PLANT LONG-CHAIN ISOPRENOID ALCOHOLS (POLYPRENOOLS) DECREASE HEPATOTOXICITY AND NEUROTOXICITY CAUSED BY ISONIAZID, AN ANTI-TUBERCULOSIS DRUG

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Summary

Objective. Treatment of tuberculosis (TB) with medications such as isoniazid may lead to antibacterial resistance, hepatotoxic and neurotoxic effects. The development of other methods of treatment for TB is needed. Ropren® is an established hepatoprotector and neuroprotector and was tested in isoniazid rat and mouse models.

Methods. Analysis of liver function was performed in rats by measuring alanine aminotransferase (ALT), aspartate transaminase, (AST), total and direct bilirubin and alkaline phosphatase (AP). The condition of the liver (density, colour, elasticity and the condition of the front edge) was also recorded and hematoxylin and eosin stained sections were analysed. The distribution of dystrophic changes in hepatocytes was determined. Neurotoxicity was evaluated in mice by measuring the onset of seizures and the subsequent mortality rate of mice.

Results. Ropren® (30 mg/kg, given concomitantly with isoniazid, total daily dose 75 mg/kg, over 14 days) protected the liver of rats from isoniazid toxicity. Loss of body weight in rats given 30 mg/kg doses of Ropren® was significantly less than in the control group and in the groups given 10 or 15 mg/kg doses of Ropren®. AST levels in rats given Ropren® (10, 15 and 30 mg/kg) were similar to levels in the control group. ALT levels decreased significantly in rats given 30 mg/kg of Ropren®. AP decreased significantly in rats given 10 and 15 mg/kg of Ropren®. The AP level in rats given 30 mg/kg Ropren® also decreased, but this was not statistically significant. Total and direct bilirubin levels were similar in treated and control rats. Rat livers from the control and experimental groups showed reduced elasticity and a colour change. Rats given 30 mg/kg Ropren® had a decrease in the weight index compared to the control group. There was a significant decrease in the degree of dystrophy in hepatocytes in rats given Ropren®. Hepatoprotective effects were more pronounced at 10 and 30 mg/kg Ropren®. Ropren® (20 and 100 mg/kg) given 40 minutes prior to isoniazid (200 mg/kg) delayed the onset of seizures in mice and improved survival rate.

Conclusion. Ropren® reduced isoniazid toxicity and had a protective effect on the liver. Ropren (20 mg/kg) also had a neuroprotective effect. Ropren® should be considered as an adjuvant treatment for TB in humans, where it could be used concomitantly with current established therapies.

Keywords: Ropren®, isoniazid, polyprenols, isoprenoid alcohols, tuberculosis, liver, hepatoprotector, neurotoxicity.

* Иллюстрации к статье – на цветной вклейке в журнал
Modern phthisiology uses prolonged and intensive chemotherapy for the treatment of tuberculosis (TB), as well as medications that reduce the side effects caused by these therapies. Liver damage caused by medications used to treat TB is a serious and frequent complication faced by people with this disease. Many studies have outlined the toxic effects of anti-TB drugs, including isoniazid [1–4]. Indeed, concomitant treatment with drugs such as rifampin or pyrazinamide has been shown to increase the risk of hepatotoxicity [2,4,5]. Drug-induced liver damage can also be age-associated, with older people more susceptible to the hepatotoxic effects of treatment [4,6].

Isoniazid, a chemotherapy commonly used to treat TB has been linked to acute liver damage, acute liver failure, a temporary increase in serum amino transferase and an increase in bilirubin levels [1,7–9]. Studies have shown that hepatotoxicity is caused by accumulation of toxic intermediates in the liver when isoniazid is metabolised. Isoniazid is acetylated by the liver enzyme N-acetyltransferase and people who are ‘slow acetylators’ are at higher risk of drug-induced liver damage [8,10].

Neurotoxic effects caused by isoniazid (including seizures, encephalopathy, memory loss and peripheral neuropathy) have also been seen in people undergoing TB treatment [2,6,11–13]. The neurotoxic effects of isoniazid, a derivative of hydrazine, have been attributed to the inhibition of enzymes (such as pyridoxine phosphokinase) that play a role in the synthesis of gamma-aminobutyric acid (GABA). GABA inhibits decarboxylase glutamic acid by having an antagonistic effect on pyridoxal phosphate (a co-enzyme of decarboxylase glutamic acid). Isoniazid disrupts the metabolism of pyridoxal phosphate and glutamic acid by crossing the blood-brain barrier and this causes a neurotoxic effect and damage to the central and peripheral nervous system. Isoniazid also causes a decrease in the levels of GABA, an inhibitory neurotransmitter in the central nervous system. Lower levels of GABA increase the likelihood of seizures [14,15].

As the use of established drugs and treatments may lead to antibacterial resistance, hepatotoxicity and neurotoxic effects, the development of other methods of treatment for TB is essential.

Studies have shown that preparations from plants may have hepatoprotective and antimycotic effects against TB [16–20]. Indeed, the use of herbs and medicinal plants may decrease the hepatotoxic effects experienced by those undergoing treatment for TB with conventional therapies [21–24]. Preparations from pine and spruce needles have been used as treatments for respiratory infections, anemia and in cases of vitamin deficiency [25,26]. Ropren®, a substance isolated from the neutral fraction of the green verdure of Picea abies (L.) Karst (comprises 95–98 % polyprenols) [27]. Purified Ropren® is diluted in natural vegetable oil and the finished form is a solution containing 25 % polyprenols and 75 % oil base [27–30]. In the liver, polyprenols are metabolised into dolichols, which are involved in the glycosylation of membrane proteins and the formation of glycoproteins [31–33]. The presence of dolichols increases with age and studies suggest that this substance may act as a scavenger of peroxidized lipids that are in the cell membrane [34]. Other studies have shown that polyprenols have a protective effect against liver injury by carbon tetrachloride and D-Gal [35].

Ropren® is involved in oxidative phosphorylation and facilitates the restoration of hepatocyte membranes. Studies in rat models with hepatitis (caused by phenacetin and dichloroethane) showed that liver function and the morphology improved in rats given Ropren® [36]. The protective effects of Ropren® were also demonstrated in a rat model with carbon tetrachloride-induced hepatic encephalopathy [37]. Ropren® has been used for the treatment of fatty liver dystrophy, hepatitis, cirrhosis and for liver damage caused by alcohol or drugs [29,32,38,39].

Indeed, studies have also shown that Ropren® has neuroprotective effects, including antidepressant-like effects [30] and effects on myelin loss in multiple sclerosis [40,41]. Ropren® improved anxiety-like and depressive-like behaviour and memory in a rat model of Alzheimer’s disease [28,42,43] and improved cognitive behaviour in rats [27,28]. Ropren® also showed a marked neuroprotective effect in a study involving a rat model with carbon tetrachloride-induced hepatic encephalopathy, which compared the neuroprotective effects of Ropren® to Heptral® (ademetionine) [37]. The more pronounced neuroprotective effect of Ropren® was caused by changes in the activity of the dopaminergic system in the brain [37]. Moreover, Ropren® has immunomodulating and antioxidant properties [44].

Given the established therapeutic effects of Ropren®, this study investigates its potential as a prophylactic substance to reduce the hepatotoxic and neurotoxic effects when used in a concomitant regimen with a current TB treatment (isoniazid).

### Materials and methods

#### Animal ethics

All experiments were carried out in accordance with the Guide for Care and Use of Laboratory Animals, published by the National Institute of Health (National Research Council, publication no. 85–23, revised in 1996), and the Animal Welfare Committee at the Institute of Phthisiopulmonology at the Sechenov I. M. First Moscow State Medical University. The Ethics Committee for Animal Research at the Sechenov I. M. First Moscow State Medical University approved the rationale, design, and methods used in this study.

**Ropren®**

Ropren® substance (pure polyprenols or long-chain isoprenoid alcohols) was isolated from the green verdure of *Picea abies* (L.) Karst. The extraction and purification of this substance has been described previously [27]. The functional activity of the registered finished form of this substance, which contains 25 % polyprenols and 75 % oil-base, has been studied extensively [27,43,45]. Ropren® was supplied by Prenolica Limited (formerly known as Solagraf Limited).
Rat model to evaluate hepatoprotective effect of Ropren®

Animals
To study the hepatoprotective effect of Ropren® (Prenolica Limited; formerly Solagran Limited, Australia) we used 32 standardised, outbred female rats (Stolbovaya animal farm, Russian Academy of Science, Russia), aged between 10 to 12 months. The animals weighed 290–350 g at the beginning of the study and had liver damage caused by the administration of isoniazid (MosChemPharmPreparaty Ltd, Moscow, Russia).

The animals were divided into four groups (one control; Group 1 and three experimental groups; Groups 2, 3 and 4) with eight animals in each group. Five intact rats (intact female group) were also placed into a separate group and used to determine the baseline levels of the biochemical parameters.

Administration of isoniazid and Ropren®
Isoniazid (25 mg/kg in 0.5 mL) was administered orally to animals in the control group (Group 1) and the three experimental groups (Groups 2, 3 and 4) three times per day with a three-hour break between dosing (total daily dose was 75 mg/kg). Animals were given isoniazid on 14 consecutive days. The eight rats in the experimental groups (Groups 2, 3 and 4) were given 0.5 mL of Ropren® (10, 15, and 30 mg/kg diluted with refined sunflower oil to 0.5 mL) concomitantly with isoniazid over 14 days. Animals from the control group (Group 1) were given 0.5 mL of vegetable oil concomitantly with isoniazid.

Rats were fed a low-protein diet consisting of bread and water to facilitate the rapid onset of the dystrophic process. Blood samples were taken from the sublingual vein of all animals on day 15. The animals were then sacrificed and histochemical analyses were carried out.

Statistical analysis
Statistical values were expressed as mean ± standard error of the mean. The significant differences between the mean values of the groups were determined using the Student t-test and the Fisher criterion test.

Blood biochemistry
Rat blood serum was analysed using a clinical chemical analyser (Sapphire 400; Japan) and the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total and direct bilirubin and alkaline phosphatase (AP) were determined.

Liver morphology and histochemistry
Pathomorphological analysis was used to evaluate the macroscopic condition of the liver (density, colour, elasticity and the condition of the front edge). The weight index (ratio of liver to body weight), an index of liver growth, was also calculated. Histological sections were taken and stained with hematoxylin and eosin. The distribution of dystrophic changes in hepatocytes was evaluated with the precise point counting method using the G. G. Avtandilov planimetric ocular grid [46] and a light microscope (×400 magnification; Leica-DM 2500, Leica Microsystems Pty Ltd, Germany).

Dystrophic changes in hepatocytes were scored using the following scale: 4 points – presence of large and small vacuoles taking up 70 % of the cytoplasm; 3 points – presence of large and small vacuoles taking up between 50 % and 70 % of the cytoplasm; 2 points – presence of large and small vacuoles taking up between 25 % and 50 % of the cytoplasm; 1 point – presence of large and small vacuoles taking up 25 % or less of the cytoplasm.

Mouse model to evaluate the neuroprotective effect of Ropren®

Animals
To study the neuroprotective effect of Ropren® we used a neurotoxic mouse model. Forty-nine sexually mature standardised, outbred white mice (Stolbovaya animal farm, Russian Academy of Science, Russia) were used in this study. The animals weighed 25–30 g and were housed in standard cages, under standard environmental conditions and had access to food and water ad libitum. Each animal was housed in a separate cage.

For the experiment, the mice were divided into the following groups: Group 1 – five male and five female mice given isoniazid (200 mg/kg) only; Group 2 – eight male and seven female mice given isoniazid (200 mg/kg) + Ropren® (20 mg/kg); Group 3 – six male and six female mice given isoniazid (200 mg/kg) + Ropren® (100 mg/kg).

Administration of isoniazid and Ropren®
Isoniazid (200 mg/kg in 0.5 mL) was given as a single dose intragastrically via a gavage. This corresponded to LD_{50} for mice. Isoniazid tablets (0.3 g; MosChemPharmPreparaty LLC, Russia) were diluted in water to the appropriate concentration.

Ropren® (20 mg/kg and 100 mg/kg in a 0.5 mL oil solution) was also administered intragastrically, 40 minutes before isoniazid was administered to the animals.
A rat model was used to study the hepatoprotective effect of Ropren®. Liver damage was induced in rats by the administration of isoniazid, a common anti-tuberculosis drug. The significance of the differences in the data were determined using non-parametric methods, namely the Pearson’s chi-square test with Yates correction. The elasticity of the liver is a good indicator of the health of this organ. Diseased livers or those damaged by toxins are stiffer than healthy livers. Post-mortem examination of the livers from the rats from the control group (Group 1) and experimental groups (Groups 2, 3 and 4) showed a reduction in the elasticity of the liver and a slight change to the color (pale, yellowish hue).

<table>
<thead>
<tr>
<th>Rat group (n=37)</th>
<th>Ropren* (mg/kg)</th>
<th>Total bilirubin (µmol/L)</th>
<th>Direct bilirubin (µmol/L)</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>Alkaline phosphatase (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (control)</td>
<td>0</td>
<td>4.45±0.18</td>
<td>2.90±0.10</td>
<td>10.05±0.52**</td>
<td>137.45±0.64**</td>
<td>390.1±35.21</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>4.18±0.29</td>
<td>2.40±0.08</td>
<td>8.60±0.96</td>
<td>133.52±4.18</td>
<td>244.00±24.62*</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>4.16±0.29</td>
<td>2.51±0.12</td>
<td>8.79±0.61</td>
<td>138.14±5.08</td>
<td>229.30±20.64*</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>5.30±0.45</td>
<td>2.81±0.04</td>
<td>6.48±0.94*</td>
<td>135.80±3.83</td>
<td>306.10±20.50</td>
</tr>
<tr>
<td>Intact untreated</td>
<td>0</td>
<td>4.79±0.37</td>
<td>2.59±0.13</td>
<td>7.73±0.7</td>
<td>92.30±3.04*</td>
<td>306.10±24.35</td>
</tr>
</tbody>
</table>

Seizures and mortality rate
Once the mice were given isoniazid they were continuously monitored for the first six hours. They were then monitored twice a day for a total of 10 days. Their behaviour (activity and grooming), the onset of tonic-clonic seizures (in minutes) and the mortality rate was recorded.

Statistical analysis
The significant differences in the data were determined using non-parametric methods, namely the Pearson’s chi-square test with Yates correction.

Results and discussion
Ropren® (30 mg/kg) decreased liver damage caused by isoniazid treatment of rats
A rat model was used to study the hepatoprotective effect of Ropren®. Liver damage was induced in rats by the administration of isoniazid.

Physiology of rats
In general, there was deterioration in the appearance of the rats (all groups) at day 4 of the study along with an observed decrease in the ability of all animals. By the end of the experiment, all rats had a decrease in body weight. Control rats (Group 1) lost 8 % of their body weight while rats given 10 mg/kg of Ropren® (Group 2) lost 7.2% and those given 15 mg/kg of Ropren® (Group 3) lost 7.3%. Even though they were given the same low protein diet, the rats given 30 mg/ml Ropren® (Group 4) lost 4.3 % of their body weight, whereas the intact rats (intact female group) lost 2 % of their body weight.

To summarise, rats given 30 mg/ml of Ropren® (Group 4) lost approximately half the percentage body weight compared to rats in the control group (Group 1). Further statistical studies of these changes might be useful.

Biochemical analysis of female rat serum
When compared with the intact female group of rats, the animals in the control group (Group 1) had a statistically significant increase in ALT (10.05 U/L and 7.73 U/L, respectively; p<0.05) and AST levels (137.45 U/L and 92.30 U/L, respectively; p<0.01) (Table 1). The AST levels in rats given Ropren® (Groups 2, 3 and 4) did not differ greatly from the level found in the rats from the control group (Group 1) (Table 1).

ALT levels in the rats decreased in the experimental groups (Groups 2, 3 and 4) when compared to the control group (Group 1), although the only statistically significant decrease occurred in Group 4 rats given 30 mg/kg Ropren® (6.48 U/L and 10.05 U/L, respectively; p<0.05) (Table 1).

There was an increase in the AP level when control rats (Group 1) were compared to the animals in the intact female group (390.1 U/L and 306.1 U/L, respectively), although this increase was not statistically significant (Table 1). On the other hand, rats from the groups given 10 mg/kg and 15 mg/kg Ropren® (Groups 2 and 3, respectively) showed a statistically significant decrease in AP levels when compared to the control group (Group 1) (244 U/L and 229.3 U/L compared to 390.1 U/L, respectively; p<0.05) (Table 1). The AP level in rats given 30 mg/kg Ropren® (Group 4), also decreased, although this was not statistically significant (Table 1).

The levels of total and direct bilirubin were relatively similar across all groups of animals (Table 1). A slight increase in the level of total bilirubin was observed in the rats given 30 mg/kg Ropren® (Group 4) but this was not statistically significant.

The results showed that generally, 30 mg/kg Ropren® offered some protection to the liver from the toxic effect of isoniazid.

Morphological and histochemical analysis of rat liver
The elasticity of the liver is a good indicator of the health of this organ. Diseased livers or those damaged by toxins are stiffer than healthy livers. Post-mortem examination of the livers from the rats from the control (Group 1) and experimental groups (Groups 2, 3 and 4) showed a reduction in the elasticity of the liver and a slight change to the color (pale, yellowish hue).

There was a statistical difference between the weight index of the control group (Group 1) (3.38±0.06) of rats when compared to the intact group (3.08±0.05) (p<0.05). Among the three experimental groups, rats given 30 mg/kg Ropren® (Group 4) had a weight index similar to the intact group and lower than the control group (Group 1) (p<0.05). Rats given 10 mg/kg Ropren®
(Group 2) had a weight index lower than the control group (Group 1 but higher than the intact group, while those given 15 mg/kg Ropren® (Group 3) had a higher weight index than both the control (Group 1) and intact groups (p<0.05) (Table 2).

Histological analysis of the livers of rats from the control group (Group 1) showed that the overall architecture of the liver (lobular structure) was preserved. However, there was a difference in the level of protein and fat dystrophy in the liver samples. Most of hepatocytes in the centre and on the periphery of the lobes had marked protein and fatty dystrophy with small, medium and large vacuoles. There were a few hepatocytes with two nuclei (Figure 1*). Morphometric analysis showed that 65.4 % of hepatocytes showed signs of dystrophy (Table 3). The degree of dystrophy (as measured with the Avtandilov planimetric ocular grid; see Materials and methods) was 3.25 points (Table 3).

In the liver sections stained with hematoxylin and eosin, there was a moderate amount of protein and lipid dystrophy in some of the hepatocytes found predominantly on the periphery of the lobes (Figures 2 and 3*).

Hepatocytes in the centre of the sections had preserved clear borders and eosinophilic weakly stained cytoplasmic granules (Figures 2 and 3*).

Rats given Ropren® (Groups 2, 3 and 4) had a decrease in hepatocytes showing signs of dystrophy compared to those in the control group (Group 1) (Table 3). In particular, rats given 10 mg/kg (Group 2) and 30 mg/kg Ropren® (Group 4) (41.8 and 56.7 %, respectively) had a statistically significant decrease (p<0.01) in hepatocytes showing signs of dystrophy compared to the control (Group 1) (65.4 %). Although there was a decrease in hepatocytes showing signs of dystrophy in rats given 15 mg/kg Ropren® (Group 3), this change was not statistically significant (Table 3).

All groups of rats given Ropren® (Groups 2 and 3; p<0.05 and 4 p<0.01) showed a statistically significant decrease in the degree of dystrophy (as measured with the Avtandilov planimetric ocular grid) when compared to the control group (Group 1) (Table 3).

Overall, administration of Ropren® to rats lessened the effects of liver damage caused by isoniazid. The hepatoprotective effects of Ropren® were more pronounced at the 10 and 30 mg/kg doses.

**Ropren® decreases severity of seizures and the number of deaths in mice treated with isoniazid**

A mouse model was used to study the neuroprotective effect of Ropren®.

### Behavioral and clinical characteristics of mice

When mice were given isoniazid (200 mg/kg), they showed some changes in behaviour. Between 20 and 40 minutes after administration of isoniazid, the mice exhibited an increase in excitement level, an increase in motor activity and the onset of tonic-clonic seizures. Fifty per cent of the mice died of acute respiratory insufficiency that developed at the peak of the tonic-clonic seizures. The animals that survived became motionless and did not consume any food or water.

Increased behavioural excitement, increased motor activity and the presence of tonic-clonic seizures stopped after 24 hours. The mice were monitored for 10 days and during this period, no changes in behaviour were observed and no more animals died.

The time of onset of seizures and the mortality rate of mice differed among the four experimental groups (Table 4). Mice given isoniazid (200 mg/kg) alone experienced the onset of seizures after 45 to 60 minutes of administration of this medication. Half of the mice in this group died (Table 4). In comparison, mice given Ropren® (20 or 100 mg/kg) 40 minutes prior to isoniazid (200 mg/kg), did not have seizures until between 80 and 100 minutes after administration of the substance (Table 4). Approximately 13 % of mice died in the group given isoniazid (200 mg/kg) and Ropren® (20 mg/kg). This result was statistically significant (p<0.05) when compared to the group of mice given isoniazid (200 mg/kg) alone (Group 1) and when compared to the isoniazid (200 mg/kg) and Ropren® (100 mg/kg) group (Group 3).

Approximately 33 % of the mice in the isoniazid (200 mg/kg) and Ropren® (100 mg/kg) group died (Table 4). Ropren® was more effective at the lower dose (20 mg/kg) than at the higher dose (100 mg/ml) and this is consistent with findings from our other neurotoxicity studies.

Administering Ropren® as a prophylactic delayed the onset on clonic-tonic seizures in mice and improved the survival rate of the animals. Ropren® has the capacity to reduce the neurotoxic effects of isoniazid and have a protective effect.

### Table 2

<table>
<thead>
<tr>
<th>Rat group (n=37)</th>
<th>Ropren® (mg/kg)</th>
<th>Weight index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (control)</td>
<td>0</td>
<td>3.38±0.06**</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>3.26±0.01</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>3.56±0.13**</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>3.08±0.07*</td>
</tr>
<tr>
<td>Intact untreated</td>
<td>0</td>
<td>3.08±0.05</td>
</tr>
</tbody>
</table>

* On color insert.
plant long-chain isoprenoid alcohols (polyprenols) decrease hepatotoxicity and neurotoxicity caused by isoniazid… | экспериментальное обоснование применения ропрена…

<table>
<thead>
<tr>
<th>Rat group (n=32)</th>
<th>Ropren* (mg/kg)</th>
<th>Hepatocytes showing signs of dystrophy (%)</th>
<th>Dystrophic changes in hepatocytes (points*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (control)</td>
<td>0</td>
<td>65.40±2.5</td>
<td>2.70±0.4*</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>41.80±3.4**</td>
<td>2.70±0.46*</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>56.70±5.6</td>
<td>2.84±0.2*</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>42.70±3.8**</td>
<td>1.85±0.18**</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mouse group (n=49)</th>
<th>Test substance and concentration</th>
<th>Onset of seizures (minutes)</th>
<th>Mortality rate (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n=10)</td>
<td>Isoniazid (200 mg/kg)</td>
<td>45–60</td>
<td>5 (50.0 %)</td>
</tr>
<tr>
<td>2 (n=15)</td>
<td>Isoniazid (200 mg/kg) + + Ropren* (20 mg/kg)†</td>
<td>80–100</td>
<td>2 (13.3 %)*#</td>
</tr>
<tr>
<td>3 (n=12)</td>
<td>Isoniazid (200 mg/kg) + + Ropren* (100 mg/kg)†</td>
<td>80–100</td>
<td>4 (33.3 %)</td>
</tr>
</tbody>
</table>

**Table 3.** Morphometric analysis of rat liver.

* statistically significant (p<0.05) when compared to control group; ** statistically significant (p<0.01) when compared to control group.

# Dystrophic changes in hepatocytes were scored using the following scale: 4 points – presence of large and small vacuoles taking up 75% of the cytoplasm; 3 points – presence of large and small vacuoles taking up between 50% and 70% of the cytoplasm; 2 points – presence of large and small vacuoles taking up between 25% and 50% of the cytoplasm; 1 point – presence of large and small vacuoles taking up 25% or less of the cytoplasm.

**Table 4.** The effects of Ropren* on neurotoxic side effects caused by isoniazid treatment of mice.

† Ropren* was administered 40 minutes before isoniazid; * statistically significant (p<0.05) when compared to Group 1; # statistically significant (p<0.05) when compared to Group 3.

**Conclusion**

The results from the studies described in this manuscript show that Ropren* can reduce the toxic effects of isoniazid and have a protective effect on the liver. Ropren* (20 mg/kg) can also have a protective effect on the neurological system. Ropren* should be considered as an adjuvant treatment for TB in humans, where it could be used in conjunction with current established therapies to decrease toxic side effects.

**Conflict of interest**

Dr Soultanov is an academic scientist involved in decades of research into substances from conifer needles in Russia. He is a Director and shareholder of Prenolica Limited (formerly Solagran Limited), which is the company that is commercialising the technology. Prenolica Limited supplied the Ropren* that was used in the studies described in this manuscript. Dr Trusov is the Director of the Medical Department of Solagift Pty Ltd in Tomsk, Russia. The remaining authors declare that there is no conflict of interest in this research.

**Acknowledgments**

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**References**


К статье
Экспериментальное обоснование применения ропрена для профилактики гепатотоксических и нейротоксических реакций, вызванных изониазидом (Plant long-chain isoprenoid alcohols (polyprenols) decrease hepatotoxicity and neurotoxicity caused by isoniazid, an anti- tuberculosis drug) – p. 102–109

Figure 1.
Morphological changes in the liver of rats treated with isoniazid only. Hematoxylin and eosin staining. Magnification x400. Note the presence of hepatocytes with two nuclei.

Figure 2.
Morphological changes in the liver of rats treated with Ropren® (10 mg/kg). Hematoxylin and eosin staining. Magnification x400. There was moderate protein and lipid dystrophy, predominantly on the periphery of the lobes. Hepatocytes in the centre of the sections had preserved clear borders and weakly stained eosinophilic cytoplasmic granules.

Figure 3.
Morphological changes in the livers of rats treated with Ropren® (30 mg/kg). Hematoxylin and eosin staining. Magnification x400. There was moderate protein and lipid dystrophy, predominantly on the periphery of the lobes. Hepatocytes in the centre of the sections had preserved clear borders and weakly stained eosinophilic cytoplasmic granules.

К статье
Инвазивный аспергиллез с поражением органов пищеварения: описание клинического случая аспергиллеза толстой кишки и результаты проспективного исследования (стр. 124–127)

Рисунок 1.
Послеоперационный материал (резецированный участок слепой кишки). Язва в области купола слепой кишки 3х4 см.

Рисунок 2.
Гистологический препарат (окраска по Гомори-Гроккоту). Гифы мицелия, ветвящиеся под острым углом, x200