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The ameliorating effect of *Filipendula hexapetala* extracts on hepatorenal toxicity of cisplatin

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ABSTRACT

The effects of the methanolic extracts of *Filipendula hexapetala* Gilib. aerial parts (FHA) and roots (FHR) against cisplatin induced kidney and liver injuries in rats were investigated as well as determination of genotoxicity and antigenotoxicity of the extracts. Treatment with FHA and FHR significantly decreased levels of urea, uric acid, serum transaminases, alkaline phosphatase and γ-glutamyl transferase, and increased the content of total protein. In addition, treatment with the extracts significantly attenuated the cisplatin-induced oxidative stress in kidney and liver tissues by increasing catalase and superoxide dismutase activities and the content of reduced glutathione and decreasing the content of thiobarbituric acid reactive substances (TBARS). The histopathological studies confirmed the protective effects of the extracts against cisplatin-induced kidney and liver injuries. The extracts ameliorated cisplatin-induced genotoxicity. These results suggest that *F. hexapetala* extracts are effective nephro- and hepatoprotective agents, with potential to reduce oxidative stress and ameliorate cisplatin-induced nephro- and hepatotoxicity.

1. Introduction

There has been an increasing interest in the health enhancing role of functional foods or physiologically-active food compounds. One of the most important traits of some functional food ingredients, in addition to their nutritional values, is their physiological benefit. Many recent studies have reported that components of plant-based diet play a very important role in health promotion (Crozier, Jaganath, & Clifford,

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2009; Farzaneh & Carvalho, 2015). Malignancies are one of the most frequent problems as the consequences of a fast pace life in modern society, unhealthy diet, and everyday stress. A fascinating array of plant and food-derived putative anticarcinogens, which provide aroma, colour and flavour to our diet and may promote good health, was discovered in recent years. Different natural dietary components have a cancer preventive potential which are able to suppress tumour-specific metabolic pathways, but they can also improve efficiency of chemotherapeutics, alleviate the adverse side effects of chemotherapy, and detoxify the body of chemotherapeutics. Thus, cancer patients are advised to have the proper nutrition with needful nutrients already before and during the chemotherapy in order to ensure the best health possible (Gerhauser, 2013; Sak, 2012). Phenolic compounds are the most dominant bioactive compounds identified in plant foods with antioxidant properties and free radical scavenging activities. The consumption of foods rich in phenolic compounds is associated with various physiological effects, such as preventing cancer and some chronic diseases as well as alleviating the harmful effect of drugs and chemotherapeutics (Quirós-Sauceda et al., 2014).

One of the most used anticancer drugs is an inorganic complex called cisplatin – *cis*-[$Pt(NH_3)_2Cl_2$] (*cis*-diamminedichlorplatinum(II)). Cisplatin (CP) in organism interacts with deoxyribonucleic acids causing interstrand and intrastrand crosslinking with local denaturation of the DNA chain (Chirinoa & Pedraza-Chaverri, 2009; Gómez-Ruiz, Maksimović-Ivanić, Mijatović, & Kaluđerović, 2012; Kelland, 2007). Besides this very important anticancer effect of cisplatin, there are also many undesirable side effects such as vomiting, digestive tract disorders, and toxicity like nephrotoxicity, hepatotoxicity, neurotoxicity and ototoxicity (Chirinoa & Pedraza-Chaverri, 2009; Kelland, 2007; Longo, Gervasi, & Lubrano, 2011). Side effects of CP arise because this complex has a huge affinity for sulphur-containing compounds, and that novel compounds are generally responsible for mentioned toxic effects in the organism (Crul, Schellens, Beijnent, & Maliepaard, 1997; Martin, 1999). Besides that, CP use in cancer chemotherapy may be responsible for secondary malignancies (Misra & Choudhury, 2006; Nersesyan & Muradyan, 2004).

The main interest of research is now to find a drug that provides excellent anticancer effect, with little or no harmful effect on the organism. Also, many research efforts are focused on finding new compounds or formulations which could reduce or prevent the negative effects of anticancer drugs, especially CP side effects. Today, beside many synthesized drugs, large amounts of medicinal plants which can be used as foods or food ingredients still play a key role in the prevention and treatment of different diseases. It has been reported that many plants and phenolic compounds as their constituents, possess the protective role against CP toxicity. For example, extracts of *Zingiber officinale* (Ajith, Nivitha, & Usha, 2007), *Aloe barbadensis* (Chatterjee, Mukherjee, & Nandy, 2012), and grape seed (Yousef, Saad, & El-Shennawy, 2009) showed nephroprotective effects in CP-induced toxicity. Also, natural compounds like rosmarinic acid (Domitrović, Potoćnjak, Crnčević-Orlić, & Škoda, 2014), *β*-caryophyllene (Horváth et al., 2012), rutin (Arjumand, Seth, & Sultana, 2011) and curcumin (Waseem & Parvez, 2013) attenuate or ameliorate CP-induced nephro- and hepatotoxicity.

Filipendula hexapetala Gilib. (Rosaceae), dropwort, is a perennial herb (up to 80 cm high) with pinkish-white flowers and characteristic tuberous roots, found in dry grasslands of Europe and Asia (Tucakov, 1973). Vračarić et al. (1990) reported that this plant is edible and can be used as a functional food. Young spring leaves can be used in salad, and later on only as cooked vegetables. They have a specific taste, so it is better to mix them with other wild vegetables. The tuberous roots have a bittersweet taste that resembles bitter almonds. They can be eaten fresh and prepared in a lot of different ways, also in combination with other tuberous plants for making porridge and bread, among others. Usage of dropwort in traditional medicine is based on the diuretic, astringent, antirheumatic and anti-inflammatory properties of this plant. Also, it was used for treating breathlessness, sore throats, congestion and kidney-problems (Maksimović, Petrović, Pavlović, Kovačević, & Kukić, 2007; Radulović et al., 2007). In our previous work, we demonstrated high antioxidant potential and antimicrobial activity of *F. hexapetala* aerial part and root extracts. The extracts also showed good stability under different pH and thermal conditions (Katanić, Mihailović et al., 2015). These results suggested that it is necessary to obtain more detailed studies and to evaluate the *in vivo* activity of *F. hexapetala*.

The present study aimed to characterize the phenolic compounds present in the extracts of aerial parts (FHA) and roots (FHR) of *F. hexapetala*. Also, based on traditional usage of *F. hexapetala*, we evaluated the degree of protective activity of *F. hexapetala* extracts on *in vivo* cisplatin-induced nephrotoxicity and hepatotoxicity, with determination of *in vivo* genotoxic effect and antigenotoxic potential.

2. Materials and methods

2.1. Chemicals

All chemicals and reagents were of analytical grade and purchased from Sigma Chemical Co. (St. Louis, MO, USA), Aldrich Chemical Co. (Steinheim, Germany) and Alfa Aesar (Karlsruhe, Germany). Cisplatin was purchased from Sigma-Aldrich Co. Commercial reagent kits for determination of total protein (TP), creatinine (CRE), urea (UR), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma glutamyl transferase (γGT) activities were provided by BioSystems S.A. (Barcelona, Spain). Gallic acid, vanillic acid, kaempferol and quercetin were purchased from Sigma–Aldrich, caffeic acid was purchased from Merck KGaA (Darmstadt, Germany), (+)-catechin and ellagic acid from Serva (Heidelberg, Germany), hyperoside and rutin from Carl Roth (Karlsruhe, Germany), epicatechin from Thermo Fisher Scientific (Geel, Belgium) and spiraeoside from Extrasynthese (Genay, France). HPLC-grade acetonitrile, water and trifluoroacetic acid (Merck, Darmstadt, Germany) were used in HPLC analyses. All spectrophotometric measurements were performed on UV–VIS double beam spectrophotometer Halo DB-20S (Dynamica GmbH, Switzerland).

2.2. Plant material and preparation of the extracts

F. hexapetala Gilib. was collected at the locality Šumarice (Kragujevac, Central Serbia), during the flowering season (May

2013). A voucher specimen (No. 111/013) was deposited in the Herbarium of the Department of Biology and Ecology, Faculty of Science, University of Kragujevac, Kragujevac, Serbia, after the identification of species. The air-dried aerial parts and roots (60 g each) of *F. hexapetala* were fine powdered and separately macerated for 24 h with methanol for three times (300 mL each) at room temperature. After filtration, the extracts were concentrated using a rotary evaporator under reduced pressure to obtain dry extracts without traces of methanol. The percentage yields of dry extracts of *F. hexapetala* were 20.52% (w/w) for FHA and 31.82% (w/w) for FHR. The extracts were dissolved in normal saline prior to the *in vivo* pharmacological study. The concentrations used in the experiments and HPLC analysis were based on the dry weight of the extracts.

2.3. HPLC analysis of phenolic compounds

The HPLC system Prominence (Shimadzu, Kyoto, Japan) consisted of a system controller (CBM-20A, Shimadzu), a column oven CPO-20AC (Shimadzu) and a solvent delivery pump with a degasser (DGU-20A5, Shimadzu) with a photodiode array detector (SPD-M20A, Shimadzu) that monitored the wavelengths 190–800 nm. The responses of the detectors were recorded using LC Solution software version 1.24 SP1. The chromatography was performed using a Kinetex® C18 column (10 cm × 4.6 mm I.D., 2.7 μm particle size) produced by Phenomenex (Torrance, CA, USA). The chromatographic conditions were identical to the previously reported method (Katanić, Boroja et al., 2015). The tentative identification of phenolic acids and flavonoids was performed by comparing retention times and absorption spectra of unknown peaks with reference standards as well as co-chromatography with added standards. For quantification of phenolic acids in the extracts, calibration curves were prepared for gallic acid, caffeic acid, vanillic acid, ellagic acid, (+)-catechin, (−)-epicatechin, quercetin, hyperoside, rutin, spiraeoside and kaempferol. Eight mass concentrations of 100, 50, 25, 12.5, 6.25, 3.125, 1.5625 and 0.78125 μg/mL of standard solution were prepared. For further identification of phenolic acids and flavonoid glycosides, the extracts were hydrolysed to obtain the free phenolic acids and flavonoid aglycons by modifying a method described by Engida et al. (2013).

2.4. Test animals

Male albino Wistar rats (230 ± 20 g) used in this study were obtained from the Animal House of Military Medical Academy, Belgrade, Serbia. All the animals were maintained under standard laboratory conditions of constant temperature (24 ± 2 °C), relative humidity (50 ± 15%), 12 h light/12 h dark cycle, and allowed free access to food and water. All animal procedures were in compliance with the EEC Directive (86/609/EEC) on the protection of animals used for experimental and other scientific purposes, and were approved by the Ethical Committee for the Use of Laboratory Animals of the Institute for Biological Research "Siniša Stanković", University of Belgrade.

2.5. Cisplatin toxicity experimental design

The animals were randomly divided into ten groups containing five rats in each. Based on previous studies of Sahu et al. (2011, 2013), nephro- and hepatotoxicity were induced by intraperitoneal (i.p.) administration of cisplatin dissolved in normal saline at the dose of 7.5 mg per kg body weight (b.w.).

The experimental design was performed as follows: *Group I* served as the negative control (NC) and normal saline was administered orally for 10 days and a single injection (i.p.) of 0.5 mL isotonic saline was administered on the 5th day. *Group II* served as the cisplatin control or positive control (PC), normal saline was administered orally (p.o.) for 10 days and a single intraperitoneal (i.p.) injection of cisplatin (7.5 mg/kg, i.p.) dissolved in normal saline was administered on the 5th day. The animals in *groups III–V* received for 10 days the aerial part extract of *F. hexapetala* (FHA) dissolved in normal saline at 100, 200 and 400 mg per kg b.w. doses p.o., respectively, and the rats in *groups VI–VIII* were administrated for 10 days with the root extract of *F. hexapetala* (FHR) dissolved in normal saline at 100, 200 and 400 mg per kg b.w. doses p.o., respectively. A single dose of cisplatin (7.5 mg/kg, i.p.) was administrated to animals in groups III–VIII on the 5th day, 1 hour prior to extract dose. *Groups IX* and *X* served as extracts control groups and were administrated with FHA and FHR dissolved in normal saline at a concentration of 400 mg per kg b.w. p.o., respectively, for 10 days. Twenty four hours after last treatment, body weight of rats was recorded and then the animals were sacrificed and blood samples were collected immediately. The kidneys and livers were immediately removed and weighed for the organ weight ratio calculation. The relative weight of organs (%) was calculated as g/100 g body weight. Organs were dissected into two halves, one for biochemical analysis and genotoxic and antigenotoxic effects of extracts and the other was fixed in 4% formalin and kept for histopathological assessment.

2.6. Determination of serum biochemical markers

To obtain the serum, blood samples were collected, placed for 45 min at room temperature and then centrifuged at 2988 × *g* for 10 min at 4 °C in a Sorval SS-34 rotor (DJB Labace Ltd., Newport Pagnell, Buckinghamshire, UK). Serum biochemical markers of renal and hepatic injury: urea (UR), creatinine (CRE), uric acid (UA), alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (γGT) and total proteins (TP), were estimated using BioSystems commercial kits (Biosystems S.A., Barcelona, Spain) according to the manufacturer's manual and Roche/Cobas Mira automated analyser (Roche Diagnostic Limited, Rotkreuz, Switzerland). Determination of serum biochemical markers (UR, CRE, UA) was based on spectrophotometric measurement. Total protein content was measured spectrophotometrically using the Biuret test for proteins. Spectrophotometric determination of ALT, AST, ALP and γGT was based on kinetic evaluation of enzyme activity.

2.7. Determination of kidney and liver antioxidant markers

Kidney and liver tissue homogenates (10%) were prepared in phosphate buffer saline (50 mM, pH 7.4) and then centrifuged at 1968 × *g* for 15 min at 4 °C. The supernatants were used for the spectrophotometric assays for determination of glutathione (GSH) level (Ellman, 1959), superoxide dismutase (SOD)

(Misra & Fridovich, 1972) and catalase (CAT) (Góth, 1991) activities. The level of thiobarbituric acid-reactive substance (TBARS) was determined in the kidney and liver tissues by the method of Ohkawa, Ohishi, and Yagi (1979). The TBARS values were then calculated using the standard curve of malondialdehyde (MDA). Total protein concentrations were determined using the commercial assay kit, using bovine serum albumin as a standard.

2.8. Histopathological examination

Kidney and liver sections were fixed in 4% formalin in phosphate buffered solution for 24 h. After that, dehydrated pieces of kidney and liver tissues were embedded in paraffin wax, cut into 4–6 μm thick sections using a microtome, stained with haematoxylin-eosin and observed under a microscope for histopathological changes in the kidney and liver. Photographs of each slide were taken at 40× or 100× magnifications.

2.9. Genotoxic and antigenotoxic effects

2.9.1. Determination of DNA damage by the alkaline comet assay

DNA damage was measured using the alkaline comet assay according to Singh, McCoy, Tice, and Schneider (1988). The liver and kidney samples were excised and smaller fragments were transferred on ice. The DNA was electrophoresed for 30 min at 300 mA and 30 V, whereupon the alkali was neutralized with 0.4 M Tris–HCl, pH 7.4, three times for 5 min and fixed for 5 min in absolute alcohol, air-dried and stored at room temperature. Immediately before analysis, the slides were stained with 80 μL of ethidium bromide (20 μg/mL) and covered with cover slip.

2.9.2. Data scoring and photomicrographs

Comets were visualized and captured with 40× objective lens of fluorescence microscope Nikon (Ti-Eclipse) attached to the CCD camera. One hundred comet images per slide were randomly captured and analysed. Only cells that had a clear line around them were scored. Also, all comets with nearly all the DNA in the tail or with a very wide tail were excluded from the analysis, since they could represent dead cells (Hartmann & Speit, 1997). The visual classification method of Collins (2004) was applied to assess the extent of DNA damage. Cells were scored from 0 (undamaged) to 4 (maximally damaged), according to tail intensity (size and shape). A total comet score and the percentage of reduction (%R) in the comet score in the treatments with extracts showing antigenotoxicity was calculated according to Manoharan and Banerjee (1985) and Waters, Brady, Stack, and Brockman (1990) using the following formula:

$$\%\ \text{Reduction} = \frac{a-b}{a-c} \times 100$$

where a corresponds to the mean score observed in the treatment with cisplatin (positive control), b corresponds to the mean score observed in pretreatment with the extracts prior to cisplatin and c corresponds to the mean score in the negative control.

2.10. Statistical analysis

The data were expressed as mean ± S.E.M. Statistical evaluation of the data was performed by one-way analysis of variance (ANOVA). Statistical analysis was performed using the IBM SPSS statistical software package, version 20 for Windows. The results were considered to be statistically significant at $p < 0.05$.

3. Results

3.1. Phytochemical results

The phenolic compounds in two extracts of *F. hexapetala* were identified and quantified by HPLC analysis. In Fig. 1, HPLC chromatograms for the extracts before (A, B) and after hydrolysis (C, D) are shown and individual quantification of phenolic compounds is presented in Table 1. Six polyphenolic compounds were identified in the aerial part extract (FHA): gallic acid, ellagic acid, epicatechin, quercetin, hyperoside and spiraeoside. Convincingly the most dominant phenolic compound in the FHA extract was flavonol epicatechin (64.36 mg/g dry weight of extract). Also, FHA extract was rich in quercetin derivatives, spiraeoside (21.80 mg/g) and hyperoside (14.87 mg/g). In the root extract of *F. hexapetala* (FHR) only two phenolic compounds were identified, catechin and epicatechin, of which catechin was present in a significant amount (14.44 mg/g). After hydrolysis of the *F. hexapetala* extracts some more compounds were identified. After the hydrolysis, higher concentrations of gallic acid and ellagic acid in FHA extract were recorded, as well as the presence of caffeic acid in regard to the not hydrolysed extract. Moreover, in hydrolysed FHA extract, concentration of epicatechin was significantly decreased and catechin presence was not recorded. Also, in FHA were identified the flavonoid aglycons quercetin and kaempferol. After hydrolysis of the root extract three phenolic acids (gallic, ellagic and vanillic acids) were identified.

3.2. Effects of the extracts on serum biochemical markers

As shown in Table 2, the body weight changes of all the groups treated with FHA and FHR extracts were significantly higher ($p < 0.05$) compared to the group of animals which was treated with cisplatin only. The effects of different doses of FHA and FHR extracts on serum biochemical markers in CP-treated rats were studied (Table 2). After a single injection of cisplatin, serum activities of AST, ALT, ALP and γGT enzymes in the positive control (Group II) were significantly increased ($p < 0.05$) compared to the normal control group. The urea and uric acid values were also significantly increased in the CP-treated group compared to the normal control group ($p < 0.05$), while the level of TP was significantly ($p < 0.05$) decreased. Treatments of the animals with different doses of FHA (100, 200, and 400 mg/kg b.w.) and FHR (100, 200, and 400 mg per kg b.w.) significantly reduced ($p < 0.05$) the levels of ALT, AST, ALP and γGT, as well as the levels of UR, UA and increased the levels of total proteins (TP), as compared to the positive control group. However, it was noticed that groups treated with the extracts and CP had higher creatinine values compared to both the negative and

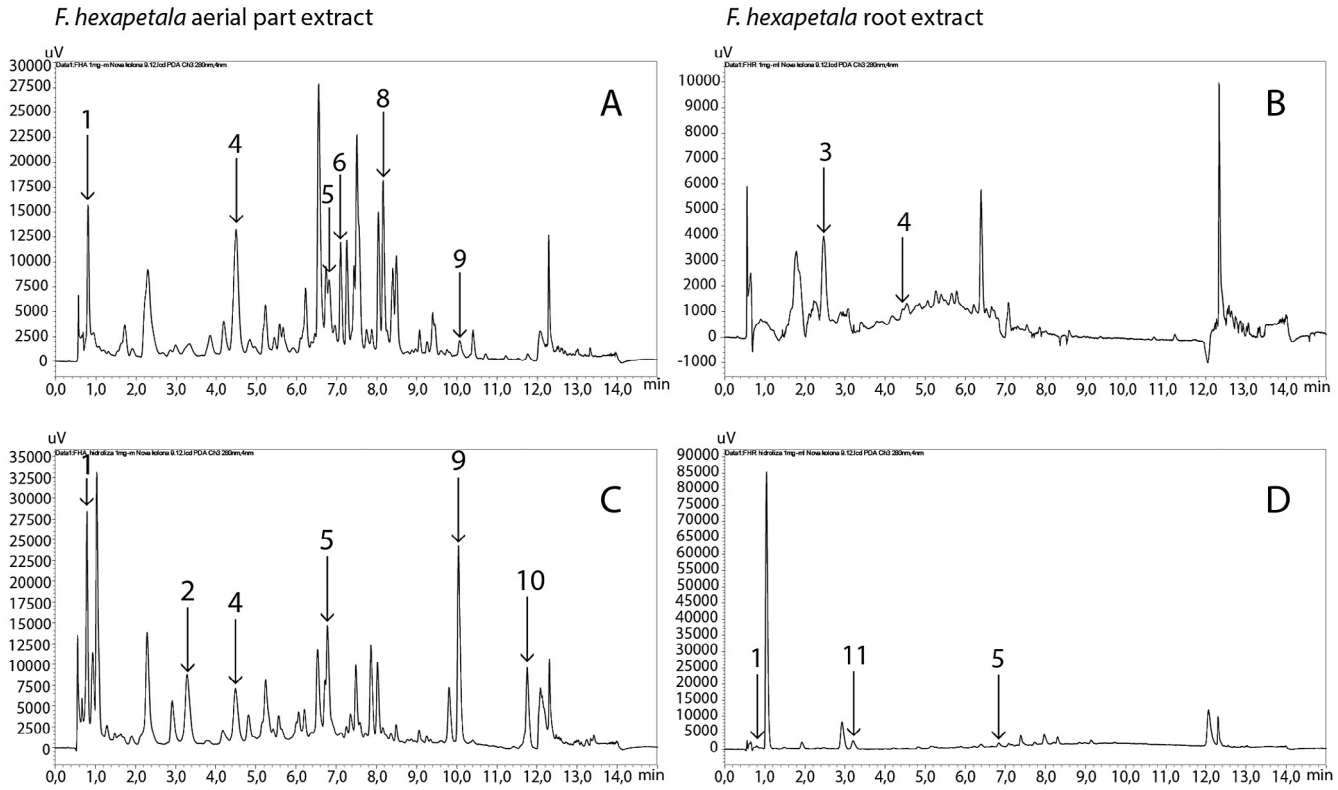

Fig. 1 – HPLC profiles of *Filipendula hexapetala* methanolic extracts before (A, B) and after hydrolysation (C, D). Detection was performed at 280 nm. Peaks identification: 1 – gallic acid; 2 – caffeic acid; 3 – (+)-catechin; 4 – (-)-epicatechin; 5 – ellagic acid; 6 – hyperoside; 7 – rutin (not detected); 8 – spiraeoside; 9 – quercetin; 10 – kaempferol, 11 – vanillic acid.

positive control groups. The groups treated only with the highest dose of FHA and FHR (400 mg/kg b.w.) showed significant ($p < 0.05$) reduction of AST and γGT activities, while extracts did not change TP levels ($p > 0.05$) compared to the untreated control (group I). Groups treated only with extracts in high concentration showed elevated levels of UR, CRE and UA compared with the untreated control group, but levels of UR and UA were significantly ($p < 0.05$) lower compared with positive control (group II).

3.3. *Antioxidant CAT and SOD enzyme activities and GSH and TBARS levels*

The effects of various doses of FHA and FHR extracts on the activities of CAT and SOD and the levels of GSH and TBARS in the kidneys and livers of CP-treated animals are presented in Fig. 2. CP treatment significantly decreased activity of CAT and SOD, as well as the level of GSH in kidney tissue compared to the normal group ($p < 0.05$). Regarding the TBARS level in

Table 1 – Phenolic compounds (mg/g) in *F. hexapetala* aerial part and root methanolic extracts, before and after hydrolysis (mean ± SD).[a]

Compound	Before hydrolysis		After hydrolysis	
	FHA	FHR	FHA	FHR
Gallic acid	6.21 ± 0.08	–	12.46 ± 0.03	0.44 ± 0.01
Ellagic acid	8.83 ± 0.04	–	14.20 ± 0.07	4.59 ± 0.06
Caffeic acid	–[b]	–	8.30 ± 0.02	–
Vanillic acid	–	–	–	3.28 ± 0.04
(–)-Catechin	–	14.44 ± 0.12	–	–
(+)-Epicatechin	64.36 ± 0.11	0.86 ± 0.06	30.75 ± 0.08	–
Hyperoside	14.87 ± 0.05	–	–	–
Rutin	–	–	–	–
Spiraeoside	21.80 ± 0.02	–	–	–
Quercetin	4.49 ± 0.03	–	42.15 ± 0.05	–
Kaempferol	–	–	7.06 ± 0.03	–

[a] Results are based on the dry weight of the extracts.
[b] Not detected.

Table 2 – Effects of *F. hexapetala* extracts on body weight (b.w.), kidney to body weight ratio and liver to body weight ratio and serum biochemical parameters of CP-treated rats.[a]

Groups	Body weight change (%)	Kidney/b.w. ratio × 1000	Liver/b.w. ratio × 1000	Kidney function tests			Liver function tests				
				UR (mmol/L)	CRE (μmol/L)	UA (μmol/L)	ALT (U/L)	AST (U/L)	ALP (U/L)	γGT (U/L)	TP (g/L)
I	27.33 ± 3.12	8.61 ± 0.12	29.32 ± 1.24	6.50 ± 0.30	42.8 ± 1.00	50.05 ± 3.75	56.45 ± 2.81	135.61 ± 10.49	64.54 ± 7.81†	49.38 ± 16.92	66.51 ± 5.60
II	5.60 ± 1.01*	8.80 ± 0.32	32.96 ± 2.03*	9.10 ± 2.70*	49.5 ± 1.51*	86.90 ± 3.62*	91.66 ± 6.98*	172.85 ± 25.31*	179.47 ± 13.62*	124.18 ± 24.53*	60.05 ± 2.08
III	9.72 ± 2.32†	8.02 ± 0.21†	28.25 ± 3.67†	6.55 ± 0.75†	49.9 ± 7.81	54.9 ± 12.10†	55.87 ± 1.99†	107.09 ± 16.32†	102.09 ± 11.14†	34.15 ± 4.30†	65.13 ± 11.58
IV	20.00 ± 2.48†	9.64 ± 0.34†	31.71 ± 4.05	7.62 ± 0.40†	54.7 ± 2.65†	54.05 ± 9.05†	67.22 ± 2.32†	142.01 ± 12.26†	125.69 ± 11.09†	54.72 ± 5.97†	71.15 ± 13.01†
V	10.67 ± 1.64†	8.35 ± 0.42	33.32 ± 2.86	7.72 ± 1.40†	55.65 ± 6.25†	50.01 ± 8.33†	55.59 ± 2.14†	104.76 ± 18.04†	127.19 ± 10.67†	36.48 ± 6.79†	74.22 ± 4.23†
VI	12.35 ± 2.75†	8.28 ± 0.28†	41.33 ± 3.49†	7.71 ± 1.00†	52.80 ± 4.60†	65.5 ± 9.90†	51.79 ± 3.87†	153.65 ± 17.46†	131.07 ± 14.54†	58.96 ± 6.21†	70.31 ± 1.59†
VII	14.81 ± 3.62†	8.31 ± 0.17	34.38 ± 4.65	9.00 ± 0.15	52.55 ± 2.65†	52.25 ± 2.85†	69.84 ± 2.43†	103.30 ± 19.36†	129.08 ± 8.77†	17.19 ± 2.36†	70.21 ± 6.13†
VIII	12.35 ± 2.83†	8.48 ± 0.35	38.06 ± 3.87†	8.25 ± 0.55†	53.70 ± 0.70†	57.95 ± 3.75†	53.83 ± 2.24†	131.82 ± 19.76†	119.18 ± 1.98†	38.32 ± 4.06†	74.65 ± 3.38†
IX	35.71 ± 4.61*,†	7.75 ± 0.49*,†	39.33 ± 2.74*,†	8.75 ± 0.95*,†	49.75 ± 0.95*	60.00 ± 1.80*,†	41.03 ± 1.56*,†	98.81 ± 13.51*,†	98.51 ± 6.82*,†	17.93 ± 3.89*,†	69.47 ± 0.52†
X	30.43 ± 3.59*,†	8.33 ± 0.31	43.52 ± 2.33*,†	8.90 ± 0.10*	51.20 ± 0.70*	73.20 ± 0.40*,†	51.51 ± 2.10†	97.77 ± 10.16*,†	108.70 ± 2.91*,†	38.59 ± 3.31*,†	68.43 ± 0.77†

[a] Values represent mean ± SEM from three independent experiments, n = 5 rats per group.

* $p < 0.05$ when compared with the negative control group.

† $p < 0.05$ when compared with the cisplatin group.

I – Control group; II – CP 7.5 mg/kg b.w., i.p.; III – FHA 100 mg/kg b.w. + CP; IV – FHA 200 mg/kg b.w. + CP; V – FHA 400 mg/kg b.w. + CP; VI – FHR 100 mg/kg b.w. + CP; VII – FHR 200 mg/kg b.w. + CP; VIII – FHR 400 mg/kg b.w. + CP; IX – FHA 400 mg/kg b.w.; X – FHR 400 mg/kg b.w.

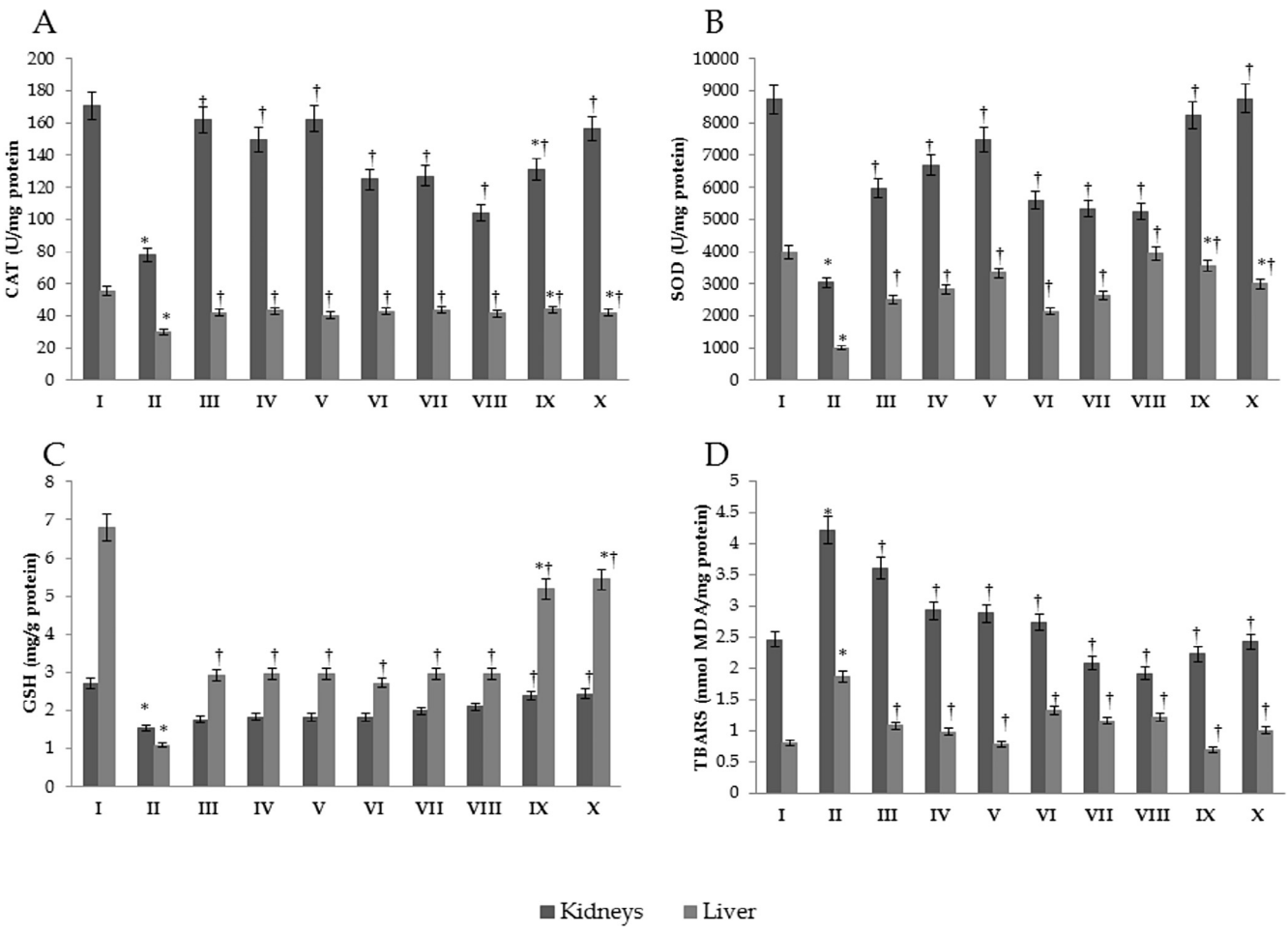

Fig. 2 – Effects of *F. hexapetala* extracts on the levels of renal and hepatic CAT (A), SOD (B), GSH (C) and MDA (D) after cisplatin treatment in rats. I – Control group; II – CP 7.5 mg/kg b.w., i.p.; III – FHA 100 mg/kg b.w. + CP; IV – FHA 200 mg/kg b.w. + CP; V – FHA 400 mg/kg b.w. + CP; VI – FHR 100 mg/kg b.w. + CP; VII – FHR 200 mg/kg b.w. + CP; VIII – FHR 400 mg/kg b.w. + CP; IX – FHA 400 mg/kg b.w.; X – FHR 400 mg/kg b.w. Data represent means ± S.E.M. n = 5 animals in each group. *$p < 0.05$ when compared with the negative control group; †$p < 0.05$ when compared with the cisplatin group.

kidneys, there was a significant increase of the TBARS values in the group which received only cisplatin ($p < 0.05$). The treatment with FHA and FHR extracts on the other hand significantly prevented the decrease of enzymatic activity and GSH level in kidneys. Higher activity of CAT and SOD in kidney tissue was observed in groups treated with CP in combination with FHA extract, especially in the concentration of 400 mg/kg b.w. (Fig. 2A and B), although the enzyme activities in groups treated with FHR were not negligible and were significantly different compared to the CP-group ($p < 0.05$). Also, high activity of the enzymes in kidney tissues has been noted in the groups treated only with extracts in a concentration of 400 mg/kg b.w. compared with the CP-group. The enzyme activities in kidneys of animals from these two groups were not significantly different ($p > 0.05$) compared to the normal control (group II). Only exception was CAT activity in the group treated only with FHA (400 mg/kg), which was significantly different ($p < 0.05$) compared to both the normal and positive control groups. As presented in Fig. 2C, GSH level in the kidneys of the animals treated with CP only was much decreased and significantly different regarding normal control group ($P < 0.05$). In the groups treated with CP and the extracts, the kidney GSH levels were slightly increased, but not significantly different from the CP-group ($P > 0.05$). The groups which were treated only with the highest dose of the extracts showed much higher levels of GSH, which were significantly different compared with the CP-group ($P < 0.05$), but not significantly different from the normal control group ($P > 0.05$). As shown in Fig. 2D the treatment with CP caused a very high level of TBARS in rats' kidneys. The highest dose of FHA and especially FHR extract (400 mg/kg b.w.) significantly decreased ($P < 0.05$) the CP-induced TBARS levels in the kidneys. Meanwhile, a similar trend in TBARS level was observed in the liver homogenates with the difference regarding FHA-treated groups where a strong decrease of TBARS level ($p < 0.05$) compared to the CP-group was noticed. The highest activity CAT (Fig. 2A) was observed in the liver homogenates of the groups treated with a medium dose of both extracts (200 mg/kg b.w.). In the groups treated with extracts and CP, the activity of SOD grown in a dose-dependent manner is shown in Fig. 2B. The CAT and SOD activities, in liver tissues of the groups treated with the highest dose of the extracts only, were much higher than in the CP-treated group ($p < 0.05$), but also

significantly different ($p < 0.05$) compared to the normal control group. Considering the GSH level in the CP-treated group, it was much decreased, compared to the normal control group (Fig. 2C). The groups treated with extracts and a single dose of CP showed a significant increase in GSH level ($p < 0.05$) compared to the CP-group.

3.4. *Histopathological and morphological examination of kidneys and livers*

The different groups of rats were studied in cellular architecture of the kidney and liver tissues by histopathological analysis, which is presented in Table 3. The induction of nephro- and hepatotoxicity by cisplatin and tissue protective effects of *F. hexapetala* extracts are also supported by histological observations as was evident from the levels of blood and tissue biochemical parameters. The photomicrographs of the kidney and liver tissues are presented in Fig. 3 (I–X) and Fig. 4 (I–X), respectively.

The photomicrographs of kidney tissues from the normal control group (Fig. 3I) showed the normal architecture of cells with weak congestion and interstitial oedema in some samples. Also, some samples from this group were diagnosed with weak desquamation and hydropic degenerescence in the tubular epithelium. Histopathological findings of the CP-group showed degenerating tubular structures with vacuolization and loss of tubular architecture and contained eosinophilic materials in the lumen. Also, there were marked congestion, glomerular sclerosis and atrophy distension of capsular space as well as interstitial haemorrhaging, oedema and inflammatory infiltrate. The administration of FHA and FHR for 10 days (100, 200 and 400 mg/kg b.w.) and treatment with CP showed mild to moderate presence of degenerating tubular and glomerular changes (Fig. 3III–VIII). It was observed that the highest dose of FHA and FHR (400 mg/kg b.w.) without CP revealed predominant normal kidney morphology with occasional mild tubular and glomerular damage (Fig. 3IX–X).

The liver tissue of rats in the normal control group showed a normal architecture of hepatic cells with mild congestion in some of the samples (Fig. 4I). Tissue samples of the livers of the animals treated with a single dose of CP (Table 3 and Fig. 4II) demonstrated significant evidence of injury with marked congestion, sinusoidal dilatation, ballooning degeneration followed by infiltration of lymphocytes, leucocytes and macrophages as well as focal necrosis. A moderate Kupffer cell hyperplasia and fibrosis of portal areas were also observed. The groups treated with FHA and FHR in different concentrations showed less expressed histopathological changes of the liver tissue. As shown in Table 3, FHA in a dose of 100 mg/kg b.w. showed significant reduction of liver tissue injury caused by CP administration, taking into account that in groups treated only with the highest dose of the extracts (groups IX and X, 400 mg/kg b.w.) some mild changes of the tissue structure were observed.

3.5. *Genotoxicity and antigenotoxicity of the extracts*

The results for the antigenotoxicity assay conducted using cisplatin in combination with different concentrations of *F. hexapetala* extracts are presented in Table 4. DNA migration in the liver was clearly shown to be increased in the presence of CP, 7.5-fold above the values in the negative control. Oral administration of FHA (100 mg/kg/day) for 10 days with a single dose of CP decreased the levels of DNA damage elevated by CP treatment by 36.1%. Treatments with FHA in concentrations of 200 and 400 mg/kg and CP showed a reduction in the extent of DNA damage compared with treatment with CP with %R of 33.1 and 24.4%. Also, a reduction in the extent of DNA damage was found for the FHR extract, with the largest effect being observed in the treatment with 100 and 200 mg/kg, with the percentage reduction of 30.1% and 23%, respectively. However, no significant reduction was observed for treatment combining higher concentrations of FHR extract and CP. These results indicate the absence of a dose–response correlation, since the lower concentration was found to be more effective and a gradual increase of *F. hexapetala* concentration did not result in a proportional increase in the reduction of CP-induced genotoxicity. Although the extracts presented a moderate protector effect, statistically the comet scores were not reduced to the levels of the negative control and the reductions obtained were always less than 50% of the positive control level. The frequencies of DNA damage in the rat liver treated only with 400 mg/kg FHA or FHR extracts are described in Table 4 (groups IX and X). Although the mean total scores were significantly different from the negative control, genotoxic effect of both extracts was significantly lower than that of CP.

Table 4 shows the extent of DNA damage in kidney cells exposed to extracts and CP using the comet assay. While DNA migration was clearly increased with CP over the negative control in kidneys, no relevant reduction of DNA damaging effects of CP occurred in the presence of the *F. hexapetala* extracts from the roots and aerial parts in the range of concentrations tested. No reduction in DNA damage induced by CP was observed in treatment of FHA and FHR extracts (400 mg/kg b.w.) prior to and after CP administration, with scores close to 230 similar to those in the positive control treated with CP alone. In the analysis of the comet class distribution, damaged cells were concentrated in class 2 in all treatments with CP and FHA or FHR extracts. The values of the mean total scores in kidneys obtained after treatment only with 400 mg/kg b.w. of *F. hexapetala* methanolic extracts (Table 4, groups IX and X) were statistically different from negative control (group I) but lower than that observed in the CP control group (group II).

4. Discussion

Cisplatin (CP) is presently one of the most effective cytostatic agents in the treatment of a wide range of solid tumours, including cancers of the head and neck, lung, ovary, testis, bladder, cervix and endometrium (Badary, Abdel-Maksoud, Ahmed, & Owieda, 2005; İşeri, Ercan, Gedik, Yükse, & Alican, 2007). Therapeutic effects of CP on cancer cells occur primarily through reactive oxygen species (ROS)-mediated induction of apoptosis (Bragado, Armesilla, Silva, & Porras, 2007). In spite of its significant antitumour activity, administration of CP is usually associated with serious injurious effects since ROS are extremely deleterious and cause damage to cell structures and interrupt a wide range of cellular functions. The two major

Table 3 – Effects of *F. hexapetala* extracts on morphological parameters of rat kidneys and livers after the CP treatment (n = 5).[a,b]

Histopathological parameters of kidneys

Groups[b]	Congestion	Hydropic degenerescence in tubular epithelium	Glomerular atrophy distension of capsular space	Glomerular sclerosis	Interstitial inflammatory infiltrate	Necrosis of tubular epithelial cells	Interstitial oedema	Interstitial haemorrhaging	Desquamation of tubular epithelium	Eosinophilic materials in tubular lumen
I	+[a]	+	–	–	–	–	+	–	+	–
II	+++	+++	+++	++	++	++	+++	+++	+++	+++
III	++	++	+	+	+	+	++	++	++	++
IV	+	++	+	+	+	+	++	+	++	++
V	++	++	++	+	+	++	++	++	++	++
VI	+	++	+	+	+	+	++	+	++	++
VII	++	++	++	+	+	++	++	++	++	++
VIII	+	++	+	+	+	+	++	+	++	++
IX	+	+	+	+	–	+	+	+	+	+
X	+	+	+	+	–	+	+	+	+	+

Histopathological parameters of liver

Groups[b]	Congestion	Sinusoidal dilatation	Ballooning degeneration	Kupffer cell hyperplasia	Infiltration of lymphocytes, leucocytes and macrophages	Focal necrosis	Fibrosis
I	+	–	–	–	–	–	–
II	+++	+++	+++	++	+++	+++	++
III	+	+	+	+	+	+	–
IV	++	++	+	++	++	++	+
V	++	++	++	+	+	+	+
VI	++	++	++	++	++	++	–
VII	+	+	+	++	+	+	–
VIII	++	++	++	++	++	++	+
IX	+	+	++	+	+	+	–
X	+	+	++	+	+	+	–

[a] (–), absent; (+), mild; (++), moderate; (+++), marked.

[b] I – Control group; II – CP 7.5 mg/kg b.w., i.p.; III – FHA 100 mg/kg b.w. + CP; IV – FHA 200 mg/kg b.w. + CP; V – FHA 400 mg/kg b.w. + CP; VI – FHR 100 mg/kg b.w. + CP; VII – FHR 200 mg/kg b.w. + CP; VIII – FHR 400 mg/kg b.w. + CP; IX – FHA 400 mg/kg b.w.; X – FHR 400 mg/kg b.w.

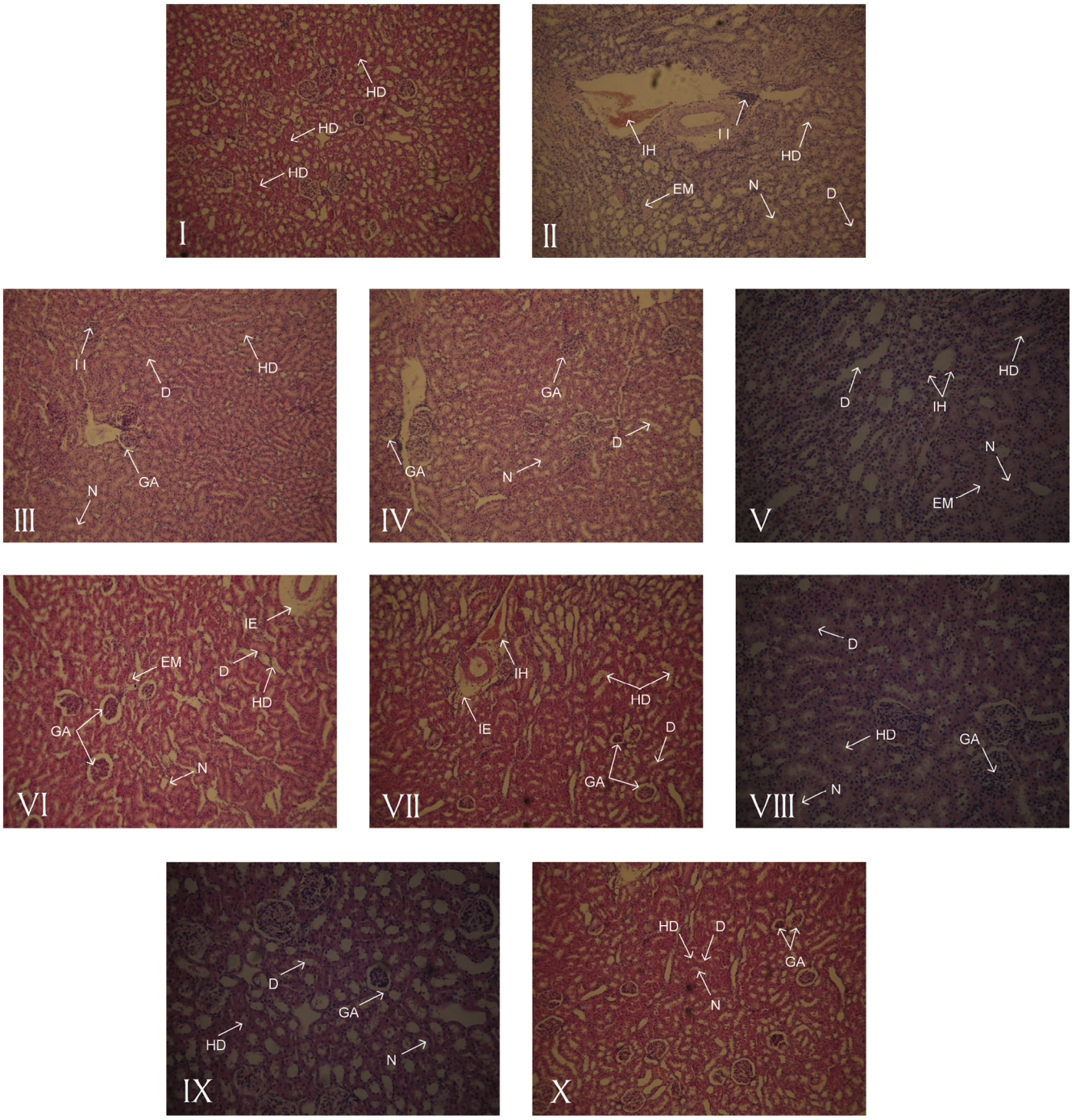

Fig. 3 – Photomicrographs of kidney sections from: I – Control group; II – CP 7.5 mg/kg b.w., i.p.; III – FHA 100 mg/kg b.w. + CP; IV – FHA 200 mg/kg b.w. + CP; V – FHA 400 mg/kg b.w. + CP; VI – FHR 100 mg/kg b.w. + CP; VII – FHR 200 mg/kg b.w. + CP; VIII – FHR 400 mg/kg b.w. + CP; IX – FHA 400 mg/kg b.w.; X – FHR 400 mg/kg b.w. H & E, original magnification 40× or 100×. Arrows: C – congestion; HD – hydropic degenerescence in tubular epithelium; GA – glomerular atrophy distension of capsular space; II – interstitial inflammatory infiltrate; N – necrosis of tubular epithelial cells; IE – interstitial oedema; IH – interstitial haemorrhaging; D – desquamation of tubular epithelium; EM – eosinophilic materials in tubular lumen.

groups of cellular antioxidant systems (non-enzymatic, e.g. GSH, vitamin C; and enzymatic, e.g. CAT, SOD) are responsible for the defence of the organism (Chirinoa & Pedraza-Chaverri, 2009). Recent studies showed that the oxidative stress is the main reason for CP-induced toxicity, whereby the depletion of reduced glutathione (GSH) occurs, followed by the increased levels of some markers of oxidative stress, like TBARS, hepatic transaminases (ALT, AST), and reduction in the level of antioxidant enzymes (CAT, SOD) (Dasari & Tchounwou, 2014; Yilmaz et al., 2004).

In our study, a single dose of CP (7.5 mg/kg b.w.) exerted in prominent nephrotoxicity which is indicated through

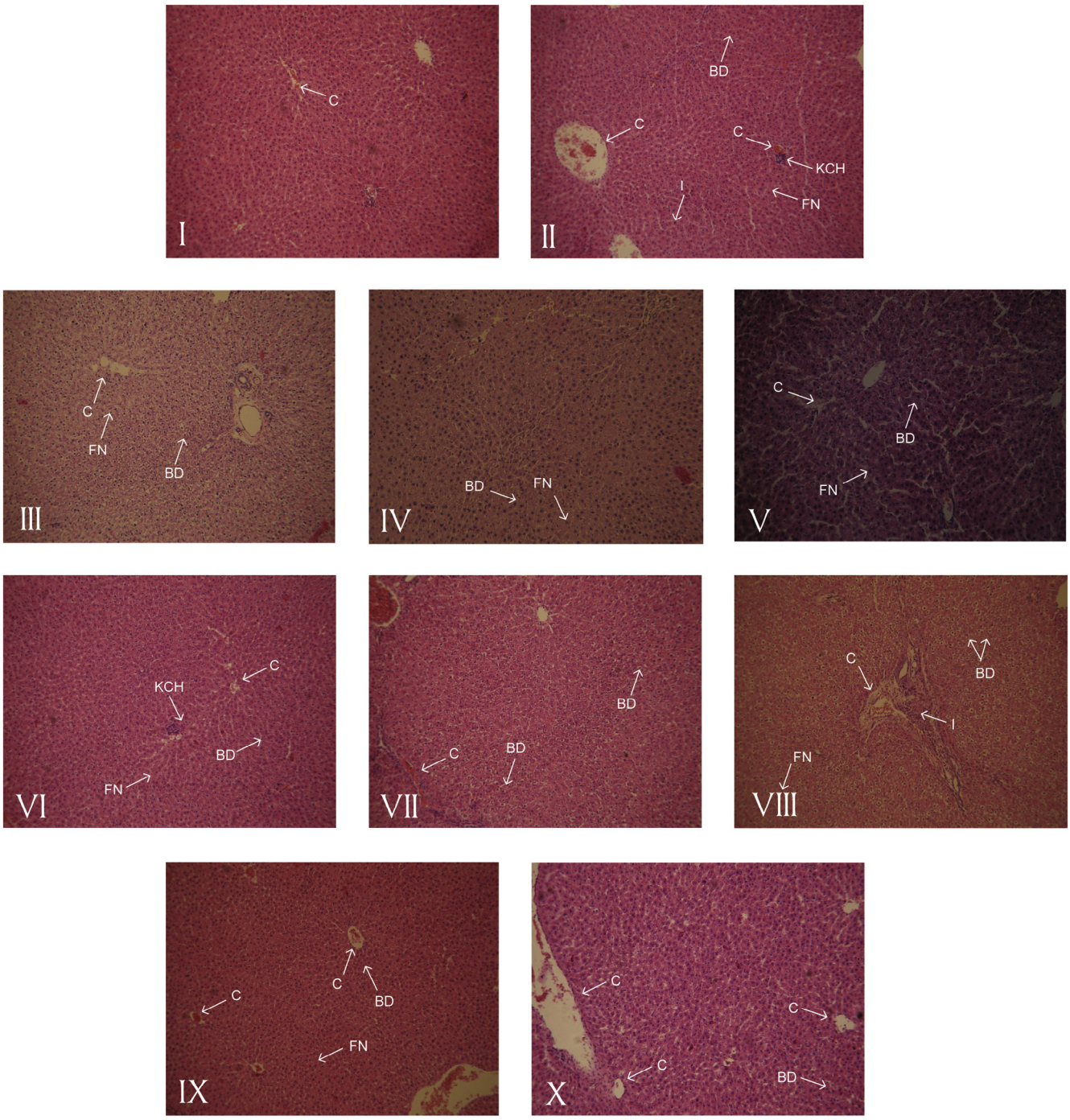

Fig. 4 – Photomicrographs of liver sections from: I – Control group; II – CP 7.5 mg/kg b.w., i.p.; III – FHA 100 mg/kg b.w. + CP; IV – FHA 200 mg/kg b.w. + CP; V – FHA 400 mg/kg b.w. + CP; VI – FHR 100 mg/kg b.w. + CP; VII – FHR 200 mg/kg b.w. + CP; VIII – FHR 400 mg/kg b.w. + CP; IX – FHA 400 mg/kg b.w.; X – FHR 400 mg/kg b.w. H & E, original magnification 40× or 100×. Arrows: C – congestion; BD – ballooning degeneration; KCH – Kupffer cell hyperplasia; I – infiltration of lymphocytes, leucocytes and macrophages; FN – focal necrosis.

significant increase of serum urea and uric acid levels. Hepatotoxicity was also observed by the significant increase of liver function parameters in the serum. Animals treated with CP showed a decrease in body weight which could be induced by gastrointestinal toxicity (Sahu et al., 2011). Treatment of the rats with *F. hexapetala* extracts in three different doses (100, 200 and 400 mg/kg b.w.) for 10 consecutive days, starting 5 days before CP administration, exerted significant protection of injurious CP effects on levels of kidney and liver intoxication parameters. In the livers and kidneys of rats in the CP-treated group, significant decrease in the activity of CAT and SOD was observed, compared to the negative control group. Meanwhile, treatment with *F. hexapetala* extracts was able to markedly alleviate CP effect and enhance the activity of both

Table 4 – Detection of DNA damage using the comet assay in livers and kidneys of rats exposed to the aerial part and root extracts of *F. hexapetala*.

Groups	Comet class					Total score[a]	% R
	0	1	2	3	4		
Liver							
I	76.2 ± 0.3	23.8 ± 0.2	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	23.8 ± 1.9	/
II	0.00 ± 0.00	53.2 ± 2.1	21.3 ± 1.3	20.2 ± 0.2	5.3 ± 0.3	177.7 ± 2.1*	/
III	0.00 ± 0.00	82.8 ± 1.2	13.8 ± 0.6	1.7 ± 1.2	1.7 ± 1.2	122.2 ± 0.8*,†	36.1
IV	0.00 ± 0.00	79.3 ± 0.3	15.9 ± 1.1	3.2 ± 1.1	1.6 ± 0.6	126.8 ± 4.8*,†	33.1
V	0.00 ± 0.00	70.1 ± 0.7	20.8 ± 0.2	7.8 ± 0.1	1.3 ± 0.7	140.2 ± 3.3*,†	24.4
VI	0.00 ± 0.00	77 ± 1.4	16.4 ± 0.5	4.9 ± 0.4	1.7 ± 1.8	131.3 ± 2.1*,†	30.1
VII	0.00 ± 0.00	71.2 ± 0.8	18.6 ± 0.3	6.8 ± 1.3	3.4 ± 1.4	142.3 ± 0.2*,†	23
VIII	0.00 ± 0.00	59.5 ± 1.3	21.8 ± 0.8	15.8 ± 0.2	2.9 ± 0.3	162.5 ± 2.5*,†	9.9
IX	31.3 ± 0.21	42.7 ± 0.81	16.7 ± 0.12	9.4 ± 0.7	0.00 ± 0.00	104.2 ± 1.01*,†	
X	24.1 ± 0.15	48.2 ± 0.6	15.7 ± 0.53	12.05 ± 0.23	0.00 ± 0.00	115.7 ± 0.52*,†	
Kidneys							
I	71.2 ± 0.1	28.8 ± 0.4	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	28.8 ± 0.1	/
II	0.00 ± 0.00	24.1 ± 1.2	55.2 ± 0.3	12.1 ± 0.8	8.6 ± 0.3	205.2 ± 0.3*	/
III	0.00 ± 0.00	30.1 ± 1.2	50.5 ± 1.3	11.7 ± 0.6	7.8 ± 1.2	197.1 ± 1.1*	4.6
IV	0.00 ± 0.00	21.3 ± 0.5	55.1 ± 0.2	22.5 ± 0.3	1.1 ± 0.2	203.3 ± 0.5*	1.1
V	0.00 ± 0.00	3.9 ± 0.7	68.6 ± 0.8	25.5 ± 0.4	1.9 ± 0.3	225.5 ± 0.2*,†	/
VI	0.00 ± 0.00	32.3 ± 0.7	58.1 ± 0.3	6.4 ± 1.2	3.2 ± 0.9	180.6 ± 1.2*,†	13.9
VII	0.00 ± 0.00	32.9 ± 0.4	52.3 ± 1.1	10.3 ± 1.1	4.5 ± 1.3	186.4 ± 0.4*,†	10.7
VIII	0.00 ± 0.00	19.4 ± 1.4	52.3 ± 1.5	16.4 ± 2.1	11.9 ± 2.2	220.8 ± 1.1*,†	/
IX	11.1 ± 0.43	33.3 ± 0.17	37.1 ± 0.12	14.8 ± 0.4	3.7 ± 1.02	166.6 ± 0.32*,†	
X	19.1 ± 0.23	19.1 ± 0.7	28.6 ± 0.43	33.3 ± 1.01	0.00 ± 0.00	176.2 ± 0.14*,†	

[a] Values represent mean ± SEM from three independent experiments, n = 5 rats per group.
* $p < 0.05$ when compared with the negative control group.
† $p < 0.05$ when compared with the cisplatin group.
I – Control group; II – CP 7.5 mg/kg b.w., i.p.; III – FHA 100 mg/kg b.w. + CP; IV – FHA 200 mg/kg b.w. + CP; V – FHA 400 mg/kg b.w. + CP; VI – FHR 100 mg/kg b.w. + CP; VII – FHR 200 mg/kg b.w. + CP; VIII – FHR 400 mg/kg b.w. + CP; IX – FHA 400 mg/kg b.w.; X – FHR 400 mg/kg b.w.

CAT and SOD in rat tissues. One of the most important constituents of non-enzymatic defence against oxidative stress is reduced glutathione (GSH), but the metabolism of some compounds is initiated by their conjugation with GSH. It has been reported that CP is capable of forming CP–GSH conjugates, which have been isolated from cells treated with CP and from the serum of CP-treated rats (Mistry, Lee, & McBrien, 1989). Our results showed a significant decrease of GSH levels in kidney and liver tissues of CP-treated rats. While the treatment of the rats with the extracts helped in recovering the GSH levels in liver tissues, they were unable to significantly alleviate the GSH level in kidneys. The reason for these results could be the increased formation of CP–GSH conjugates in the renal tissue. One of the main degradation products in lipid oxidation is MDA which is often determined as an indicator of oxidative stress (Niki, 2014). As expected, treatment with FHA and FHR extracts prevented the rise of TBARS (MDA equivalents) content in kidneys and liver tissues. The mechanism of *F. hexapetala* extracts protection against CP harmful effects could be based on antioxidative protection and the neutralization of ROS. Tested extracts also significantly improved liver and kidney functions which were disrupted with the applied dose of CP, characterized by a reduced release of liver intracellular enzymes into the bloodstream and normalization of kidney function by regulation of the urea and uric acid levels. The increased activities of hepatic cell marker enzymes in serum, which indicated oxidative damage and breakdown of hepatic cell membrane structure, were decreased by the administration of extracts, implying that *F. hexapetala* may effectively stabilize the hepatic cell membrane and prevent the leakage of intracellular enzymes. These hepatoprotective effects of extracts also could be attributed to their antioxidative properties, because *F. hexapetala* showed, in our previous investigations, significant radical scavenger and antioxidant activities (Katanić, Mihailović et al., 2015). The study of Ćebović and Maksimović (2012) also confirmed the hepatoprotective effect of *F. hexapetala* flower extract in carbon tetrachloride-induced liver toxicity where treatment with extract significantly increased the levels of antioxidant parameters in the liver tissue of treated animals, which correlates with our findings. Comparative histopathological analysis of the kidney and liver tissues of CP-treated rats showed that CP induces extensive morphological changes. FHA and FHR extracts were able to markedly attenuate the degree of changes caused by CP only, like congestion, glomerular atrophy and sclerosis, interstitial oedema and haemorrhaging in kidney tissue, and congestion, ballooning degeneration, focal necrosis and fibrosis in liver tissue. Identification of inhibitors of mutation is useful for the identification of anticarcinogenic compounds, since mutagens are directly or indirectly related to carcinogenesis. In the present study, three different *F. hexapetala* doses were tested regarding their capacity to protect DNA from damage in the liver and kidneys. DNA migration was clearly shown to be increased in the presence of CP compared with the negative control in liver

and kidneys. The lowest concentrations of the extracts were the most effective and showed a potential antigenotoxic action against the CP effects in liver and kidney tissues. However, there was no reduction of CP-induced DNA damage in the kidneys after the treatment with the extracts at doses of 400 mg/kg b.w. which indicates that the treatment with FHA and FHR at the highest doses increases the genotoxicity of CP. The extracts at the highest dose also caused DNA damage in liver versus the control group, but this effect was lower than the effect in kidneys or effect of CP alone. There is no study on *F. hexapetala* co-genotoxicity so far. Using the *Drosophila* wing somatic mutation and recombination test (SMART) similar co-genotoxic effects have been observed by Patenković, Stamenković-Radak, Nikolić, Marković, and Anđelković (2013) for water infusion of *Gentiana lutea* L. in co- and post-treatments with methyl methanesulphonate (MMS) which indicates that the extracts of some plants may increase negative effects of genotoxic agents.

Despite all of the obtained promising results of rat treatment with *F. hexapetala* extracts along with a single dose of CP, there is a concern about some of the histopathological and genotoxic results in groups IX and X. These two groups were treated only with the highest dose of FHA and FHR, without CP. The treatments provoke several mild tissue damages in the kidneys and liver, and the genotoxicity level in kidneys was comparable to the group treated only with CP. Therefore, particular attention should be paid to the dosage of the *F. hexapetala* extracts for using them in some products for preventing CP-toxicity. Nevertheless, the obtained results present the ameliorating effect of FHA and FHR in all applied concentrations on CP-induced toxicity.

The alleviating effect of *F. hexapetala* extracts is probably due to their phenolic composition. Many recent findings suggested that a wide range of phenolic compounds from plant origin, as well as plant extracts, possess very good biological properties, especially as antioxidants in oxidative stress related diseases (Saeidnia & Abdollahi, 2013). Those that are particularly significant for this study certainly are nephro- and hepatoprotective effects in CP-induced toxicity (Ajith et al., 2007; Alqasoumi, 2014; Arjumand et al., 2011; Yousef et al., 2009). HPLC analysis showed that the main phenolic compound of FHA is epicatechin, which biological properties are well studied. The beneficial effect of epicatechin is related to prevention of oxidative stress damage *in vivo* (Fraga & Oteiza, 2011; Spencer et al., 2001). Also, in FHA we quantified two quercetin glycosides, hyperoside and spiraeoside, and quercetin itself in small amount. After hydrolysis of the FHA extract, the quantity of quercetin was much higher due to hydrolysation of its glycosides. The FHA extract contained some more unidentified quercetin derivates since the amount of quercetin after hydrolysis was much higher than the summary amount of quercetin from hydrolysis of the spiraeoside and hyperoside together. Quercetin is a flavonoid well-known for its *in vivo* antioxidant potential, including hepatoprotective activity (Liu et al., 2010). Sanchez-Gonzalez, Lopez-Hernandez, Perez-Barriocanal, Morales, and Lopez-Novoa (2011) showed that quercetin also possesses nephroprotective activity against CP-induced toxicity without compromising its antitumour activity. One of the mutual compounds in hydrolysed FHA and FHR extracts is ellagic acid, which has been proven for the prevention of CP-induced oxidative stress in liver and heart tissues (Yüce, Ateşşahin, Çeribaşi, & Aksakal, 2007). The amount of ellagic acid increased after hydrolysis of the extracts, which implicates that the extracts contain ellagic acid derivates, e.g. ellagitannins. This is correlated with our recent findings that *F. hexapetala* aerial parts and roots contain high amount of tannins (Katanić, Mihailović et al., 2015).

5. Conclusion

The present study has demonstrated that two extracts of *F. hexapetala* (FHA and FHR) containing bioactive phenolic compounds, especially phenolic acids and quercetin glycosides can significantly alleviate the negative effects of cisplatin administration. These findings indicate that the extracts could remedy liver and kidney function as seen through the decrease of serum biochemical parameters, normalization of tissue oxidative stress parameter levels and reduction of histopathological changes in the kidneys and liver. At the same time, the extracts in the concentration of 100 mg/kg b.w. were found to reduce CP-induced DNA damage of kidneys and liver tissues and this dose showed a potential antigenotoxic action against the CP effects. According to these results, *F. hexapetala* extracts at lower concentrations can be successfully applied as human antigenotoxic agents. The administration of *F. hexapetala* could be a useful approach as an adjuvant therapy in the use of CP for cancer treatment, although further investigations are still required to completely evaluate the protective effect of the aerial part and root extracts of this plant on nephro- and hepatotoxicity of cisplatin and obtaining the appropriate therapeutic dose.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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