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IN VIVO PROTECTIVE EFFECTS OF MAURITIANIN

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ABSTRACT

Mauritianin is a rare flavonoid isolated from the overground parts of *Astragalus monspessulanus* subsp. *monspessulanus.* The aim was to investigate mauritianin *in vivo* for possible hepatoprotective, nephroprotective and neuroprotective activity in a model of carbon tetrachloride-induced liver damage. Thirty six Wistar rats were used and allocated to six experimental groups, treated orally: non-treated (control); treated with mauritianin alone (10 mg/kg); treated with silymarin alone (100 mg/kg); CCl_4-treated; pre-treated with mauritianin for 7 days, then given CCl_4 and subjected to curative treatment with mauritianin (10 mg/kg) for another 14 days; and treated with silymarin (100 mg/kg, 7 days), given CCl_4, then for the next 14 days treated with silymarin (100 mg/kg). The reduced glutathione level (GSH), malonedialdehyde production (MDA), and the activity of ethylmorphine-*N*-demethylase (EMND) were measured in liver homogenate. A histopathological examination was performed on the livers, brains and kidneys of all animals. Administered alone, mauritianin had no toxicity. Both biochemical and pathoanatomical findings were similar to those in the silymarin-treated group. CCl_4 caused severe organ damage – increased MDA production, decreased GSH and EMND levels, lipid accumulations in the liver, pericellular oedemas in the brain and interstitial haemorrhages, accompanied by necrotic changes in the kidneys. In animals pre-treated with mauritianin, exposed to CCl_4 and treated 14 days more, minimal biochemical and pathological changes were found, compared to the latter group. A well-preserved histoarchitectonic was found, commensurable to observation of the organs of silymarin and CCl_4 treated rats.

Key words: *in vivo* study; rats; mauritianin; hepatoprotective; neuroprotective; nephroprotective

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INTRODUCTION

In recent years, there have been trends to establish hepato-, neuro- and other protective effects of plant extracts and biologically active substances, including flavonoids (Sahreen *et al*., 2011). Mauritianin (kaempferol-3-*O*-(2,6-di-*O*-*α*-L-rhamnopyranosyl)-*β*-D-galactopyranoside) is a rare flavonoid, isolated for the first time from *Lysimachia mauritiana* (Primulaceae) (Yasukawa and Takido, 1987) and later – from other plants, including representatives of genus *Astragalus* L. (Fabaceae) (El-Mawla and Attia, 2002; Khalfallah *et al*., 2011; Krasteva *et al*., 2015). It has been increasingly investigated to detect potentially significant pharmacological effects. In an *in vivo* experiment on kaempferol and its glycosides (including mauritianin), antitumor-promoting activity was demonstrated in two-stage carcinogenesis induced by 7,12-dimethylbenz[a]anthracene (DMBA) and 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in mice. The compounds showed significant suppression of TPA-induced tumour formation. Cell-mediated immunosuppression was interrupted by treatment with mauritianin and myricetin, but not with kaempferol and naringenin, although chemically similar (Yasukawa *et al*., 1990). Mauritianin increased delayed-type TPA-suppressed susceptibility in mice, which may enhance immune resistance to cancer (Nishibe *et al*., 1996). The compound, isolated from *Rinorea anguifera* (Violaceae), was tested for antitopoisomerase activity and was found to stabilize the topoisomerase I-DNA complex in concentration from 1μM to 100 μM (Ma *et al*., 2005). Mauritianin, derived from the leaves of *Swartzia apetala* var. *glabra* (Fabaceae), showed high antimicrobial activity against *Candida albicans* (de Araújo *et al*., 2013). Mauritianin, isolated from Bolivian *Chenopodium quinoa* (Amaranthaceae), exhibited antithyrosinase and antioxidant activity (Navia *et al*., 2020). The flavonoid exhibited also antiviral activity, including against COVID-19 (Owis *et al*., 2020).

In our previous studies mauritianin was isolated from the overground part of *A. monspessulanus* subsp. *monspessulanus* and exhibited statistically significant

neuro- and hepatoprotective activity on various *in vitro* models of toxicity – isolated rat hepatocytes and brain synaptosomes (Krasteva *et al*., 2015; Kondeva-Burdina *et al*., 2019). Hepatocytes, treated with the flavonoid on *t*-BuOOH-induced oxidative stress model, showed reduced lactate dehydrogenase (LDH) release by 60%, compared to the untreated group; reduced malonedialdehyde (MDA) production – by 56%, and increased reduced glutathione (GSH) levels – by 220% compared to 200% in hepatocytes, treated with silybin (Krasteva *et al*., 2015). On 6-hydroxydopamine (6-OHDA)-induced oxidative stress in synaptosomes, mauritianin administration preserved synaptosomal viability by 69% and prevented GSH depletion by 140% (Kondeva-Burdina *et al*., 2019). The aim of the study was to confirm the proven *in vitro* protective effects of mauritianin in an *in vivo* model of toxicity with CCl_4.

MATERIALS AND METHODS

Plant material, extraction and isolation of mauritianin: Overground parts of *A. monspessulanus* were collected in June 2019 from Rhodopi Mountain, Bulgaria. The species was identified by Dr. D. Pavlova from the Department of Botany, Faculty of Biology, Sofia University, where a voucher specimen was deposited (№ SO-107533). The procedure for isolation and structural elucidation of mauritianin was described previously (Krasteva *et al*., 2015). The air-dried and powdered plant material was exhaustively extracted with 80% MeOH under reflux and the concentrated extract was successively partitioned between $CHCl_3$, EtOAc, and *n*-BuOH. The resulting *n*-BuOH extract was subjected to column chromatography (CC) over Diaion HP-20 (H_2O-MeOH, 100:0→0:100, v/v) to obtain nine main fractions (A-I). Fraction C was separated by CC over Sephadex LH-20 (eluent MeOH) and six subfractions (C1-C6) were obtained. Subfraction C4 was purified by repeated low pressure liquid chromatography (LPLC) over octadecylsilica gel (C_{18}) (MeOH-H_2O, 40:60, v/v). The fraction was purified by isocratic semi-preparative HPLC over RPC_{18} (MeCN-H_2O, 16:84, v/v) to give mauritianin (655 mg, 97.5% HPLC purity). The compound was identified by comparing its NMR and MS data with the literature (Krasteva *et al*., 2015).

Chemicals: All the reagents used were of analytical grade. Carbon tetrachloride, trichloroacetic acid (TCA), silymarin, beta-nicotinamide adenine dinucleotide 2`-phosphate reduced tetrasodium salt (NADPH), reduced glutathione (GSH), thiobarbituric acid (TBA), $ZnSO_4$, and $Ba(OH)_2$ were purchased from Sigma (Taufkirchen, Germany). 2,2-dinitro-5,5-dithiodibenzoic acid (DTNB) was obtained from Merck (Darmstadt, Germany).

Animals: The studies were performed on male Wistar rats (N=36) with body mass of 200-250 g. The rats were housed in Plexiglas cages (3 per cage) in a 12/12 light/dark cycle under standard laboratory conditions (ambient temperature 20 °C ± 2 °C and humidity 72% ± 4%) with free access to water and standard pelleted rat food 53-3, produced according ISO 9001:2008. The animals were purchased from the National Breeding Centre, Slivnitsa, Bulgaria. Seven days' acclimatization was allowed before the commencement of the experiment and a veterinary physician monitored the health of the animals regularly. Vivarium (certificate of registration of farm № 0072/01.08.2007) was inspected by the Bulgarian Drug Agency in order to check the husbandry conditions (№A-11-1081/03.11.2011). The Bulgarian Food Safety Agency (BFSA) and the Institutional Animal Care Committee at Medical University of Sofia (KENIMUS) approved the study protocol and an Ethic clearance (No 200/23.03.2020) was issued for the study by BFSA. The principles stated in the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS 123) (Council of Europe, 1991) were strictly followed throughout the experiment.

Experimental design: The rats were randomly allocated into six experimental groups, each consisting of six animals (*n*=6).
Group 1 - control animals treated with saline (500 ml/kg, p.o., 21 days).
Group 2 - animals treated with mauritianin (10 mg/kg, p.o., 21 days). The dose is 1/200 of the LD_{50} (rats) = 2000 mg/kg (Simeonova *et al*., 2019).
Group 3 - animals treated with silymarin (100 mg/kg, p.o., 21 days) (Habbu *et al*., 2008).
Group 4 - animals treated with CCl_4 (10% solution in olive oil, 1.25 ml/kg, p.o.) (Ahn *et al*., 2007).
Group 5 - animals treated with mauritianin (10 mg/kg, p.o., 7 days). On the seventh day, 90 min after treatment, the animals were given CCl_4 p.o. (10% solution in olive oil, 1.25 ml/kg), then for the next 14 days they were treated with mauritianin at a dose of 10 mg/kg.
Group 6 - animals treated with silymarin (100 mg/kg, p.o., 7 days). On the 7th day, 90 min after gavage, the animals were treated p.o. with CCl_4 (10% solution, 1.25 ml/kg), then treated with silymarin at a dose of 100 mg/kg for the next 14 days.

Reduced glutathione level: GSH was assessed by measuring the non-protein sulfhydryls after precipitation of proteins with trichloroacetic acid (TCA), followed by measurement of thiols in the supernatant by the DTNB reagent (Bump *et al*., 1983). Briefly, livers were homogenised in 5% TCA (1:3) and centrifuged for 20 min at 4000 x g. The reaction mixture contained 0.05 ml supernatant, 3 ml 0.05 M phosphate buffer (pH 8) and 0.02 ml DTNB reagent. GSH level was expressed as nmol/g wet tissue.

MDA assay: Lipid peroxidation was determined by measuring the production rate of thiobarbituric acid reactive substances (TBARS), expressed as MDA equivalents (Polizio and Pena, 2005). Briefly, livers were homogenised with 0.1 M phosphate buffer, pH 7.4 (1:3). Homogenates were mixed with 1 ml 25% (TCA) and 1 ml 0.67% thiobarbituric acid (TBA). Samples were then mixed thoroughly, heated for 20 min in a boiling water bath, cooled and centrifuged at 4000 x g for 20 min. The absorbance of supernatant was measured at 535 nm against a blank that contained all the reagents except the tissue homogenate. MDA concentration was calculated using a molar extinction coefficient of $1.56x10^5$ M^{-1} cm^{-1} and expressed in $nmol.g^{-1}$ wet tissue.

Activity of ethylmorphine-N-demethylase (EMND): The enzyme activity was evaluated by the formation of formaldehyde, trapped in the solution as semicarbazone, and measured by a colorimetric method with Nash reagent, at 412 nm. The incubation was in 25 ml Erlenmeyer flasks at 37 ºC in a water bath with constant shaking for 20 min. The reaction was initiated with a NADPH-generating mixture. After incubation, the reaction was stopped with 1 ml of 15% $ZnSO_4$ and 1 ml of 4.5% $Ba(OH)_2$. The mixture was centrifuged at 4000 x g for 20 min. Enzyme activity was expressed in nmol/min/mg protein (Cheol Park *et al*., 2004).

Protein content was measured by Lowry's method (Lowry *et al*., 1951). Analyses were performed in triplicate and the mean values were taken.

Histopathological examination: Samples were taken from the liver, brain and kidneys, fixed in 10% buffered formalin, dehydrated with increasing concentration of ethanol, and embedded in paraffin. Thin sections (5 µm) were stained with haematoxylin/eosin (H&E) for general histological features determination (Bancroft and Gamble, 2002) and observed under light microscope Euromex BioBlue (Arnhem, The Netherlands).

Statistical analysis: Statistical analysis was performed using ANOVA statistic technique. Results were expressed as mean ± SEM, for six rats in each group. The experimental groups were compared using the Kruskal–Wallis variance analysis test and a *post-hoc* analysis using Mann–Whitney *U* test was performed. Values of $p \leq 0.05$ were considered statistically significant.

RESULTS

The large quantity of the flavonoid mauritianin, obtained in the present study allowed the *in vivo* investigation. Both biochemical and histopathological effects of the compound were observed for the first time in CCl_4-induced hepatotoxicity in rats.

Markers for antioxidant and hepatoprotective effect: Administered alone, mauritianin and silymarin did not change MDA output and GSH levels. On EMND activity, both substances lead to a statistically significant decrease in the enzyme activity. Mauritianin decreased it by 23% and silymarin - by 32%, compared to the control (untreated animals) (Table 1).

Table 1. Effect of mauritianin and silymarin administered alone on GSH level, MDA production and EMND activity.

Group	GSH, nmol/g Liver	MDA, nmol/g Liver	EMND, nmol/min/mg protein
Control	6.10 ± 0.04	3.91 ± 0.09	0.365 ± 0.03
Mauritianin	6.35 ± 0.05	4.11 ± 0.08	0.280 ± 0.02 **
Silymarin	5.98 ± 0.08	4.09 ± 0.07	0.247 ± 0.01 **

$^{**}p<0.01$ *vs* control (non-treated animals)

Administered alone, CCl_4 increased MDA production by 46%, decreased GSH levels by 37% and EMND activity by 52%, compared to untreated animals. In combination with CCl_4, mauritianin exhibited a hepatoprotective effect, comparable to the effect of silymarin, reducing the production of MDA respectively: mauritianin - by 22% and silymarin - by 31%, relative to CCl_4; kept the activity of EMND, respectively: mauritianin - by 20%, and silymarin - by 29%, relative to CCl_4 and preserved the level of GSH, respectively: mauritianin - by 32%, and silymarin - by 39%, compared to CCl_4 (Table 2).

Table 2. Effect of mauritianin and silymarin in combination with CCl_4 on GSH level, MDA production and EMND activity.

Group	GSH, nmol/g liver	MDA, nmol/ g Liver	EMND, nmol/min/mg protein
Control	6.10 ± 0.04	3.91 ± 0.09	0.365 ± 0.03
CCl_4	3.85 ± 0.03 **	5.72 ± 0.09 **	0.177 ± 0.02 **
CCl_4 + mauritianin	5.08 ± 0.06 ++	4.45 ± 0.07 ++	0.213 ± 0.02 ++
CCl_4 + silymarin	5.37 ± 0.07 ++	3.92 ± 0.08 ++	0.229 ± 0.03 ++

$^{**}p<0.01$ *vs* control (non-treated animals); $^{++}p<0.01$ *vs* animals, treated only with CCl_4

Macroscopic and pathomorphological examination: Macroscopic examination of the livers of the animals from the control group revealed normal anatomical structure, without visible lesions. The livers were located

behind the diaphragm and occupied the central area of the cranial abdomen. The livers were brownish-red in colour, with smooth capsule, and had a well-defined lobar structure (Fig. 1A).

Microscopically, the liver parenchyma had a normal architecture. The lobules had a centrally located terminal hepatic venule, around which liver hepatic trabeculae and sinusoids were radially located. The trabeculae were composed of morphologically unaltered hepatocytes (Fig. 2A).

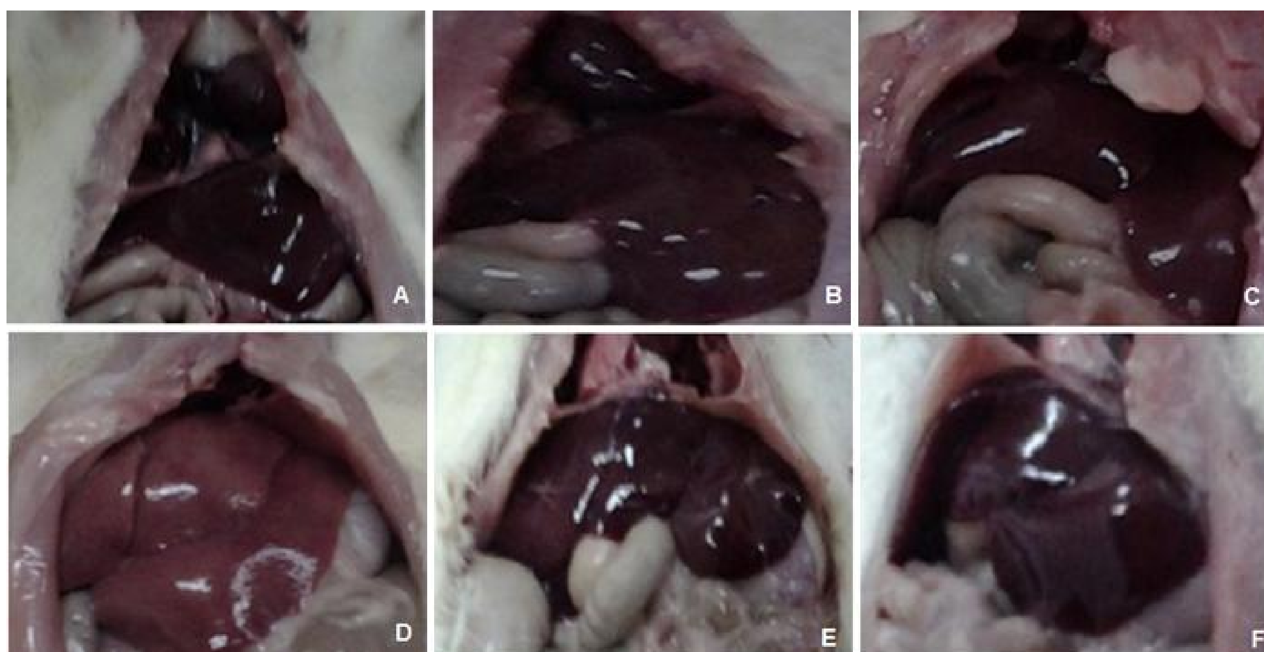

Figure 1. Macroscopic view of the livers (n=6, in six experimental groups). A: Normal anatomical and topographic location and unchanged morphological structure; B and C: Preserved anatomo-topographic and normal morphological structure; D: Enlarged liver with rounded edges and terracotta colour; E and F: Preserved anatomo-topographic and normal morphological structure.

In all animals from the groups treated with mauritianin and silymarin alone, the macroscopic and histological picture of the livers was similar to those from the control group (Fig. 1 B and C; Fig. 2 C, D, E, F). The livers of the rats treated with CCl_4 were enlarged, with rounded edges and a terracotta-like colour (Fig. 1 D). On palpation they were brittle with smearable consistency.

In the rats treated with CCl_4 the microscopy revealed diffuse lipid accumulations in the hepatocytes. Lipid droplets of various sizes and regular round shapes in the cytoplasm of hepatocytes, displacing the cell nuclei to the periphery (Figs. 2 G and H) were observed. Preserved histoarchitectonics of the liver lobules in animals treated with CCl_4 and mauritianin was observed. Single chaotically located lipid accumulations in some places (Fig. 2 I and J) were found as well. In the animals treated with CCl_4 and silymarin there were no changes in the liver tissue. Lipid accumulations in the cytoplasm of some hepatocytes in the centrilobular and midzonal zones were found (Fig. 2 K and L).

Histological examination of the cerebellum of animals from the control group showed a normal cerebral cortex consisting of three layers (Fig. 3A and B). The most superficial was the molecular layer, composed mainly of neuropyl. The granular layer was observed near the white matter of the brain. Between these, a well-defined section, consisting mainly of Purkinje's cells, was found.

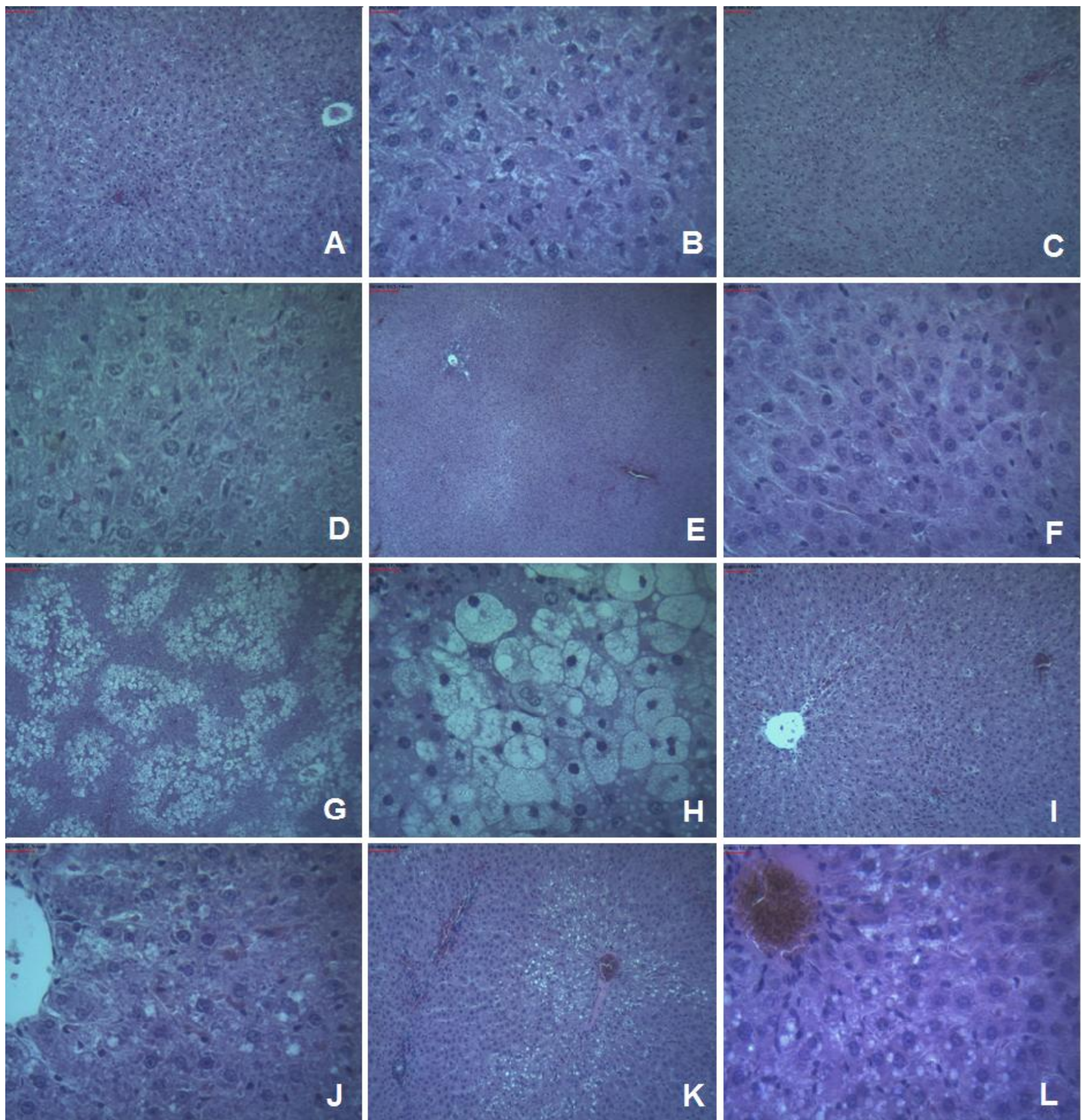

Figure 2. Histopathological examination of rat livers (n=6, in six experimental groups). A: Normal histological architecture of lobular liver structure (100x); B: Normal structure of hepatocytes (400x); C: Normal histoarchitecture of liver lobes (100x); D: Normal hepatocyte structure (400x); E: Normal histological structure of liver parenchyma (40x); F: Normal histological structure of liver parenchyma (400x); G: Massive lipid droplets accumulated in lobular liver structure (40x); H: Accumulations of lipid droplets in cytoplasm of hepatocytes and dislocation of nuclei (400x); I: Single lipid accumulations in hepatocytes (100x); J: Single lipid accumulations located in centrilobular zone (400x); K: Lipid accumulations in centrilobular and midzonal liver areas (100x); L: Lipid droplets in cytoplasm of hepatocytes located around central vein (400x).

The histological structure of the cerebellum of the animals from the groups treated only with mauritianin and silymarin was similar to that of the control (Fig. 3 C, D, E and F). In animals treated with CCl_4 many pericellular oedemas were observed in the cerebellar structure, mainly at the level of white matter (Figs. 3 G and H). Pyknotic changes were found in the cortex in some of the Purkinje's cells (Fig. 3 H).

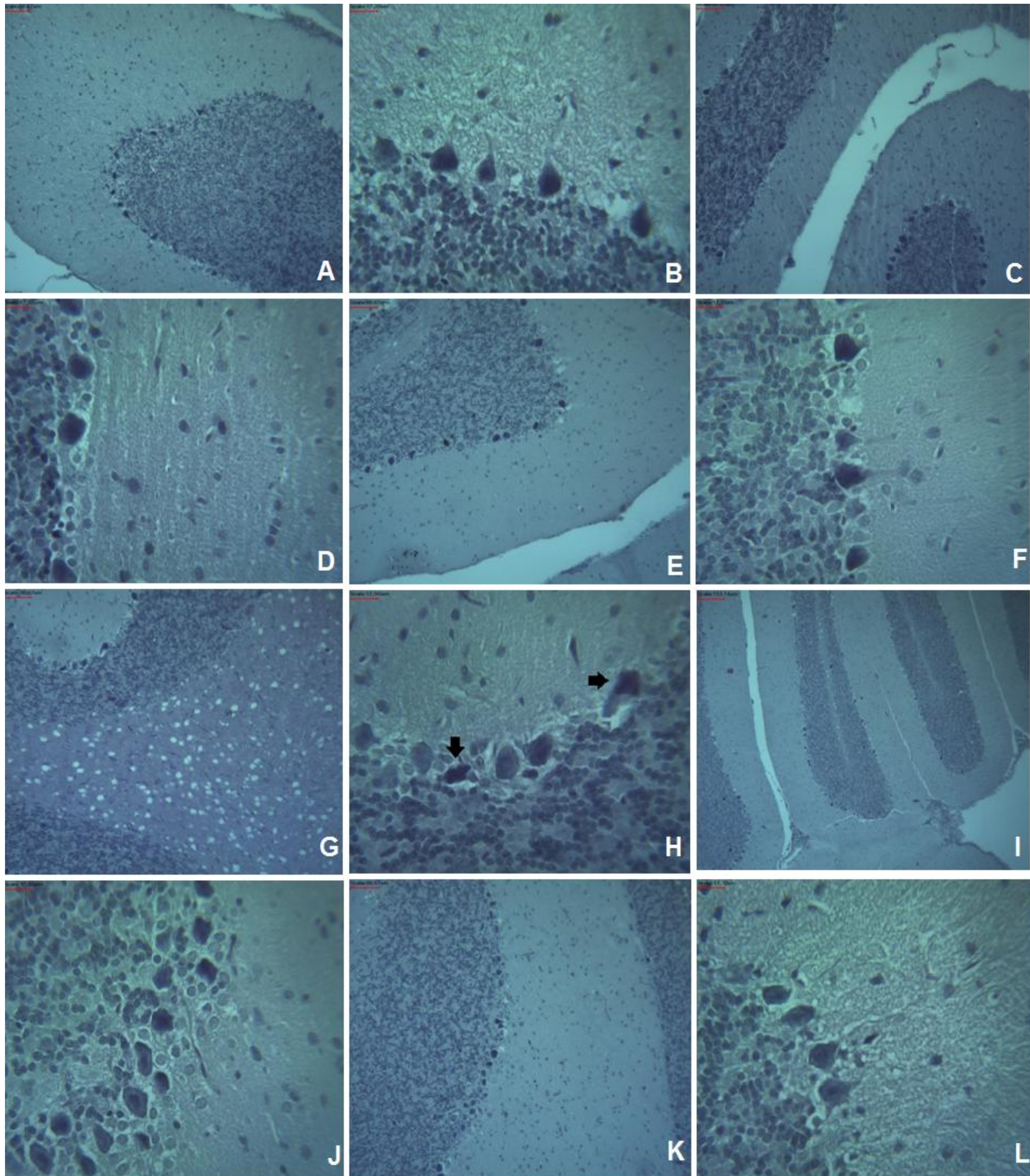

Figure 3. Histopathological examination of cerebellum of rats (n=6, in six experimental groups). A: Normal histoarchitectonics of cerebellum (100x). B: Normal appearance of granular, Purkinje's and molecular layers of cerebellum (400x) C: Unchanged histological structure of cerebellum (100x). D: Preserved histological structure of layers of cerebellum (400x). E: Unchanged microscopic structure of cerebellum (100x). F: Normal appearance of layers of cerebellum (400x). G: Presence of multiple pericellular oedema in corpus medullare of cerebellum and pyknotic changes in some of the Purkinje's cells (100x). H: Presence of pyknotic changes in some of Purkinje cells (400x I: Preserved histological structure of the cerebellum and single pericellular oedematous changes (40x). J: Preserved histological structure of layers of the cerebellum (400x). K: Unchanged histostructure of cerebellum and single pericellular oedema (100x). L: Preserved microscopic appearance of layers of cerebellum (400x).

In the groups treated with CCl_4 and mauritianin a preserved histological structure was established. Single pericellular oedematous changes were seen in some places (Fig. 3 I), but the Purkinje's layer was unchanged (Fig. 3 J). The observed histological finding was similar in the animals from groups challenged with CCl_4 and then protected with either silymarin or mauritianin (Fig. 3 K and L).

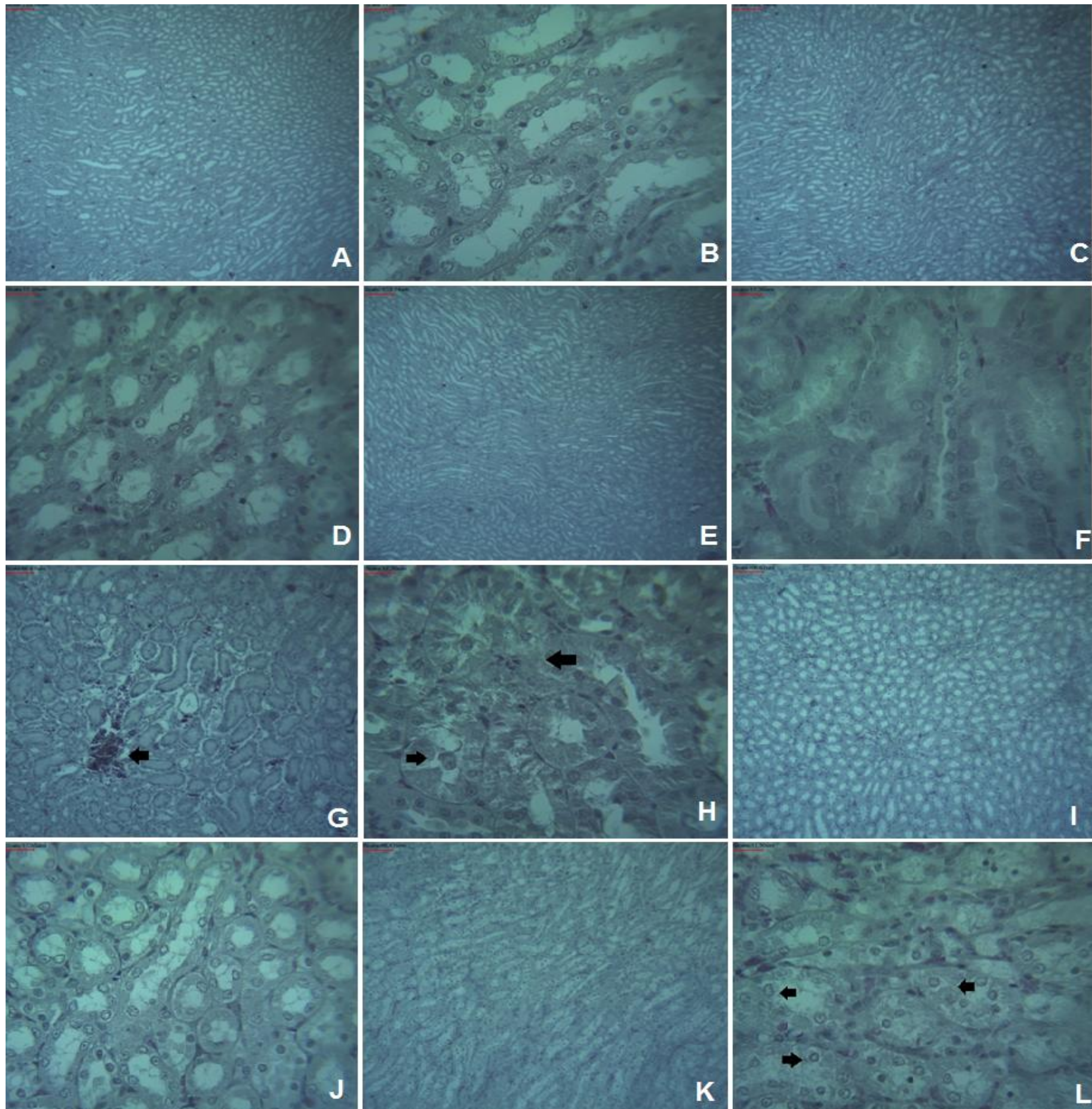

Figure 4. Histopathological examination of rat kidneys (n=6, in six experimental groups). A: Normal histological structure of parenchyma (40x). B: Normal histoarchitectonics of renal tubules (400x). C: Unchanged microscopic structure of renal parenchyma (40x). D: Unchanged microscopic structure of renal tubules (400x). E: Unchanged histological structure of kidney (40x). F: Unchanged histological structure of renal tubules (400x). G: Presence of haemorrhages in interstitial space (100x). H: Presence of dystrophic-necrotic changes in epithelial layer of the renal tubules (400x). I: Preserved histological structure of kidney, with accumulation of mononuclear cells in interstitium (40x). J: Preserved structure of epithelial layer of renal tubules (400x). K: Preserved histological structure of renal parenchyma (100x). L: Presence of granular appearance of cytoplasm of some epithelial cells of renal tubules (400x).

The microscopic examination of the kidneys in the control group revealed normal histological architecture with unaltered glomerular structures, tubules, interstitium and blood vessels (Fig. 4 A and B). In the animals from groups treated only with mauritianin and silymarin the findings were similar to those of the control (Fig. 4 C, D, E and F). In rats treated with CCl_4, haemorrhages were found in the interstitial spaces (Fig. 4 G). In the epithelium of the renal tubules degenerative or necrotic changes, accompanied by desquamation and disintegration of single epithelial cells were observed (Fig. 4 H). In the kidneys of animals treated with CCl_4 and mauritianin, a preserved structure as well as accumulation of mononuclear cells in the interstitial space were found (Fig. 4 I) and the tubular structures were unchanged (Fig. 4 J). In animals treated with CCl_4 and silymarin a preserved histological structure was also observed (Fig. 4 K). Single mononuclear cells in the interstitium alongside dystrophic changes in some of the epithelial cells of the renal tubules were found (Fig. 4 L).

DISCUSSION

Liver damage, caused by CCl_4, is a result of its oxidative biotransformation, which produces reactive metabolites such as the trichloromethyl radical ($•CCl_3$) and its oxidized form, the trichloromethyl peroxy radical ($CCl_3OO•$) (Weber *et al*., 2003). They react with membrane lipids and initiate lipid peroxidation processes in cell membranes. Possible mechanisms for the action of *t*-BuOOH are related to depletion of GSH cells, as well as oxidation of -SH groups (important for the action of mitochondrial enzymes and/or the integrity of the mitochondrial membrane) induced by membrane lipid peroxidation (Drahota *et al*., 2005). In this regard, the protective effects of an *n*-BuOH extract, pure compounds as well as a purified saponin mixture from *A. monspessulanus* subsp. *monspessulanus* in different toxicity models were studied. *In vitro*/*in vivo* models of hepatotoxicity induced by CCl_4 and *t*-BuOOH, as well as non-enzymatic lipid peroxidation with Fe^{2+}/ascorbic acid, were used in order to observe changes in some biochemical parameters, as well as a decrease in the activity of enzyme systems involved in antioxidant defence (Kondeva-Burdina *et al*., 2015; Simeonova *et al*., 2015). The results show that CCl_4 statistically significantly increased the production of MDA (a major marker of lipid peroxidation), LDH activity, and depleted the amount of GSH in cells. CCl_4 adversely affects the antioxidant defence system by reducing the activity of antioxidant enzymes: catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST). Both mauritianin and silymarin, administered alone, did not show statistically significant pro-oxidant effects, while in a model of intoxication an antioxidant activity was observed, expressed in decreased MDA production and LDH activity, preservation of GSH in cells and increased activity of antioxidant enzymes to an extent that was commensurate with silymarin. These results are an evidence of the antioxidant and membrane stabilizing properties of mauritianin and correlate with protective effect of other flavonoids on these parameters (Deqing *et al*., 1994; Wang *et al*., 1996; Kumar *et al*., 2009; Verma and Khosa, 2010; Gupta *et al*., 2011). Oral administration of CCl_4 in mammals induces lipid accumulation in hepatocytes, liver degeneration, centrilobular necrosis and in isolated cases – inflammatory changes (Faroon, 2005). This pathological model is exploited by researchers to prove *in vivo* hepatoprotective effects of flavonoids (Mistry *et al*., 2013; Hismiogullari *et al*., 2014). Liver lesions were not found for the control group and the animals treated with either mauritianin or silymarin alone. The histological observation is significant for the non-toxic nature of the tested flavonoid. In animals exposed to CCl_4 the typical histological alteration were visible in the liver – diffuse and disseminated, mainly perivascular lipid accumulations in hepatocytes and degenerative-necrotic changes in the nuclei of the cells. In the animals, treated with mauritianin and CCl_4, a well-preserved liver histoarchitectonic was found, that was commensurable to the microscopic observation of the livers from rats of the group treated with silymarin and CCl_4. Pathological findings presented correlate with results proving the hepatoprotective activity of other flavonoids (Mistry *et al*., 2013; Hismiogullari *et al*., 2014).

The central nervous system is extremely sensitive to free radical damage. New molecules developed as potential antioxidants with neuroprotective action and aimed at the treatment of acute or chronic neurological diseases must necessarily have the ability to pass blood-brain barrier after systemic administration. The metabolism of flavonoids and their passage into the system circulation after oral resorption is proved (Pardridge, 2001). Antioxidant molecules such as GSH, ascorbic acid, vitamins A and E and some enzymes (i.e. SOD, GPx, and CAT) protect neurons from oxidative stress and from reactive oxygen species (ROS). A very important function of GSH in the brain tissue is its ability to react and to scavenge in a non-enzyme mediated manner endogenous and exogenous ROS (Burton *et al*., 1985). Cerebral oxidative stress induced by CCl_4 is due to depletion of GSH, and increased MDA levels. Therefore, toxicity is due to suppression of antioxidant defence mechanisms accompanied by increase in lipid peroxidation (LPO) (O'Brien *et al*., 2000). Our results support data from the literature for the protective effects of flavonoids on different neurotoxicity models (Pardridge, 2001; O'Brien *et al*., 2000). Elevated LPO was observed when CCl_4 was given to rats, expressed through high levels of MDA, while levels of GSH were

reduced. This negative effect was definitely ameliorated in the groups pre-treated with mauritianin, with a decrease in LPO in brain tissue, as evidenced by decreased MDA production, and restored GSH levels, nearly to control values. Significant cerebroprotective effect could be assigned to mauritianin, expressed in decrease of LPO and improved cellular antioxidant status of the brain. Single hepatotoxic doses of CCl_4 cause oxidative stress impairing all brain structures of rats (Ritesh *et al*., 2015). Histological alterations, expressed as haemorrhages, demyelinisation, and damage of Purkinje's cells were consistent to those reported after CCl_4 ingestion in rats (Faroon, 2005). Our findings add to the well-defined neuroprotective effect of flavonoids, in addition to their antioxidant properties (Andersen and Markham, 2005).

The kidneys are also a target organ of impairment after CCl_4 intoxication, although not as well pronounced as the liver (Faroon, 2005). A number of authors have investigated the nephroprotective effect of plant-derived flavonoid-containing extracts in a CCl_4 model of renal impairment. Serum urea and creatinine levels and histological changes in the renal parenchyma, mainly expressed in dystrophic-necrotic changes in the epithelium of the proximal renal tubules, are considered to be the main criteria for the degree of renal impairment (Olagunju *et al*., 2009; Shah *et al*., 2017). Lesions expressed in oedema and granulation of the cytoplasm of the epithelial cells of the proximal tubules with necrosis. Flavonoids are considered to positively affect renal function and their protective effect is already known (Vargas *et al*., 2018). Our findings confirm this information and support the hypothesis of the mechanism – improvement of the antioxidant defence by ROS scavenging. In the experimental animals treated with mauritianin and CCl_4, normal histoarchitectonics was registered, with preserved tubules and interstitial structure. Some mononuclear cells were visible – the microscopic finding was similar to that of kidneys from animals, treated with a combination of silymarin and CCl_4.

Conclusion: Hepatoprotective, neuroprotective and nephroprotective effects of mauritianin were established *in vivo*, alone and in CCl_4 toxicity model. Pathological evaluation revealed a lack of abnormal lipid accumulation in hepatocytes and the absence of alternative and hemodynamic changes in the brain and kidneys, which confirmed the results and showed a better protective effect compared to that of silymarin, but that should be proven by some further experimental studies.

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Conflict of Interest: The authors declare no conflict of interest amongst themselves.

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