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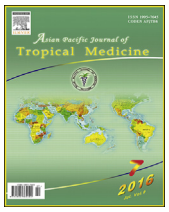

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Hepatoprotective properties of oleanolic and ursolic acids in antitubercular drug-induced liver damage

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ABSTRACT

Objective: To estimate to what extent the mixture of ursolic acid and oleanolic acid, in addition to the antitubercular standard regime, affects the hepatotoxicity profile.
Methods: Liver injury was induced in male BALB/c mice by administering, per os and daily for 11 weeks, a combination of anti-Tubercular (anti-TB) agents Rifampicin (10 mg/kg), Isoniazid (10 mg/kg), and Pyrazinamide (30 mg/kg). The ursolic acid and oleanolic acid mixture at doses of 100 or 200 μg/mouse/day was subcutaneously injected throughout the entire study period (11 weeks). Biochemical and hematological analysis was supplemented by liver histological examination.
Results: Animals treated with the mixture of triterpenic acids exhibited significantly decreased aspartate transaminase and alanine aminotransferase levels and amelioration of the histopathological alterations produced by the anti-TB drugs.
Conclusions: The triterpene mixture was able to prevent the steatosis induced by the anti-TB drugs.

1. Introduction

Drug-induced liver toxicity is a serious potential adverse effect produced by the currently used anti-Tubercular (anti-TB) chemotherapeutic regimen containing Isoniazid (INH), Rifampicin (RIF) and Pyrazinamide (PZA). All of these anti-TB drugs are potentially hepatotoxic, but when administered in combination, their toxic effects are enhanced in a synergistic manner [1,2]. The precise mechanisms of INH and RIF hepatotoxicity are not fully understood, but hepatocyte injury and death are most likely due to toxic hydrazine derivatives and free radicals that are responsible for oxidative stress, lipid peroxidation, and choline deficiency, leading to the lowering of phospholipid protein synthesis and the consequential alteration in cell wall configuration, reduced glutathione levels and the activation of Cytochrome P_{450} 2E1 (CYP2E1) [3,4].

The conversion of monoacetyl hydrazine (AcHz), a metabolite of INH, into a toxic metabolite via cytochrome P_{450} leads to hepatotoxicity. On the other hand, RIF promotes the cytochrome P_{450} enzyme, increasing the production of toxic metabolites from AcHz. The plasma half-life of AcHz is shortened and the compound is quickly converted into its active metabolites. Furthermore, RIF can increase the metabolism of INH to isonicotinic acid, which is hepatotoxic. PZA is responsible for severe hepatohypersensitivity reactions and, in combination with INH and RIF, increases the incidence of hepatotoxicity [4–7].

The search for hepatoprotectors to prevent risk situations in patients with tuberculosis (TB), who require treatment with anti-TB is an issue of growing importance. The literature points to the hepatoprotective effects of some synthetic compounds, such as N-acetylcysteine [8], reamberine, remaxol and ademethionine [9]. At the same time, a group of naturally occurring compounds has been reported as potential hepatoprotective agents against the toxic effects of anti-TB, with the effects of silymarin [10–12], curcumin and resveratrol [12–14], observed as remarkable. The search for hepatoprotective agents that avoid antitubercular drug-induced damage is mandatory, and this is reflected in the large number of medicinal plant extracts undergoing validation. The list of the latter is extensive and among these *Silybum marianum*, the well-researched plant for the treatment of liver

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diseases is highlighted. The active components of the species (silybin A and B, isosilybin A and B silybin and other minor compounds) are found in the seed's extract, denominated silymarin, which possesses hepatoprotective effects against the toxic action of RIF [15] and which has been proposed as a dietary supplement for patients treated with anti-TB [11]. In a randomized, controlled clinical trial, *Curcuma longa* and *Tinospora cordiflora*, in addition to the standard anti-TB regime, very significantly prevented, in terms of incidence, the duration and severity of hepatotoxic episodes in patients with TB [3]. Some medicinal plants extracts are proposed as hepatoprotectors, such as garlic (*Allium sativum*) [16] and the root extracts from *Punica granatum* and *Cassia auriculata* [17,18], *Cnidoscolus chayamansa* [19], *Vitex negundo* [20], *Hibiscus vitifolius* [21], *Pisonia aculeate* [22], *Mikania scandens* [23], *Moringa oleifera* [24], *Asteracantha longifolia* [25] and others [26].

With regard to the mixture of triterpenes ursolic acid or oleanolic acid (UA/OA), the natural product-of-interest in the on-going study, *in vivo* anti-TB activity was already demonstrated in an experimental mouse model of progressive pulmonary TB, and a significant reduction of bacterial loads and pneumonia with a higher expression of Interferon gamma (IFN-γ) and Tumor necrosis factor alpha (TNF-α) in the lungs was described. The authors concluded that the antimicrobial activity of the mixture is concomitant to an immune-stimulatory effect [27].

The *in vivo* antitubercular activity of these natural compounds has been patented [28] and the results obtained here comprise a breakthrough in understanding the simultaneous role of these triterpene acids as both anti-TB and hepatoprotective agents.

On continuing the study of these compounds that offer potential as anti-TB drugs, the hepatoprotective effect was subjected to evaluation in an experimental model of liver damage induced with the commonly used anti-TB first-line drugs RIF/INH/PZA.

2. Material and methods

2.1. Chemical compounds

The mixture of UA/OA was obtained by chemical fractionation of the methanolic extract of *Bouvardia ternifolia* aerial parts. The purification procedure was performed following the methodology described previously [29] and was structurally characterized by mass spectra and protonic nuclear magnetic resonance spectrometric data by comparison with those previously reported by this author. The drugs INH, RIF, PZA, ultra-pure olive oil and sodium carboxymethyl cellulose were purchased from Sigma–Aldrich and the kits used for determination of blood chemistry parameters were purchased from Randox Co.

2.2. Animals' treatment

In the present study, male Balb/C mice weighing (25 ± 2) g were used and were obtained from CMN-SXXI Bioterium, Mexico City. The mice were maintained in pathogen-free housing in plastic cages during a 7 d conditioning period prior to the performance of the experiments, under laboratory conditions [12 h/12 h light/dark cycles; temperature (25 ± 2) °C; humidity 45%–55%], with rodent chow food and water *ad libitum*. The experiments were performed following the Statutes of the International Committee for the Care and Use of Laboratory Animals and Mexican Official Norm (NOM-062-ZOO-1999) revised in 2001. All experimental protocols complied with the Animal Care Committee of the Hospital de Especialidades at the CMN-SXXI, IMSS.

As was previously reported, subcutaneous (*s.c.*) administration of the UA/OA mixture in models of acute toxicity, median Lethal dose (LD_{50}) was >2 g/kg in mice and rats and sub-acute toxicity (for 28 d) did not cause lethality or alterations in blood chemistry parameters or to histological changes in liver and kidney [29]. Taking this data into account, doses of 100 and 200 μg (*s.c*) were administered daily to the mice.

Hepatotoxic damage was induced with a combination of anti-TB drugs composed of INH (10 mg/kg), RIF (10 mg/kg) and PZA (30 mg/kg) dissolved in Isotonic saline solution (ISS), which was intragastrically (*i.g*) administered daily for 11 weeks. Doses of anti-TB drugs were the same as those reported for the *in vivo* assay [27]. The UA/OA mixture was dissolved in ultra-pure olive oil (Sigma) and was administered *s.c.* together with the anti-TB drugs for 11 weeks. A special #2 cannula was utilized to facilitate *i.g.* administration of anti-TB drugs. Assessment of the hepatoprotective potential of UA/OA was guided by the methodology reported in references 21 and 48, with some modifications.

Healthy mice were randomly assigned to six groups of eight animals each as follows: Group I (negative control) with vehicle (ISS *i.g.* and ultra-pure olive oil *s.c.*); Group II (positive control) were treated with anti-TB drugs (RIF/INH/PZA) administered *i.g.* via; Groups III and IV received UA/OA in doses of 100 and 200 μg/mouse/day by *s.c.* via, respectively; Group V animals were administered with anti-TB drugs and UA/OA at a dose of 100 μg/mouse/daily and Group VI received anti-TB drugs and UA/OA at a dose of 200 μg/mouse/day.

During the experimental period, the animals were observed for signs of morbidity and mortality. The weight of all mice was recorded from day zero and every 7 d thereafter throughout the experiment until week 11. Animals that died during the experimental period were dissected and the organs were obtained, weighed and observed macroscopically.

2.3. Hematology and serum biochemistry

After the last treatment, the animals were left to fast for 12 h and blood samples were collected by means of retro-orbital sinus puncture without the use of anesthesia. Tubes with EDTA were used for hematological testing and without anticoagulant for biochemical analysis. Hematological analysis was performed using a Beckman Coulter Cell Counter and the following parameters were determined: Total Red blood cell count; Hemoglobin, Hematocrit; Mean corpuscular volume; Mean corpuscular hemoglobin concentration; Mean corpuscular hemoglobin; Total platelet count, Total White blood cell count and a white-cell differential study was also performed to evaluate lymphocytes, segmented neutrophils, eosinophils, monocytes, and basophils.

Biochemical parameters with the commercially RANDOX kits were obtained with Selectra Analyser (Vitalab 2 model) automated equipment. In accordance with the manufacturer's instructions, the following parameters were determined: glucose; creatinine; urea; the liver marker enzymes Serum glutamic

oxaloacetic transaminase or Aspartate aminotransferase (AST), Serum glutamic pyruvic transaminase or Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP).

2.4. Histopathological evaluation

The mice were sacrificed by cervical dislocation and necropsy was carried out soon after death for macroscopic examination of liver, kidneys and spleen. Tissue biopsies from these organs were fixed in 10% formalin, processed and embedded in paraffin. The paraffin block was cut into (4–5) μm slices with a rotary microtome and stained with Hematoxylin and Eosin (H&E), following the procedure described previously in 27. The samples were examined under a light microscope with particular attention paid to organs exhibiting: microscopic findings in liver such as steatosis, necrosis, microabscesses, fibrosis, portal linfoide inflammation and centrolobulillar hydropic degeneration; in spleen was hematopoiesis and kidney tubular necrosis and hydropic tubular changes.

2.5. Statistical analysis

SigmaPlot ver. 12.0 software (2011–2012) was employed for analysis of results and graphic elaboration. Data are presented as mean and Standard error of the mean ± SEM. Values of body weight (BW) gain values were submitted to a bifactorial Analysis of variance (ANOVA) and to a post hoc Student–Newman–Keuls (SNK) test. Results of $P < 0.05$ were considered significant. For hematological and biochemical data analysis, one-way ANOVA was employed with a post hoc SNK test, in which $P < 0.05$ was considered significant. Finally, relative organ weights in treated mice and Hematocrit data and the Kruskal–Wallis test (ANOVA on ranks) were carried out with a post hoc SNK test, in which relevant outcomes were those with a value of $P < 0.05$.

3. Results

3.1. Body weight of the animals

In the course of 11 experimental weeks, a gradual increase in BW gain in all groups was observed from day 28; however, up the end of the study and in animals treated with the anti-TB (Group II), this parameter was significantly lower than that presented by the remaining groups, determining one half of those values registered for control group animals, which received only the vehicle (3.54 g *vs.* 6.00 g). BW gain in Groups III and IV mice, which were injected with UA/OA in 100 and 200 μg doses, exhibited behavior close to that of the control group animals. With respect to Group V and VI mice treated with UA/OA at 100 and 200 μg doses in addition to anti-TB drugs, BW gain with the 200 μg dose demonstrated slightly lower values than the controls (6.08 g), although at the end of the study, BW gain was close to that of the control group (6.33 and 5.95 g, respectively) (Figure 1).

3.2. Relative organ weights

Significant differences in the percentage of organ weight/BW ratios were found only in the case of liver; therefore, we refer to the particular case of this organ. Compared with the control group (relative liver weight, 4.05%), an increase to 4.78% was calculated for the group treated with anti-TB drugs. Relative liver weights in mice treated only with 100 or 200 μg UA/OA were similar to those of the control groups (4.06% and 4.12%, respectively). Mice treated with anti-TB drugs in addition to 100 μg UA/OA exhibited a relative liver weight of 4.12%, similar to that of the control group; in contrast, a different behavior was observed when the dose of UA/OA to 200 μg. In this latter case, relative liver weight increased to 4.74%, a value similar to that of the group administered anti-TB drugs only (Figure 2).

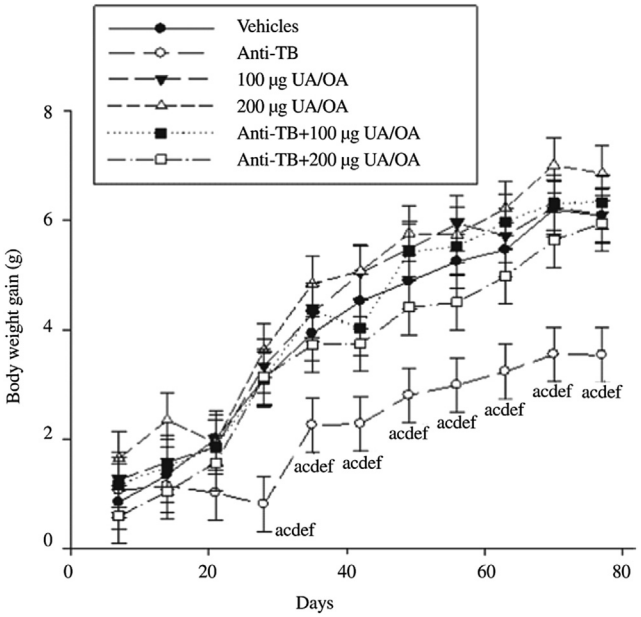

Figure 1. Effect of the UA/OA mixture on body weight gain in anti-TB-induced hepatotoxicity mouse model.
Data are mean ± SEM. Bifactorial statistical ANOVA of repeated measures, post hoc SNK test ($P < 0.05$); [a]*vs.* Vehicles; [b]*vs.* Anti-TB; [c]*vs.* 100 μg UA/OA; [d]*vs.* 200 μg UA/OA; [e]*vs* anti-TB + 100 μg UA/OA; [f]*vs.* Anti-TB + 200 μg UA/OA; Anti-TB (RIF, INH, PZA); $n = 8$.

3.3. Hematological and biochemical parameters

Hematological parameters fell within the range of those determined for control mice and mean values among groups

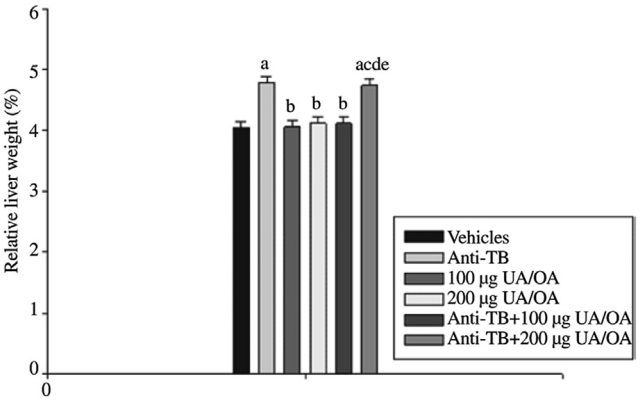

Figure 2. Effect of the UA/OA mixture on % relative weight in anti-TB-induced hepatotoxicity mouse model.
Data are mean ± SEM. Statistical analysis, Kruskal–Wallis test, ANOVA, on ranks, post hoc SNK test ($P < 0.05$); [a]*vs.* Vehicles; [b]*vs.* Anti-TB; [c]*vs.* 100 μg UA/OA; [d]*vs.* 200 μg UA/OA; [e]*vs.* Anti-TB + 100 μg UA/OA; [f]*vs.* Anti-TB + 200 μg UA/OA; Anti-TB (RIF, INH, PZA); $n = 8$.

were not statistically significant (data not shown). Regarding evaluation of biochemical parameters, in none of the groups tested were statistical differences established in serum glucose and creatinine (parameters determining renal function), although urea concentration in controls (62.140 ± 1.319) mg/dL increased slightly (16.33%) in the anti-TB group (75.29 ± 2.19) mg/dL. In contrast, urea levels in mice treated with both anti-TB and UA/OA at the previously mentioned doses showed values of (56.800 ± 1.151) mg/dL and (64.90 ± 2.06) mg/dL, respectively, whereas the group treated only with UA/OA at 100 and 200 μg doses, showing values of (65.51 ± 2.97) and (68.42 ± 1.86) mg/dL respectively had plasma concentrations close to those of the controls (Figure 3).

Transaminase values in the control group was AST (155.91 ± 2.67) UI/L and ALP (45.27 ± 2.59) UI/L. Evaluation of the liver function of the anti-TB group showed an increase in both serum transaminase levels: AST (180.67 ± 3.85) UI/L and ALT (86.33 ± 3.79) UI/L; this increase diminished significantly when the animals received anti-TB drugs and 100 μg of UA/OA (AST 100.33 ± 2.86 UI/L; ALT 55.42 ± 4.25 UI/L), although the group that received anti-TB plus 200 μg of UA/OA showed a high values with respect to the control: AST (188.87 ± 1.87) UI/L and ALT (98.00 ± 3.17) UI/L, these values were similar to those of the anti-TB group. Results are illustrated in Figures 4 and 5. Furthermore, the serum concentration of ALP in the anti-TB group showed the highest value with respect to negative controls (182.33 *vs.* 161.09) UI/L. On comparison with the controls, all treated groups of animals had higher ALP concentrations: UA/OA treatment at doses of 100 and 200 μg yielded values of 174.67 and 177.91 UI/L, respectively, close to those of the groups with UA/OA at 100 and 200 μg and the anti-TB challenge (172.25 and 176.75) UI/L (Figure 6).

3.4. Histopathological analysis

The liver histological analysis revealed steatosis and increased apoptosis only in the group submitted to anti-TB drugs for 11 weeks (Figure 7A). Fatty accumulation was not observed in the livers of control animals (Figure 7B), nor were liver alterations detected in groups treated with 100 and 200 μg UA/OA (Figure 7C), nor in animals receiving the anti-TB challenge in addition to 100 or 200 μg UA/OA (Figure 7D). No changes in the kidney microscopic examination were identified. Splenic hematopoiesis was similar in mice with and without treatment.

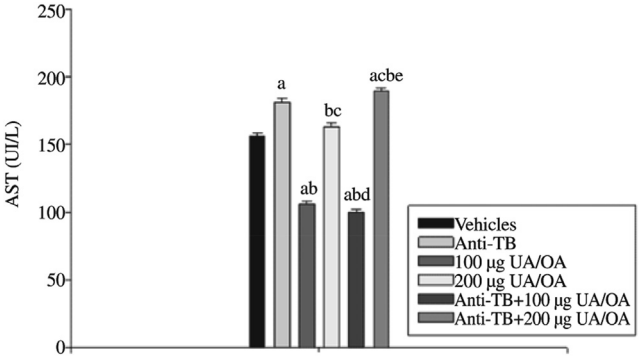

Figure 4. Effect of UA/OA mixture on serum AST concentration in anti-TB-induced hepatotoxicity mouse model.
Data are mean ± SEM. Statistical analysis, one-way ANOVA post hoc SNK test ($P < 0.05$); [a]*vs.* Vehicles; [b]*vs.* Anti-TB; [c]*vs.* 100 μg UA/OA; [d]*vs.* 200 μg UA/OA; [e]*vs.* Anti-TB + 100 μg UA/OA; [f]*vs.* Anti-TB + 200 μg UA/OA; Anti-TB (RIF, INH, PZA); $n = 8$.

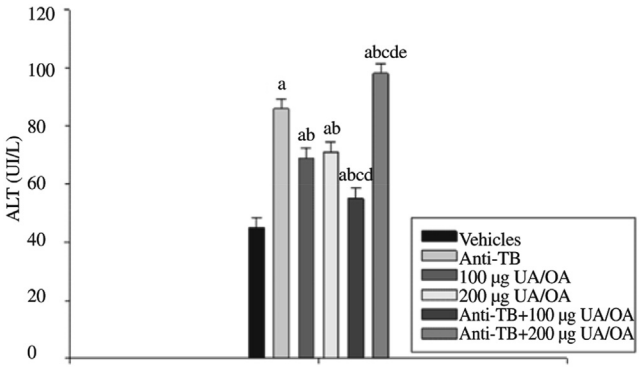

Figure 5. Effect of UA/OA mixture on serum ALT concentration in anti-TB-induced hepatotoxicity mouse model.
Data are mean ± SEM. Statistical analysis, one-way ANOVA, post hoc SNK test ($P < 0.05$); [a]*vs.* Vehicles; [b]*vs.* Anti-TB; [c]*vs.* 100 μg UA/OA; [d]*vs.* 200 μg UA/OA; [e]*vs.* Anti-TB + 100 μg UA/OA; [f]*vs.* Anti-TB + 200 μg UA/OA; Anti-TB (RIF, INH, PZA); $n = 8$.

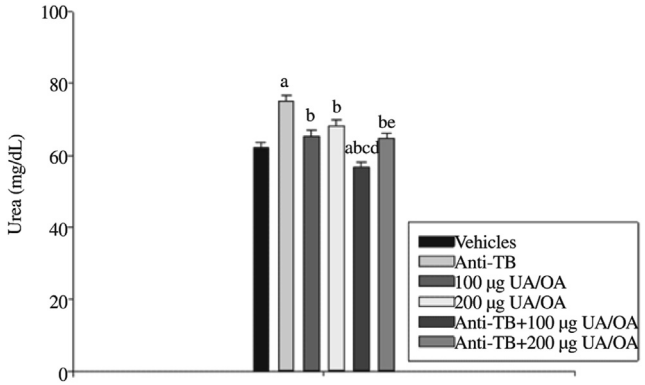

Figure 3. Effect of UA/OA mixture on serum urea concentration in anti-TB-induced hepatotoxicity mouse model.
Data are mean ± SEM. Statistical analysis, one-way ANOVA, post hoc SNK test ($P < 0.05$); [a]*vs.* Vehicles; [b]*vs.* Anti-TB; [c]*vs.* 100 μg UA/OA; [d]*vs.* 200 μg UA/OA; [e]*vs.* Anti-TB + 100 μg UA/OA; [f]*vs.* Anti-TB + 200 μg UA/OA; Anti-TB (RIF, INH, PZA); $n = 8$.

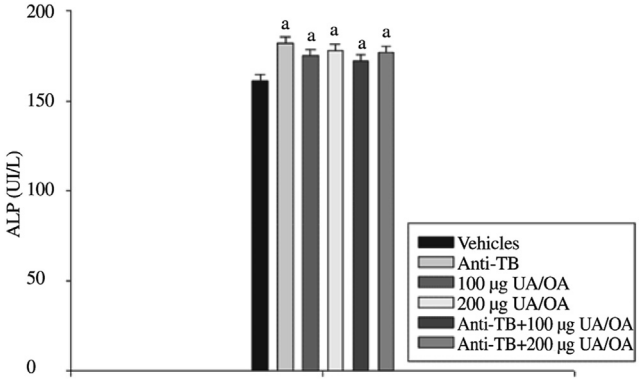

Figure 6. Effect of UA/OA mixture on serum ALP concentration in anti-TB-induced hepatotoxicity mouse model.
Data are mean ± SEM. Statistical analysis, one-way ANOVA, post hoc SNK test ($P < 0.05$); [a]*vs.* Vehicles; [b]*vs.* Anti-TB; [c]*vs.* 100 μg UA/OA; [d]*vs.* 200 μg UA/OA; [e]*vs.* Anti-TB + 100 μg UA/OA; [f]*vs.* Anti-TB + 200 μg UA/OA; Anti-TB (RIF, INH, PZA); $n = 8$.

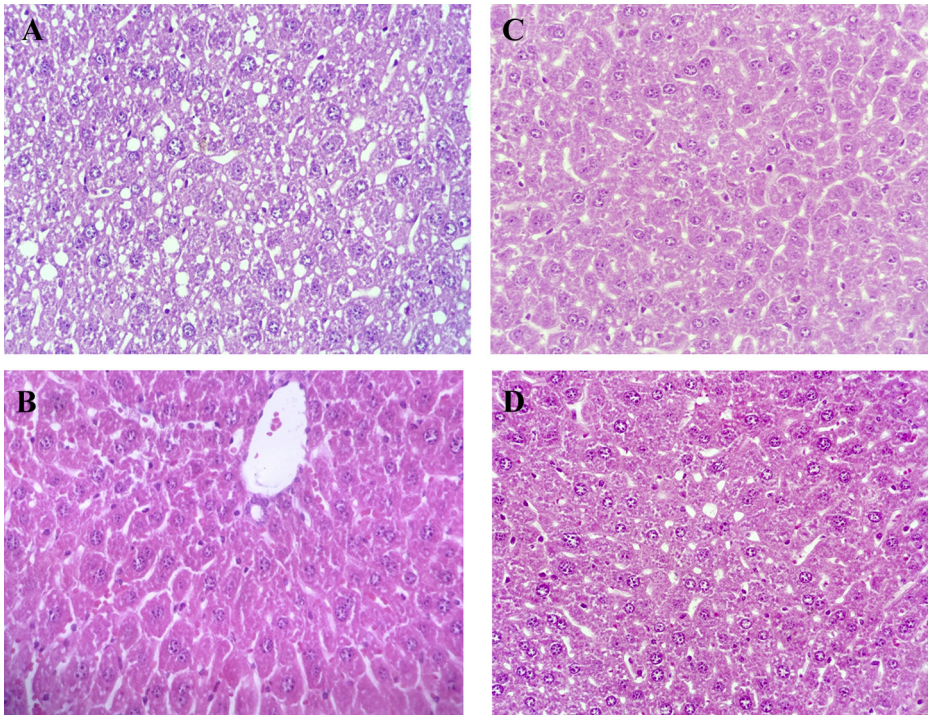

Figure 7. Liver histology, mice groups with anti-TB (A), control (B), mixture of UA/OA at 100 mg/mice (C) and anti-TB plus mixture of UA/OA at 100 mg/mice (D).

4. Discussion

Evidences both *in vitro* and *in vivo* suggest that UA possesses multi-fold biological properties, including anti-inflammatory, anti-oxidative, hipolipidemic and a hepatic metallothionein-inducer property, hepatoprotector effect, as well as other significant effects [29–32]. The same profile can be noted regarding OA, because this triterpene possesses promising pharmacological activities, such as an anti-inflammatory agent, an antioxidant, a cardioprotector, an antidiabetic and as a hepatoprotector [33–39].

Since the report of Jeong [39] concerning the protective effect of OA against Carbon tetrachloride (CCl_4)-induced hepatotoxicity in mice, studies on the hepatoprotector effect of both terpenoids against various drugs that induce hepatotoxicity have appeared in the scientific literature. Studies on *in vivo* experimental models have shown the effects of OA and UA as protectors of acute liver injury induced by CCl_4, acetaminophen, paracetamol, ethanol and D-galactosamine, being UA the most active drug [32,40–44]. Clinical trials in China showed that oral administration of OA for 3 months or more in patients with acute and chronic liver diseases decreased serum aminotransferase levels, symptoms and the occurrence of cirrhosis in cases of patients with chronic hepatitis [33,45].

The hepatoprotective effect of OA and UA against liver damage induced by anti-TB drugs lengthens the list of the multiple biological activities possessed by these triterpenes, highlighting the already reported antimycobacterial activity and antitubercular effect of the mixture [27,46]. The majority of endo- and exo-genous substances are biotransformed in the liver and when reactive products are generated, and they alter the functional and structural integrity of the organ [4,5]. Drug use is a major cause of hepatotoxicity and severe conditions such as liver cirrhosis or hepatocellular carcinoma may occur, a well-known example comprising the hepatotoxicity caused by anti-TB agents [13,19]. Hepatotoxic effects in anti-TB therapy with first-line agents are considered unique among liver problems because all these show dependence on the toxicity of the drug used and the regime established [12,47].

The therapeutic value, efficacy, and toxicity of drugs are parameters that are evaluated as a first step in animals with experimentally induced liver damage. The animal model employed in the present study induced hepatotoxicity in Balb/C mice by means of anti-TB agents commonly used in humans, a combination of RIF, INH and PZA [48]. It is well-known that alterations in anti-TB-induced BW usually reflect physiological changes in liver function. Throughout the experiment (11 weeks), the gradual increase of BW gain in mice treated with anti-TB drugs was significantly lower than those registered in the other evaluated groups (a 58% reduction at the end of the study). This behavior was not reproduced in mice treated with anti-TB drugs plus 100 or 200 μg of UA/OA, meaning that the triterpene mixture supported the normal growth of animals. On the other hand, significant differences in the relative organ weights were found only in liver samples. In this case, the group treated with anti-TB drugs showed a higher relative weight gain (4.78%) with respect to that of the control group (4.05%), but the parameter decreased when the anti-TB group in addition received 100 μg UA/OA (4.12%); in contrast to this, a different behavior was observed on increasing the UA/OA dose to 200 μg; in this case, the triterpene mixture did not act in the same manner. In this regard, studies-in-progress are currently being conducted to clarify this response.

High levels of urea are due to protein metabolism, which takes place in the liver and normally occurs when hepatotoxicity is induced [49,50]. A slight increase in urea levels was determined in the anti-TB group, and administration of UA/OA (100 and 200 μg) returned this parameter to values close to those of the control group, with the 100 μg concentration being the more

effective of the two. According to Awofeso [51] hepatotoxicity is being a serious adverse complication of anti-TB therapy, which ranges from asymptomatic elevation of serum transaminases to acute liver failure. First-line anti-TB drugs raise the levels of hepatic enzymes AST and ALT and liver biopsies reveal lobular hepatitis [4,19]. RIF is a powerful enzyme inducer that enhances the hepatotoxicity of INH and PZA. Hepatotoxicity was detected in 1%–2% of patients treated with this drug, and high values of liver enzymes transaminases AST/ALT and ALP in plasma were reported [19,52]. An augmented level of these hepatic markers in serum may indicate cellular leakage and loss of functional integrity of the cell membrane in liver as a result of the oxygen free radicals produced by anti-TB drugs [7,53,54].

Our results after 11 treatment weeks assume the initiation of a hepatotoxic process in the group of mice treated with anti-TB, because serum transaminases AST and ALT were significantly increased. Administration of 100 μg UA/OA as a unique agent produced an important decrease of AST concentration; an effect of the same magnitude was detected in mice receiving anti-TB drugs in addition to the triterpene mixture. The effect of UA/OA on ALT concentrations was also appreciated, although with less intensity.

Mice treated with anti-TB drugs initiated a steatosis process. Histological liver analysis showed, in samples obtained from animals administered the UA/OA mixture (100 or 200 μg) and those treated with UA/OA plus the anti-TB challenge, a similar architecture to that of the controls group. In the case of the anti-TB-treated group, histological observations support the biochemical findings, in that liver slides clearly depicted morphological alteration related with fatty accumulation. From this analysis, it is possible to support that treatment with the UA/OA mixture was able to avoid hepatic lesions induced by RIF/INH/PZA administration and nearly completely prevented the development of liver steatosis. Evaluation of the UA/OA hepatoprotector effect in the experimental model described showed that 100 μg offers better protection against damage caused by anti-TB drugs.

While it is not possible to conclude, in this preliminary trial, the underlying mechanism of the UA/OA hepatoprotector effect against standard anti-TB drugs, we may assume the suppression of Nuclear factor-kappa beta (NF-kB) activation, inhibition of Cytochrome P_{450} 2E1 (P4502E1) expression and activity, as reported for the hepatoprotector effect of OA against CCl_4 [39], was enhanced hepatic-glutathione regeneration capacity [41], or the upregulation of metallothionein expression mediated by TNF-α and IL-6 shown *in vitro* [34,54], without dismissing the background that these triterpenes possess as strong antioxidants [55]. It is important to mention that additional studies are underway to evaluate the hepatoprotector effects of the triterpene mixture in this same model (Balb/c mice). Animals were administered by oral via and with more prolonged treatment periods (4 months). Another study currently being developed is that of evaluating the hepatoprotector effect of the triterpene (UA/OA) mixture are administered by oral via but with higher doses of RIF/INH/PZA (30, 30, and 90 mg/kg, respectively).

The results showed that UA/OA mixture was able to prevent the steatosis induced by anti-TB drugs when was co-administered daily during 77 d by *s.c.* The triterpene mixture UA/OA favored BW gain and reduced levels of AST and ALT in animals receiving anti-TB plus triterpenes. The animals groups that only received triterpene mixture or vehicle didn't showed steatosis, and no alteration was observed on BW gain in these groups. The values of AST, ALT and ALP were slightly higher respect to vehicle group but lower than the group treated with anti-TB drugs. We have previously shown that the mixture of UA/OA has antitubercular activity and now we are demonstrating that this mixture protects against damage caused by the basic drugs to treat TB (RIF/INH/PZA).

Conflict of interest statement

The authors declare that they have no competing interest.

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