EVALUATION OF THE HEPATOPROTECTIVE ACTIVITY OF LEAF EXTRACTS OF *MIMOSA PUDICA* ON WISTAR RATS

Abstract

The liver is an important metabolic organ of the body that can be subjected to conditions with serious repercussions. The management of hepatic pathologies is very often complex and sometimes requires transplants, hence the interest in the development of new strategies, including plant-based drugs to address liver related challenges. *Mimosa pudica*, of the Mimosaceae family, has been described in Ayurvedic medicine for treating many ailments and moreover liver disease which is the aim of this study. After performing an extraction following the standard procedures, an *in vivo* evaluation of the hepatoprotective activity of *M.pudica* leaf extracts on Wistar rats was conducted using standard assays, which consisted of the dosage of biochemical parameters such as Aspartate amino transferase (AST), Alanine amino transferase (ALT), Alkaline phosphate (AP), Creatinine, total proteins, total cholesterols, triglycerides, albumin, high density lipoproteins-cholesterol (HDL-cholesterol), low density lipoproteins-cholesterol (LDL-cholesterol) uric acid, albumin, urea, malondialdehyde (MDA), Superoxide dismutase (SOD), Glutathione, Catalase and histopathological analysis of rat's liver. Analysis of biochemical parameters showed a significant reduction in the liver damage in rats treated with *M. pudica* leaf extracts, and less marked liver damage as compared to the positive control group was a promising indicator of hepatoprotection. Histopathology analysis showed no observable damage of organs treated with the plant extracts

Keywords: *Mimosa pudica*, hepatoprotection, leaf extract, Wistar rats

INTRODUCTION

The liver is an organ involved in several body functions including coagulation; detoxification of xenobiotics; metabolism of different metabolites as well as the control of their concentration [1]. This explains why the slightest liver disease could have serious consequences on human health. Liver diseases can be caused by, among other things: viral infections (hepatitis B and C) and drugs. In Europe, in the year 2019, approximately 29 million people died from liver diseases mainly by excessive alcohol consumption representing 3% of all death for the same year [2].

In Africa, hepatitis B and C virus infections affect more than 70 million people (60 million and 10 million respectively) with 90% of people living with these infections [3]. The management of these pathologies is most often expensive and not accessible for developing countries and especially for their landlocked areas. To this end, it is therefore important to explore new therapeutic sources, including plant-based drugs, which constitute an important source of molecules with therapeutic activities. Herbal medicines represent a special and important form of traditional medicine in which the practitioner specializes in the use of planta to treat different ailments [4]. The WHO estimates that 70 to 95% of the population in developing countries would use traditional medicine, more precisely phytotherapy, to solve their health problems [5]. Traditional medicine has been for many years a therapeutic source

for people's health problems around the world, including various methods such as the use of plants [6].

Mimosa pudica, a plant of the Mimosaceae family, has been described in Ayurvedic medicine as having many pharmacological properties, in traditional medicines it is used since a long time to treat hypertension, hyperglycemia, depression, insomnia, intestinal parasite, inflammation, malaria, asthma, diarrhea, hepatotoxicity [7]. *M.pudica* are found in many countries like Mexico, Brazil, India, Bangladesh, China, Indonesia, Madagascar, South America, tropical Africa [8]. It is a plant with short thorny branches, glandular hairs; bipinnate leaves, sensitive to the touch; flower head globular, lilac-pink; stem erect, slender, prickly and well branched; campanulate calyxes; petals crenate towards the base; pods 1.5 to 2.5 cm long narrowly spiny on sickle sutures [9]. In Ayurvedic medicine it has as therapy against leprosy, dysentery, vaginal and uterine affections, inflammation, asthma, fatigue and liver affection [9]. This study focuses on the hepatoprotective activity of leaf extracts of this plant.

MATERIALS AND METHODS

Plant material

The study was carried out in the laboratory of preclinical animal studies and toxicology of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé I, and the collection of samples was done within the campus of the same Faculty from November 2021 to march 2022. The plant material consists of leaves of *Mimosa pudica*. This species was identified at the National Herbarium of Cameroon in comparison with herbarium specimen N 57673/HNC. The harvested leaves were dried at room temperature away from direct sunlight likely to modify the molecules they contain and the dried leaves were ground into a powder with an electric robotic blender.

Extraction techniques used for the preparation of the leaf extracts were conducted using four methods:

- Maceration: here we used two different solvents: water and ethanol. 100g of *M.pudica* leaf powder was weighed. 300ml of solvent was added to the powder and the mixture was left at room temperature for 48 hours, while stirring regularly. After 48 hours, the mixture was filtered to separate the waste using Whatman filter paper No 1 [10].

- Infusion: The same principle as in maceration was used, except for the fact that in this case the solvent used was boiled water at 100 ºC [10].

- The decoction: in this method the water-leaf powder mixture was heated for 30 min in a water bath and cooled at room temperature ±25 ºC. Once cooled, the waste was separated and the concentrated extract was decanted and filtered [10].

Animal Material

Albino male Wistar rats (*Rattus norvegicus*) having masses between 80-100 g, 12 months weeks, were used. They were raised in the animal facility of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé I

Hepatoprotective activity evaluation

Distribution of animals

42 rats were used, divided into 7 groups of 6 rats each. The control group 1 received distilled water. The positive control group 2 received ethanol to induce hepatotoxicity [11]. Test groups 3-6 were treated with both ethanol and their corresponding plant extract while Group 7 was treated with ethanol and silymarin a reference hepatoprotective medication. [12]. All the groups were treated for a period of 28 days. Table 1 shows the distribution and treatment that each group of rats received during the experiment:

Table I : distribution and treatment of the different animals in the study groups

Groups	Treatment
Group 1	Healthy control, receives 2ml of distilled water
Group 2	Positive control, receives a 40% ethanol at a dose of 2ml/100g body weight for 28 days
Group 3	Receives 300mg/kg of extract obtained by ethanolic maceration + 2ml/100g of body mass of a 40% ethanol
Group 4	Receives 300mg/kg of extract obtained by aqueous maceration + 2ml/100g body weight of a 40% ethanol
Group 5	Receives 300mg/kg of extract obtained by infusion + 2ml/100g of body weight of a 40% ethanol
Group 6	Receives 300mg/kg of extract obtained by decoction + 2ml/100g of body mass of a 40% ethanol
Group 7	Receives silymarin at a dose of 300mg/kg + 2ml/100g body weight of a 40% ethanol

Preparation of solutions to be administered

Extract solutions

According to the OECD guidelines N°420: Acute Oral Toxicity – Fixed Dose Procedure, which was applied in this study, each animal received not more than 2ml/100g concentration of extract and. the following formula was used to determine the concentration to be administered:

$$Volume\ to\ be\ administered(ml) = \frac{\left[dose\ \left(\frac{mg}{Kg}\right) x\ animal\ weight\ (Kg)\right]}{weight\ concentration\ (\frac{mg}{kg})}$$

From this formula we could therefore determine the mass of extract required (weight concentration) in order to administer in 1ml of solution:

$$weight\ concentration\ \left(\frac{mg}{ml}\right) = \frac{\left[Dose\ \left(\frac{mg}{Kg}\right) x\ animal\ weight(Kg)\right]}{Volume\ to\ be\ administred\ (ml)}$$

Preparation of silymarin

Silymarin was the reference drug of choice used for the treatment of chronic liver disease and liver cirrhosis. This drug was used as a reference as it effectively prevents multiple liver disease mechanisms and ensure protection, regeneration, and rejuvenation of the liver. The preparation of the silymarin solution followed the same OECD guidelines N°420: Acute Oral Toxicity – Fixed Dose Procedure. So, in order to administer 300mg/Kg we must take 750 mg of silymarin. The specialty used in this study was Silybon® 140 mg tablet of which 6 tablets were crushed and dissolved in 25ml of distilled water. We prepared 40 % ethanol solution while respecting the volume limitations in the OECD guidelines. We prepared the 40% ethanol from a 96% ethanol solution and administered 2ml/Kg to each rat.

Induction of hepatotoxicity

In order to induce hepatotoxicity, the animals received 2ml/100g body weight of 40% ethanol for 28 days. During this period the rats were maintained on a standard laboratory diet. At 29 days, the rats were sacrificed by cervical dislocation and their blood, kidneys and liver were collected for further biochemical and histological analysis.

Quantification of biochemical parameters

Liver and kidney function parameters such as AST [13], ALT [14], AP [15], Creatinine[16], total proteins[17], total cholesterols [18], triglycerides[19], albumin[20], HDL-cholesterol [18], low LDL-cholesterol [18], uric acid [21], urea [22]. This was carried out using a commercial assay kit (Chronolab SYS S.L. Avenida diagonal 609, planta 10, DRU28 Barcelona. Spain).

Quantification of Malondialdehyde, Superoxide dismutase, Glutathione, Catalase

Malondialdehyde assay

Malondialdehyde is a carbonyl compound resulting from the decomposition of fatty acid hydroperoxides. It reacts with thiobarbituric acid to give pink colored chromophores whose concentration is determined by absorbance at 500 nm [23].

Reduced Glutathione dosage

2,2'-Dithio -5,5'bis (2-nitrobenzoic) acid reacts with the SH groups of glutathione to form a colored complex which absorbs at 412 nm [24].

Catalase Assay

Hydrogen peroxide is broken down in the presence of catalase. This destroyed peroxide binds to potassium dichromate to form a green precipitate of unstable perchloric acid, which is then destroyed by heat to form a green complex which exhibits an absorption maximum at 570 nm. The catalase activity is proportional to the optical density of the complex was obtained from the calibration curve of hydrogen peroxide [25].

Histopathological analysis

Histology allows the preparation of tissues/organs for observation under the microscope. The techniques used are those described by Cannet.

Statistical analysis

The various results obtained were expressed by parameters such as the mean, standard deviation and mode. Comparison between groups was performed using the analysis of variance (ANOVA) followed by the post hoc Dunnett test using statistical analysis software Graph Pad Instat version 5.0.

Results

Hepatoprotective activity evaluation

Assessment of zootechnical parameters

Table 2 summarizes the different data obtained during the evaluation of zootechnical parameters. No interpretation of zootechnical parameter results

Table 2: Assessment of the zootechnical parameters of all study groups

Groups	Food intake	Water intake	Weight gain
Healthy group	118,88 ± 46,39	124,31 ± 38,85	42,83 ± 13,44
Positive group	109,81 ± 48,09	122,35 ± 38,26	49,00 ± 5,29
Aqueous maceration	122,80 ± 61,77	90,31 ± 25,19**	49,00 ± 9,54
Hydroethanolique maceration	105,27 ± 40,53	116,04 ± 40,41	62,33 ± 4,16
Decoction	112,00 ± 48,78	95,42 ± 30,57*	45,25 ± 5,74
Infusion	114,27 ± 66,37	89,23 ± 32,63**	45,00 ± 5,00
Sylimarine	132,86 ± 66,60	69,00 ± 30,23***	47,33 ± 15,50

Values are expressed in terms of mean ± standard deviation, (n = 3). The comparison between the groups is carried out using the test of variance (ANOVA) followed by Dunnet's post hoc test; *p<0.05, **p<0.01; ***p < 0.001 is considered significant compared to the control.

Biochemical parameters

Table 3 indicates the treatment of animals with plant extracts followed by the administration of ethanol at a dose of 1ml/100g.There is an increase in AST, which is a marker found in skeletal muscle cells and cardiac cells as well as in liver cells, in the negative control group and a non-significant decrease with a p-value > 0, 05 in the groups having received the various preparations of the extract, and reference medicinal product while significant reduction was observed in the healthy control group with a p-value <0.01.

The treatment of animals with plant extracts followed by the administration of ethanol at a dose of 1ml/100g shows an increase in ALT, which is a marker found in liver and kidney cells in the negative control group and a non-significant decrease with a p-value > 0.05 in the groups having received the various preparations of the extract, and reference medicinal product while its concentration is significantly reduced in the healthy control group with a p-value < 0.05.

The administration of the extracts and the reference drug did not significantly affect the serum albumin level and the much greater decrease in the negative control group could indicate liver, kidney and intestinal damage with a p-value > 0.05.

The administration of the extract shows a greater amount of total protein in the negative control group indicating inflammation and a significant decrease in the groups having received the extract as well as the reference drug.

The administration of the extracts followed by the administration of ethanol leads to a rise in the creatinine level much higher in the negative control group and significantly reduced in the healthy control groups, the decoction, the infusion and the aqueous extract and a non-significant decrease in the hydro-ethanolic extract.

The administration of the extracts followed by the ethanol treatment leads to an increase in the total cholesterol level in the negative control group and a non-significant decrease in the other groups with a p-value > 0.05.

On the other hand, we found a high quantity of triglycerides and LDL-cholesterol in the negative control group and a significant decrease in all the other groups.

With regard to HDL-cholesterol, a decrease is observed in the negative control group and a non-significant increase in the healthy control groups and those that received the plant extracts.

The administration of ethanol resulted in an increase in lipid peroxidation in the group that received only distilled water, a non-significant decrease in the groups that received the extract and a significant decrease in the healthy control group. A decrease in the endogenous antioxidants superoxide dismutase, catalase and glutathione is observed in the negative control group and an increase in the positive control groups and the groups that received different fractions of the extract.

Table3: Effects of extracts on liver damage

Parameters	Healthy group	Negative control	Hydro ethanolic	Infusion	Decoction	Silymarin	Aqueous maceration
AST (µmole/min/ml)	11,77 ± 4,53	2,60 ± 0,98**	9,76 ± 1,81	5,78 ± 2,72	6,77 ± 0,31	6,33 ± 0,93	5,54 ± 0,64
ALT (µmole/min/ml)	8,92 ± 4,30	2,74 ± 0,87*	6,70 ± 1,33	4,91 ± 2,00	7,56 ± 1,43	3,71 ± 0,93	4,74 ± 1,95
AP (µmole/min/ml)	33,48 ± 8,05	2,86 ± 0,79***	9,71 ± 3,07***	5,51 ± 0,96***	7,28 ± 0,89***	3,71 ± 2,03***	5,96 ± 2,63***
Albumin (g/dl)	18,54 ± 7,84	28,1 ± 1,29	20,31 ± 9,31	28,53 ± 3,96	25,47 ± 1,15	24,30 ± 10,03	24,29 ± 3,07
Total proteins (mg/dl)	31,46 ± 9,22	5,33 ± 2,11***	13,61 ± 3,61*	8,52 ± 4,31***	9,39 ± 0,58**	6,58 ± 1,96***	7,81 ± 1,48***
CrEatinin (µmole/ml)	8,17 ± 1,89	2,13 ± 0,48***	6,83 ± 0,58	6,38 ± 0,48	4,50 ± 1,41**	3,17 ± 0,29***	5,33 ± 0,29*
Total cholesterol (mmol/l)	66,70 ± 38,10	31,12 ± 18,43	34,22 ± 25,03	21,27 ± 1,11	52,03 ± 33,51	12,61 ± 0,54	10,27 ± 3,40
Triglycerides (mmol/l)	103,05 ± 22,03	22,47 ± 11,87***	29,72 ± 14,82***	35,32 ± 2,49***	53,33 ± 0,73**	12,27 ± 1,11***	13,04 ± 0,50***
HDL –Cholesterol (mmol/ml)	0,009 ± 0,008	0,03 ± 0,02	0,02 ± 0,01	0,01 ± 0,002	0,02 ± 0,01	0,02 ± 0,003	0,02 ± 0,0005
LDL-Cholesterol (mmol/l)	51,50 ± 11,02	3,22 ± 1,09***	14,84 ± 7,40	16,98 ± 1,70*	26,65 ± 0,37***	5,35 ± 0,17***	6,51 ± 0,26***
MDA (µmole/l)	11,66 ± 4,35	3,91 ± 0,86**	7,17 ± 1,86	6,66 ± 0,59	6,81 ± 2,72	6,56 ± 1,68	6,30 ± 0,72
Catalase (µmole/min/ml)	80,92 ± 5,94	123,90 ± 18,56	104,89 ± 39,66	109,63 ± 50,33	106,21 ± 31,06	112,29 ± 31,06	158,30 ± 10,68

SOD (µmole/mg)	288,08 ± 110,93	369,07 ± 4,36	365,89 ± 7,23	362,94 ± 23,10	361,67 ± 15,13	347,08 ± 15,41	369,91 ± 3,02
Glutathione (µmole/mg)	2,73 ± 1,67	8,76 ± 0,48*	5,58 ± 2,04	7,05 ± 2,19	7,80 ± 2,94	8,06 ± 3,01	7,30 ± 1,45

The results are expressed as mean ± standard deviation with n = 5; Data analysis was performed using the ANOVA test, followed by Turkey Kramer's post hoc multiple comparison test. The differences were considered significant from the p-value *p<0.05; **p<0.01. ***p<0.001.

Histopathological study

Histological analysis of the liver of the different groups of animals did not show any major changes in the internal structure of the liver, normal architecture of the centrilobular vein, the sinusoids and hepatocytes in the groups treated with the plant extract and the reference drug. Obstruction of the central vein in the negative control group which may suggest fatty liver (Figure 1).

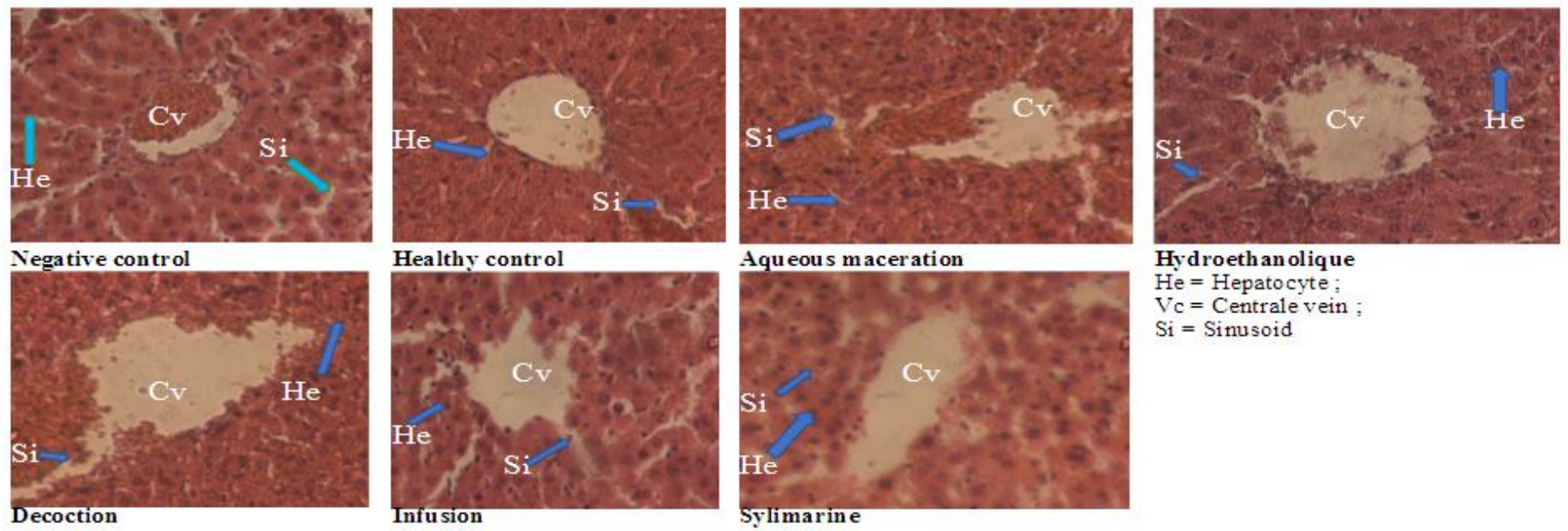

Figure 1: Histological sections of the liver of animals from the different study groups

Discussion

Hepatoprotective plants can be classified into three categories: hepatotoxin antagonists, hepatic protectors and healing promoters. A large number of plants have demonstrated hepatoprotective properties with approximately 160 active substances from 101 plants [26].

The evaluation of liver function is generally done by measuring biochemical parameters in the serum, namely ALT, AST, ALP, glutamyl aminotransferase (GGT) and total bilirubin [27]. ALT is an enzyme present mainly in liver cells but also in muscles, adipose tissue, intestine, colon, prostate and brain in lower concentrations. The main function of this enzyme is to catalyze the transfer reaction of an amine group from L-alanine to α-ketoglutarate with the production of L-glutamate and pyruvate in the liver. In the case of liver disease, the liver cells are affected and lead to an increase in serum ALT [28], the origin of which may be infectious, drug-induced, autoimmune or by ingestion of chemical substances such as ethanol. Ethanol is a substance capable of inducing hepatotoxicity by reducing the oxygen supply and thus promoting the formation of free radicals responsible for oxidative stress [29]. Silymarin was the reference drug of choice used in this study for the treatment of chronic liver disease and liver cirrhosis. This drug was used as a reference as it effectively prevents multiple liver disease mechanisms and ensure protection, regeneration, and rejuvenation of the liver [30]. AST allows the transfer of an amine group from L-aspartate to α-ketoglutarate to produce oxaloacetate and L-glutamate [31]. The evaluation of the hepatoprotective activity at 300mg/kg of extracts revealed a significant reduction in ALT which is a marker of hepatic cytolysis but also of AP marker of biliary damage but also of bone damage [32], AST, and total protein. These results are greater in rats treated with the aqueous extract. This is in agreement with the results obtained by Kumaresan et al [33] in 2015. This significant reduction could be explained by the presence of polyphenolic compound having antioxidant properties and protecting the liver from alterations. Elevated serum ALT is considered to be more specific for liver damage [34]. This reduction of serum transaminases can be explained by a mechanism inducing an increase in biomolecules contributing to the protection of hepatocytes by acting as an anti-inflammatory, anti-apoptotic agent or free radical scavenger [35].

Reactive oxygen species are biochemical substances produced during various aerobic processes which can cause alterations of macromolecules such as lipids, proteins, DNA and which are the basis of diseases such as cancers, arthritis rheumatoid [36]. The dosing of antioxidant parameters revealed a significant increase in antioxidant markers in vivo and all the more marked in rats treated with the aqueous extract. These results are in agreement with those obtained by Nazeema et al [37] year? in their study on the anti-hepatotoxic and antioxidant defense potential of *Mimosa pudica*. The reduction of lipid peroxidation responsible for liver damage could explain the results obtained. Substances such as flavonoids and many others compounds contained in leaf extracts could justify this reduction of oxidative stress by induction of endogenous antioxidant enzymes, inhibition of lipid peroxidation and also by free radical scavenging [38].

In the histopathological section of the liver, we noted a normal architecture of the centrilobular vein, sinusoids and even that of the hepatocytes in the groups treated with the extracts and an obstruction of the central vein in the negative control group which could suggest hepatic steatosis. This does not correspond to the results obtained by Rajendran et al

[39] in 2009 in their study on the hepatoprotective activity of *M.pudica* leaves. This difference can be explained by the use of a different hepatotoxicity inducer in the present study, which is ethanol. Several models of hepatotoxicity induction with different substances are proposed depending on the desired liver damage. Thus, we will distinguish substances which will cause hepatic lesions characteristic of nonalcoholic fatty liver disease, hepatocellular carcinoma, liver cirrhosis, liver fibrosis and substances like carbon tetrachloride are more indicated for fibrosis and cirrhosis models [40].

Conclusion

The present study which had as its objective, to evaluate the hepatoprotective activity of *Mimosa pudica* leaves extracts showed that the extracts leaves possess hepatoprotective power on rats treated with ethanol with a much greater power with the extract obtained by aqueous maceration. The main limitation of this study is the impossibility of determining the therapeutic range of plant extracts due in particular to a lack of *M. pudica* leaves. Further studies on the elucidation of the mechanism of action of hepatoprotection as well as the molecules or families of molecules involved should be considered.

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