum levels of transaminases, alkaline phosphatase and γ-GT; with the production of changes in liver tissue particularly around central vein cells and hepatocellular vacualization and sinusoidal dilation and lymphocyte infiltration.
Cisplatin ability to induce changes in ALT and AST activity may be a secondary incident resulting from cisplatin-induced liver damage and resulting in hepatocyte leakage **(Yadav, 2015)**.

Liver oxidative stress markers and histology

Table 3 presents values for Liver antioxidant status of the different groups.

Table 3 *Anacyclus valentinus* ethanolic extract (EEA) effect on malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) tumor necrosis factor alpha (TNF-α) and nitric oxide (NO) in rats dosed with cisplatin.
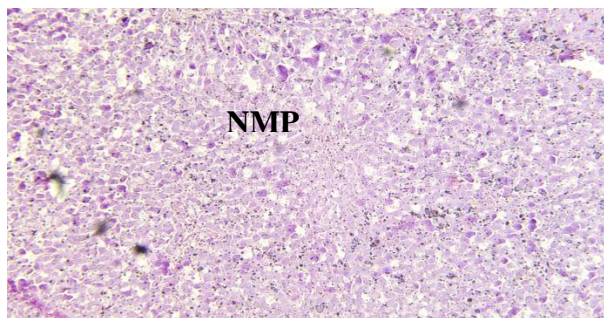
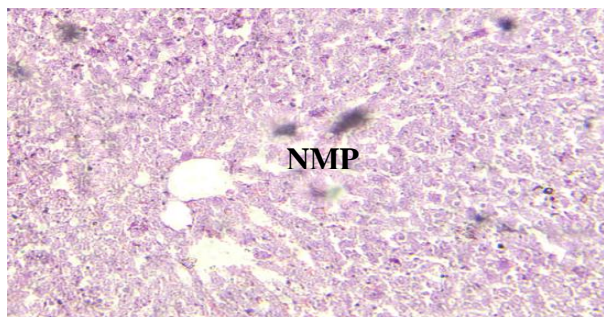| Parameters	Groups			
	G1	G2	G3	G4
MDA liver (nmol/ml)	0.93 ± 0.01	3.22 ± 0.12[a]	0.80 ± 0.02	1.26 ± 0.35[b]
SOD liver (U/cg Hb)	46.61 ± 0.81	12.83 ± 0.76 [a]	75.52 ± 0.85	35.93 ± 1.13[b]
CAT liver (U/mg Hb)	101.72 ± 1.33	28.11 ± 0.68 [a]	109.76 ± 0.85	97.41 ± 0.43[b]
GSH-Px liver (U/g Hb)	84.97 ± 0.69	15.06 ± 0.41 [a]	93.96 ± 0.86	82.88 ± 0.88[b]
TNF-α (ng/g)	47.00 ± 0.61	105.49 ± 0.94 [a]	39.8 ± 0.67	54.20 ± 0.53[b]
NO (µmol/g)	5.45 ± 0.25	25.32 ± 0.24 [a]	4.23 ± 0.19	6.12 ± 0.09[b]

Parameters expressed as mean ± SEM (n=6).
[a]Significant difference from the control group (G1) ($p < 0.05$). [b]Significant difference from the G2 group ($p < 0.05$). G1: control ; G2: 7 mg/ Kg of cisplatin; G3: 200 mg / kg of EEA (28 days); G4: 200 mg / kg of EEA (28 days) then 7 mg /kg of cisplatin.

Our findings showed a significant decrease (P<0.05) in SOD, CAT and GSH-Px levels in the liver of G2 (rats exposed to 7 mg/Kg CP) by -72.47%, -72.36% and -82.27%, as opposed to G1. Hepatic antioxidant enzymes activity highly augmented (P<0.05) in rats treated by *Anacyclus valentinus* namely G4 (treated with 200 mg/Kg of EEA and CP at the same time), where an increase in SOD (+23.09 U/cg), CAT (69.29 U/mg) and GSH-Px (67.82 U/g) was recorded compared to rats in group G2.

Intravenous injection of CP in G2 significantly increased MDA and NO levels (p<0.001) in the liver by 71.11% and 78.47%, respectively, compared to (G1). In the same context, a significant reduction (p<0.001) separately of -1.96 nmoL/mg and -19.20 μmoL/g in hepatic MDA and NO was observed in G4 (which were dosed CP and EEA at the same time) compared to G2.

Histopathological section of the liver of rats in G1 and G3 showed a normal histological appearance of hepatic parenchyma, centrilobular veins and hepatocytes (Figs. 2a, 2c). While the livers of G2 exposed only to 7 mg/Kg CP (G2) showed vascular congestion, an inflammatory reaction with microvacuolar steatosis and chronic hepatitis in the liver (fig. 2b). On the other side, G4 (rats received the EEA and CP at the same time) showed a more or less normal parenchyma consisting of normal hepatocytes and sinusoids, with mild microvacuolar steatosis (Fig. 2d).
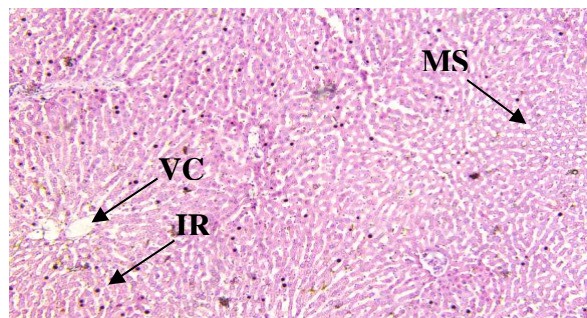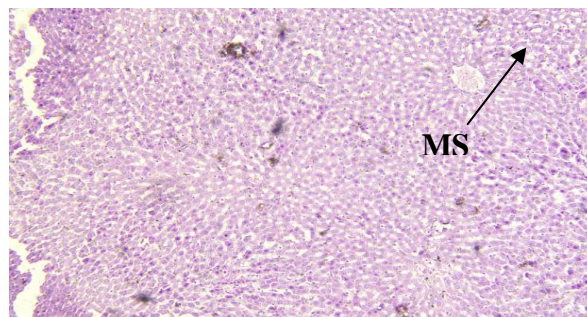
A

B

C

D

Figure 2 Microscopic examination of hepatic tissue (HE x 100) of groups G1 (A), G2 (B): vascular congestion, an inflammatory reaction and microvacuolar steatosis, G3 (C) and G4 (D): microvacuolar steatosis. G1 , G2: cisplatin 7 mg/ Kg ; G3:EEA 200 mg / Kg (over a 28 day period); G4: EEA 200 mg / Kg (over a28 day period) then cisplatin 7 mg /Kg
NMP: normal hepatic parenchyma, IR: inflammatory reaction, MS: microvacuolar steatosis, VC: vascular congestion.

Oxidative stress is commonly known to be a disbalance between reactive oxygen species (ROS), reactive nitrogen species (RNS) production and antioxidant capacity (**Ścibior et al., 2018**). Under stressful conditions, antioxidants serve as a defense mechanism against free radicals and control ROS. Antioxidants restrain oxidation and antioxidant deficiencies in the body (**Alexander et al., 2019**).

Oxidative stress is one of the most important states of liver tissue induced by cisplatin. The O_2^- generated by cisplatin is transformed into H_2O_2 by SOD and detoxified by CAT or GSH-P$_X$ by conversion into H_2O and O_2 (**Bentli et al., 2013**). According to **Youcef et al. (2009)**, cisplatin toxicity mechanism relates to the depletion of the antioxidant defense system. Experiments showed that a high decrease in antioxidant activities and increase in free radicals is typical under chemotherapy regimens, namely to cisplatin treatment.

Cisplatin stimulates the production of ROS by damaged mitochondria, which decreases the production of antioxidants and increases the production of free radicals (**Omar et al., 2016**) including NO. MDA is a lipid peroxidation product namely resulting from the toxic effects of active ROS. ROS is a result of the oxidation of unsaturated fatty acids in cell membranes (**Cagin et al., 2015**).

Markers of inflammation

The results presented in Table 3 show a very high level of TNF-α (+55%) in G2 (CP 7 mg/Kg injected three times) as opposed to (G1). Contrastingly, EEA dosing (200 mg/Kg for a 21 days period) and EEA injection to G3 showed a significant decrease (-51.29 ng/g) by report to the G2. In group G3 (EEA dosed / 200 mg/Kg), the level of TNF-α (39.8 ng/g) was close to that of the control group (47 ng/g).

Based on our results shown in Figure 3, there was a significant increase (P<0.05) of +50% PGE2 in G2 (injected with CP only) compared to the rats of the first group G1. In addition, there was a 42% decrease in prostaglandin E2 in the rats of group G4 (which received 200 mg/Kg of EEA and CP) in comparison to group G2. Administration of the EEA to rats in the G3 group improved PGE2 levels compared to the control group.
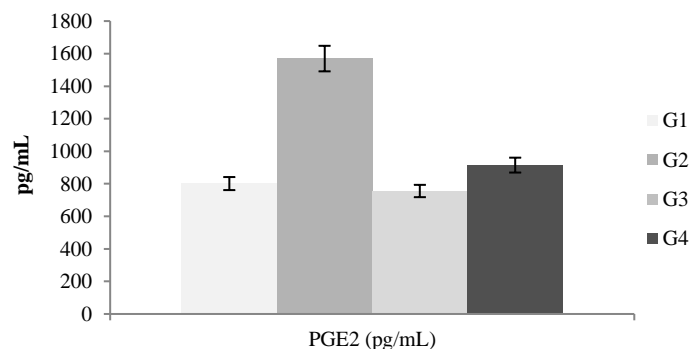

Figure 3 Effect of ethanolic extract of *Anacyclus valentinus* (EEA) on prostaglandin E2 (PGE2) in Wistar rats dosed by cisplatin. Parameters expressed as mean ± SEM (n=6).

Increased amounts of PGE2 are produced under several pathological conditions, including inflammation and tissue damage (**Elkhouly et al., 2012**). TNF-α, is a major mediator in several immunological and inflammatory responses and in a number of pathological conditions (**Huang et al., 2005**). It is essential in initiating an effective inflammation process against infection .Their excessive production may lead to organ system dysfunction and mortality (**Cao et al., 2012**).

Our experimental data show that *Anacyclus valentinus* ethanolic extract caused in rats a significant reduction (P<0.05) in hepatic parameters, hepatic malondialdehyde, NO free radical and biomarkers of inflammation, and induced a significant increase in oxidative enzymes (SOD, CAT , GSH-Px) ,together with histopathological alteration disappearance.

The therapeutic effect of the ethanolic extract of *Anacyclus valentinus* against cisplatin-induced hepatotoxicity is due to the fact that it is rich in phenolic

compounds (thymol, ferulic acid, caffeic acid, etc.) and flavonoids (luteolin, galangin, catechin, quercetin, etc.).

Polyphenols, including flavonoids, are bioactive compounds with a number of biological activities (**González et al., 2011**). The hepatotoxicity of cisplatin is hypothesized via the oxidative stress dependent mechanism mediated through generating reactive oxygen species (ROS) (**Palipoch et al., 2014**) and decreasing the antioxidant defense system, including antioxidant enzymes (**Kart et al., 2010**). Polyphenols exert an anti-inflammatory actions through various mechanisms: radical scavenging, metal chelation, NADPH oxidase inhibition (NOX), mitochondrial respiratory chain tempering , inhibition of enzymes involved in the production of ROS such as xanthine oxidase, together with endogenous antioxidant enzymes SOD positive regulation (**Yahfouf et al., 2018**), CAT and GSH-Px (**Zhang and Tsao, 2016**). Flavonoids help inhibit enzymes in arachidonic acid metabolism through lowering inflammatory mediators release derived from this pathway. They are likely to inhibit prostaglandin biosynthesis by warding off phospholipase A2 and cyclooxygenase enzymes (**Maleki et al., 2019**).

CONCLUSION

Our study demonstrates that the aerial parts of *Anacyclus valentinus* contain polyphenols and flavonoids. It also revealed that cisplatin produces hepatotoxicity by increasing hepatic and inflammatory markers and the NO free radical; triggering lipid peroxidation and reducing antioxidant enzyme activities. Administration of ethanolic extract of *Anacyclus valentinus* to rats beneficially reduced cisplatin toxicity.

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