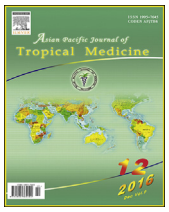
HOSTED BY
ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage: http://ees.elsevier.com/apjtm



Review http://dx.doi.org/10.1016/j.apjtm.2016.10.010

Medical plant extracts and natural compounds with a hepatoprotective effect against damage caused by antitubercular drugs: A review

María Adelina Jiménez-Arellanes✉, Gabriel Alfonso Gutiérrez-Rebolledo, Mariana Meckes-Fischer, Rosalba León-Díaz

Unidad de Investigación Médica en Farmacología, UMAE Hospital de Especialidades, Centro Médico Nacional-Siglo XXI, Instituto Mexicano del Seguro Social, México City, Mexico

ARTICLE INFO

Article history:
Received 11 Aug 2016
Received in revised form 13 Sep 2016
Accepted 10 Oct 2016
Available online 9 Nov 2016

Keywords:
Medicinal plants
Hepatoprotective effect
Antitubercular drugs-induced hepatotoxicity
Natural compounds

ABSTRACT

Drug-induced liver injury encompasses a spectrum of diseases ranging from mild biochemical abnormalities to acute liver failure; example of this scenery is hepatotoxicity caused by the first-line antituberculous drugs isoniazid, rifampin and pyrazinamide, which are basic for treatment of drug-sensible and drug-resistant tuberculosis. In the search for pharmacological alternatives to prevent liver damage, antitubercular drugs have been the subject of numerous studies and published reviews, a great majority of them carried out by Asian countries. At the same time, hepatoprotectors from plant source are now emerging as a possible alternative to counteract the toxic effects of these therapeutic agents. The present review aims to highlight the most recent studies on the subject, based information published in scientific databases such as Scopus and PubMed.

1. Introduction

Tuberculosis (TB) is a disease that affects one third of the world's population; in 2014, nearly 9.6 million cases were reported and close to 2 million deaths [1–3]. At present, there is an alarming increase of multidrug-resistant-TB (MDR-TB) and of extended drug-resistant-TB (XDR-TB) cases; the former are resistant to RIF, PZA and INH (basic drugs) and XDR cases are resistant to RIF, INH, fluoroquinolones and to a second-line injectable drug (amikacin, capreomycin or kanamycin). The World Health Organization (WHO) TB Report 2012 indicates that 4% of new cases and 20% of retreated cases are MDR and that <20% receive adequate treatment [2,4]. On the other hand, around 60% of the patients with MDR-TB are cured and 10% become XDR-TB; of the latter, only 10% are cured [2–5].

RIF, INH and PZA are basic for treating sensitive or mono-resistant TB, and these mainly cause liver damage, as well as neuropathy, hypersensitivity, nephrotoxicity, nausea, vomiting and gastritis. The hepatotoxicity incidence depends on the population studied, treatment time and factors such as age, malnutrition, alcoholism, diabetes mellitus, arthritis, HIV/AIDS, host genetic, exposure to other drugs, *etc* [2,4,6–8]. For the treatment of sensitive TB, a multi-therapy is used, based on four first-line drugs (RIF, INH, PZA, EMB or STR) for 2 months and a mixture of RIF/INH for 8 months. For latent TB, RIF/INH is administered for 3 months or RIF/PZA for 4 months; this treatment causes 2.5%–13% of liver damage, respectively. When the INH is administered alone for 9 months, the hepatotoxicity increases to 1.6% and it increases to 2.6% when RIF/INH mixture is used [8–10].

MDR-TB is treated with up to eight drugs, including first and second line (amikacin, capreomycin, fluoroquinolones, cycloserine, ethionamide, *etc*), for a period of 8–30 months; in this case, the treatment causes severe liver damage in >69% of patients. Subsequently, secondary effects provoke non-adherence to treatment and contribute to treatment failure, propitiating the appearance of drug resistance (DR) [8,9]. It is noteworthy that for contacts of MDR-TB cases, the PZA/EMB combination or fluoroquinolones are employed for treatment [4,11,12].

✉First and corresponding author: María Adelina Jiménez-Arellanes, Unidad de Investigación Médica en Farmacología, Hospital de Especialidades, Centro Médico Nacional Siglo XXI (CMN-SXXI), Instituto Mexicano del Seguro Social (IMSS), Edif. CORSE 2do piso, Av. Cuauhtémoc 330, Col. Doctores, Deleg. Cuauhtémoc, 06720 México, D.F., Mexico.
Tel: +52 55 56276900x21367, +52 55 63950472
E-mails: adelinajim08@prodigy.net.mx, adelinaj@unam.mx

Peer review under responsibility of Hainan Medical University.
Foundation project: Part of this manuscript was supported by grant from the Instituto Mexicano del Seguro Social, projects FIS/IMSS/PROT/G15/1414.

Most xenobiotics, such as drugs, are biotransformed in the liver; this organ is the most affected when the substance is metabolized and generates more toxic products, such as free radicals (FR). This alters the structural integrity and functionality of the liver, generates inflammation, steatosis, induces hepatitis, liver fibrosis, nonalcoholic cirrhosis, necrosis and even hepatocellular carcinoma, and these are among the causes of drug withdrawal from the market [13–16].

The majority of epidemiological studies on hepatotoxicity have been conducted in Europe, Asia and the U.S., and incidence varies among the different regions of the world. The proportion is higher in developing countries as compared with developed countries; for example, it has been reported that in India there is a higher incidence of adverse effects, the mixture of RIF/INH/PZA causing up to 30% of hepatotoxicity, while in other countries this percentage is 23% [17,18]. This type of study has also been carried out in Sub-Saharan Africa, but the incidence of hepatotoxicity has not been reported [8,12].

Since the discovery of INH (1952), the incidence of hepatotoxicity is present in 2% of patients and 20% of these show an increase in the values of hepatic enzymes, such as glutamic oxaloacetic transaminase (GOT) or aspartate aminotransferase (AST) and glutamic pyruvic transferase (GPT) or alanine aminotransferase (ALT) and alkaline phosphatase (ALP) [19]. With the introduction of RIF (in 1963), cases of hepatitis were more frequent, but the increase was greater when the mixture INH/RIF was employed for TB treatment [11]. In the decade of the 1950s, PZA was introduced, being the most active drug against MDR *Mycobacterium tuberculosis* strains, but it is more hepatotoxic, and cases of hepatotoxicity increased when the mixture of INH/RIF/PZA was used, reaching values of up to 60% [8,11,12,20].

The pathogenesis of the hepatotoxicity caused by the INH/RIF mixture is not yet very clear. It generates very reactive compounds such as FR, these favoring the development of oxidative stress (OS), lipid peroxidation and choline deficiency. Phospholipoprotein synthesis and integrity of the cellular membrane of the hepatocytes are altered. Moreover, the levels of glutathione present in them are reduced [11,12,20].

Although the mechanism by which anti-TB drugs induce hepatotoxicity is not yet fully elucidated, it is known that INH and RIF are metabolized by diverse hepatic enzymes of the P_{450} cytochrome family [12]. RIF is an inducer of CYP2D6 and CYP3A4 isoforms of the cytochrome while INH induces CYP2E1 [20–22]. These generate toxic metabolites, such as hydrazine (toxic metabolite of INH), giving rise to OS in humans as well as in animals, and also generate hepatic necrosis. Likewise, INH inhibits cytochrome P_{450} 1A2 reductase, which is involved in the detoxification of its metabolite (hydrazine); therefore, the hepatotoxicity of this drug increases [12,17,23]. Moreover, it has been asserted that RIF increases the biotransformation of INH by stimulating the liver's enzymatic system; in turn, affecting the toxic metabolites of the INH and increasing OS. Furthermore, PZA, once metabolized, becomes pyrazinoic acid (PA), which causes granulomatous hepatitis [12]. In addition, there is strong evidence that host genetic factors influence individual susceptibility to develop hepatotoxicity by anti-TB, such as polymorphisms in *NAT2*, *CYP2E1* and *GST* genes, this is a topic that is getting much attention. Similarly, the effects of GST polymorphisms on genetic susceptibility to anti-TB damage have been reported, particularly for *GSTM1* and *GSTT1* genes [24].

Due to the fact that the three main anti-TB drugs (RIF, INH, and PZA) are those that cause the greatest liver damage and cannot be substituted today, research is being conducted aimed at preventing or reducing the adverse side effects by using herbal extracts and/or natural compounds isolated from these, with a hepatoprotective effect. Among natural products, we can mention silymarin, resveratrol, vitamins E and C, polyphenols and garlic, among others, which do not interfere with the anti-TB effect of the drugs. It is noteworthy that the inhibition of cytochrome P_{450} in its isoform CYP2E1, together with the antioxidant effect of these substances, is beneficial and is the most common mechanism of herbal remedies and isolated natural compounds. Therefore, they are the most frequently recommended substances for protection from the hepatotoxicity induced by anti-TB drugs [17,21,25–27].

In the present paper, an exhaustive search was carried out on the hepatoprotective effect of extracts and/or compounds obtained from medicinal plants against liver damage induced by anti-TB drugs (RIF/INH or RIF/INH/PZA mixture) in preclinical models, *in vivo* and *in vitro*. The main scientific sources consulted were the Scopus and PubMed databases. In this manuscript we describe 101 references published from the year 2000 to date. The key words employed included medicinal plants, hepatoprotective effect, antitubercular drugs-induced hepatotoxicity and natural compounds.

It is noteworthy that in the literature there are numerous papers concerned with the hepatoprotective effect of extracts and compounds obtained from medicinal plants against the liver damage induced by several chemical substances, such as carbon tetrachloride (CCl_4), ethanol (EtOH), acetaminophen (or paracetamol), among others; but there is a scarcity of research that describes this effect against the damage generated by the administration of anti-TB drugs -RIF/INH/PZA- [26].

2. Plants extract with hepatoprotective effect

The ethanol (EtOH) extract from the leaves of *Cnidoscolus chayamansa* administered orally demonstrated a protective effect in Wistar rats against the hepatotoxicity induced by the mixture of RIF/INH (100 mg/kg each), this extract diminishing AST, ALT and ALP levels. The observed effect was similar to that of the positive control (silymarin, 2.5 mg/kg), the authors attributing this protection to the flavonoids present in the plant extract [27].

The aqueous extract of *Allium sativum* bulbs (fresh garlic homogenate, 0.25 g/kg/d) generates a hepatoprotective effect against the sub-acute liver damage induced by the mixture of INH/RIF (50 mg/d, each) administered by oral via, half an hour before anti-TB drugs over a period of 28 d in Wistar rats. The results showed that ALT, AST and total bilirubin levels were reduced in animals receiving the garlic extract and RIF/INH, with respect to the group where only RIF/INH was administered. The authors also observed an increase in the glutathione level and a low level of lipid peroxidation; the effect observed was attributed to the presence of thiosulfinates, steroids, terpenes, flavonoids and other phenols present in garlic [17]. *Allium sativum* (250 mg/kg, oral via), administered by 28 days also protects from liver injury caused only with INH (50 mg/kg); the effect observed was similar to that of silymarin (200 mg/kg) employed as a positive control [28].

Another scientific paper report the evaluation of four extracts [petroleum ether, Chloroform ($CHCl_3$), Methanol (MeOH) and

the aqueous] obtained from *Hibiscus vitifolius* roots in a model of hepatotoxicity induced with the mixture of INH (7.5 mg/kg), RIF (10 mg/kg) and PZA (35 mg/kg) in Wistar rats. The MeOH extract, followed by the aqueous extract administered orally exhibited significant hepatoprotective activity on attenuating toxic effects in the liver produced by anti-TB drugs. Biochemical parameters and reduction of hepatocellular necrosis were very similar to the positive control silymarin (100 mg/kg); the authors contribute the antioxidant effect of the extract to the presence of flavonoids [14].

The EtOH (95%) extract from the aerial part of *Asteracantha longifolia* (*A. longifolia*; syn. *Hygrophila auriculata*) exhibited a hepatoprotective effect on male Sprague–Dawley rats against the damage induced with the mixture of INH/RIF (50 mg/kg each), the EtOH extract was administered at dose of 500 mg/kg/d, by oral via for 28 d. The authors found that this extract restored ALT, AST and ALP levels and accelerated the regeneration of hepatic cells [29]. The hepatoprotective effect has also been described of the aqueous extract of *A. longifolia* (complete plant and root) against the acute damage caused by CCl_4 and acetaminophen [30–32]. The plant's MeOH extract also showed a hepatoprotective effect against damage caused by acetaminophen [33]. The alkaloid-rich fraction from the MeOH extract of *A. longifolia* demonstrated a hepatoprotective effect *in vitro* model [human hepatocellular carcinoma (HepG2)] and *in vivo* (in Wistar rats) on inducing injury with CCl_4; the protective effect observed similar to that of silymarin [34].

A similar study was performed on Wistar rats treated with the EtOH (50%) extract from *Ziziphus oenoplia* (L.) Mill. roots in a hepatotoxicity model induced by INH/RIF (50 mg/kg each); the extract was administered orally for 21 d. The results at doses of 300 mg/kg were very similar to those of the control group (silymarin, 100 mg/kg) in restoring the serum levels of AST, ALT, ALP and bilirubin [35].

The hepatoprotective effect is also reported by the acetonic (70%) extract from the fruit of *Punica granatum* (*P. granatum*) against the hepatotoxicity induced by the mixture of INH/RIF (50 mg/kg each, administered by intraperitoneal -*i.p.*- via). The INH/RIF was co-administrated with 400 mg/kg of the extract for 15 d on male Wistar rats by oral via; the authors found that the extract diminished OS by reducing lipoperoxidation. *P. granatum* inactivates FR and increasing the activity of the antioxidant enzymes SOD, CAT, GST and Gluthathione Peroxidase (GPx), these enzymes constituting the most important endogenous antioxidant defense systems which limit the toxicity associated with the FR formed during damage induced by anti-TB drugs [22]. Likewise, the EtOH extract of *P. granatum* (peel) and of *Vitis vinifera* (seeds) administered on male Wistar rats for 12 weeks confers protection from the hepatocellular injury caused by diethylnitrosamine [36]. The MeOH (95%) extract from *Pisonia aculeata* (250 and 500 mg/kg) suspended in acacia gum at 5% demonstrated a protective effect in Wistar rats against injury induced with RIF and INH (100 and 50 mg/kg, respectively), normalizing AST, ALT, ALP and total bilirubin levels, inhibiting cytochrome P_{450}, augmenting nicotinamide adenine dinucleotide phosphate (NADPH) levels and diminishing lipid peroxidation, the effect being close to that generated by the control of silymarin [25].

Cissampelos pareira (EtOH extract) is another medicinal plant with hepatoprotective activity against damage liver induced by INH/RIF (50 mg/kg each) on Wistar rat; this extract was tested at 100 200 and 400 mg/kg by intraperitoneal via administered for 28 d. In this case, the author used silymarin (200 mg/kg) as positive control. The result showed that this extract reduces the levels of SGPT, SGOT, SALP, total protein, albumin, total bilirubin; the effect was similar to that showed by silymarin and was dose-dependent [37]. The EtOH extract of *Cissampelos pareira* was also active against CCl_4 induced hepatotoxicity damage [38]. The EtOH extract of *Cucumis trigonus* (fruit) was tested as hepatoprotective agents against liver damage induced by RIF/INH (at 50 mg/kg each) in Wistar rats. This extract was tested at 100 250 and 500 mg/kg, administered for 21 d by *i.p.* route. The group that received 500 mg/kg of extract showed similar levels of SGOT, SGPT, SALP and GGTP to the silymarin group. Total bilirubin, conjugated bilirubin, unconjugated bilirubin, total protein, albumin and globulin levels were better in the extract-treated group (500 mg/kg); in this group, the histological analyses revealed a normal liver architecture [39]. Other plants species such as *Mentha peprita*, *Origanum vulgare* and *Pimpinella anisum* and mixture of these were tested against damage induced by INH (150 mg), RIF (300 mg), PZA (500 mg) and ETB (500 mg) on Sprague–Dawley rats. The anti-TB mixture was administered 30 min prior to each extract or the plant mixture and the administration was performed during 30 d. At the end, the results showed that the hepatoprotective effect comprised silymarin > polyherbal > *Mentha peprita* > *Origanum vulgare*, *Pimpinella anisum* [40].

On the other hand, among the scarce investigations carried out on patients, there is a clinical study conducted on patients diagnosed with TB, who were co-administered for 4 weeks with a polyherbal mixture [*Phyllanthus niruri* (*P. niruri*), *Curcuma xanthorrhiza* and *Curcuma longa* (*C. longa*)] together with TB multi-therapy. The authors found that the polyherbal mixture prevents the rise in ALT with respect to those treated with anti-TB drugs alone; thus, the authors concluded that this polyherbal mixture possesses a hepatoprotective effect [41].

The capsule (500 mg) with a polyherbal preparation known as Livina was tested in a clinical trial in TB patients. Livina comprise of *Picrorhizha kurroa*, *P. niruri*, *Andrographis paniculata*, *Cichorium invitybus*, *Tephrosia purpurea*, *Solanum dulcamara*, *Crenum aciaticum*, *Astonia seholanis* (50 mg each), *Holarrhave antidysenteric*, *Tinospora cordifolia* (*T. cordifolia*), *Terminala chebula* and *A. longifolia* (25 mg each). Two capsules of Livina were administered twice daily after meals for six months. The levels of SGOT, SGPT, ALP were lower in Livina treated group with respect to the placebo group at weeks 4 and 8 after treatment. The authors suggest that Livina was efficacious against hepatic dysfunction caused by anti-TB in patients with TB [42]. Another species that has been evaluated as a hepatoprotective agent against damage induced with EtOH 40% administered for 21 d or with a mixture of INH (27 mg/kg), RIF (54 mg/kg) and PZA (135 mg/kg) administered for 21 d in Wistar rats is the MeOH extract from the *Cassia auriculata* root. This extract was administered at 300 and 600 mg/kg by oral via during 30 d in both assays. It was observed that the *Cassia auriculata* extract reduced AST, ALT, ALP, total bilirubin and total cholesterol levels and stimulated the activity of the endogenous enzymatic systems, such as CAT, GPx and SOD. The authors concluded that the hepatoprotective effect is due to maintaining the integrity of the hepatocyte cellular membrane, reducing the concentration of liver enzymes in serum and increasing the endogenous antioxidant effect of the organisms. These latter results were

confirmed with histopathological studies [43]. The EtOH (95%) extract from *Moringa oleifera* leaves administered orally during 45 d at 150, 200 and 250 mg/kg also demonstrated a significant protective effect against the damage induced by INH, RIF and PZA (at doses of 7.5, 10, and 35 mg/kg, respectively) on Wistar rats; in this study, the authors employed silymarin as a positive control (200 mg/kg). The authors found that AST, ALT and ALP, total bilirubin, cholesterol and triglycerides levels were similar in the extract group treated with 250 mg/kg and in the silymarin group, also increase the level of antioxidants enzyme and showing a reduction of lipid peroxidation in this group [44,45].

Furthermore, the MeOH extract of *Annona squamosa* leaves showed a hepatoprotective effect on RIF/INH –induced liver damage (100 mg/kg each) in Wistar rats; treatment was administered during 21 d orally. In this study, the authors employed silymarin as positive control. The extract of *Annona squamosa* at 250 and 500 mg/kg, reduced ALT, AST, ALP, gamma-glutamyl transpeptidase (GGT), protein and total bilirubin levels. The GSH levels increased in group treated with extract and silymarin. This hepatoprotective effect showed by the extract was similar to that exhibited by silymarin [46]. The result of a study conducted in Dunkin-Hartley guinea pigs with liver damage caused by INH (50 mg/kg), RIF (100 mg/kg) and PZA (300 mg/kg) and treated for 21 d with 200 mg/kg of each extract of *C. longa*, *Ocimum sanctum*, *T. cordifolia* and *Ziziphus mauritiana*; revealed that these plants possess a good hepatoprotective effect. In the extract treated group showed normal architecture in the liver histology, no steatosis, no inflammation, and no triaditis or necrosis was observed. The most active plants among these were *C. longa* and *T. cordifolia* [11]. These latter two species (*C. longa* and *T. cordifolia*) were evaluated in patients with TB; the results indicated that patients treated with anti-TB drugs and these medicinal species showed a reduction in the incidence of hepatotoxicity (2/316 patients) as compared with the group treated with anti-TB drugs alone (27/192 patients). The proportion of hepatitis also diminished, as well as AST, ALT and bilirubin levels in patients treated with medicinal plants; thus, the authors concluded that this mixture of plants exerts a hepatoprotective effect *in vivo*. Similarly, *Ocimum sanctum* is a hepatoprotector against the liver damage caused by acetaminophen on rats [47]; while *T. cordifolia* and *Ziziphus vulgaris* are hepatoprotectors against the damage caused by CCl_4 [48,49]. Oral administration during one month of 1 mL/kg of the decoction extract from *Artemisia vulgaris* (*A. vulgaris*) (prepared with 1 g leaves/mL water) was given on male and female Wistar rats with hepatotoxicity damage induced by antitubercular drugs (RIF, 54 mg/kg/d; INH, 27 mg/kg, d; PZA, 135 mg/kg/d). *A. vulgaris* leaves were collected at different seasonal time. The result showed that *A. vulgaris* collected in the May–June period exerts a better hepatoprotective effect in this model [50].

The hepatoprotective effect of the aqueous extract from *Tamarindus indica* fruit (250 and 500 mg/kg) was evaluated against liver damage induced with INH (50 mg/kg) and RIF (100 mg/kg) in Wistar rats; this extract decreased the liver enzyme (AST, ALT and ALP) level as well as bilirubin and TBARS in serum. Additionally, the extract at 500 mg/kg increased the activity of the antioxidant systems (GSH, SOD and CAT) with simultaneously lowered values of lipid peroxidation. The effect observed was better at 500 mg/kg than at lower dosis (250 mg/kg); finally, the microscopic structure of hepatocytes was similar to that shown by silymarin after 14 d of administration [51]. In addition, tablets with decoction of *Tamarindus indica* leaves showed a hepatoprotective effect against CCl_4 liver damage [52]. The *Cuscuta reflexa* (aerial parts) MeOH extract is another medicinal plant with significative hepatoprotective effect against hepatotoxicity induced by RIF (100 mg/kg) and INH (100 mg/kg). This extract was administered in Albino rats at 100, 200 and 400 mg/kg by *i.p.* via, and silymarin was employed as positive control. In this assay, the MeOH extract showed that ALT, ASP, ALP, γ-GT, total bilirubin and total proteins levels were similar to those of the silymarin group; these results were confirmed with histological analyses [53].

The EtOH extract from whole *Solanum xanthocarpum* was also tested on Wistar rats. This extract was administered at doses of 125 and 250 mg/kg during 28 d and liver damage was induced with INH/RIF (50 mg/kg of each); silymarin at 100 mg/kg was utilized as positive control. The EtOH extract showed a hepatoprotective effect through regulation to normal values of liver enzyme (ALP, AST and ALT) levels in serum; in addition, it increased antioxidant activity in liver (SOD, CAT and GSH). The authors associated this beneficial effect with the content of secondary metabolites such as alkaloids and flavonoids, which decrease the oxidative damage on liver cells [54]. On the other hand, the EtOH extract from *Solanum xanthocarpum* (fresh and matured fruits) at 100 200 and 400 mg/kg was administered by *i.g.* via in the Wistar rats during 35 d. Hepatotoxicity damage was induced with INH/RIF/PZA (7.5, 10 and 35 mg/kg each) and silymarin was used as positive control (100 mg/kg). This extract reduce the lipid peroxidation (LPO) levels, restored the endogenous antioxidant system (GSH, SOD and CAT), reduced hepatocellular necrosis and inflammatory cell infiltration; this effect was dose dependent [55]. Kalpu *et al.* evaluated, in the same hepatotoxicity model, the protective activity of aqueous extract of a polyherbal Indian formulation known as Vasaguduchyadi Kwatha (a mixture of eight medicinal plants) on liver injury induced by the administration of anti-TB drugs (INH 27 mg/kg, RIF 54 mg/kg and PZD 135 mg/kg) in Wistar rats during 60 d. The results showed that at a 5.04 mL/kg dose, the polyherbal remedy decreased liver enzyme (ALT and AST) and bilirubin levels in serum, as well as attenuated hepatocellular necrosis and led to a reduction of inflammatory cell infiltration [56].

Another polyherbal Indian formula known as Hepatoplus™ [the capsule contains: *Phyllanthus amarus* (whole plant) 100 mg, *Eclipta alba* (leaves) 50 mg, *Tephrosia purpurea* (leaves) 30 mg, *C. longa* (rhizome) 30 mg, *Picrorhiza kurroa* (root) 20 mg, *Withania somnifera* (root) 100 mg, *Pinius succinifera* (amber) 37.50 mg, *Pistacia lentiscus* (resinous exudates) 25 mg, *Orchis mascula* (seed) 25 mg and *Cycas circinalis* (flower) 62.50 mg] was evaluated against anti-TB-induced liver toxicity (INH/RIF 50 mg/kg each) in Sprague Dawley rats at a dose of 50 and 100 mg/kg for 30 d. LIV 52 (each tablet contains *Capparis spinosa* 32 mg, *Cichorium intybus* 32 mg, *Mandur bhasma* 32 mg, *Solanum nigrum* 32 mg, *Terminalia arjuna* 32 mg, *Cassia occidentalis* 16 mg, *Achillea millefolium* 16 mg and *Tamarix gallica* 16 mg) was used as positive control at 100 mg/kg. Hepatoplus™ exhibited a good protection against oxidative damage by increasing antioxidant defenses (GPx, CAT, SOD and GSH), and also restored serum levels of liver enzymes; although the hepatoprotective effect was dose dependent, the effect observed was similar to the control LIV52 [57].

This polyherbal formulation (100 mg/kg) administered during 30 d in Sprague Dawley male was effective against oxidative damage generated by anti-TB-induced hepatotoxicity, decreasing LPO levels and DNA damage by one half in the liver cells. Additionally, the gene expression of caspases and oxidases such as CYP2E1 was regulated by the co-administration of Hepatoplus; the activity and levels of antioxidant defenses such as GPx, CAT, SOD and GSH were increased in the hepatocytes of rats treated with the polyherbal remedy. In this case, the authors used LIV 52 (100 mg/kg) as positive control [58].

Another study, rats treated with Hepatoplus™ revealed normal architecture of liver cells and demonstrated the *in vitro* hepatoprotective effect of the same polyherbal formulation on liver cell lines against oxidative-induced damage through a mixture of INH/RIF (30 ng/mL). The cell line was treated with three concentrations of Hepatoplus™ (50, 100 and 200 ng/mL). Liver cell lines treated with Hepatoplus™ were protected from oxidative stress and maintained a normal antioxidant profile and liver marker enzymes in a dose-dependent manner. The authors concluded that Hepatoplus™ protects hepatocytes from OS and apoptosis [59].

The EtOH extract from the aerial parts of *Acanthospermum hispidum* (400 mg/kg) was evaluated for 28 d against liver injury induced by anti-TB drugs (RIF 40 mg/kg, INH 27 mg/kg, PZA 66 mg/kg and EMB 53 mg/kg) on Wistar rats. This plant extract reduced the level of liver enzymes in serum and microscopic analysis revealed that liver tissue was regenerated [60]. The same beneficial effect was observed when mice with INH-induced liver injury (100 mg/kg) were treated with *Saccharum officinarum* juice (15 mL/kg) during 30 d, provoking a decrease in serum bilirubin, ALT, AST and ALP levels [61].

In a recent study carried out by Wali *et al.* in 2015, the hepatoprotective activity of the propolis EtOH extract from Kashmir Himalaya (200 and 400 mg/kg) was evaluated against oxidative liver damaged induced by the INH/RIF mixture (100 mg/kg, each) on rats for 14 d. The result showed that propolis at both doses regenerated the liver tissue and modulated oxidative liver injury markers; it also demonstrated a decrease of liver damage caused by INH/RIF. The authors correlated this hepatoprotective effect shown by propolis with its metabolites, such as flavonoids and phenolic acids, through their antioxidant potential, demonstrating this capacity by DPPH inhibition *in vitro* [62].

The aqueous extract from *Azadirachta indica* was administered with anti-TB drugs (INH/RIF/PAZ 27/54/135 mg/kg/d) during 30 d on Wistar rats by oral via. The author found that this extract prevented the biochemical changes in serum; the levels of bilirubin, protein, ALT, AST and ALP were similar to those of the silymarin control group [63]. On the other hand, the MeOH extract from *Ficus religiosa* leaves was tested against liver damage induced with INH/RIF (100 mg/kg, each) in Wistar albino rats; the extract was tested at 100, 200 and 300 mg/kg and was administered during 21 d by oral via. LIV 52 was use positive control. The result revealed that the MeOH extract reduced SGPT and SGOT levels at similar values to those of the LIV 52 group but total protein, albumin ad ALP levels were similar to the INH/RIF group. Histological analysis of the liver showed protection, because the animal group treated with MeOH extract shown a normal hepatic architecture [64]. This extract showed a hepatroprotector effect against liver damage induced with paracetamol [64].

Many other plant species such as *Ferula asafoetida*, *Momordica charantia*, *Nardostachys jatamansi*, *Launeae procumbens*, *Terminalia catappa* [65], *Ficus palmata*, *Vitis vinifera* [66–69]; *Andrographis paniculada*, *Phyllacanthus emblica*, *P. niruri*, *Thymus vulgaris* [70]; *Citrus paradisi*, *Vaccinium* spp., *Cactus pear*, *Opuntia ficus-indica*, *Matricaria chamomilla*, *Camomilla recutita*, *Hibiscus esculentus* [71]; *Juniperus phoenocea* [72]; *C. longa* [73,74]; essential oil and MeOH extract from *Rosmarinus officinalis* [75–78] and others plants extract, have been evaluated as hepatoprotective agents against damage induced by CCl_4, EtOH, D-galactosamine, and/or acetaminophen. However, their protector effect is unknown against damage caused by anti-TB drugs [79,80].

3. Pure natural compounds with hepatoprotective effect

Scarce studies have been carried out that are focused on evaluating the protective effect of some of the compounds obtained from medicinal plants with regard to damage caused by anti-TB drugs. To our knowledge, only silymarin, curcumin, naringenin, resveratrol and N-trans-caffeoyldopamine have been evaluated. It is noteworthy that the hepatoprotective effect of certain natural compounds against damage caused by EtOH, CCl_4, paracetamol and/or acetaminophen has been described. Antioxidant activity comprises the principal mechanism of action of several substances possessing a hepatoprotective effect [27].

Silymarin is a standardized mixture with high antioxidant power, a mechanism by which it is thought to exert its hepatoprotective effect on damage produced by FR generated by anti-TB drugs, EtOH, acetaminophen, CCl_4 and others. Silymarin is obtained from *Silybum marianum* and is frequently used for the treatment of liver diseases worldwide; it is endowed with antioxidant, anti-inflammatory, immunomodulatory, antiproliferative, antiviral, and antifibrotic activity [21]. It contains at least seven flavolignans, the most important of which comprise silybin A, silybin B, silydianin, silycristin and isosilybin A and B, among others. Silybin A is the most important compound and represents 50%–70% of the extract of silymarin, being absorbed 20%–50%.

A study was carried out on Wistar rats with liver damage caused by the administration of RIF/INH (100 and 50 mg/kg, respectively) and with the mixture of RIF/INH/PZA (100, 50 and 350 mg/kg, respectively), co-administered for 14 d with silymarin (200 mg/kg). The results showed that silymarin protects the liver damage caused by the mixture of anti-TB drugs and it regenerates in liver, the biochemical changes induced by the mixture of RIF/INH or RIF/INH/PZA. This effect is due to that silymarin reduced ALT and AST levels to normal values and also reduced serum albumin, total protein and bilirubin values. In addition, the liver of the animals under study that received anti-TB drugs and silymarin did not induce steatosis, necrosis, or fibrosis. The authors suggest that silymarin can be employed as a nutritional supplement in patients treated with anti-TB drugs [21]. Furthermore, it has been demonstrated that silymarin (150 mg/kg) administered orally possesses a hepatoprotective effect similar to that of N-acetyl-cysteine in the case of acute injury caused by acetaminophen [81]. It is noteworthy that silymarin is frequently employed as a positive control in the search for substances with a hepatoprotective effect. Silymarin has also exhibited a beneficial effect on anti-TB-induced hepatotoxicity in rabbits (INH 50 mg/kg). At a 50 mg/kg dose administered during 6 months, bilirubin and ALT

serum levels were reduced with respect to anti-TB group [82]. The *in vivo* hepatoprotective effect of silymarin has been reported in a double-blind study of patients with TB and its treatment (INH, 5 mg/kg/d; RIF, 10 mg/kg/d; PZA, 25 mg/kg/d and EMB, 15 mg/kg per day). The treated group received silymarin (140 mg tablet, 3 times a daily) during 2 months, while the placebo group received tablets similar in appearance to those of silymarin. The author found that silymarin protects the liver damage caused by anti-TB drugs without demonstrating an adverse effect. It was observed that serum ALP, GGT, ALT and AST values were higher than those shown by the placebo group. Also, it was described that SOD restoration can comprise a mechanism which explains the beneficial effect exhibited by silymarin [10].

Another natural compound that showing a hepatoprotective effect *in vivo* is the flavonoid naringenin; in the case of damage caused by CCl_4 in mice, the authors observed that naringenin restores ALT and AST levels and improves the activity of SOD and GPx, avoiding lipid peroxidation and, in turn, avoiding hepatocyte necrosis, steatosis, and fibrosis [83,84]. This flavone also provides protection from the liver damage caused by dimethylnitrosamine in rats, against the damage generated by acetaminophen in mice and against the chronic damage (60 d) caused by EtOH. The authors report that naringenin restores ALT, AST, ALP, bilirubin, albumin and total proteins serum levels and, reducing the lipid oxidation in the liver [85,86]. It is noteworthy that naringenin possesses significant antioxidant and hepatoprotective activity; however, to date, its hepatoprotector effect against anti-TB drugs induced damage was not been described.

In a study *in vitro* with the HepG2 cell line, the authors tested the hepatoprotective effect of the mixture of silymarin, curcumin and N-acetylcysteine against the liver damage caused with the INH/RIF/PZA mixture. The results revealed that the mixture of these natural compounds increases cell viability, maintains cell morphology and does not alter mitochondrial activity [18]. The protective effect *in vivo* against liver damage caused by anti-TB drugs is not described.

Curcumin is another natural compound in which the *in vivo* hepatoprotective effect has been demonstrated (Wistar rats) against liver damage caused by acetaminophen; the authors report that it diminishes the expression of MetalloProteinase 8 (MMP-8) and increases the expression of genes encoding antioxidant enzymes in the liver [87]. This compound also demonstrated a hepatoprotective effect against acute and subacute liver damage caused by CCl_4 [88,89], paraquat [90], EtOH *in vitro* and *in vivo* [91,92] and dimethylnitrosamine [93]; however, the hepatoprotective effect against damage induced by anti-TB drugs has not been described.

The mixture of Picroliv, curcumin and ellagic acid demonstrated a protective effect against the acute liver damage caused by CCl_4 and paracetamol in mice and rats [88,94]. However, elucidation is still lacking on its protective effect against the liver damage caused by anti-TB multi-therapy.

Resveratrol is another natural compound that has been evaluated against the acute liver damage caused by the mixture of INH (50 mg/kg) and RIF (100 mg/kg) in male Balb/C mice. This compound, at a dose of 100 mg/kg, was administered 30 min prior to the RIF/INH mixture during 3 d. The results showed that resveratrol reduced AST and ALT serum levels by 36% and 58%, respectively, with regard to animals treated only with anti-TB drugs alone; the authors also observed that glutathione and CAT levels were higher in the Resveratrol/RIF/INH-treated group with respect to the RIF/INH group; likewise, the group that received resveratrol-RIF/INH exhibited lower myeloperoxidase values (19%) than the RIF/INH group. Through histological analysis, the authors observed less microvesicular steatosis and apoptosis in the livers of animals that received resveratrol-RIF/INH with respect to the group that was administered anti-TB drugs. The authors recommend the use of this substance to revert the damage caused by anti-TB drugs. This compound also protects against liver damage caused by acetaminophen and EtOH [95].

N-trans-Caffeoyldopamine isolated from *Capsicum annuum*, *Theobroma cacao* and *Lycium chinense* was tested at 2.5 mg/kg against oxidative damage induced by anti-TB drugs (INH/RIF 100 mg/kg each) on male Wistar rats. This natural compound showed a protective effect due to its good antioxidant activity, increasing SOD and GSH levels in hepatic tissue and significantly decreasing liver-enzyme levels (AST, ALT and ALP), as well as the lipid peroxidation in liver. It is therefore, suggested that N-trans-Caffeoyldopamine can provide a definite protective effect against acute hepatic injury caused by INH/RIF in rats, which may mainly be associated with its antioxidative effect. This compound inhibited LPO through the CYP4502E1 down-regulation [96].

On the other hand, the triterpenes Oleanolic Acid (OA, 3-β-hydroxy-olea-12-en-28-oic acid) and Ursolic Acid (UA, 3-β-hydroxyurs-12-en-28-oic acid) possess an important hepatoprotective effect against the liver damage caused by EtOH, CCl_4, D-galactosamine, acetaminophen, cadmium, bromobenzene, phalloidin, thioacetamide and other hepatotoxic substances [97–100]. In China, clinical-phase experiments have been conducted on patients with acute and chronic hepatitis, who were treated with OA administered by oral via during 3 months or more. The authors found that the compound demonstrated a beneficial effect with respect to the placebo group; this compound diminished AST and ALP serum levels, as well as cirrhosis in cases of chronic hepatitis. Recently, was published the results on the hepatoprotective effect of the mixture of oleanolic and ursolic acid (OA/UA, obtained from *Bouvardia ternifolia*) against liver injury induced with INH (10 mg/kg), RIF (10 mg/kg) and PZ (30 mg/kg) in BALC/c mice. The animals received by subcutaneous via this OA/UA mixture at 100 and 200 mg/mouse daily during 11 weeks. The results showed that this mixture generates an increase on body weight gain compared to the anti-TB-drugs group, also reduced the level of ALT and AST. In addition, histological analysis of livers from anti-TB group showed a steatosis and increased apoptosis, these effects was no detected in anti-TB plus OA/UA group [101]. Some naturally-occurring compounds such as saikosaponin, isolated from *C. longa*, quercetin, anthocyanin, esculin, retinol, α-Tocopherol and lycopene have been evaluated as hepatoprotective agents against damage caused by CCl_4, EtOH and acetaminophen [1,16]. However, to date, the beneficial effect of these substances against injury caused by anti-TB drugs remains unknown.

4. Conclusions

The combination of RIF, INH and PZA is considered a basic drug for treating TB, but these induce hepatotoxicity favoring treatment suspension and the appearance of MDR cases. On the other hand, TB and HIV co-exist in the same population,

increasing the risk of TB 6–50 fold. In view of this scenario, the medicinal plants and compounds obtained from them comprises a necessary alternative to consider in the search of hepatoprotective agents for managing liver damage produced by anti-TB drugs. In this regard, we can mention that, to date, there are few plant species that have been evaluated *in vivo* against the liver damage induced by anti-TB drugs and that only six natural compounds (silymarin, curcumin, N-trans-caffeoyldopamine, OA/UA mixture and resveratrol) have been described as hepatoprotective compounds. Currently, only three articles, to our knowledge, have described the hepatoprotective effect of polyherbal preparation in patients with TB, and evaluation of the hepatoprotective effect of pure compounds has not been performed. The results found show that these medicinal species and compounds do indeed exert a hepatoprotective effect and that they can be candidates for use in minimizing the liver damage caused by anti-TB drugs.

Conflict of interest statement

We declare that we have no competing interests.

Acknowledgments

Part of this manuscript was supported by a grant from the Instituto Mexicano del Seguro Social (IMSS), project FIS/IMSS/PROT/G15/1414.

References

[1] García A, Bocanegra-García V, Palma-Nicolás JP, Rivera G. Recent advances in antitubercular natural products. *Eur J Med Chem* 2012; **49**(1): 1-23.
[2] Günther G. Multidrug-resistant and extensively drug-resistant tuberculosis: a review of current concepts and future challenges. *Clin Med (London)* 2014; **14**(3): 279-285.
[3] WHO (World Health Organization). *Global tuberculosis report*. 20th ed. 2015 [Online] Available from: http://apps.who.int/iris/bitstream/10665/191102/1/9789241565059_eng.pdf [Accessed on 15th June, 2016]
[4] Brigden G, Nyang'wa BT, du Cros P, Varaine F, Hughes J, Rich M, et al. Principles for designing future regimens for multidrug-resistant tuberculosis. *Bull World Health Organ* 2014; **92**(1): 68-74.
[5] Zager EM, McNerney R. Multidrug-resistant tuberculosis. *BMC Infect Dis* 2008; **8**(1): 10-15.
[6] Njoku DB. Drug-induced hepatotoxicity: metabolic, genetic and immunological basis. *Int J Mol Sci* 2014; **15**(4): 6990-7003.
[7] Aithal GP, Watkins PB, Andrade RJ, Larrey D, Molokhia M, Takikawa H, et al. Case definition and phenotype standardization in drug-induced liver injury. *Clin Pharmacol Ther* 2011; **89**(1): 806-815.
[8] Tostmann A, Boeree M, Aarnoutse R, de Lange W, van der Ven A, Dekhuijzen R. Antituberculosis drug-induced hepatotoxicity: concise up-to-date review. *J Gastroenterol Hepatol* 2008; **23**(2): 192-202.
[9] Ginsberg AM. Tuberculosis drug development: progress, challenges, and the road ahead. *Tuberculosis* 2010; **90**(3): 162-167.
[10] Luangchosiri C, Thakkinstian A, Chitphuk S, Stitchantrakul W, Petraksa S, Sobhonslidsuk A. A double-blinded randomized controlled trial of silymarin for the prevention of antituberculosis drug-induced liver injury. *BMC Complement Altern Med* 2015; **15**(1): 334.
[11] Adhvaryu MR, Reddy NM, Vakharia BC. Prevention of hepatotoxicity due to anti-tuberculosis treatment: a novel integrative approach. *World J Gastroenterol* 2008; **14**(30): 4753-4762.
[12] Senousy BE, Belal SI, Draganov PV. Hepatotoxic effects of therapies for tuberculosis. *Nat Rev Gastroenterol Hepatol* 2010; **7**(10): 543-556.
[13] Abdulaziz-Bardi D, Halabi MF, Abdullah NA, Rouhollahi E, Hajrezaie M, Abdulla MA. *In vivo* evaluation of ethanolic extract of *Zingiber officinale* rhizomes for its protective effect against liver cirrhosis. *Biomed Res Int* 2013; **1**(1): 918460.
[14] Samuel AJ, Mohan S, Chellappan DK, Kalusalingam A, Ariamuthu SI. *Hibiscus vitifolius* (Linn.) root extracts show potent protective action against anti-tubercular drug induced hepatotoxicity. *J Ethnopharmacol* 2012; **141**(1): 396-402.
[15] Tejeda CF. Hepatotoxicidad por fármacos. *Rev Clin Med Fam* 2010; **3**(3): 177-191.
[16] Vitaglione P, Morisco F, Caporaso N, Fogliano V. Dietary antioxidant compounds and liver health. *Crit Rev Food Sci Nutr* 2004; **44**(7–8): 575-586.
[17] Pal R, Vaiphei K, Sikander A, Singh K, Rana SV. Effect of garlic on isoniazid and rifampicin-induced hepatic injury in rats. *World J Gastroenterol* 2006; **12**(4): 636-639.
[18] Singh M, Sasi P, Gupta VH, Rai G, Amarapurkar DN, Wangikar PP. Protective effect of curcumin, silymarin and N-acetylcysteine on antitubercular drug-induced hepatotoxicity assessed in an *in vitro* model. *Hum Exp Toxicol* 2012; **31**(8): 788-797.
[19] Devarbhavi H. An update on drug-induced liver injury. *J Clin Exp Hepatol* 2012; **2**(3): 247-259.
[20] Ramappa V, Aithal G. Hepatotoxicity related to anti-tuberculosis drugs: mechanisms and management. *J Clin Exp Hepatol* 2013; **3**(1): 37-49.
[21] Eminzade S, Uraz F, Izzettin FV. Silymarin protects liver against toxic effects of anti-tuberculosis drugs in experimental animals. *Nutr Metab* 2008; **5**(18): 1-8.
[22] Yogeeta S, Ragavender HRB, Devaki T. Antihepatotoxic effect of *Punica granatum* acetone extract against isoniazid- and rifampicin-induced hepatotoxicity. *Pharm Biol* 2007; **45**(8): 631-637.
[23] Liu K, Lu J, Gao Z, Klaassen CD, Ma X. Role of CYP3A in Isoniazid metabolism in vivo. *Drug Metab Pharmacokinet* 2014; **29**(2): 219-222.
[24] Ambreen K, Sharma R, Singh KP, Abbas M, Kumar S. Association of *GSTM1*, *GSTT1* and *CYP2E1* gene polymorphisms with antituberculosis drug induced hepatotoxicity in North Indian population. *Int J Health Sci Res* 2014; **4**(2): 149-160.
[25] Anbarasu C, Rajkapoor B, Kalpana J. Protective effect of *Pisonia aculeata* on rifampicin and isoniazid induced hepatotoxicity in rats. *Int J Phytomed* 2011; **3**(1): 75-83.
[26] Jaeschke H, Williams CD, McGill MR, Xie Y, Ramachandran A. Models of drug-induced liver injury for evaluation of phytotherapeutics and other natural products. *Food Chem Toxicol* 2013; **55**(1): 279-289.
[27] Kulathuran PK, Chidambaranathan N, Mohamed H, Jayaprakash S, Narayanan N. Hepatoprotective activity of *Cnidoscolus chayamansa* against rifampicin and isoniazid induced toxicity in Wistar rats. *Res J Pharm Biol Chem Sci* 2012; **3**(2): 577-585.
[28] Nasim I, Sadiq M, Jehangir A. Hepatoprotective effect of garlic (*Allium sativum*) and milk thistle (silymarin) in isoniazid induced hepatotoxicity in rats. *Biomedica* 2011; **27**(1): 166-170.
[29] Lina SMM, Ashab I, Ahmed MI, Al-Amin M, Shahriar M. Hepatoprotective activity of *Asteracantha longifolia* (Nees.) extract against anti-tuberculosis drugs induced hepatic damage in Sprague-Dawley rats. *PhOL* 2012; **3**(1): 13-19.
[30] Hewawasam RP, Jayatilaka KA, Pathirana C, Mudduwa LK. Protective effect of *Asteracantha longifolia* extract in mouse liver injury induced by carbon tetrachloride and paracetamol. *J Pharm Pharmacol* 2003; **55**(10): 1413-1418.
[31] Shailajan S, Chandra N, Sane RT, Menon S. Effect of *Asteracantha longifolia* Nees. against CCl_4 induced liver dysfunction in rat. *Indian J Exp Biol* 2005; **43**(1): 68-75.
[32] Shanmugasundaram P, Venkataraman S. Hepatoprotective and antioxidant effects of *Hygrophila auriculata* (K. Schum.) and

Heine acanthaceae root extract. *J Ethnopharmacol* 2006; **104**(1–2): 124-128.
[33] Shivashangari KS, Ravikumar V, Devaki T. Evaluation of the protective efficacy of *Asteracantha longifolia* on acetaminophen-induced liver damage in rats. *J Med Food* 2004; **7**(2): 245-251.
[34] Raj VP, Chandrasekhar RH, Rao MC, Rao VJ, Nitesh K. *In vitro* and *in vivo* hepatoprotective effects of the total alkaloid fraction of *Hygrophila auriculata* leaves. *Indian J Pharmacol* 2010; **42**(2): 99-104.
[35] Rao CV, Rawat AK, Singh AP, Singh A, Verma N. Hepatoprotective potential of ethanolic extract of *Ziziphus oenoplia* (L.) Mill roots against antitubercular drugs induced hepatotoxicity in experimental models. *Asian Pac Trop Med* 2012; **5**(4): 283-288.
[36] Kumar AK. Protective effect of *Punica granatum* peel and *Vitis vinifera* seeds on DEN-induced oxidative stress and hepatocellular damage in rats. *Appl Biochem Biotechnol* 2015; **175**(1): 410-420.
[37] Balakrishman S, Khurana BS, Singh A, Kaliappan I, Dubey G. Hepatoprotective effect of hydroalcoholic extract of *Cissampelos pareira* against rifampicin and isoniazid induced hepatotoxicity. *Cont J Food Sci Technol* 2012; **6**(1): 30-35.
[38] Surendran S, Eswaran MB, Vijayakumar M, Rao CV. *In vitro* and *in vivo* hepatoprotective activity of *Cissampelos pareira* against carbon-tetrachloride induced hepatic damage. *Indian J Exp Biol* 2011; **49**(12): 939-945.
[39] Gopalakrishnan SB, Kalaiarasi T. Hepatoprotective activity studies of *Cucumis trigonus* ROXB against rifampicin-isoniazid-induced toxicity in rats. *Eur J Pharm Med Res* 2015; **2**(6): 141-146.
[40] Ali ZY. Biochemical evaluation of some natural products against toxicity induced by anti-tubercular drugs in rats. *N Y Sci J* 2012; **5**(10): 69-80.
[41] Rachmawati E, Nurrochmad A, Puspita Sari I. Assessment of hepatoprotective effect of polyherbal combination of *Phyllanthus niruri* (meniran), *Curcuma xanthorrhiza* (wild ginger), and *Curcuma longa* (turmeric) against liver dysfunction due to anti-tuberculosis drugs. In: *New development of pharmaceutical care in a pharmacogenetic and pharmacogenomic approach: proceeding the international conference pharmaceutical care*. Malang: Department of Pharmacy, University of Muhammadiyah Malang; 2014, p. 4.
[42] Gulati K, Ray A, Vijayan VK. Assessment of protective role of polyherbal preparation Livina, against anti-tubercular drug induced liver dysfuntion. *Indian J Exp Biol* 2010; **48**(1): 318-322.
[43] Jaydeokar AV, Bandawane DD, Bibave KH, Patil TV. Hepatoprotective potential of *Cassia auriculata* roots on ethanol and antitubercular drug-induced hepatotoxicity in experimental models. *Pharm Biol* 2014; **52**(3): 344-355.
[44] Pari L, Kumar NA. Hepatoprotective activity of *Moringa oleifera* on antitubercular drug-induced liver damage in rats. *J Med Food* 2002; **5**(3): 171-177.
[45] Kumar NA, Pari L. Antioxidant action of *Moringa oleifera* Lam. (drumstick) against antitubercular drugs induced lipid peroxidation in rats. *J Med Food* 2003; **6**(3): 255-259.
[46] Thattakudian S, Uduman MS, Sundarapandian R, Muthumanikkam A, Kalimuthu G, Parameswari S, et al. Protective effect of methanolic extract of *Annona squamosa* Linn. in isoniazid-rifampicin induced hepatotoxicity in rats. *Pak J Pharm Sci* 2011; **24**(2): 129-134.
[47] Lahon K, Das S. Hepatoprotective activity of *Ocimum sanctum* alcoholic leaf extract against paracetamol-induced liver damage in albino rats. *Pharm Res* 2011; **3**(1): 13-18.
[48] Ebrahimi S, Ashkani-Esfahani S, Emami Y, Riazifar S. Hepatoprotective effect of *Ziziphus vulgaris* on carbon tetrachloride (CCl_4) induced liver damage in rats as animal model. *GMJ* 2013; **2**(3): 88-94.
[49] Kumar V, Modi PK, Saxena KK. Exploration of hepatoprotective activity of aqueous extract of *Tinospora cordifolia*- an experimental study. *Asian J Pharm Clin Res* 2013; **6**(1): 87-91.
[50] Mitra P, Ghosh T, Mitra PK. Seasonal variation in hepatoprotective activity of Titeypati (*Artemisia vulgaris* L.) leaves on antitubercular drugs induced hepatotoxicity in rats. *SMU Med J* 2016; **3**(1): 763-774.
[51] Amir M, Khan MA, Ahmad S, Akhtar M, Mujeeb M, Ahmad A, et al. Ameliorating effects of *Tamarindus indica* fruit extract on anti-tubercular drugs induced liver toxicity in rats. *Nat Prod Res* 2015; **30**(6): 715-719.
[52] Rodríguez-Amado JR, Lafourcade-Prada A, Escalona-Arranz JC, Pérez-Rosés R, Morris-Quevedo H, Keita H, et al. Antioxidant and hepatoprotective activity of a new tablets formulation from *Tamarindus indica* L. *Evid Based Complement Alternat Med* 2016; **2016**: 3918219.
[53] Balakrishman B, Sagameswaran B, Bhaskar VH. Effect of methanol extract of *Cuscuta reflexa* aerial parts on hepatotoxicity induced by antitubercular drugs in rats. *Int J App Res Nat Prod* 2010; **3**(1): 18-22.
[54] Verma P, Paswan S, Singh SP, Shrivastva S, Rao CV. Assessment of hepatoprotective potential of *Solanum xanthocarpum* (whole plant) Linn. against isoniazid & rifampicin induced hepatic toxicity in Wistar rats. *Elixir Appl Bot* 2015; **87**(1): 35578-35583.
[55] Hussain T, Gupta RK, Khan MS, Hussain MD, Arif MD, Hussain A, et al. Evaluation of antihepatotoxic potential of *Solanum xanthocarpum* fruit extract against antitubercular drugs induced hepatopathy in experimental rodents. *Asian Pac J Trop Biomed* 2012; **2**(6): 454-460.
[56] Kalpu NK, Ashok BK, Shukla VJ, Prajapati P, Ravishankar B. Hepatoprotective activity of Vasaguduchyadi Kwatha - a compound herbal formulation against antitubercular drugs (isoniazid + rifampicin + pyrazinamide) induced hepatotoxicity in albino rats. *Pharma Sci Monit* 2015; **6**(3): 73-84.
[57] Sankar M, Rajkumar J, Sridhar D. Hepatoprotective activity of hepatoplus on isoniazid and rifampicin induced liver damage in rats. *Indian J Pharm Sci* 2015; **77**(1): 556-562.
[58] Sankar M, Rajkumar J, Devi J. Hepatoprotective activity of hepatoplus on isonaizid and rifampicin induced hepatotoxicity in rats. *Pak J Pharm Sci* 2015; **28**(3): 983-990.
[59] Sankar M, Rajkumar J, Sridhar D. Effect of hepatoplus on isoniazid and rifampicin induced hepatotoxicity in liver cell lines. *Int J Pharm Pharm Sci* 2015; **7**(5): 215-219.
[60] Himaja N. Comparative study of hepatoprotective activity of *Acanthospermum hispidum* plant extract and herbal niosomal suspension against anti-tubercular drug induced hepatotoxicity in rats. *Asian J Pharm Clin Res* 2015; **8**(5): 256-259.
[61] Khan SW, Tahir M, Lone KP, Munir B, Latif W. Protective effect of *Saccharum officinarum* L. (sugar cane) juice on isoniazid induced hepatotoxicity in male albino mice. *J Ayub Med Coll Abbottabad* 2015; **27**(2): 346-350.
[62] Wali AF, Avula B, Ali Z, Khan IA, Mushtag A, Rehman MU, et al. Antioxidant, hepatoprotective potential and chemical profiling of propolis ethanolic extract from Kashmir Himalaya region using UHPLC-DAD-QToF-MS. *Biomed Res Int* 2015; **2015**: 393462.
[63] Kale BP, Kothekar MA, Tayade HP, Jaju JB, Mateenuddin M. Effect of aqueous extract of *Azadirachta indica* leaves on hepatotoxicity induced by antitubercular drugs in rats. *Indian J Pharmacol* 2003; **35**(3): 177-180.
[64] Parameswari SA, Chetty CM, Chandrasekhar KB. Hepatoprotective activity of *Ficus religiosa* leaves against isoniazid+rifampicin and paracetamol induced hepatotoxicity. *Pharmacogn Res* 2013; **5**(4): 271-276.
[65] Gao J, Tang X, Dou H, Fan Y, Zhao X, Xu Q. Hepatoprotective activity of *Terminalia catappa* L. leaves and its two triterpenoids. *J Pharm Pharmacol* 2004; **56**(11): 1449-1455.
[66] Liu T, Zhao J, Ma L, Ding Y, Su D. Hepatoprotective effects of total triterpenoids and total flavonoids from *Vitis vinifera* L. against immunological liver injury in mice. *Evid Based Complement Alternat Med* 2012; **2012**: 969386.
[67] Madi AA, Farouk EM. Lipid-lowering and hepatoprotective effects of *Vitis vinifera* dried seeds on paracetamol-induced hepatotoxicity in rats. *Nutr Res Pract* 2015; **9**(1): 37-42.
[68] Orhan DD, Orhan N, Ergun E, Ergun F. Hepatoprotective effect of *Vitis vinifera* L. leaves on carbon tetrachloride-induced acute liver damage in rats. *J Ethnopharmacol* 2007; **112**(1): 145-151.

[69] Sharma SK, Vasudeva N. Hepatoprotective activity of *Vitis vinifera* root extract against carbon tetrachloride-induced liver damage in rats. *Acta Pol Pharm* 2012; **69**(5): 933-937.
[70] Grespan R, Aguiar RP, Giubilei FN, Fuso RR, Damiao MJ, Silva EL, et al. Hepatoprotective effect of pretreatment with *Thymus vulgaris* essential oil in experimental model of acetaminophen-induced injury. *Evid Based Complement Alternat Med* 2014; **2014**: 954136.
[71] Alqasoumi SI. 'Okra' *Hibiscus esculentus* L. A study of its hepatoprotective activity. *Saudi Pharm J* 2012; **20**(2): 135-141.
[72] Alqasoumi SI, Farraj AI, Abdel-Kader MS. Study of the hepatoprotective effect of *Juniperus phoenicea* constituents. *Pak J Pharm Sci* 2013; **26**(5): 999-1008.
[73] Salama SM, Abdulla MA, AlRashdi AS, Ismail S, Alkiyumi SS, Golbabapour S. Hepatoprotective effect of ethanolic extract of *Curcuma longa* on thioacetamide induced liver cirrhosis in rats. *BMC Complement Alternat Med* 2013; **13**(1): 56-73.
[74] Sengupta M, Sharma GD, Chakraborty B. Hepatoprotective and immunomodulatory properties of aqueous extract of *Curcuma longa* in carbon tetrachloride intoxicated Swiss albino mice. *Asian Pac J Trop Biomed* 2011; **1**(3): 193-199.
[75] Soria-Fregozo C, Miranda-Beltrán M, Flores-Soto ME, Pérez-Vega MI, Rodríguez-Rodríguez RY, López-Velázquez AL, et al. Protective effect of *Rosmarinus officinalis* L. on the expression of the glutamate transporter (GLT-1) and neuronal damage in the frontal cortex of CCl_4-induced hepatic damage. *J Med Plant Res* 2012; **6**(49): 5886-5894.
[76] Sotelo-Félix JI, Martínez-Fong D, Muriel De la Torre P. Protective effect of carnosol on CCl_4-induced acute liver damage in rats. *Eur J Gastroenterol Hepatol* 2002; **14**(9): 1001-1006.
[77] Sotelo-Félix JI, Martínez-Fong D, Muriel P, Santillán RL, Castillo D, Yahuaca P. Evaluation of the effectiveness of *Rosmarinus officinalis* (Lamiaceae) in the alleviation of carbon tetrachloride-induced acute hepatotoxicity in the rat. *J Ethnopharmacol* 2002; **81**(2): 145-154.
[78] Rašković A, Milanović I, Pavlović N, Ćebović T, Vukmirović S, Mikov M. Antioxidant activity of rosemary (*Rosmarinus officinalis* L.) essential oil and its hepatoprotective potential. *BMC Complement Alternat Med* 2014; **14**(1): 225-234.
[79] Dandagi PM, Patil MB, Mastiholimath VS, Gadad AP, Dhumansure RH. Development and evaluation of hepatoprotective polyherbal formulation containing some indigenous medicinal plants. *Indian J Pharm Sci* 2008; **70**(2): 265-268.
[80] Feroz Z, Khan RA, Mahayrookh A. Hepatoprotective effect of herbal drug on CCl_4 induced liver damage. *Pak J Pharm Sci* 2013; **26**(1): 99-103.
[81] Kazemifar AM, Hajaghamohammadi AA, Samimi R, Alavi Z, Abassi E, Asl MN. Hepatoprotective property of oral silymarin is comparable to N-Acetyl cysteine in acetaminophen poisoning. *Gastroenterol Res* 2012; **5**(5): 190-194.
[82] Jahan S, Khan M, Imran S, Sair M. The hepatoprotective role of silymarin in isoniazid induced liver damage of rabbits. *J Pak Med Assoc* 2015; **65**(6): 620-623.
[83] Esmaeili MA, Alilou M. Naringenin attenuates CCl_4-induced hepatic inflammation by the activation of an Nrf2-mediated pathway in rats. *Clin Exp Pharmacol Physiol* 2014; **41**(6): 416-422.
[84] Hermenean A, Ardelean A, Stan M, Hadaruga N, Mihali CV, Costache M, et al. Antioxidant and hepatoprotective effects of naringenin and its β-cyclodextrin formulation in mice intoxicated with carbon tetrachloride: a comparative study. *J Med Food* 2014; **17**(6): 670-677.
[85] Lee MH, Yoon S, Moon JO. The flavonoid naringenin inhibits dimethylnitrosamine-induced liver damage in rats. *Biol Pharm Bull* 2004; **27**(1): 72-76.
[86] Lv Y, Zhang B, Xing G, Wang F, Hu Z. Protective effect of naringenin against acetaminophen-induced acute liver injury in metallothionein (MT)-null mice. *Food Funct* 2013; **4**(2): 297-302.
[87] Soliman MM, Abdo-Nassan M, Ismail TA. Immunohistochemical and molecular study on the protective effect of curcumin against hepatic toxicity induced by paracetamol in Wistar rats. *BMC Complement Alternat Med* 2014; **14**(1): 457-468.
[88] Girish C, Pradhan SC. Hepatoprotective activities of picroliv, curcumin, and ellagic acid compared to silymarin on carbon-tetrachloride-induced liver toxicity in mice. *J Pharmacol Pharmacother* 2012; **3**(2): 149-155.
[89] Park EJ, Jeon CH, Ko G, Kim J, Sohn DH. Protective effect of curcumin in rat liver injury induced by carbon tetrachloride. *J Pharm Pharmacol* 2000; **52**(4): 437-440.
[90] Han W, Wu D, Lu Y, Wang L, Hong G, Qiu Q, et al. Curcumin alleviated liver oxidative stress injury of rat induced by paraquat. *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi* 2014; **32**(5): 352-356.
[91] Lee H, McGregor RA, Choi MS, Seo KI, Jung UJ, Yeo J, et al. Low doses of curcumin protect alcohol-induced liver damage by modulation of the alcohol metabolic pathway, CYP2E1 and AMPK. *Life Sci* 2013; **93**(18–19): 693-699.
[92] Naik RS, Mujumdar AM, Ghaskadbi S. Protection of liver cells from ethanol cytotoxicity by curcumin in liver slice culture in vitro. *J Ethnopharmacol* 2004; **95**(1): 31-37.
[93] Farombi EO, Shrotriya S, Na HK, Kim SH, Surh YJ. Curcumin attenuates dimethylnitrosamine-induced liver injury in rats through Nrf2-mediated induction of heme oxygenase-1. *Food Chem Toxicol* 2008; **46**(4): 1279-1287.
[94] Girish C, Koner BC, Jayanthi S, Rao KR, Rajesh B, Pradhan SC. Hepatoprotective activity of picroliv, curcumin and ellagic acid compared to silymarin on Paracetamol induced liver toxicity in mice. *Fundam Clin Pharmacol* 2009; **23**(6): 735-745.
[95] Nicoletti NF, Rodrigues-Junior V, Santos AA, Leite CE, Dias AC, Batista EL, et al. Protective effects of resveratrol on hepatotoxicity induced by isoniazid and rifampicin via SIRT1 modulation. *J Nat Prod* 2014; **77**(10): 2190-2195.
[96] Wu ZR, Bai ZT, Sun Y, Chen P, Yang ZG, Zhi DJ, et al. Protective effects of the bioactive natural product N-trans-Caffeoyldopamine on hepatotoxicity induced by isoniazid and rifampicin. *Bioorg Med Chem Lett* 2015; **25**(22): 5424-5426.
[97] Balanehru S, Nagarajan B. Protective effect of oleanolic acid and ursolic acid against lipid peroxidation. *Biochem Int* 1991; **24**(5): 981-990.
[98] Liu J, Liu Y, Parkinson A, Klaassen CD. Effect of oleanolic acid on hepatic toxicant-activating and detoxifying systems in mice. *J Pharmacol Exp Ther* 1995; **275**(2): 768-774.
[99] Saraswat B, Visen PK, Agarwal DP. Ursolic acid isolated from *Eucalyptus tereticornis* protects against ethanol toxicity in isolated rat hepatocytes. *Phytother Res* 2000; **14**(3): 163-166.
[100] Saravanan R, Viswanathan P, Viswanathan-Pugalendi K. Protective effect of ursolic acid on ethanol-mediated experimental liver damage in rats. *Life Sci* 2006; **78**(1): 713-718.
[101] Gutiérrez-Rebolledo GA, Siordia-Reyes GA, Meckes-Fischer M, Jiménez-Arellanes A. Hepatoprotective properties of oleanolic and ursolic acids in anti-tubercular drug-induced liver damage. *Asian Pac J Trop Med* 2016; **9**(7): 644-651.