

Lepidium meyenii Walp (red maca) supplementation prevents acrylamide-induced oxidative stress and liver toxicity in rats: Phytochemical composition by UHPLC–Q/Orbitrap/MS/MS

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Research Article

Keywords: Lepidium meyenii, Oxidative stress, Malondialdehyde, Lipid peroxidation, acrylamide, UHPLC-ESI-MS/MS

Posted Date: April 4th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1499211/v1>

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Abstract

Lepidium meyenii Walp (red maca) is a high Andean plant cultivated since the time of the Incas, and has innumerable therapeutic properties. The study aims to identify its phytochemical composition using UHPLC–Q/Orbitrap/MS/MS, and to evaluate its effects on acrylamide-induced oxidative stress. The lyophilized aqueous extract of red maca (LAqE-RM) was orally administered in doses of 1 and 2 g/kg body weight for 4 weeks. Malondialdehyde (MDA) levels in erythrocytes, brain and liver, as well as hepatic levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined. Administration of acrylamide for 2 weeks and 4 weeks significantly increased ($p < 0.001$) MDA levels in erythrocytes, brain, and liver. However, LAqE-RM was able to prevent ($p < 0.001$) an increase in MDA levels in all tissues studied. Likewise, the groups treated with LAqE-RM presented significantly ($p < 0.001$) lower levels of ALT and AST compared to the control. Treatment with LAqE-RM ameliorated the acrylamide-induced oxidative stress by reducing MDA levels in erythrocytes, brain, and liver, and by reducing liver levels of ALT and AST in a dose dependent manner. Twenty-five secondary metabolites were identified and characterized from LAqE-RM based on UHPLC mass spectrophotometry, these include; carbolines, alkamides, fatty acids and macamides, which are probably involved in their antioxidant protective role.

Introduction

Acrylamide is considered by the International Agency for Research on Cancer as a probable class 2A human carcinogen [1], and a chemical highly reactive substance [2]. Studies have showed that acrylamide are involved in kidney, endometrial and ovarian cancer [3]. The mechanism involved in acrylamide induced cancer is still not clear, but genotoxic and non-genotoxic pathways have been suggested [4]. This substance is absorbed from the gastrointestinal tract, transported to various organs, and metabolized by two pathways. First, direct conjugation with glutathione, and second, by the formation of glycidamide via cytochrome P450 and subsequent conjugation with glutathione, with both pathways possibly inducing genotoxicity on the deoxyribonucleic acid [5].

The Swedish National Food Administration reported the presence of acrylamide in food in the year 2002 [6], obtained as a by-product of the cooking process, when reducing sugars react with asparagine during the Maillard reaction [7]. Acrylamide concentration are reportedly influenced by cooking temperature, cooking time, moisture content, and the amount of reducing sugar and asparagine in raw foods [8–10]. Recently, a higher dietary acrylamide intake was significantly associated with poor physical performance, as well as a possible risk factor for sarcopenia [11].

Acrylamide induces a disruption of proteins, lipids, nucleic acids functions, activation of the apoptotic pathway and oxidative stress in the nervous system [12, 13]. Oxidative stress is considered as an imbalance between pro and antioxidant species, and plays a crucial role in the development of age-related diseases [14]. And Acrylamide also impairs the antioxidant system, causing elevation of reactive substances derived from thiobarbituric acid, glutathione S-transferase superoxide dismutase in plasma, and decreased glutathione in liver, kidney, and brain [15]. Polyunsaturated fatty acids in cell membranes are extremely sensitive to increased ROS [16] and lipid peroxidation of these membranes leads to generation of products, such as malondialdehyde (MDA). ROS also causes detrimental to DNA, proteins, and certain enzymes [16, 17].

The use of complementary and alternative medicine (CAM) has increased among patients with different morbidities, as well in the general population for the general health improvement and maintenance [18]. The *Cruciferae* family (*Brassicaceae*) has 350 genera and more than 2,500 species distributed throughout the world, among them, the most important is *Lepidium meyenii* Walp, known as 'maca'. Up to thirteen varieties of maca are described with different biological properties [19]. Since Inca times, this herbaceous plant is cultivated at altitudes between 3,800 and 4,500 meters above sea level (m.a.s.l.), and is used by the native communities for its high nutritional and medicinal value [20]. Currently, maca is no longer only consumed in the areas of the central Andes, where it is produced, but its use has spread throughout the country [21]. However, despite the species having at least five chemical markers of the macamide class, differences in chemical profile are often observed between maca varieties and according to geographic origin, and thus, chemical analysis of the sample is always necessary in studies of biological/pharmacological activities, when an explanation of the mechanism of action is desired [22].

In this study, we sort to understand the protective effect of *Lepidium meyenii* Walp (red maca) on acrylamide-induced oxidative stress in whole organism, such as rat brain, liver, and plasma, and the possible medicinal components involved.

Material And Methods

Chemicals

2-Thiobarbituric (TBA), iron (III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), hydrochloric acid (HCl), sodium chloride (NaCl), Potassium chloride (KCl), potassium phosphate monobasic (KH_2PO_4), potassium phosphate dibasic (K_2HPO_4), butylhydroxytoluene (BHT), pyridine, acetic acid, glycine, and acrylamide were from Merck- Perú. Trichloroacetic acid (TCA) was from Fisher Chemical, n-butanol was from Riedel-de Haën and 1,1,3,3-tetraethoxypropane was from Sigma-Aldrich (St Luis, MO, USA). Ketamine (500mg/10mL) was from Laboratorio Sanderson S.A. and saline solution (NaCl 0.9%) was from BRAUN MEDICAL.

Plant Material

Dry hypocotyls of *Lepidium meyenii* Walp (red maca, Juvens) from Carhuamayo, Junín at 4000 m.a.s.l. were used. Traditionally, dried Maca hypocotyls are boiled and served as juice. For the present study, the aqueous extract of the hypocotyls was prepared according to the traditional method [23].

Uhplc-ms-ms Chemical Identification Of The Laje-rm

An equipment UHPLC Waters XEVO TQ-XS system was used for the chemical analysis of *L. meyenii* extract (red maca). For the analysis, 10 mg of the dry material were dissolved in 2 mL of methanol and filtered through a 0.22 μm PTFE filter. Liquid chromatography was performed using an ACQUITY UHPLC HSS C18 column (100 mm $\times$ 2.1 mm, 1.8 μm , Waters) operated at 25°C. Mobile phases were 1% formic aqueous solution (A) and acetonitrile 1% formic acid (B). The gradient program time was started at 10% B at zero time, then going to 20% B for 6 min, increasing to 50% B in 1 min, then going to 55% B within 7 min, then going to

90% B in 12 min and, finally, returning to the initial conditions within 4 min. The flow rate was 0.3 mL per min, and the injection volume was 10 μ L. Detection of all compounds was performed using a triple quadrupole mass spectrometer equipped with an electrospray ion source (ESI) operated in positive and negative ionization mode. The "cone voltage" was maintained at 40 V. The drying temperature was set at 450°C and the dry gas flow rate was set at 13 L/min. Nitrogen was used as dry gas, fogging gas and collision gas. The collision energy was set to 30 eV. HRESIMS and MS/MS spectra were acquired over an m/z range from 50 to 2000 amu.

Animal Treatments

Forty-two Female Sprague-Dawley rats weighing between 150 and 200 g, kept under controlled conditions (12-h dark/12-h light cycle, 23–25 °C and 50–60% humidity) as mentioned earlier. The animals received a standard food pellet and before experiments they were fasted overnight with water *ad libitum*. The rats were randomly distributed into seven experimental groups (n = 6 per group), as shown in the following scheme:

Biochemical Procedures

Twenty-four hours after the last day of treatment, the animals were weighed and sacrificed through decapitation in accordance with the experimental protocols approved by the Ethics Committee for animal research of the Universidad Nacional de Trujillo.

Malondialdehyde (Mda) Measurements In Erythrocytes

MDA levels were quantified using a method published previously [24]. Briefly, packed erythrocytes were placed in a test tube with 1.0 mL of phosphate buffered saline (PBS, pH 7.0). The mixture was incubated in a water bath for 30 min at 37°C. Then proteins were precipitated by adding equal volumes (1.5 mL) of acetic acid (5%, v/v) and TBA (0.8%; w/v) and the mixture was incubated for 60 min at 90°C. Next, 1.0 mL of KCl (2%, w/v) and 3.0 mL of butanol/pyridine (1:10; v/v) were added. Then, the mixture was shaken and centrifuged at 3000 rpm for 10 min. Finally, MDA levels were measured spectrometrically at 532 nm.

Lipid Peroxidation In Brain

MDA levels were measured according to a previously described procedure [23]. The brain was kept at 4°C, then homogenized in ice-cold potassium phosphate buffer (20 mM, pH 7.4) containing 140 mM KCl. Subsequently, it was centrifuged at 1700 g for 10 minutes at 4°C, the supernatant was collected and incubated in a water bath at 37°C and in the dark for 60 minutes; Finally, 20 μ L of TCA (10%, w/v) were added and it was centrifuged at 960 g for 10 minutes at 4°C. 1 mL of the supernatant obtained above was taken and 2 mL of TBA (0.67%, w/v) was added, the mixture was incubated at 100°C for 60 minutes and after cooling on ice for 15 minutes, 4 mL of n-butanol/pyridine (15:1, v/v) and 0.5 mL of distilled water were added. The supernatants of each of the test tubes were centrifuged at 960 g for 10 minutes at 4°C, the supernatant was removed, and the reading was performed at 532 nm.

Lipid Peroxidation In Liver

Initially, the liver was perfused with ice-cold 0.9% NaCl via the portal vein before homogenization. 2 g of the sample was crushed and homogenized with 50.0 mM of phosphate buffer (pH 7.4) and centrifuged at 10000 rpm for 10 min at 4°C. Then, 50 µL of phosphate buffer (50 mM, pH 7.4), 1 mL of 10% (w/v) of TCA and 450 µL of supernatant were transferred into a new tube and centrifuged at 10000 rpm for 10 min at 4°C. To measure malondialdehyde levels, 1 mL of TBA (0.67%; w/v) and 1.0 mL of supernatant were taken and mixed. The mixture was kept in a boiling water bath for 30 min. 4 mL of n-butanol and pyridine (15:1; v/v) was added after the mixture was cooled down. The values were read using spectrophotometer at 532 nm [24].

Transaminases Levels

Blood samples were centrifuged immediately after extraction at 10,000 rpm for 10 minutes. The supernatant serum was extracted and frozen. Once all the samples were collected, the serum was thawed at room temperature and transaminases were quantified using a commercial kit for ALT (alanine aminotransferase) and AST (aspartate aminotransferase). The results were indicated in IU/L.

Statistical Analysis

GraphPad Prism software (San Diego, CA 92037, USA) was used, *n* represents the number of animals studied, and values were expressed as the mean ± standard deviation (SD). For the statistical analysis of the groups, a one-way or two-way ANOVA was used as appropriate, followed by a Bonferroni *post hoc* test. A value *p* of < 0.05 was considered statistically significant.

Results And Discussion

Characterization of the LAqE-RM by UHPLC–Q/Orbitrap/MS/MS

Twenty-five compounds were identified in the chromatogram of the LAqE-RM (Fig. 2, Table 1). **Fig. S1 (a-h) (Supplementary materials)** shows spectra of compounds detected as examples.

Methyltetrahydro-β-carbolines

Compounds **1–3** were characterized as methyltetrahydro-β-Carbolines. Peaks **1, 2 and 3** with a pseudomolecular ion [M + H] at *m/z*: 233, 231 and 235 are tentatively identified as (1R,3S)-1-methyltetrahydro-beta-5,6-hydridecarboline-3-carboxylic acid, (1R,3S)-1-methyltetrahydro-beta-5,6-hydridecarboline-3-carboxylic acid and (1R,3S)-1-methyltetrahydro-beta-5,6-hydridecarboline-3-carboxylic acid, presenting in common the fragment *m/z* = 91 amu, related to the C₂H₄NH₂ from methyltetrahydro ring and its attached COOH [25].

Alkamides

Alkamides were represented by the substances **6**, **10**, **13** and **14**. Pseudomolecular ions $[M + H]$ at m/z : 227, 291, 353 and 357 were identified as 1-benzyl-2-propane-4,5-dimethylimidazilium, 1,3-dibenzyl-2,4,5-trimethylimidazilium, 1,3-dibenzyl-2-phenyl-4,5-dimethylimidazilium and 1,3-dibenzyl-2-pentyl-4,5-dimethylimidazilium, respectively [25]. Substances **6** and **10** presented the characteristic loss of benzylic function ($m/z = 91$ amu), at $m/z = 135$ and 200 amu, respectively, while the substances **13** and **14** gave the fragments $m/z = 185$ and 277 amu, related to the loss of benzylic and phenyl groups and pentyl group, respectively.

Fatty Acids

Substances **7**, **8**, **11** and **14–18** were identified as 16-hydroxy-9-oxo-10E,12E,14E-octadecatrienoic acid, 9,16-dioxo-10E,12E,14E-octadecatrienoic acid, 9-hydroxy-10E,12Z,15Z-octadecatrienoic acid, 13-oxo-9Z,11E-octadecadienoic acid, 13-oxo-9E,11E-octadecadienoic acid, 9-oxo-10E,12Z-octadecadienoic acid, 9-oxo-10E,12E-octadecadienoic acid and 5-oxo-6E,8E-octadecadienoic acid, respectively. The two first fatty acids presented pseudomolecular ions $[M + H]$ at m/z : 309 and 307, while the other substances presented ions $[M + H]$ at m/z 295, according to the literature [26]. The substances **14** and **15** presented characteristic fragments at m/z : 124 amu, related to the fragmentation between the two double bonds and loss of COOH, and 189, related to consequently loss of C_5H_5 . The substances **16** and **17** presented the fragment at m/z : 151 amu, related to the fragmentation between the two double bonds and loss of COOH. The same type of fragmentation is also seen in the substance **18**, generating a fragmentation at m/z : 95 amu, followed by the loss of methyl terminal group (m/z : 81 amu). These fragmentation mechanisms were also observed for the substances **7**, **8** and **11**, generating the fragments at m/z : 185 and 171 amu.

Macamides

The compounds **19–25** were identified as the macamides N-benzyl-13-oxo-9Z,11E-octadecadienamide, N-benzyl-13-oxo-9E,11E-octadecadienamide, N-benzyl-9-oxo-10E,12Z-octadecadienamide, N-benzyl-9-oxo-10E,12E-octadecadienamide, N-benzyl-5-oxo-6E,8E-octadecadienamide, presenting pseudomolecular ions $[M + H]$ at m/z : 384, and N-(3-Methoxybenzyl)-(9Z,12Z,15Z)-octadecatrienamide and N-benzyl-9-oxo-12E-octadecenamide, with pseudomolecular ions $[M + H]$ at m/z : 398 and 386, respectively [26]. The substances **19–23** presented two characteristic fragments at m/z : 91 and 108, which in turn, came from the cleavages at amide group, including or not the nitrogen atom. The substance **24** generated the same mechanism of fragment, which gave the m/z : 124 and 138 amu. In its turn, the substance **25** presented the fragment $m/z = 260$, related to the loss of C_9H_{16} from the cleavage between the carbonyl group and adjacent carbon.

Other Compounds

The substance **4** was identified as the benzyl isothiocyanate, presenting a pseudomolecular ion $[M + H]$ at m/z : 150, with fragments at m/z : 91 and 77 amu, related to the loss of NCS group and the consequently loss of CH_2 , respectively. The substance **5** was tentatively identified as N-(3-hydroxy-benzyl)-2Z-fivecarbonacrylamide, with a pseudomolecular ion $[M + H]$ at m/z : 180, and a consequently loss of water,

generating the $m/z = 162$ amu. The next loss of fivecarbonacrylamide group is also seen with the fragment $m/z = 82$ amu [25, 27]. Furthermore, the substance **9** was identified as 3-benzyl-1,2-dihydro-N-hydroxypyridine-4-carbaldehyde, presenting a pseudomolecular ion $[M + H]$ at m/z : 216 and the fragments at m/z : 198 and 91 amu, related to the loss of water and the benzylic group, respectively [25].

Table 1
Chemical composition of the LAqE-RM extracts by UHPLC-MS-MS

N°	Compounds	Rt	[M + H] (m/z)	Main Fragments (m\z)	Ref.
1	(1R,3S)-1-methyltetrahydro-beta-5,6-hydridecarboline-3-carboxylic acid	5.30	233	187, 91	[25]
2	(1R,3S)-1-methyltetrahydro-carboline-3-carboxylic acid	5.71	231	143, 91	[25]
3	(1R,3S)-1-methyltetrahydro-beta-5,6,7,8-dihydridecarboline-3-carboxylic acid	7.29	235	189, 91	[25]
4	Benzyl isothiocyanate	7.93	150	91, 77	[27]
5	N-(3-hydroxy-benzyl)-2Z-fivecarbonacrylamide	8.21	180	162, 82	[25]
6	1-benzyl-2-propane-4,5-dimethylimidazilium	8.88	227	135, 91	[25]
7	16-hydroxy-9-oxo-10E,12E,14E-octadecatrienoic acid	9.64	309	277, 185, 124, 91	[26]
8	9,16-dioxo-10E,12E,14E-octadecatrienoic acid	9.75	307	292, 215, 185, 121, 91	[26]
9	3-benzyl-1,2-dihydro-N-hydroxypyridine-4-carbaldehyde	9.83	216	198, 91	[25]
10	1,3-dibenzyl-2,4,5-trimethylimidazilium	9.84	291	200, 91	[25]
11	9-hydroxy-10E,12Z,15Z-octadecatrienoic acid	10.09	295	277, 175, 121, 93, 81	[26]
12	1,3-dibenzyl-2-phenyl-4,5-dimethylimidazilium	11.07	353	185, 171, 91	[26]
13	1,3-dibenzyl-2-pentyl-4,5-dimethylimidazilium	12.56	347	277, 199, 91	[25]
14	13-oxo-9Z,11E-octadecadienoic acid	20.24	295	277, 189, 135, 124	[25]
15	13-oxo-9E,11E-octadecadienoic acid	20.38	295	277, 189, 135, 124	[26]
16	9-oxo-10E,12Z-octadecadienoic acid	20.72	295	277, 217, 151, 124	[26]
17	9-oxo-10E,12E-octadecadienoic acid	20.90	295	277, 217, 151, 124	[26]
18	5-oxo-6E,8E-octadecadienoic acid	21.29	295	277, 95, 81	[26]
19	N-benzyl-13-oxo-9Z,11E-octadecadienamide	23.01	384	367, 283, 277, 260, 108, 91	[26]
20	N-benzyl-13-oxo-9E,11E-octadecadienamide	23.15	384	367, 283, 277, 260, 108, 91	[26]
21	N-benzyl-9-oxo-10E,12Z-octadecadienamide	23.63	384	367, 277, 260, 108, 91	[26]

N°	Compounds	Rt	[M + H] (m/z)	Main Fragments (m\z)	Ref.
22	N-benzyl-9-oxo-10E,12E-octadecadienamide	23.82	384	367, 277, 260, 108, 91	[26]
23	N-benzyl-5-oxo-6E,8E-octadecadienamide	23.86	384	277, 91	[26]
24	N-(3-Methoxybenzyl)-(9z,12z,15z)-octadecatrienamide	24.20	398	275, 138, 124, 89	[26]
25	N-benzyl-9-oxo-12E-octadecenamide	24.72	386	259, 91	[26]

As shown in Table 1, macamides are N-benzylamides of long-chain fatty acids (LCFAs) and are the key bioactive components of red maca. Therefore, its improvements in neural cell viability are attributed to the prevention of mitochondrial membrane depolarization and reduced induction of ROS [28]. Also, there have been reported that supplementation with N-benzyloleamide and N-benzylinoamide of *L. meyenii* significantly reduced MDA content in liver and brain, thus protecting against oxidative damage induced by prolonged exercise [29]. Another previous study suggested that *L. meyenii* exhibits strong antioxidant activity and inhibition of lipid peroxidation in rats with hereditary hypertriglyceridemia [30]. A recent study showed that red maca aqueous extract decreased serum MDA levels in rats, both at sea level and at high altitude [20].

LAqE-RM Prevents Pro-oxidant Effects Induced By Acrylamide

To the best of our knowledge, this study is the first to report that red maca prevents oxidative stress induced by acrylamide in the whole rat organism. We found that treated group with LAqE-RM for 4 weeks significantly ($p < 0.001$) decreased MDA levels induced by acrylamide exposure (4 mg/Kg body weight) for 2 weeks. For erythrocytes (1.0 g/kg: 3.47 ± 0.09 nmol/mL and 2.0 g/kg: 2.92 ± 0.08 nmol/mL vs. control: 5.55 ± 0.12 nmol/mL; Fig. 3a), brain (1.0 g/kg: 4.72 ± 0.16 nmol/g tissue and 2.0 g/kg: 3.77 ± 0.12 nmol/g tissue vs. control: 5.7 ± 0.35 nmol/g tissue; Fig. 3c) and liver (1.0 g/kg: 3.91 ± 0.18 nmol/mL and 2.0 g/kg: 2.85 ± 0.42 nmol/mL vs. control: 5.83 ± 0.29 nmol/mL; Fig. 3e).

Even after a 4-week treatment with acrylamide, LAqE-RM significantly ($p < 0.001$) decreased lipid peroxidation on erythrocyte (1.0 g/kg LAqE-RM: 5.5 ± 0.07 nmol/mL and 2.0 g/kg LAqE-RM: 3.76 ± 0.07 nmol/mL vs. control: 6.85 ± 0.08 nmol/mL; Fig. 3b), brain (1.0 g/kg LAqE-RM: 5.98 ± 0.24 nmol/g tissue and 2.0 g/kg LAqE-RM: 5.14 ± 0.09 nmol/g tissue vs. control: 8.63 ± 0.52 nmol/g tissue; Fig. 3d) and liver (1.0 g/kg LAqE-RM: 4.86 ± 0.28 nmol/mL and 2.0 g/kg LAqE-RM: 3.93 ± 0.07 nmol/mL vs. control: 6.97 ± 0.06 nmol/mL; Fig. 3f). Therefore, these findings and above strongly suggest that the treatment with red maca prevents oxidative stress induced by acrylamide *in vivo*. Zhang et al. [31] demonstrated that the polysaccharides of *L. meyenii* could have high antioxidant activity *in vitro* and *in vivo*. The authors based their conclusions on the observed increase of superoxide dismutase, glutathione peroxidase and glutathione S-transferase, leading to the decrease of MDA.

Red maca has a higher percentage of glucosinolates compared to other varieties [32], among the main glucosinolates found we have; glucosinalbin, glucotropaeolin, glucolimnanthin and glucoalyssin [33–35],

which has been reported for their antioxidant effect [36].

Since treated group with acrylamide presented the highest MDA levels compared to the naïve group, it confirmed the pro-oxidant effects of acrylamide on the erythrocyte, brain, and liver of rats (Fig. 3). Previous studies reported that acrylamide increases oxidative stress characterized by a significant increase in ROS and MDA levels, as well as an increase in superoxide dismutase and catalase activities [37, 38]. Exposure to acrylamide can attenuate glutathione (GSH) content and elevate MDA level and damage different tissues like brain and liver, showing an important role of oxidative stress in acrylamide-induced toxicity [13].

LAqE-RM Prevents Liver Toxicity Effects Induced By Acrylamide

Additionally, two parameters were used to evaluate liver toxicity: alanine aminotransferase (ALT) and aspartate aminotransferase (AST). ALT is more sensitive and specific as an indicator of liver cell integrity and function; therefore, a higher ALT level is an excellent predictor to indicate liver damage [39]. Meanwhile, AST is found equally abundant in liver and cardiac cells, thus elevated AST can detect liver or cardiac damage, or both [39].

In the present study, acrylamide (4 mg/kg body weight) significantly increased ALT and AST levels (Fig. 4) compared to the naïve group. In agreement with previous studies, these data show that acrylamide impairs several biochemical parameters, such as transaminase levels, which are directly related to liver dysfunction [40, 41].

In contrast, treated group with LAqE-RM for 4 weeks significantly ($p < 0.001$) decreased ALT and AST levels induced by acrylamide exposure (4 mg/kg body weight) for 2 weeks. Rats treated with the LAqE-RM had significantly ($p < 0.001$) lower levels of ALT (1.0 g/kg LAqE-RM: 44.37 ± 0.64 IU/L and 2.0 g/kg LAqE-RM: 36.37 ± 0.88 IU/L vs. control: 64.77 ± 0.91 IU/L; Fig. 4a) and AST (1.0 g/kg LAqE-RM: 40.61 ± 0.61 IU/L and 2.0 g/kg LAqE-RM: 34.75 ± 0.51 IU/L vs. control: 50.69 ± 1.1 IU/L; Fig. 4c) when acrylamide was given post-treatment for 2 weeks.

In similar way, after 4 weeks exposure of rats to acrylamide, LAqE-RM pretreatment significantly ($p < 0.001$) reduced liver damage, as evidenced by low ALT levels (1.0 g/kg LAqE-RM: 56.2 ± 0.96 IU/L and 2.0 g/kg LAqE-RM: 41.13 ± 0.56 IU/L vs. control: 83.07 ± 1.62 IU/L; Fig. 4b) and AST (1.0 g/kg LAqE-RM: 53.06 ± 0.55 IU/L and 2.0 g/kg LAqE-RM: 38.61 ± 0.59 IU/L vs. control: 72.89 ± 0.64 IU/L, Fig. 4d).

On the other hand, a directly proportional correlation can be seen between the increase in ALT and AST levels and the increase in MDA levels in the liver (Fig. 5).

This protective effect on liver function was dose dependent. Polysaccharides from *L. meyenii* have been shown to possess hepatoprotective activity, reducing AST and ALT levels in mice with alcohol-induced liver injury [31]. Pretreatment with *L. meyenii* significantly suppressed the production of aminotransferases and inflammatory cytokines, through a molecular mechanism involving the NF-Kb, IFN- γ /STAT1 and IL-6/STAT3 molecular pathways [42]. Finally, the presence of polyphenols, glucosinolates, alkamides and polysaccharides

in *L. meyenii* would be responsible for the antioxidant effect [43]; although, the alkaloids present would contribute better to the antioxidant capacity compared to phenols [44], on the other hand; a 1067.3 kDa polysaccharide called MP-1 could be directly responsible for the hepatoprotective activity of *L. meyenii* [31].

Conclusions

Treatment with *Lepidium meyenii* Walp (red maca) attenuates acrylamide-induced oxidative stress, as it reduces MDA levels in erythrocytes, brain, and liver, and prevent liver toxicity, as well as levels of ALT and AST in a dose-dependent manner. Twenty-five secondary metabolites were identified and characterized in *Lepidium meyenii* Walp (red maca) based on UHPLC mass spectrophotometry analysis. These include; carbolines, alkamides, fatty acids, and macamides. Natural product such as macamides are likely to support the protective antioxidant role of maca on oxidative stress and liver toxicity.

Declarations

Author contributions

Conceptualization and methodology: Roberto O. Ybañez-Julca, Javier Palacios; Formal analysis and investigation: Daniel Asunción-Alvarez, Ricardo Diego Duarte Galhardo de Albuquerque, Ivan Quispe-Diaz. Writing - original draft preparation: Daniel Asunción-Alvarez, Javier Palacios, Roberto O. Ybañez-Julca; Writing - review and editing: Daniel Asunción-Alvarez, Javier Palacios, Chukwuemeka R. Nwokocha. All authors have read and agreed to the published version of the manuscript.

Data Availability

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Funding

This work was supported by the Canon Minero project (R.R. N° 0262-2021/UNT) of the National University of Trujillo (UNT), and Fondecyt-Chile 1200610 to JP.

Conflicts of Interest

The authors have no competing interests to declare that are relevant to the content of this article.

Ethics approval

The experimental protocols were approved by the Ethics Committee for animal research of the Universidad Nacional de Trujillo (Res.Cons. Univ. No. 0361-2018/UNT). All of the institutional and international guidelines for the care and use of laboratory animals (NIH, 2013) were followed.

Consent to participate

Not applicable

Consent for publication

Not applicable

References

1. International Agency for Research on Cancer (IARC) (2014) IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Lyon, France
2. Semla M, Goc Z, Martiniaková M et al (2017) Acrylamide: a Common Food Toxin Related to Physiological Functions and Health. *Physiol Res* 66:205–217. <https://doi.org/10.33549/physiolres.933381>
3. Pelucchi C, Bosetti C, Galeone C, La Vecchia C (2015) Dietary acrylamide and cancer risk: An updated meta-analysis. *Int J Cancer* 136:2912–2922. <https://doi.org/10.1002/IJC.29339>
4. Besaratinia A, Pfeifer GP (2007) A review of mechanisms of acrylamide carcinogenicity. *Carcinogenesis* 28:519–528. <https://doi.org/10.1093/CARCIN/BGM006>
5. Shipp A, Lawrence G, Gentry R et al (2008) Acrylamide: Review of Toxicity Data and Dose-Response Analyses for Cancer and Noncancer Effects. *Crit Rev Toxicol* 36:481–608. <https://doi.org/10.1080/10408440600851377>
6. Tareke E, Rydberg P, Karlsson P et al (2002) Analysis of Acrylamide, a Carcinogen Formed in Heated Foodstuffs. *J Agric Food Chem* 50:4998–5006. <https://doi.org/10.1021/JF020302F>
7. Mottram DS, Wedzicha BL, Dodson AT (2002) Acrylamide is formed in the Maillard reaction. *Nat* 2002 419:6906 419:448–449. <https://doi.org/10.1038/419448a>
8. Elmore JS, Koutsidis G, Dodson AT et al (2005) The Effect of Cooking on Acrylamide and Its Precursors in Potato, Wheat and Rye. *Adv Exp Med Biol* 561:255–269. https://doi.org/10.1007/0-387-24980-X_19
9. Ishihara K, Matsunaga A, Miyoshi T et al (2005) Formation of Acrylamide in a Processed Food Model System, and Examination of Inhibitory Conditions. *Food Hyg Saf Sci* 46:33–39. <https://doi.org/10.3358/SHOKUEISHI.46.33>
10. Lukac H, Amrein TM, Perren R et al (2007) Influence of Roasting Conditions on the Acrylamide Content and the Color of Roasted Almonds. *J Food Sci* 72:C033–C038. <https://doi.org/10.1111/J.1750-3841.2006.00206.X>
11. Veronese N, Dominguez LJ, Ragusa S et al (2021) Dietary acrylamide and physical performance tests: A cross-sectional analysis. *PLoS ONE* 16:e0259320. <https://doi.org/10.1371/JOURNAL.PONE.0259320>
12. Zhu YJ, Zeng T, Zhu YB et al (2008) Effects of Acrylamide on the Nervous Tissue Antioxidant System and Sciatic Nerve Electrophysiology in the Rat. *Neurochem Res* 2008 33:2310–2317. <https://doi.org/10.1007/S11064-008-9730-9>
13. Rahbardar MG, Farmad HC, Hosseinzadeh H, Mehri S (2021) Protective effects of selenium on acrylamide-induced neurotoxicity and hepatotoxicity in rats. *Iran J Basic Med Sci* 24:1041–1049. <https://doi.org/10.22038/IJBMS.2021.55009.12331>
14. Tan BL, Norhaizan ME, Liew WPP, Rahman HS (2018) Antioxidant and oxidative stress: A mutual interplay in age-related diseases. *Front Pharmacol* 9:1162. <https://doi.org/10.3389/FPHAR.2018.01162/BIBTEX>

15. Yousef MI, El-Demerdash FM (2006) Acrylamide-induced oxidative stress and biochemical perturbations in rats. *Toxicology* 219:133–141. <https://doi.org/10.1016/J.TOX.2005.11.008>
16. Su LJ, Zhang JH, Gomez H et al (2019) Reactive Oxygen Species-Induced Lipid Peroxidation in Apoptosis, Autophagy, and Ferroptosis. *Oxid Med Cell Longev* 2019:.. <https://doi.org/10.1155/2019/5080843>
17. Del Rio D, Stewart AJ, Pellegrini N (2005) A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. *Nutr Metab Cardiovasc Dis* 15:316–328. <https://doi.org/10.1016/J.NUMECD.2005.05.003>
18. Freeman MP (2009) Complementary and Alternative Medicine (CAM): Considerations for the Treatment of Major Depressive Disorder. *J Clin Psychiatry* 70:3533. <https://doi.org/10.4088/JCP.8157SU1C.01>
19. Gonzales GF, Villaorduña L, Gasco M et al (2014) Maca (*lepidium meyenii* walp), a review of its biological properties. *Rev Peru Med Exp Salud Publica* 31:100–110. <https://doi.org/10.17843/rpmesp.2014.311.15>
20. Ybañez-Julca RO, Asunción-Alvarez D, Palacios J, Nwokocha CR (2021) Maca extracts and estrogen replacement therapy in ovariectomized rats exposed at high altitude. *Reprod Med Biol* 20:88–95. <https://doi.org/10.1002/RMB2.12357>
21. Valerio LG, Gonzales GF (2012) Toxicological Aspects of the South American Herbs Cat's Claw (*Uncaria tomentosa*) and Maca (*Lepidium meyenii*). *Toxicol Rev* 2005 241 24:11–35. <https://doi.org/10.2165/00139709-200524010-00002>
22. Pan Y, Zhang J, Li H et al (2016) Characteristic fingerprinting based on macamides for discrimination of maca (*Lepidium meyenii*) by LC/MS/MS and multivariate statistical analysis. *J Sci Food Agric* 96:4475–4483. <https://doi.org/10.1002/JSFA.7660>
23. Ybañez-Julca RO, Quispe-Díaz IM, Asunción-Alvarez D et al (2021) Antidepressant-Like Behavioral and Spatial Memory Effects in Peruvian Red Maca (*Lepidium meyenii*)-Treated Rats. *Pharmacogn J* 13:81–88. <https://doi.org/10.5530/pj.2021.13.12>
24. Benites J, Asunción-Alvarez HD, Ybañez-Julca RO et al (2019) Chemical composition by HPLC-ESI-QTOF-MS/MS: Estrogenic and antioxidant effects of *Mangifera indica* L. cv. "Kent" leave extracts on ovariectomized rats. *Boletín Latinoam y del Caribe Plantas Med y Aromáticas* 18:336–346. <https://doi.org/10.37360/BLACPMA.19.18.3.21>
25. Tafuri S, Cocchia N, Carotenuto D et al (2019) Chemical Analysis of *Lepidium meyenii* (Maca) and Its Effects on Redox Status and on Reproductive Biology in Stallions. *Mol* 2019, Vol 24, Page 1981 24:1981. <https://doi.org/10.3390/MOLECULES24101981>
26. Xia C, Deng J, Pan Y et al (2021) Comprehensive Profiling of Macamides and Fatty Acid Derivatives in Maca with Different Postharvest Drying Processes Using UPLC-QTOF-MS. *ACS Omega* 6:24484–24492. https://doi.org/10.1021/ACSOMEGA.1C02926/ASSET/IMAGES/ACSOMEGA.1C02926.SOCIAL.JPEG_V03
27. Xu YQ, Qiao SY, Wang ZQ et al (2021) Quantitative Determination of 15 Active Components in *Lepidium meyenii* with UHPLC-PDA and GC-MS. *J Anal Methods Chem* 2021:.. <https://doi.org/10.1155/2021/6333989>
28. Singh N, Barnych B, Morisseau C et al (2020) N-Benzyl-linoleamide, a Constituent of *Lepidium meyenii* (Maca), Is an Orally Bioavailable Soluble Epoxide Hydrolase Inhibitor That Alleviates Inflammatory Pain. *J Nat Prod* 83:3689–3697. <https://doi.org/https://doi.org/10.1021/acs.jnatprod.0c00938>

29. Yang Q, Jin W, Lv X et al (2015) Effects of macamides on endurance capacity and anti-fatigue property in prolonged swimming mice. 54:827–834. <https://doi.org/10.3109/13880209.2015.1087036>.
<http://dx.doi.org/103109/1388020920151087036>
30. Večeřa R, Orolin J, Škottová N et al (2007) The influence of maca (*Lepidium meyenii*) on antioxidant status, lipid and glucose metabolism in rat. *Plant Foods Hum Nutr* 62:59–63.
<https://doi.org/10.1007/s11130-007-0042-z>
31. Zhang L, Zhao Q, Wang L et al (2017) Protective effect of polysaccharide from maca (*Lepidium meyenii*) on Hep-G2 cells and alcoholic liver oxidative injury in mice. *Int J Biol Macromol* 99:63–70.
<https://doi.org/10.1016/j.ijbiomac.2017.01.125>
32. Gonzales GF, Miranda S, Nieto J et al (2005) Red maca (*Lepidium meyenii*) reduced prostate size in rats. *Reprod Biol Endocrinol* 3:1–16. <https://doi.org/10.1186/1477-7827-3-5/FIGURES/9>
33. Clément C, Diazgradós DA, Avula B et al (2010) Influence of colour type and previous cultivation on secondary metabolites in hypocotyls and leaves of maca (*Lepidium meyenii* Walpers). *J Sci Food Agric* 90:861–869. <https://doi.org/10.1002/JSFA.3896>
34. Yábar E, Pedreschi R, Chirinos R, Campos D (2011) Glucosinolate content and myrosinase activity evolution in three maca (*Lepidium meyenii* Walp.) ecotypes during preharvest, harvest and postharvest drying. *Food Chem* 127:1576–1583. <https://doi.org/10.1016/J.FOODCHEM.2011.02.021>
35. Chen L, Li J, Fan L (2017) The nutritional composition of maca in hypocotyls (*Lepidium meyenii* walp.) cultivated in different regions of China. *J Food Qual* 2017. <https://doi.org/10.1155/2017/3749627>
36. Korkmaz S (2018) Antioxidants in Maca (*Lepidium meyenii*) as a Supplement in Nutrition. In: *Antioxidants in Foods and Its Applications*. InTech
37. Pan X, Wu X, Yan D et al (2018) Acrylamide-induced oxidative stress and inflammatory response are alleviated by N-acetylcysteine in PC12 cells: Involvement of the crosstalk between Nrf2 and NF-κB pathways regulated by MAPKs. *Toxicol Lett* 288:55–64. <https://doi.org/10.1016/J.TOXLET.2018.02.002>
38. Acaroz U, Ince S, Arslan-Acaroz D et al (2018) The ameliorative effects of boron against acrylamide-induced oxidative stress, inflammatory response, and metabolic changes in rats. *Food Chem Toxicol* 118:745–752. <https://doi.org/10.1016/J.FCT.2018.06.029>
39. Djabir YY, Arsyad MA, Sartini S, Lallo S (2017) Potential Roles of *Kleinhovia hospita* L. Leaf Extract in Reducing Doxorubicin Acute Hepatic, Cardiac and Renal Toxicities in Rats. *Pharmacognosy Res* 9:168. https://doi.org/10.4103/PR.PR_129_16
40. Belhadj Benziane A, Dilmi Bouras A, Mezaini A et al (2018) Effect of oral exposure to acrylamide on biochemical and hematologic parameters in Wistar rats. *Drug Chem Toxicol* 42:157–166. <https://doi.org/10.1080/01480545.2018.1450882>
41. Rivadeneyra-Domínguez E, Becerra-Contreras Y, Vázquez-Luna A et al (2018) Alterations of blood chemistry, hepatic and renal function, and blood cytometry in acrylamide-treated rats. *Toxicol Rep* 5:1124–1128. <https://doi.org/10.1016/J.TOXREP.2018.11.006>
42. Zheng W, Du S, Tian M et al (2018) *Lepidium meyenii* Walp Exhibits Anti-Inflammatory Activity against ConA-Induced Acute Hepatitis. <https://doi.org/10.1155/2018/8982756>. *Mediators Inflamm* 2018:

43. Wang S, Zhu F (2019) Chemical composition and health effects of maca (*Lepidium meyenii*). Food Chem 288:422–443. <https://doi.org/10.1016/j.foodchem.2019.02.071>

44. Gan J, Feng Y, He Z et al (2017) Correlations between Antioxidant Activity and Alkaloids and Phenols of Maca (*Lepidium meyenii*). J Food Qual 2017:. <https://doi.org/10.1155/2017/3185945>

Figures

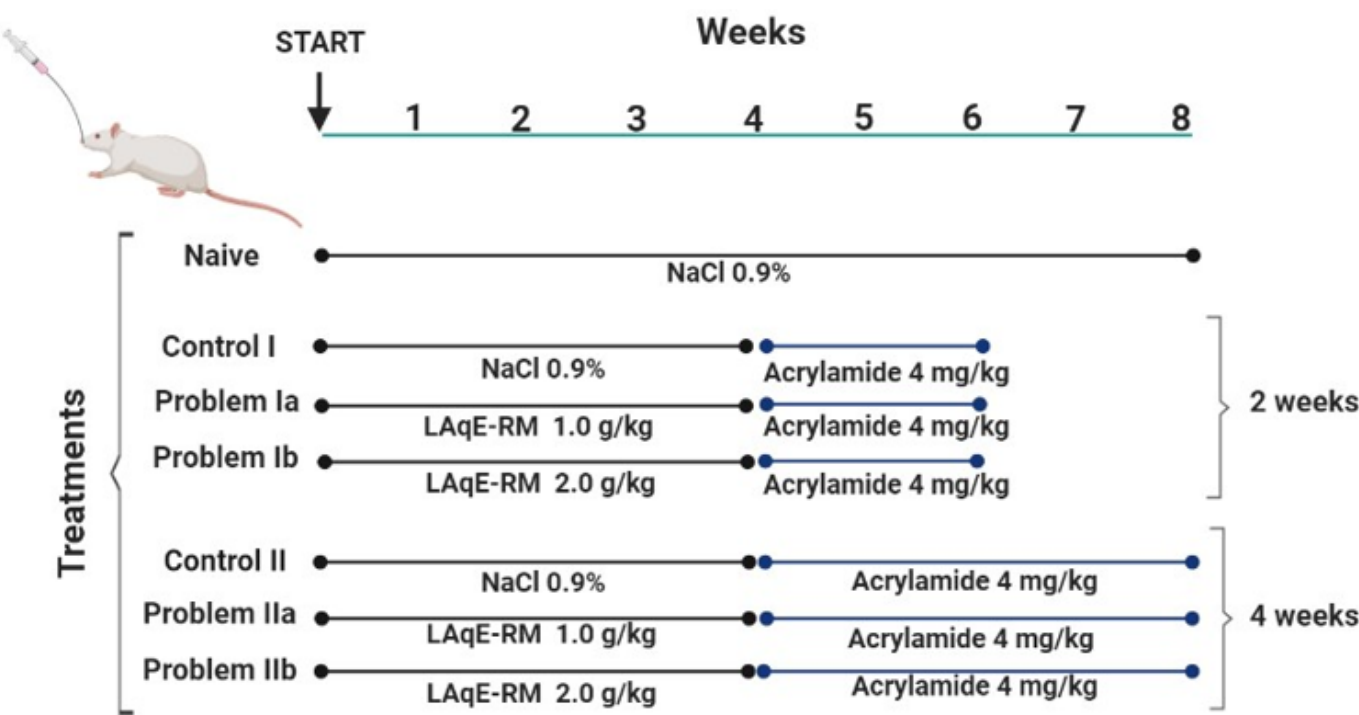


Figure 1

Scheme of treatment in the model of oxidative stress induced by acrylamide. Two protocols were carried out. First, rats treated with acrylamide for 2 weeks: Control I, Problem Ia and Problem Ib. Second, rats treated with acrylamide for 4 weeks: Control II, Problem IIa and Problem IIb. Schematic representation of treatments made in <https://biorender.com/> (accessed on 27 March 2022)

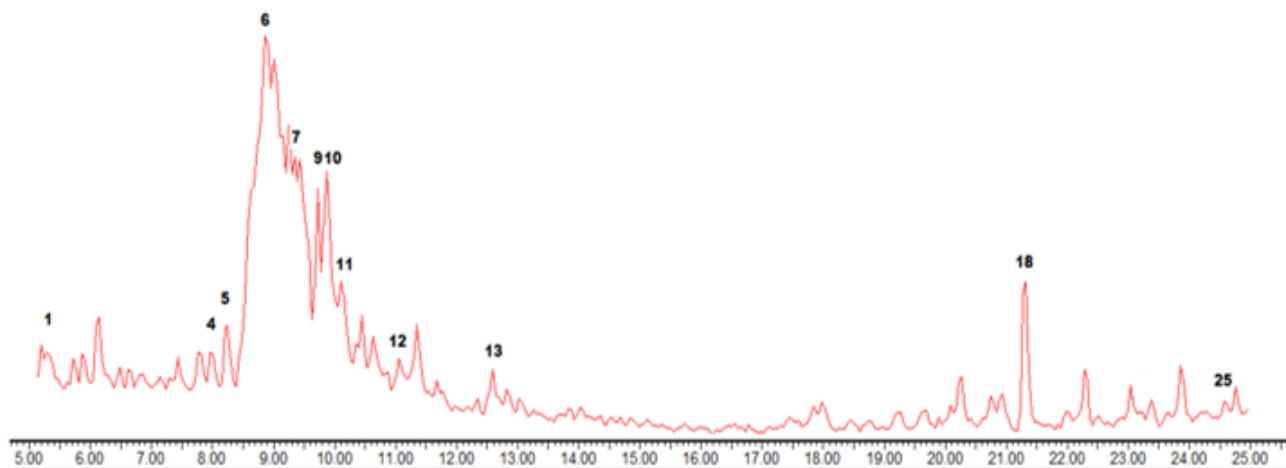


Figure 2

Chromatogram of UHPLC-MS-MS analysis of LAqE-RM presenting the main substances of each class

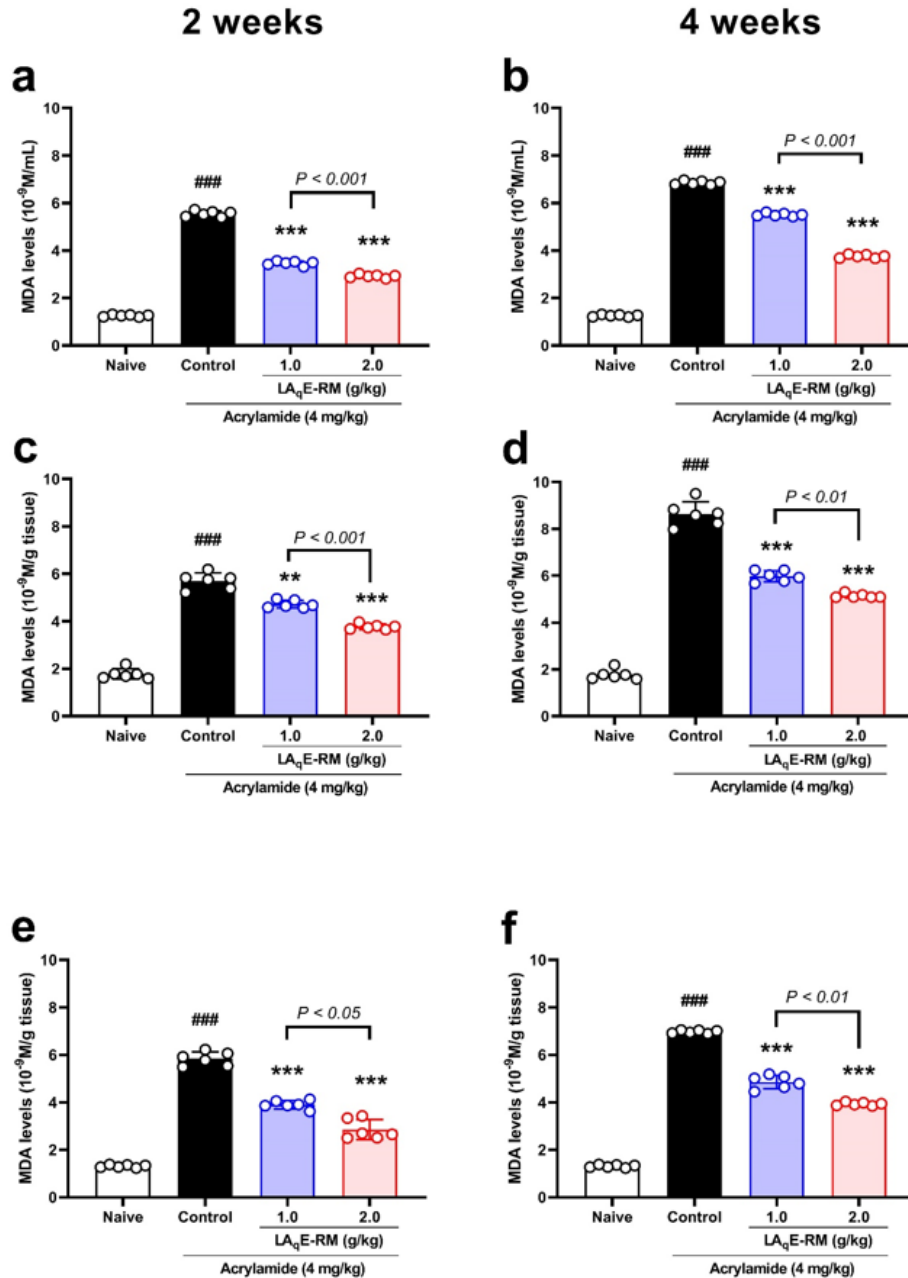


Figure 3

Effect of the LAqE-RM on acrylamide-induced oxidative stress. Animals were treated with LAqE-RM for 4 weeks and then exposed to acrylamide for 2 weeks or 4 weeks. Lipid peroxidation levels expressed as MDA in (a,b) erythrocytes, (c,d) brain and (e,f) liver. Significant differences between groups (mean $\pm$ SD). *** $p < 0.001$ compared to respective control group and ### $p < 0.001$ compared to naïve group

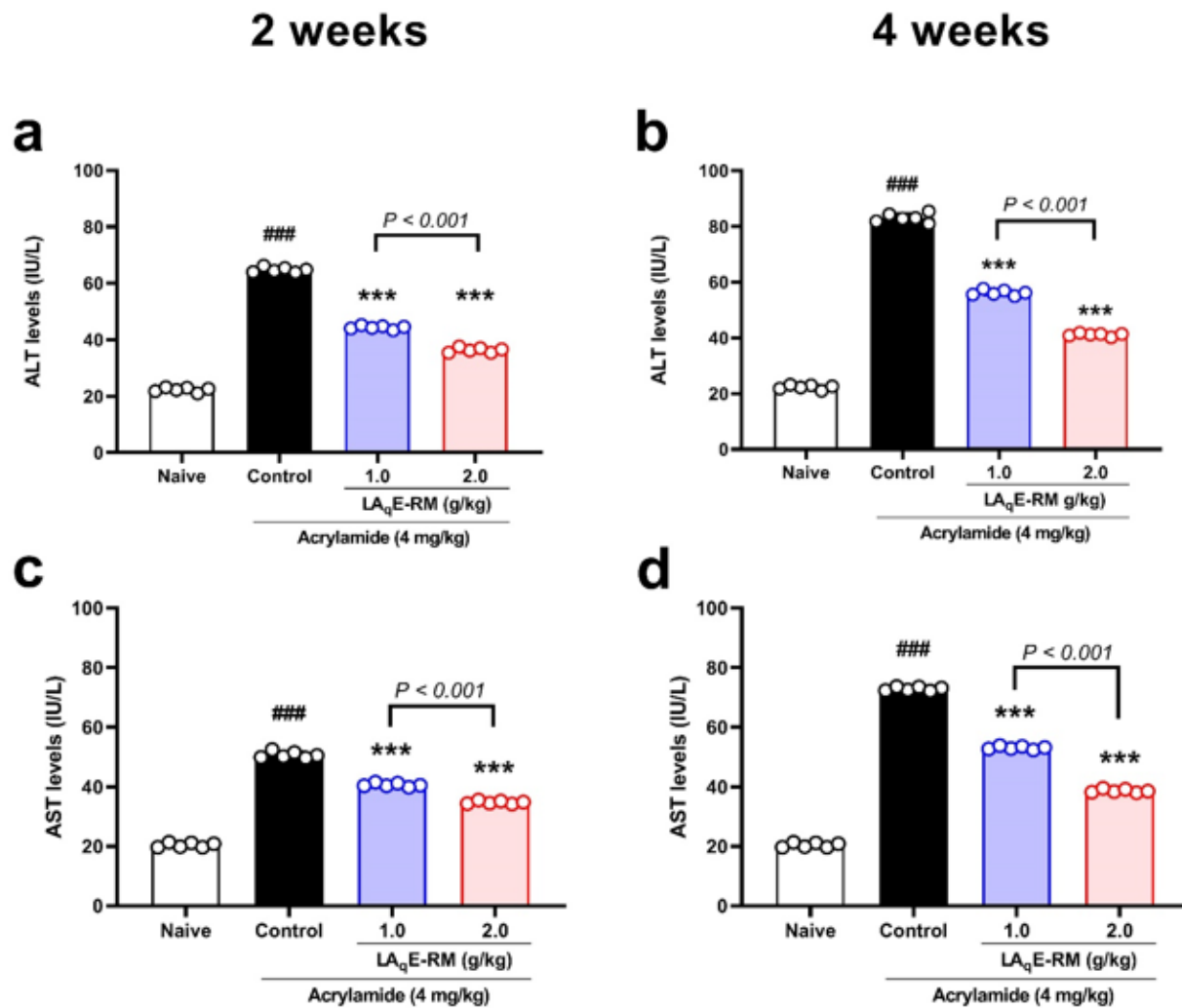


Figure 4

The LaqE-RM prevents an increase of (A) Alanine aminotransferase and (B) Aspartate aminotransferase levels on acrylamide-treated rats. Animals were treated with LaqE-RM for 4 weeks and then exposed to acrylamide for 2 weeks or 4 weeks. Significant differences between groups (mean $\pm$ SD). *** $p < 0.001$ compared to respective control group and ### $p < 0.001$ compared to naïve group

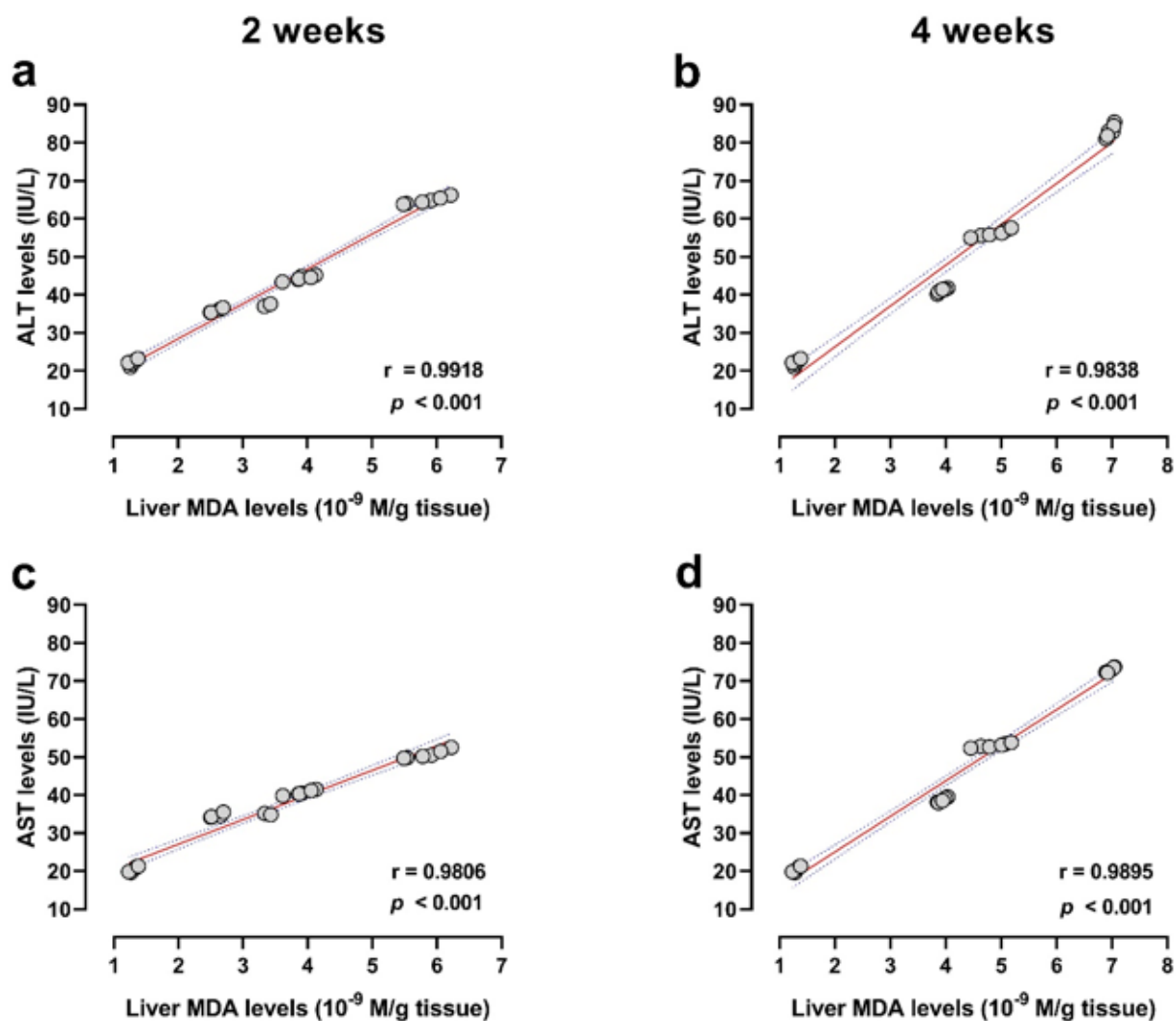


Figure 5

Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) levels and its correlation with liver malondialdehyde levels on rats exposed to acrylamide for 2 weeks (a,c) or 4 weeks (b,d). Pearson's r correlation coefficient shows a highly significant positive correlation ($p < 0.001$) between the levels of MDA and hepatic transaminases

Supplementary Files

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