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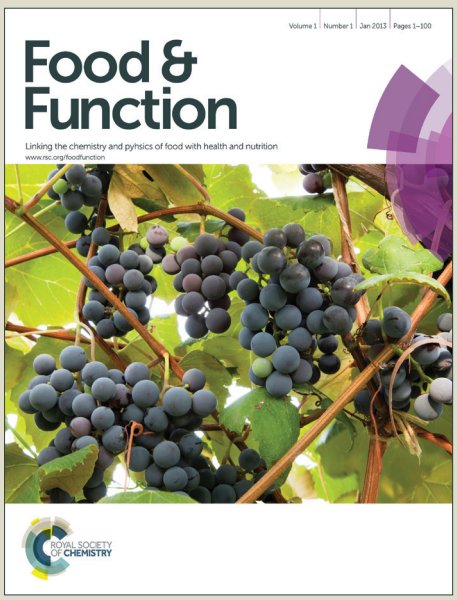

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Hepatoprotective effects of secoiridoids-rich extracts from *Gentiana cruciata* L. against carbon tetrachloride induced liver damage in rats

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Abstract

The objective of this work was to investigate the effects of the methanol extracts of *Gentiana cruciata* L. aerial parts (GCA) and roots (GCR) against carbon tetrachloride-induced liver injury in rats. Pretreatment with GCA and GCR, containing sweroside, swertiamarin and gentiopicrin in high concentrations, dose-dependently and significantly decreased levels of serum transaminases, alkaline phosphatase and total bilirubin, whereas an increase was found in the level of total protein compared with CCl_4-treated group. Moreover, oral administration of extracts significantly enhanced antioxidant enzyme activities (superoxide dismutase and

catalase), increased the content of glutathione and decreased the content of TBARS. Microscopic evaluations of liver revealed CCl_4-induced lesions and related toxic manifestations that were minimal in liver of rats pretreated with extracts at dose of 400 mg/kg b.w. The results suggest that the use of *G. cruciata* extracts have merit as a potent candidate to protect the liver against chemical induced toxicity.

Keywords: *Gentiana cruciata* L.; Hepatoprotective activity; Carbon tetrachloride; Antioxidant enzymes; Histopathology

1. Introduction

The liver is the major organ involved in the metabolism, detoxification and excretion of various endogenous and exogenous substances such as xenobiotics. Therefore, liver is one of the most frequently injured organs in the body.[1] The risk of toxic liver damage has markedly increased in recent years due to the exposure to environmental toxins, pesticides and chemotherapeutics. Many compounds, including useful drugs, can cause liver cell damage through their metabolic conversion to highly reactive substances and the generation of free radicals.[2] Despite the fact that acute and chronic liver diseases represent a global concern, modern medical treatments are often difficult to handle and have limited efficiency.[3] Herbs have recently attracted attention as health beneficial food and as source materials for drug development. They offer a potential natural health care approach that focuses on protecting and restoring the health.[4] In recent years, many researchers have become increasingly interested in medicinal and edible plant extracts that possess hepatoprotective activities. In view of pathology

of liver disease, a single drug is inadequate and sometimes with side effect, whereas a complex mixture of phytochemicals in the diet or from herbs could provide more protective and beneficial effects.[5]

Gentiana plants (Gentianaceae), with about 400 species, are distributed in Europe, Asia, America, Africa, and Australia. Plants belonging to this genus are best known for their bitter taste that is due to the secoiridoids (e.g. swertiamarin, gentiopicrin, sweroside and amarogentin). These are popular ingredients of many gastric herbal preparations and dietary supplements. *Gentiana* plants are also used in small amounts as food and beverage flavouring, in antismoking products and even used as a substitute for hops in making beer.[6] *Gentiana radix* (dried root of *Gentiana lutea* L.) is an official drug in many pharmacopoeias and it is present commercially in the form of dried fermented rhizomes and roots.[7] Caution should be exercised as to its use because it is endangered in most European countries[8] and clearly there exists a critical need for exploring another *Gentiana* species which could be used as a substitute for *Gentiana lutea* in pharmaceutical and food products. Many *Gentiana* species are known for their pharmaceutical values, such as *Gentiana cruciata* L., commonly called cross gentian.[9,10] The dried roots and above-ground parts of *G. cruciata* are consumed in the Balkan region as herbal tea or a medicinal wine for loss of appetite, as a stomachic and component in preparations showing beneficial effects in gall and liver diseases.[11,12] The chemical constituents of *G. cruciata* include the presence of bitter principles – secoiridoid-glycosides: gentiopicrin, swertiamarine and sweroside, loganic acid[8,9,13], flavone- and xanthone-C-glycosydes.[14] The major constituent, gentiopicrin, is the main active constituent which is mostly associated with the pharmacological activities of *Gentiana* plants e.g., liver-protection[15,16] and muscle relaxing.[17] Secoiridoid

glucosides, swertiamarin and sweroside, are present in various traditional medicine preparations and are reported to have hepatoprotective activity.[18,19]

According to the best of our knowledge, there are no published reports of hepatoprotective activity of *G. cruciata* and its biological activities. This study aimed to investigate the *in vivo* hepatoprotective activity of *G. cruciata* roots and aerial parts extracts against hepatotoxicity induced by CCl_4. Furthermore, we characterized by HPLC-DAD the chemical composition of *G.cruciata* extracts.

2. Materials and methods

2.1. Chemicals

All chemicals and reagents were of analytical grade and were purchased from Sigma Chemical Co. (St. Louis, MQ, USA), Aldrich Chemical Co. (Steinheim, Germany) and Alfa Aesar (Karlsruhe, Germany). Serum aspartate aminotransferase (AST), alanine aminotransaminase (ALT), alkaline phosphatase (ALP), total protein (TP) and total bilirubin (TB) estimation kits were purchased from BioSystems S.A., Barcelona, Spain.

2.2. Plant material and preparation of the extracts

Gentiana cruciata L. (GC) was collected at the locality Vikovijski kamik, Vidlič Mountain (East Serbia), during the flowering season (May 2010). Voucher specimen (No. 5493) has been deposited in the Herbarium HMD, University of Niš, Niš, Serbia, after the identification of species.

The air-dried aerial parts or roots (60 g) of *G. cruciata* were powdered using a cutter mill and separately extracted for 24 h with methanol for three times (600 ml each) at room temperature. After filtration through Whatman No. 1 filter paper, the extracts were concentrated in a rotary evaporator under reduced pressure to obtain a thick semisolid paste. The percentage yield of methanolic extracts of aerial parts (GCA) and roots (GCR) were found to be 25.0% (w/w) and 29.3% (w/w), respectively. The extracts were dissolved in normal saline prior to pharmacological study, and the concentrations used in the experiments were based on the dry weight of the extracts.

2.3. HPLC analysis of secoiridoides and xanthones

HPLC-DAD system: HPLC analyses were performed on a Hewlett-Packard HPLC system, model 1100 with DAD. The column used for secoiridoid analyses was Hypersil BDS-C18 (5 μ), 125 × 2 mm I.D. The mobile phase consisted of acetonitrile (HPLC grade, Acros Organics, Geel, Belgium) (component A) and 0.2% phosphoric acid (component B), applied in the following elution gradient: 100% to 98% B through 2 min; 98% to 90% B during next 3 min; 90% to 80% B during next 5 min and 80% to 0% B in next 10 min. The flow rate was set to 0.5 mL/min and the detection wavelength to 260 nm. Additional peak confirmation was made by peak spectral evaluation via HP Chemstation chromatographic software (Palo Alto, CA, USA), which was also used for data acquisition and method/run control.

Standard solutions preparation and data acquisition: Standard solutions were prepared by dissolving 10 mg of gentiopicrin (≥98% purity, Roth, Karlsruhe, Germany), swertiamarin and sweroside (both 98% purity, Oskar Tropitzsch, Marktredwitz, Germany) and mangiferin (≥98% purity Sigma-Aldrich, Steinheim, Germany) in 10 mL methanol. Further calibration levels were

prepared by diluting the stock with methanol. Linear regression analyses of calibration curves of these compounds revealed an excellent linearity with a correlation coefficient r=0.999, p<0.001 in each case. Total amount of compounds swertiamarin, gentiopicrin, sweroside and mangiferin in each sample was evaluated by the calculation of peak areas obtained from chromatograms using HP Chemstation chromatographic software.

2.4. Animals and experimental design

Male albino Wistar rats (220 ± 20 g) used in this study were obtained from the Animal House of Military Medical Academy, Belgrade, Serbia. All the animals were kept under standard laboratory conditions (temperature 24 ± 2 ◦C, relative humidity (50% ± 15%), and12 h light/12 h dark cycle) and allowed free access to food and water. Animal studies were approved by the Committee for Ethical Animal Care and Use of the Institute for Biological Research, Belgrade, which acts in accordance with the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (Institute for Laboratory Animal Research 1996).

Wistar rats were divided into seven groups of five animals each. First group served as normal control. *G. cruciata* extracts administered orally to different groups at the dose level of 400, 800 and 1600 mg/kg b.w. p.o. All animals were observed for toxic symptoms and mortality for 72 h.

Rats were divided into nine groups consisting of five animals in each group and treated for 7 days as follow: group I served as normal control and was daily received normal saline, and then intraperitoneally injected with 1 ml/kg b.w. olive oil. Group II served as CCl_4-hepatotoxicity control and was orally given normal saline for seven days. Group III served as standard group and received reference drug silymarin (100 mg/kg per day p.o.) for seven days

prior to CCl_4 intoxication. The animals of groups IV–VI received the aerial parts extract of *G. cruciata* (GCA) dissolved in normal saline at 100, 200 and 400 mg/kg b.w. doses p.o., respectively, and the mice in groups VI–IX were administrated with roots extract of *G. cruciata* (GCR) dissolved in normal saline at 100, 200 and 400 mg/kg b.w. doses p.o., respectively. On the last day of the treatment, the animals of groups II–IX received a single dose of CCl_4 (1:1 mixture in olive oil) at 1 ml/kg body weight intraperitoneally after 1 h of the normal saline, silymarin or *G. cruciata* extracts treatments. Twenty four hours after CCl_4 injection, all of the animals were sacrificed and blood samples were collected immediately. The livers were removed quickly and dissected to two halves, one for biochemical analysis and the other for histopathological studies.

2.5. Blood biochemical markers assay

Serum samples were obtained by centrifuging the whole blood at 5000 rpm for 10 min at 4°C in a Sorval SS-34 rotor (DJB Labace Ltd., Newport Pagnell, Buckinghamshire, UK) to obtain the serum. Serum biochemical markers of hepatic injury ALT, AST, ALP, TP and TB were estimated using BioSystems commercial kits and Roche/Cobas Mira automated analyzer.

2.6. Liver antioxidant markers assay

Liver 10% homogenates in phosphate buffer (50 mM, pH 7.4) were prepared and then centrifuged at 4000 rpm for 15 min at 4 °C. The supernatant of the liver homogenate was used for the assays of glutathione (GSH)[20], superoxide dismutase (SOD)[21] and catalase (CAT)[22] levels by a colorimetric method. The level of thiobarbituric acid-reactive substance (TBARS) was determined in liver homogenates according to the method of Ohkawa et al. (1979)[23]. The

TBARS values were then calculated using the standard curve of malondialdehyde (MDA) and expressed as nmol MDA/mg proteins. Protein concentrations were determined according to the method of Lowry et al. (1951)[24], using bovine serum albumin as standard.

2.7. Histopathological examination

Liver sections were fixed in 4% formalin in phosphate buffered solution for 24 h. After dehydration, the pieces of liver were embedded in paraffin wax, cut into 4 – 6 µm thick sections using a microtome and stained with haematoxylin-eosin. They were observed under a microscope for histopathological changes in liver architecture and photographed. Photographs of each of the slides were taken at 100 × magnification.

2.8. Statistical analysis

The data were expressed as mean ± S.E.M. Statistical evaluation of the data was performed by 1-way analysis (ANOVA). Variance homogeneity and data distribution were determined with the Levene and Kolmogorov – Smirnov tests, respectively. Post-hoc comparison between control and treated groups was performed with the T3 Dunnett's test or with the Bonferroni test when the variance was not homogeneous. Statistical analysis was performed using the SPSS statistical software package, version 13.0 for Windows. The results were considered to be statistically significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$.

3. Results

3.1. Phytochemical results

The extracts of *G. cruciata* were analyzed by HPLC–DAD in order to identify and quantify three secoiridoid compounds (sweroside, swertiamarin and gentiopicrin) and xanthone compound mangiferin. The examples of chromatograms for GCA and GCR are presented in Fig. 1. As shown in Table 1, gentiopicrin was the dominant secoiridoid glucoside in both extracts. GCR possessed significantly ($p < 0.05$) higher concentration of gentiopicrin (54.507 mg/g of extract) than GCA (19.870 mg/g of extract), while considerably ($p < 0.05$) higher concentration of sweroside was found in GCA (5.648 mg/g). In words of swertiamarin amount, there was no significant difference ($p > 0.05$) between extracts. No detectable amounts of mangiferin were found in extracts of *G. cruciata*.

3.2. Effects of extracts on blood biochemical markers

In acute toxicity studies, all the extracts were found to be safe up to 1600 mg/kg. No mortality or toxic symptoms were observed during the entire duration of the study. The effects of various doses of GCA and GCR on serum biochemical markers in CCl_4-intoxicated rats were studied (Table 2). After a single injection of CCl_4, serum activities of AST, ALT and ALP enzymes in the hepatotoxic model group (Group II) were significantly increased ($p < 0.001$). The total bilirubin values were also significantly increased in the CCl_4 group compared to the control group ($p < 0.001$), while the level of TP was significantly ($p < 0.001$) decreased. Pretreatments of animals with different doses of GCA (groups IV–VI; 100, 200, and 400 mg/kg b.w., respectively) and GCR (group VII-IX; 100, 200, and 400 mg/kg b.w., respectively) for 7 days significant ($p < 0.001$) dose-dependent reduced levels of ALT, AST and ALP as compared to the group of CCl_4-treated alone. GCR at the dose of 400 mg/kg was found to more markedly reduce the activity of AST ($p < 0.05$), ALT and ALP ($p < 0.001$) compared to GCA at the same dose. GCA and GCR also dose-dependently increased the reduced serum level of TP with effects being

significant at 200 mg/kg and 400 mg/kg. Compared to the CCl_4 group, the serum TB levels in the GCA and GCR groups decreased, especially when the dosage increased to 200 and 400 mg/kg b.w.

3.3. Hepatic antioxidant enzyme activities and GSH and TBARS levels

Figure 2 shows the effects of GCA, GCR and silymarin on the activities of CAT and SOD and the levels of GSH and TBARS in the liver of CCl_4-treated rats. CCl_4 treatment significantly decreased the GSH content and SOD and CAT activities in the liver tissue as compared with the normal group ($p < 0.001$). Considering the formation of TBARS, there was a significant increase in liver tissues of the CCl_4 group versus that of the control (Fig. 2D). As shown in Fig. 2, levels of SOD, CAT, and GSH, in liver, were up-regulated after treating with GCA and GCR (100, 200, and 400 mg/kg b.w.). However, significant ($p < 0.001$) increases activity of SOD and GSH levels caused only treatment with GCA and GCR at 400 mg/kg b.w. compared to the CCl_4-model group. GSH level for silymarin was mostly comparable and not significantly different from the value obtained from the normal group. All doses of GCA induced a significant increase in CAT activity in CCl_4-treated rats as compared with CCl_4 treatment alone, while GCR extract only significantly ($p < 0.001$) elevated the CAT activity at the dose of 400 mg/kg b.w. As shown in Fig. 2D, the highest dose (400 mg/kg b.w.) of GCA and GCR significantly ($p < 0.001$) decreased CCl_4-induced TBARS level in liver, compared to the CCl_4 group. However, there was no significant difference in TBARS levels in the groups which received GCA and GCR at the lowest doses and CCl_4-model group.

3.4. Histopathological and morphological examination of the liver

As shown in Table 3 and Fig. 3, the hepatic tissues in rats in the control group exhibited the normal cellular structure (Fig. 3I) with weak congestion and sinusoidal dilatation in some samples. Also, in some samples from this group appeared weak fibrosis. Photomicrographs of livers from the animals treated with CCl_4 (Group II, Table 3, Fig. 3II) demonstrated significant evidence of injury with marked ballooning degeneration (especially at the periphery of lobules), macrovesicular and microvesicular changes, dilated portal spaces followed by infiltration of lymphocytes, leukocytes and macrophages as well as intra-acinar infiltration of the same cells. There was focal necrosis as well as areas of piecemeal necrosis and mild fibrosis of portal areas (Fig. 3II). The hepatohistological changes induced by CCl_4 were markedly ameliorated by pretreatment with GCA or GCR in a dose-dependent manner. The groups treated with silymarin, medium and high-dose GCA and GCR correspondingly appear to relieve the pathological damages (Figs. 3III, V, VI, VIII, IX). The administration of CCl_4 along with GCA and GCR at 400 mg/kg b.w. showed near-normal appearance (Figs. 3VI, IX), suggesting that GC extracts could protect the liver from acute CCl_4-induced hepatic damage.

4. Discussion

Many *Gentiana* species have been studied for their potential positive effect on human health because of the content of many bioactive compounds with a wide range of biological activities.[25,26] Here, the methanolic extracts of *G. cruciata* (GC) have been demonstrated to have an important hepatoprotective effect against carbon tetrachloride induced liver injury. The main causes of acute liver injury by CCl_4 are free radicals, which are generated in its metabolism by the cytochrome P_{450} (CYP) system.[27] By the activation of liver CYP, CCl_4 produces the

hepatotoxic metabolites trichloromethyl free radicals (•CCl_3 or CCl_3OO•), which immediately propagate a chain of lipid peroxidation events and finally lead to the breakdown of membrane structure and the consequent leakage of hepatic cell marker enzymes into the bloodstream.[28] Administration of CCl_4 caused significant liver damage as evidenced by the altered serum and liver biochemical parameters (Table 2 and Fig. 2). The increased levels of these serum biochemical parameters were dose-dependent decreased by the administration of GCA and GCR (100−400 mg/kg b.w.), implying that GC may effectively protect the hepatocytes against the toxic effects of CCl_4. GCA and GCR at the dose of 400 mg/kg retained the levels of serum AST, ALT and ALP to near normal values when compared to normal control, and obtained values were lower than that obtained in silymarin group (100 mg/kg). The restoration of serum enzyme levels in CCl_4-treated rats pretreated with GCA and GCR indicates prevention of the leakage of intracellular enzymes by stabilizing the hepatic cell membrane. In parallel with these observations, histological examination clearly showed that CCl_4-induced focal and piecemeal necrosis, infiltration of lymphocytes, leukocytes and macrophages were lowered dramatically by treatment with GCA and GCR (Figure 3), with maximum protection at the highest dose of both extract.

One of the possible mechanisms of action of *G. cruciata* in conferring hepatoprotectivity could be attributed to its antioxidative properties. It is now increasingly clear that various mediators have been implicated in CCl_4-induced liver injury, but the role of oxidative stress and free radical damage is thought to be of particular importance.[29] Here, the administration of CCl_4 to rats sharply decreased antioxidant capacity of rat liver as evidenced by inhibiting the activities of SOD and CAT. Pretreatment with GCA or GCR at the highest dose (400 mg/kg b.w.) significantly increased the activities of these enzymes, suggesting that it could protect the two

antioxidant enzymes or activate the enzyme activity in CCl_4-damaged liver tissue. Previous studies of the mechanism of CCl_4-induced hepatotoxicity have indicated that GSH plays a key role in detoxification of the reactive toxic metabolites of CCl_4 and that hepatic necrosis begins when the GSH pool is depleted.[30] The increase in the hepatic GSH levels in the extracts (400 mg/kg b.w.) and silymarin (100 mg/kg b.w.) treated groups could be due to their effect on de novo synthesis of GSH, its regeneration, or both. The increase in TBARS level in the liver suggests enhanced peroxidation leading to tissue damage and failure of the antioxidant defense mechanisms to prevent the formation of excessive free radicals.[31] Pretreatment with GCA and GCR at the highest dose (400 mg/kg b.w) significantly reversed these changes. Administration of GCA more effectively increased activity of CAT and protect against the hepatic lipid peroxidation induced by CCl_4 compared to GCR, suggesting that it has better antioxidant properties. These results suggest that the antioxidant properties observed may be one mechanism through which GC protects against liver damage induced by CCl_4.

The HPLC assay clearly indicated that GCR contained the greatest concentration of gentiopicrin (5.45%), whereas other secoiridoids such as sweroside and swertiamarin were also identified as abundant constituents in both extracts (Table 1, Figure 1). Gentiopicrin have been reported to be able to inhibit chemically and immunologically induced hepatotoxicity in experimental animal models.[15] Lian et al. (2010b)[32] reported that gentiopicrin markedly reduced the increases in serum aminotransferase activities, lipid peroxidation and increased GSH levels, confers protection against lipopolysaccharide/D-galactosamine-induced fulminant hepatic failure in mice. Another active principle identified in GCA and GCR is swertiamarin that is known to exhibit protective effects against D-galatosamine-induced liver damage.[19] The presence these two active compounds in the methanolic extracts of *G. cruciata* may be the main contributing factor

toward its hepatoprotective activity, but a problematic aspect in understanding potential hepatoprotective events of examined extracts is that the extracts contain more unknown active chemical constituents. The total bioactivity might also depend on synergistic, antagonistic or additive interactions of molecules present in particular extracts in biological systems (cells). Since the extracts have been commonly used as a complex mixture of bioactive components in folk medicine, our goal was to examine the final effect of extracts with all interactions between the components of our extracts. Literature data and therapeutic experiences showed that a complex pathophysiological process can be influenced more effectively and with fewer or no severe side-effects by a combination of several low-dosage compounds or the corresponding extracts than by a single highly dosaged isolated compound. Phytotherapy has long followed and developed these strategies by using mono-extracts or extract combinations containing mixtures of bioactive compounds and by activating primarily self-healing and protective processes of the human body, rather than attacking and directly destroying the damaging agents. [33,34]

The findings of our study are in consistent with previous reports that *Gentiana* plants rich in secoiridods constituents, mainly gentiopicrin, are effective in protecting liver against acetaminophen- and alcohol-induced liver damage.[16,35] Regardless of the previous researches, we had no chance to compare our results with the previous ones, because of high variability in experiments *in vivo,* and inconsistent factors like treatment time and manner, the setting of studies and species induced liver damage differences etc., it is difficult to compare the present data to different studies regarding the hepatoprotective properties. Despite treatment time and manner and the different setting of studies the results of the above-mentioned studies are in agreement with our results. However, in our previous work we confirmed the hepatoprotective effects of aerial parts and root extracts of *Gentiana asclepiadea* L. in the same experimental

conditions.[36] In fact, those two plants displayed very similar hepatoprotective activities with some differences in biological activities and their chemical composition. Higher gentiopicrin content was observed in *G. asclepiadea* extracts and mangiferin identified in aerial part extract. Observed differences in chemical composition of two plants can explain that root extract of *G. asclepiadea* at the highest dose more effectively decreased activities of AST and ALT compared with *G. cruciata*, due to its genciopicrin-enriched composition. Also, both extracts of *G. asclepiadea* have shown better antioxidant activities *in vivo* and pretreatment with *G. cruciata* extracts. Regardless of the differences, we can say that *G. cruciata* possess remarkable hepatoprotective activity and could be used as a substitute for other *Gentiana* species which are endangered.

5. Conclusion

In conclusion, the results from this study clearly demonstrate that *G. cruciata* extracts contribute to prevent the important histological changes, as well as liver functionality alterations by reducing, in an important manner, many of the altered serum and liver biochemical markers of this experimental CCl_4-induced hepatotoxicity. The observed protective activity of GC may be due to the identified compound that is abundantly present in extracts and this finding could also serve as a useful reference to allow the future investigations of secoiridoids from *Gentian* plants as a novel preventative and therapeutic ingredient for the treatment of liver injury and chronic disease, responsible for hepatoprotective activity.

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Sample	Concentration (mg/g of extract)			
	Swertiamarin	Gentiopicrin	Sweroside	Mangiferin
GCA	$4.950^{a} \pm 0.677$	$19.870^{c} \pm 3.618$	$5.648^{a} \pm 1.352$	ND
GCR	$2.888^{a, b} \pm 0.109$	$54.507^{d} \pm 2.063$	$0.684^{b} \pm 0.164$	ND

Each value represents the mean ± S.E.M. of three experiments; means with superscript with different letters are significantly different at $p < 0.05$; ND, not detected

Table 2. Effects of *G. cruciata* extracts and silymarin on serum biochemical parameters of CCl_4 intoxicated rats.

Group	AST (U/L)	ALT (U/L)	ALP (U/L)	Total protein (g/L)	Total bilirubin(μmol/L)
I	152.88 ± 14.64	66.46 ± 2.81	504.43 ± 36.10	65.66 ± 1.16	1.23 ± 0.12
II	2733.03 ± 95.39†	343.85 ± 10.38†	755.10 ± 12.40†	60.08 ± 0.59†	1.75 ± 0.20†
III	748.10 ± 41.41***	231.87 ± 39.58***	575.66 ± 24.26***	62.94 ± 2.22***	1.45 ± 0.17***
IV	1072.15 ± 68.43***	179.36 ± 28.56***	613.00 ± 39.19***	60.49 ± 0.72	1.50 ± 0.36**
V	773.23 ± 52.53***	159.88 ± 18.11***	562.18 ± 49.03***	60.90 ± 0.68**	1.30 ± 0.40***
VI	459.18 ± 48.31***	122.57 ± 16.96***	556.73 ± 29.52***	62.77 ± 1.31***	1.30 ± 0.25***
VII	758.93± 49.02***	215.83 ± 63.01***	589.12 ± 29.56***	60.52 ± 0.95	1.60 ± 0.17
VIII	386.84 ± 29.30***	122.53 ± 25.06***	455.88 ± 48.46***	61.31 ± 0.82***	1.52 ± 0.11**
IX	328.20 ± 28.55***	80.70 ± 12.93***	432.13 ± 5.12***	62.53 ± 1.46***	1.45 ± 0.19***

I – Control group; II – CCl_4 (1:1 in olive oil) 1 ml/kg i.p.; III – Silymarin (100 mg/kg) + CCl_4; IV - GCA 100 mg/kg + CCl_4; V – GCA 200 mg/kg + CCl_4; VI – GCA 400 mg/kg + CCl_4; VII – GCR 100 mg/kg + CCl_4; VIII – GCR 200 mg/kg + CCl_4; IX – GCR 400 mg/kg + CCl_4;

Values are mean ± S.E.M., $n = 5$ animals in each group;† $p < 0.001$, compared to control group; $^{*}p < 0.05$ compared to CCl_4-intoxicated group; $^{**}p < 0.01$ compared to CCl_4-intoxicated group; $^{***}p < 0.001$ compared to CCl_4-intoxicated group

Table 3. Effects of *G. cruciata* extracts and silymarin on morphological parameters of rats liver after CCl_4 treatment (n = 5)

Groups	Histopathological parameters									
	Congestion	Sinusoidal dilatation	Ballooning degeneration	Micro- and macrovesicular changes	Infiltration of lymphocytes, leukocytes and macrophages	Focal necrosis	Piecemeal necrosis	Panacinar necrosis	Fibrosis	Kupffer cell hyperplasia
I	+	+	-[a]	-	-	-	-	-	+	-
II	++	++	++, mostly at the periphery of lobules	++	++	++, perivenular	++	+	++, portal	+
III	++	++	++, mostly at the periphery of lobules	+	++	+, perivenular	-	-	+, portal	+
IV	+++	++	++ central part of acinus	+	++	+ central part of acinus	++	+	++ Porto-portal	+
V	+	+	++ perivenular	-	+	+ perivenular	-	-	+ Porto-portal	+
VI	++	+	+	-	+ Intracinar and perivenular	-	-	-	+ Portal	+
VII	++	++	++	+	+	++ Periphery and central part of acinus	+	-	+ Portal	+
VIII	++	++	++	-	++	+ central part of acinus	-	-	+ Portal	+
IX	++	+	+ central part of acinus	+	+	+	-	-	+ Portal	+

[a]- , absent; +, mild; ++, moderate; +++, marked

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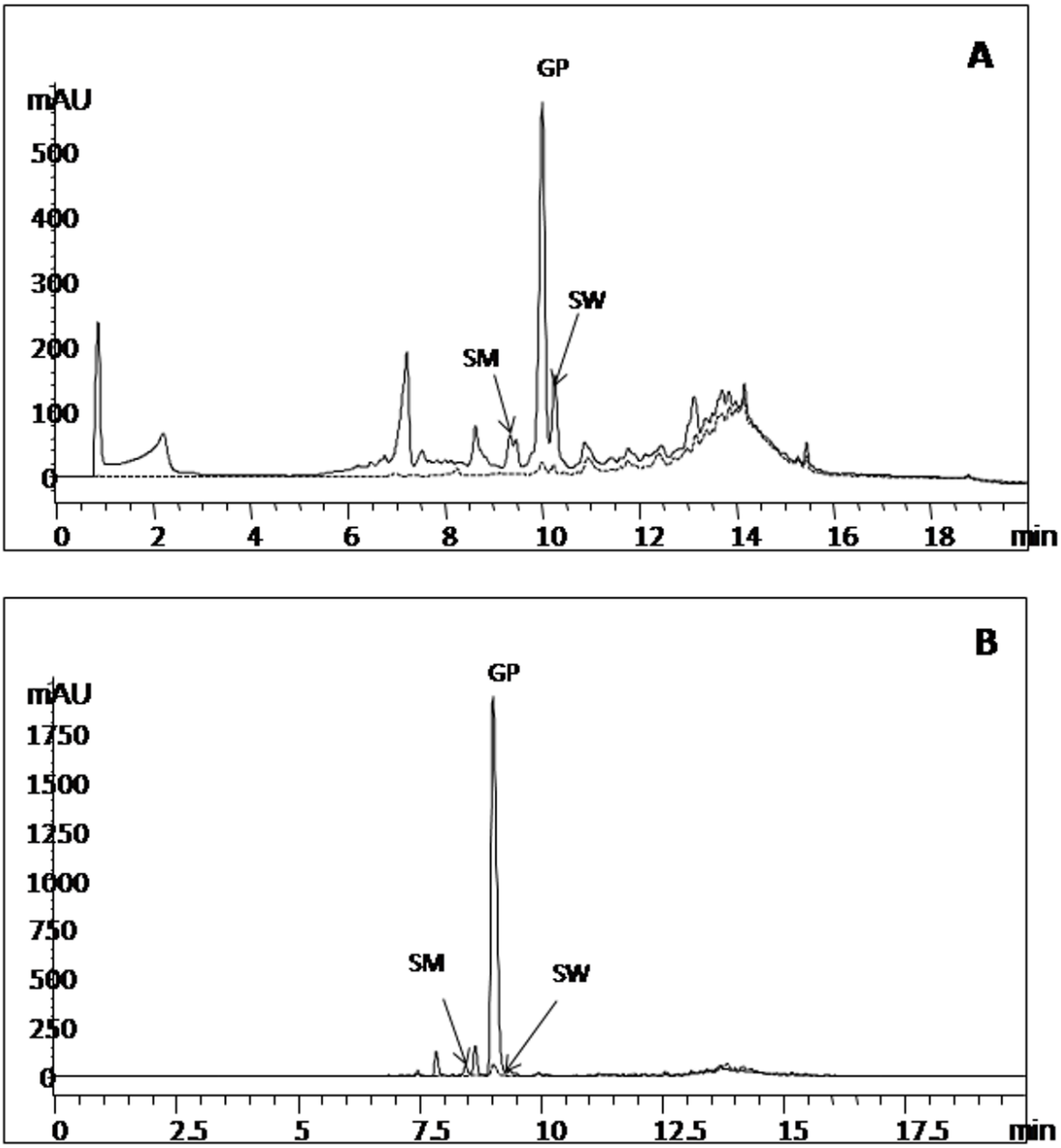
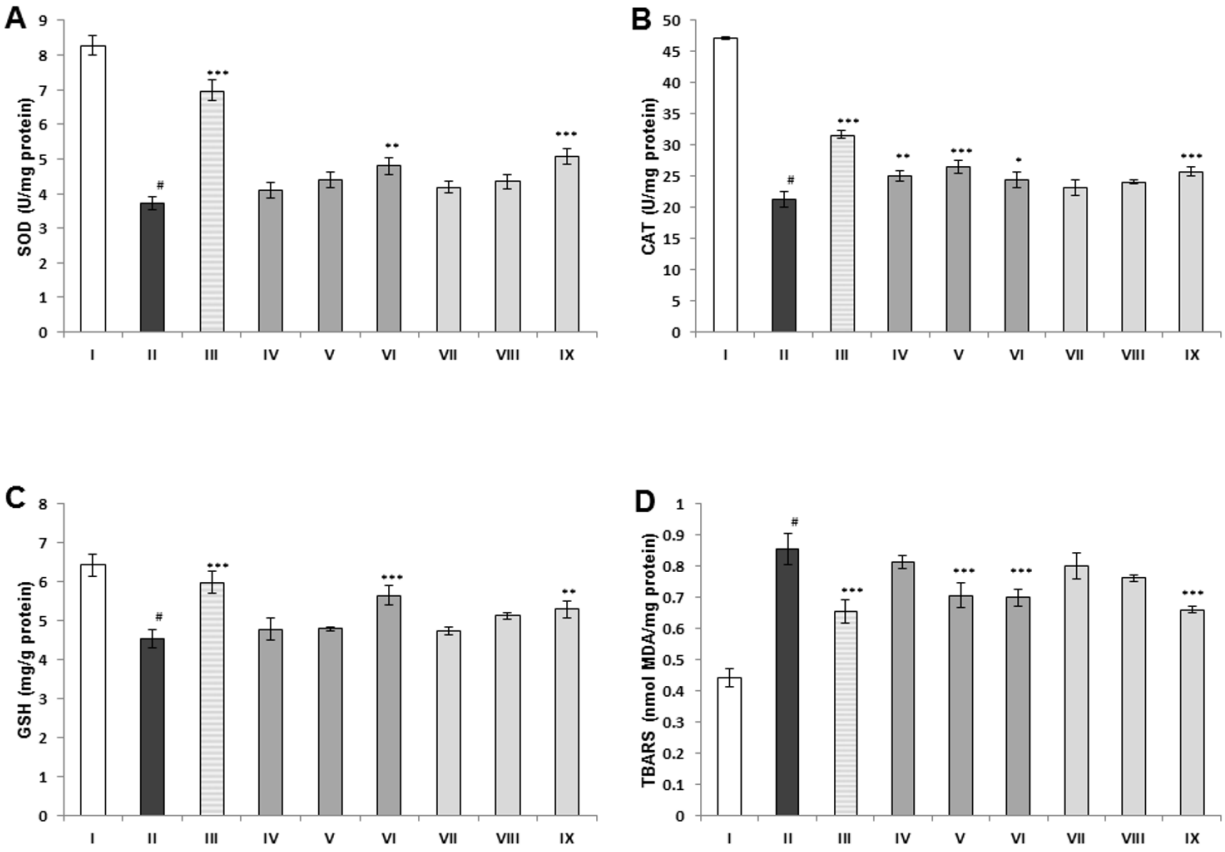


Fig. 1. Sample HPLC chromatograms of bioactive compounds (260 nm) obtained for methanolic extracts prepared from underground parts (A) and root (B) of *G. cruciata*. SM - swertiamarin, GP - gentiopicrin, SW - sweroside



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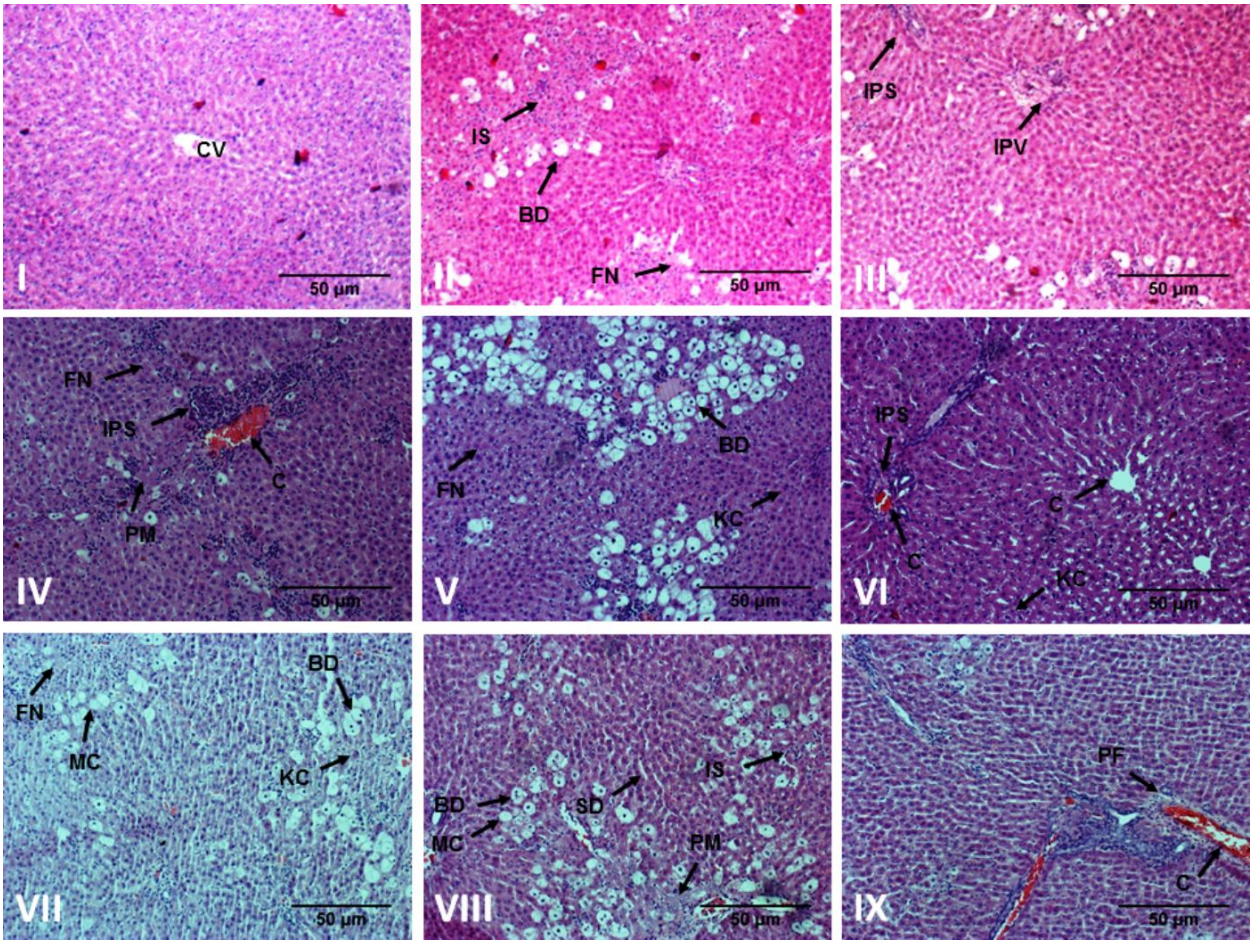

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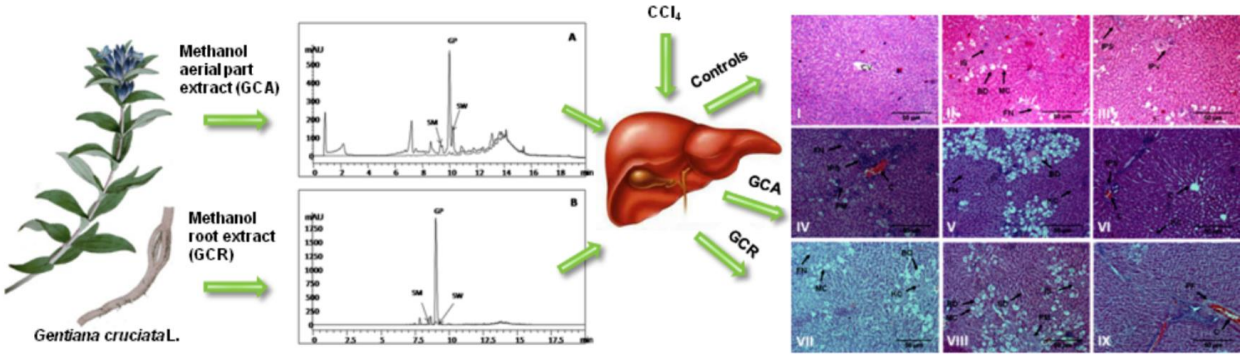
Highlights

- This study is the first report on hepatoprotective activity of *G. cruciata* extracts which contained a high content of gentiopicrin. Extracts restored serum biochemical parameters, decreased CCl_4-induced oxidative damage and CCl_4-induced liver lesions.



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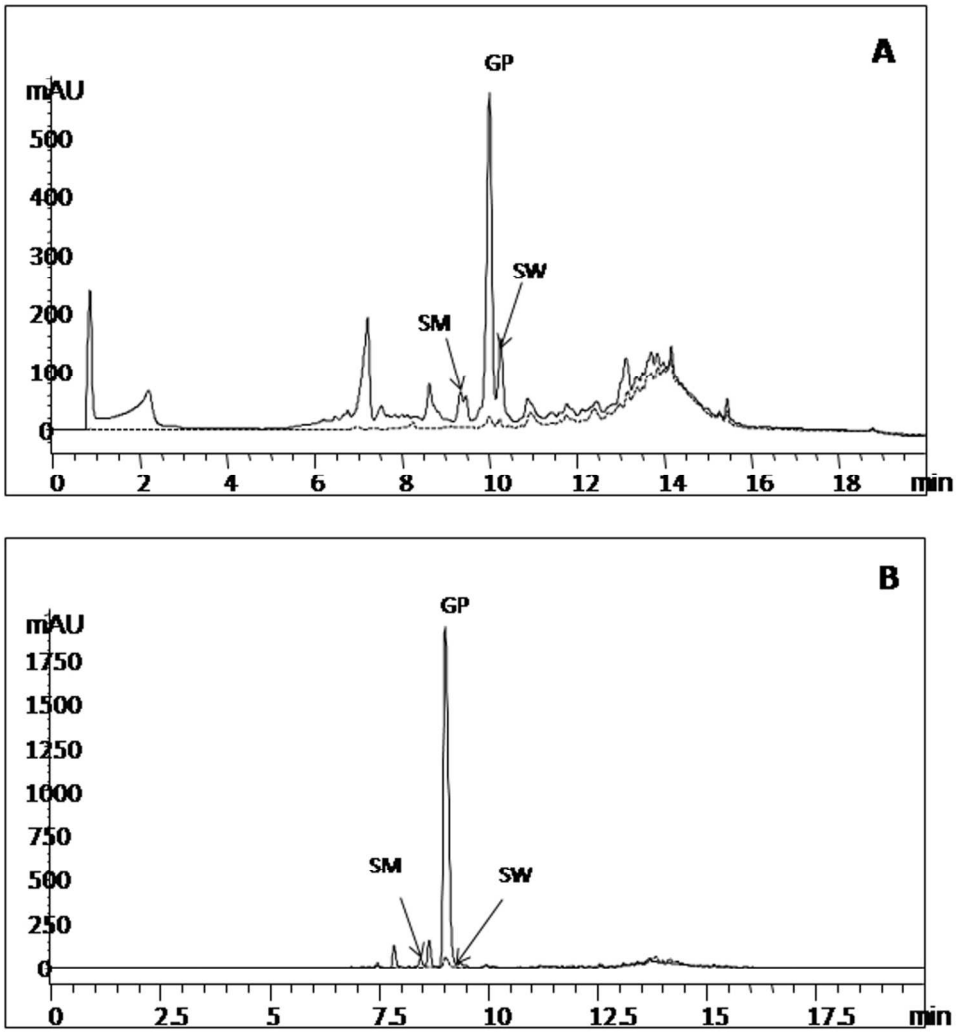

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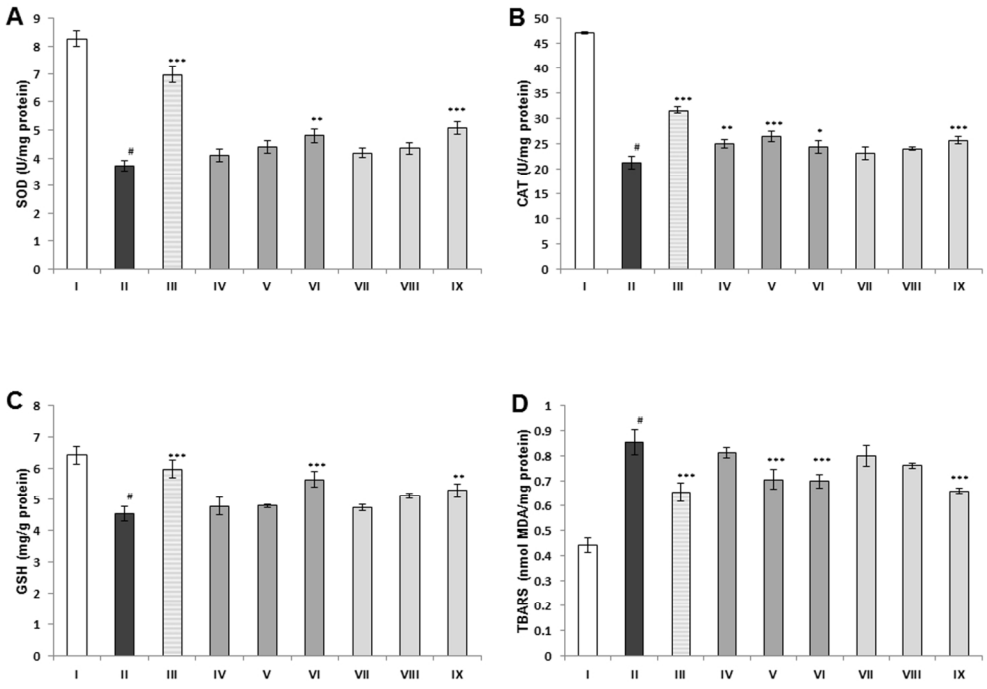
338x366mm (96 x 96 DPI)



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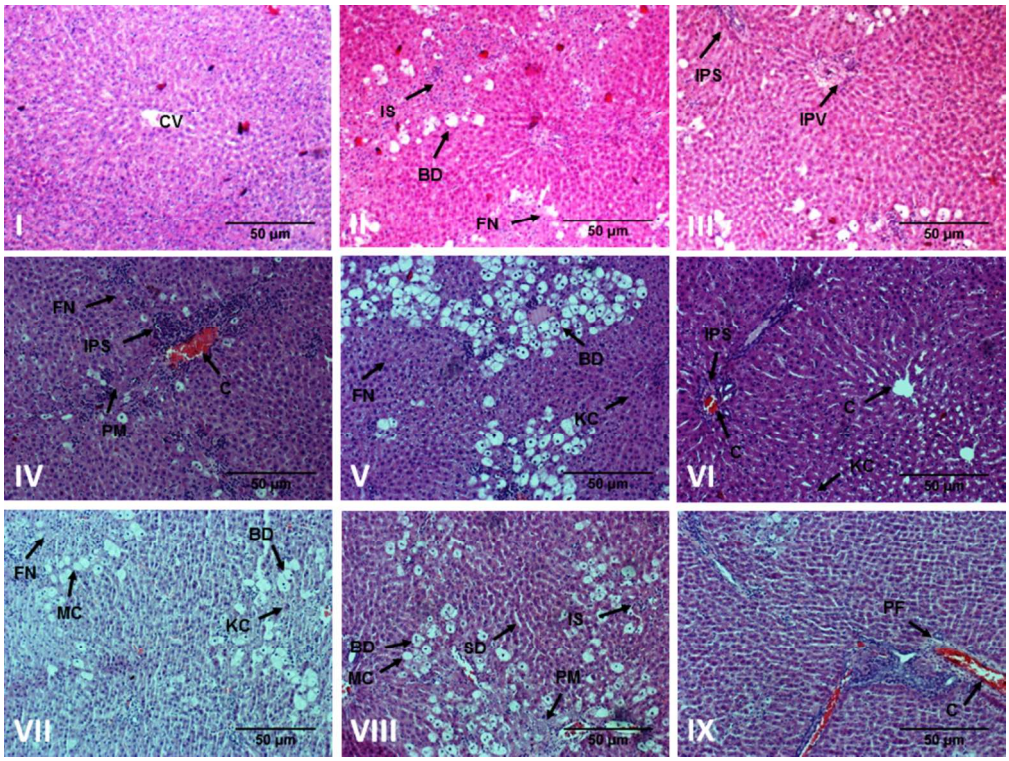

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370x277mm (96 x 96 DPI)