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Original Research Article

Potential Effects of *Ipomoea reptans Poir.* Extract on LDL, HDL levels and liver Histopathology

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Abstract

Background: *Ipomoea reptans Poir*. extract has been known to have antioxidant, antihypertensive, antidiabetic and anti-obesity activities. The content of the secondary compounds antioxidant in the extract is believed to play an important role in this mechanism also often reported that have regulating cholesterol levels.

Objective: This study aims to investigate the antioxidant activity of *I. reptans* extract on changes in LDL and HDL and liver histopathology in animal models of hypercholesterolemia that treated with *I. reptans* extract.

Methods: Twenty-five wistar rats were randomly divided into 5 groups: normal group (N), hypercholesterolemic group (Chol), *I. reptans* extract group at a dose of 200 mg/kg BW, 300 mg/kg BW and 400 mg/kgBB (IE 200; IE 300, and IE 400). Rats in group Chol and IE were induced by cholesterol in the form of a mixture of quail egg yolk and animal oil for 21 days. Then, continued with therapy with *I. reptans* extract in the IE group only according to their respective doses for 14 days. LDL and HDL levels were measured using enzymatic colorimetric, and liver organs were also taken for histopathological analysis with hematoxylin-Eosin (HE) staining.

Results: The results showed that the average LDL levels in the N, Chol, IE 200, IE 300 and IE 400 groups were 8.56 ± 1.36, 29.9 ± 1.05, 25.22 ± 4.72, 9.12 ± 0.72, and 9.22 ± 0.77 mg/dL, while the average HDL levels in the were 38.1 ± 2.24, 15.92 ± 4.39, 33.4 ± 5.91, 36.92 ± 0.47, and 42.82 ± 3.27 mg/dL respectively. Liver histopathology in the IE 300 group showed reduced fatty degeneration compared to the Chol group.

Conclusion: It can be concluded that the administration of *I.reptans* extract in hypercholesterolemia rats was able to ameliorates LDL and HDL levels, and improve liver histopathology.

Keywords: *Hypercholesterolemia; Ipomoea reptans; LDL; HDL; Liver Histopathology*

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INTRODUCTION

Hypercholesterolemia is a non-communicable disease that can cause chronic illness to death in a person. It is characterized by having low-density lipoprotein (LDL) cholesterol levels above normal or high-density lipoprotein (HDL) cholesterol in the blood below normal. Researchers calculated that increases in LDL cholesterol in 2017 caused nearly 4 million deaths, accounting for one third of deaths from ischemic heart disease and stroke. These deaths markedly decreased in Western countries while significantly increasing in Asian countries[1]. In Indonesia, it was recorded that the percentage of the population aged >15 years had LDL levels above normal by 73.8%, total cholesterol above normal by 28.8%, and low HDL levels by 24.3%.[2]. People with high blood cholesterol are at increased risk of heart disease (the leading cause of death) and stroke (the fifth leading cause of death), atherosclerosis, coronary heart disease, diabetes mellitus, and liver[3].

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The condition of hypercholesterolemia causes an increase in excess fat production in the liver so that accumulation (steatosis) occurs, leading to a more serious and even fatal condition, namely nonalcoholic steatohepatitis (NASH) and even death[4].

Currently, medicinal plants have also become a concern and not a few are exploring these plants from the medical community. This is because medicinal plants are easier to obtain and tend to have fewer side effects[5,6]. One of the plants that is very easy to cultivate, has a very affordable price and is often even consumed by Indonesian people is ground kale (*Ipomoea reptans Poir.*). This plant is often cooked and used as a companion vegetable for daily consumption by Indonesians.

In a previous study, it was concluded that the aqueous extract of *I.. reptans* family, *Ipomoea aquatica*, at a dose of 400 mg/kg had activity in controlling dyslipidemia in albino rats. Treatment with the extract was reported to be able to improve lipid profiles by lowering cholesterol and LDL levels and significantly increasing HDL levels[7]. Furthermore, in 2020, *I. reptans* extract was reported to have antioxidant, antidiabetic and anti-obesity activities[8]. The high antioxidant activity *in I. reptans* extract cannot be separated from the role of the polyphenol and flavonoid compounds contained therein. According to Laka, K. et al, flavonoids and polyphenols act as antioxidants and are proven to be able to suppress the activity of the HMG-CoA reductase enzyme so that total cholesterol biosynthesis can also be suppressed. Antioxidant activity is also able to inhibit LDL oxidation[9,10].

The role of antioxidant activity of *I. reptans* extract against hyperglycemia model rats has been extensively studied and its efficacy is known. However, until now, the role of *I. reptans* extract on cholesterol levels and improvement of liver histopathology in conditions of hypercholesterolemia has not been known. In this study, we will discuss and examine the effect of *I. reptans* extract on LDL, HDL levels and histopathological features of the liver of experimental white rats (*Rattus norvegicus*) which were made hypercholesterolemia with high cholesterol diet.

MATERIALS AND METHODS

Research Design

This research was an *in vivo* experimental study using mice as experimental animals. Male Wistar strain albino rats *(Rattus norvegicus*), body weight ranging from 150-200 grams (2-3 months) was used in this research. A total of 25 rats and habituated for 2 weeks in the Lab. Pharmacology, Pharmacy Study Program, Universitas Binawan and randomly divided into 5 groups, including: (1) Normal / N group, no intervention was given before necropsy; (2) The hypercholesterolemia/Chol group, the rat group which was given high cholesterol diet and was not treated; (3-5) Group of rats treated with *I. reptans* extract at doses of 200 mg/kgBW, 300 mg/kgBW, and 400 mg/kgBW / IE 200, IE 300, and IE400. In this group, rats that were fed a diet high in cholesterol, after hypercholesterolemia, were treated through a sonde with *I. reptans* extract according to the dose in each group. The use of experimental animals in this study has approval from the Animal Care and Use Committee of Universitas Brawijaya (Ethical Clearance Number: 055-KEP-UB-2022).

Place and Time Research

This research was conducted for 10 months from February 2022 to November 2022. The implementation of this research was in collaboration with several related laboratories. In the early stages of the research, the preparation of ground kale extract (*Ipomoea reptans poir.)* was carried out at the Balittro Test Laboratory, Bogor. While research activities using animal models, carried out in the Lab. Pharmacology, Pharmacy Study Program, Universitas Binawan. Preparation and photos of tissue histopathic preparations were carried out at the Primate Animal Study Center, IPB.

Population and Samples

The population in this study were Wistar strain white rats (*Rattus norvegicus*) with hyperglycemia which had been treated with *I. reptans* extract. While the specimens used were blood serum and liver organs from experimental animals in all groups.

Preparation of *I. reptans Extract*

Fresh leaves of *I. reptans* as much as 8 kg were washed and air-dried to obtain dried *I. reptans* leaves. Then the dried leaves of *I. reptans* were blended/mashed to obtain *I. reptans* powder. Dried *I. reptans* powder was macerated with 70% ethanol solvent, then the macerate was concentrated using a vacuum rotary evaporator and filtered to obtain a thick extract.

Thick extract was screened for phytochemicals to determine the active compounds in it. Phytochemical screening was carried out by adopting several methods that have been common and have been widely used in previous studies. The identification of alkaloid compounds was carried out using the Dragendorf test and Wagner's test. Saponin compounds were tested using the foam method. The presence of tannins and phenols in the extract was tested using gelatin and salt. The flavonoid compounds in the extract were tested using Shinoda's test/ Mg-hydrochloride reduction test. The identification of triterpenoid and steroid compounds was carried out by Salkowski's test and Libermann-Burchard's test. Meanwhile, to identify the presence of glycosides, the Keller-Killani test was carried out

Administration of High Cholesterol Diet

Giving high-fat feed was carried out using the method in previous studies with a few modifications. A mixture of 2 grams of goat oil is mixed with 1 gram of boiled quail egg yolk[11] which is dissolved in water up to 2 ml and given through a gastric tube. The administration was carried out in the Chol, IE200, IE300, and IE400 groups for 21 days.

Measurement of HDL and LDL Levels

HDL and LDL levels determination was carried out using enzymatic colorimetric measurements. The animals underwent cardiac puncture to collect blood. Blood specimens were transferred into anticoagulant-free vials and allowed to stand for 30 min to clot. Afterwards, the vials were centrifuged at 300 × g for 10 min and the

resultant serum was used for further analysis of HDL and LDL. Serum lipid levels were colorimetrically measured by routine procedures, using commercial kits from Roche and measured using a spectrophotometer. All analyzes were performed according to the manufacturer's instructions in triplicate[12–14].

Histopathological Examination

Rats from each group was randomly selected and anesthetized with ketamine. Then liver samples were taken and stored in 4% PFA. Histopathology slides were stained manually with Hematoxylen-Eosin (HE) staining. The stages of HE staining are deparaffinization, rehydration, and staining. The first staining uses hematoxylen, ±10 minutes for the color penetration of the preparation. After that it was rinsed and put in alcohol eosin dye for 5 minutes. The preparations were then immersed in distilled water to remove excess eosin. Then dehydrated with ethanol series. Next, clearing was carried out, using xylol and air-dried, then mounting (gluing) was carried out with entellan[12,15]. Histopathological slides from staining were observed microscopically at 200x magnification using Nikon Eclipse 80i DS Fi1 light microscope. The results of the tissues photograph obtained were compared to each treatment group and analyzed descriptively.

Data Analysis

The research data were analyzed using the Shapiro-Wilk normality test and the Levene's homogeneity test. LDL data is normally distributed so that it was analyzed using One-way Anova comparative test and continued with the LSD Post Hoc test. For HDL results, abnormal data is obtained so that the analysis process is carried out using Kruskal-Wallis and Mann-Whitney tests.

RESULTS

Phytochemical Test Results of *I. reptans* Extract

Based on the results of phytochemical tests, several secondary metabolites were found in the 70% ethanol extract of *I. retpans*. Secondary compounds that were positively identified in this extract included alkaloids, saponins, tannins, phenolics, flavonoids, triterpenoids, and glycosides. From the results of the phytochemical screening, it was found that the *I. reptans* extract was positive for the seven secondary compounds, and negative for the steroid content as shown in Table 1.

Tabel 1. Results of Phytochemical Screening Test of *I. reptans* Extract

No.	Secondary Compound	Results
1	Alkaloids	+
2	Saponins	+
3	Tannins	+
4	Phenolics	+
5	Flavonoids	+
6	Triterpenoids	+
7	Steroid	-
8	Glycosides	+

(+) : contained in the extract

Improvements in LDL and HDL Levels Hypercholesterolemi Animal Models Treated with *Ipomoea reptans Poir* Extract.

High cholesterol induction was carried out only in the chol group, IE200, IE300 and IE400 for 21 days. On the 22nd day, LDL and HDL measurements were carried out to ascertain whether the rats had hypercholesterolemia. The results showed that before administration of the extract, LDL levels increased significantly ($p<0.05$) in the chol, IE200, IE300 and IE400 groups respectively by 32.12 ± 1.83, 31.62 ± 1.22, 32.32 ± 2.17, and 30.28 ± 1.06 mg/dL compared to the normal group (N) with levels of 8.16 ± 0.59 mg/dL. While HDL levels were also significantly reduced ($p<0.05$) in the chol, IE200, IE300 and IE400 groups respectively, namely by 14.78 ± 1.49, 14.44 ± 1.41, 15.58 ± 1.54, and 13.44 ± 2.73 compared to the normal group 37.76 ± 1.86 mg/dL (Table 2).

After the rats experienced hypercholesterolemia, therapy with *I. reptans* extract was carried out for 14 days. Measurements of LDL and HDL levels were carried out in all groups at the end of the study. Rat blood serum samples were taken and tested for LDL and HDL levels using colorimetry according to each Kit. In LDL levels data, the results showed that post-induction of cholesterol with quail egg yolk and animal oil for 3 weeks caused LDL levels in the Chol group to increase significantly ($p<0.05$) post-induced with a mixture of cholesterol and animal oil with an average of 29.9 ± 1.05 mg/dL compared to the normal group (N) which is equal to 8.56 ± 1.36 mg/dL. Interesting results were obtained after being treated with *I. reptans* extract where, therapy with a dose of 200 mg/kg, IE200, did not cause a decrease in post-cholesterol induction LDL levels. Meanwhile, when treated with higher doses, namely doses of 300 mg/kg and 400 mg/kg, IE300 and IE400, LDL levels decreased significantly ($p <0.05$) compared to the Chol group (Figure 1), with an average LDL of 9.12 ± 0.72 mg/dL and 9.22 ± 0.77 mg/dL successively (Table 2).

For the results of HDL levels, the normal group which was not induced by cholesterol (N) had HDL levels of 38.1 ± 2.24 mg/dL. The decrease in total and LDL cholesterol levels was also followed by an increase in HDL levels (Figure 1), wherein the IE 200, IE 300 and IE 400 treatment groups had HDL levels that approached the normal group, namely 33.4 ± 5.91 mg/dL, 36.92 ± 0.47 mg/dL and 42.82 ± 3.27 mg/dL respectively. Statistically, this value increased significantly ($p<0.01$) compared to the HDL level in the chol group, which was 15.92 ± 4.39 mg/dL. Meanwhile, the normal group had HDL levels of 38.1 ± 2.24 mg/dL (Table 2).

Table 2. Average of LDL and HDL Levels of Hypercholesterolemia Rats Before and After Therapy

	LDL (mg/dL)		HDL (mg/dL)	
	Before therapy (22nd day)	**After 14 days therapy (35th day)**	**Before therapy (22nd day)**	**After 14 days therapy (35th day)**
N	8.16 ± 0.59[a]	8.56 ± 1.36[a]	37.76 ± 1.86[a]	38.1 ± 2.24[a]
Chol	32.12 ± 1.83[b]	29.9 ± 1.05[b]	14.78 ± 1.49[b]	15.92 ± 4.39[b]
IE200	31.62 ± 1.22[b]	25.22 ± 4.72[b]	14.44 ± 1.41[b]	33.4 ± 5.91[a]
IE300	32.32 ± 2.17[b]	9.12 ± 0.72[a]	15.58 ± 1.54[b]	36.92 ± 0.47 [a]
IE400	30.28 ± 1.06[b]	9.22 ± 0.77[a]	13.44 ± 2.73[b]	42.82 ± 3.27[a]

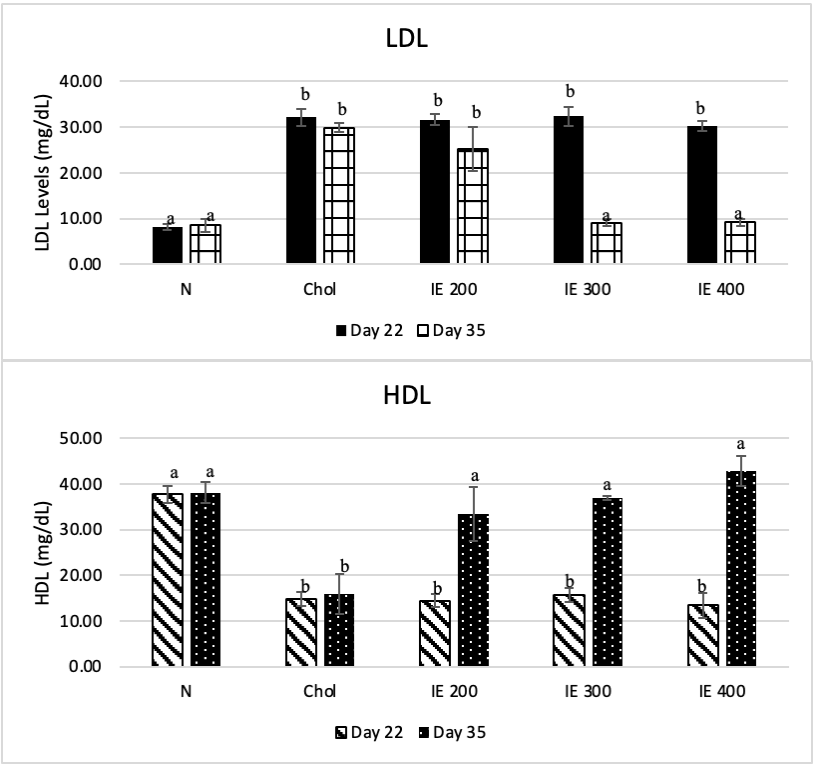

Figure 1. LDL and HDL Level Results between Before and After *I.reptans* extract Therapy

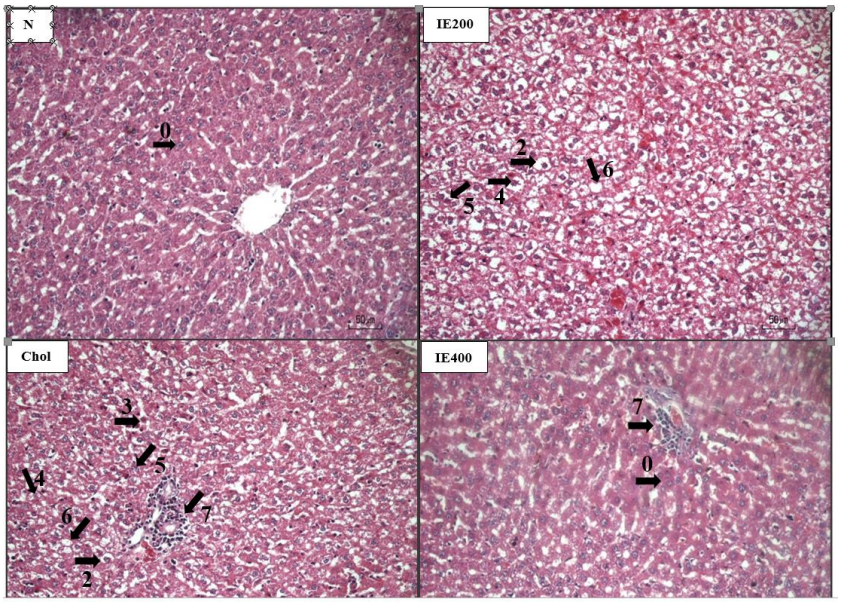

Figure 2. Histopathology of Rat Liver with Hematoxylin-Eosin Staining (200x Magnification).
Description: Normal group (N), Cholesterol group (Chol), the group treated with I. reptans dose of 200mg/kgBB (IE200) and 400 mg/kgBB (IE400); normal cells (0), microvesicular (1), ballooned cells (2), hyperchromatin cell nuclei (3), karyorrhexis (4), karyolysis (5), cells without contents (6), inflammatory cell infiltration lymphocytes (7).

Effects of *Ipomoea reptans Poir* Extract on Histopathology of Hypercholesterolemic Rat Livers

Based on the results of histopathological photos of the liver in the normal group (N) it was found that the liver cells were still normal, but with cholesterol-induced treatment in the Chol group with no therapy there was hepatocyte degeneration in the liver cells. Irregular and dilated sinusoids are seen, this is characterized by the occurrence of microvesicles, the formation of ballooned cells, and hyperchromatin cell nuclei. It can also be seen that some of the characteristics of cells experiencing necrosis include the breakdown of the pyknotic nucleus into many small basophilic particles (karyorexis), experiencing lysis (karyolysis). There is even lymphocytic infiltration of inflammatory cells. In the treatment group (IE200) it was seen that the liver cells still had fatty or degenerated hepatocytes, but there was no lymphocytic inflammatory infiltration. While the IE400 picture shows good changes, where fat degeneration is reduced, the number of normal cells appears to be more dominant and there is improvement in inflammation (Figure 2).

DISCUSSION

This study confirmed that continuous feeding of high-cholesterol diets increased LDL levels, lowered HDL levels, followed by fattening of rat liver tissue. There is an increase in LDL and a decrease in HDL in hypercholesterolemia, due to the accumulation of cholesterol in the blood due to the induction of hypercholesterolemia. Previous studies have stated that increased LDL levels and decreased HDL levels are due to excess cholesterol, which causes cholesterol build up in the body. Furthermore, cholesterol accumulation followed by free radical activity causes oxidative damage to several tissues. High cholesterol levels in the blood cause VLDL to form LDL, resulting in increased LDL in the blood. LDL levels that continue to increase make HDL depressed and cannot get rid of the excess cholesterol in the blood, so that HDL decreases[16]. This situation is in accordance with the results of this study, where there were significantly different increases in LDL levels and decreases in HDL levels in both the Chol group compared to the normal group. In previous studies, it was stated that hypercholesterolemia resulted in disturbances of lipoprotein metabolism, which included increased LDL levels and decreased HDL levels. Excess fat and cholesterol cause cylomicron to be converted into LDL by the enzyme lipoproteinlipase, this is in accordance with the statement of previous researchers who said that cholesterol binds to fat and protein and forms lipoproteins according to their respective compositions[16].

Treatment of *I.reptans* extract in hypercholesterolemic rats with three different doses of HDL levels showed a significantly different increase, but only at doses of 300 mg/kgBW and 400 mg/kgBW showed a significant difference in LDL levels compared to the Chol group. In previous studies, it was reported that *I.reptans* extract had inflammatory and antioxidant activity. Supported by previous research, the results of phytochemical screening that contained flavonoids in the form of quercetin, tannins, and saponins which function as antioxidants that can be used to lower cholesterol levels in the body[9]. These bioactive compounds can increase the synthesis of bile acids, also act as antioxidants and anti-inflammatories.

I. reptans extract therapy can increase bile acid secretion which will increase fat metabolism, as a result, excess fat will be excreted through the large intestine in the form of feces. Removed fat will lower cholesterol levels in the blood, LDL formation will also not be excessive. Antioxidant action in *I. reptans* extract serves to reduce the activity of LDL oxidation which occurs due to the accumulation of cholesterol in the blood. Antioxidants will increase HDL levels by increasing hepatic Apo A1 mRNA which plays a role in initiating Apo A1 synthesis, where Apo A1 is the main component of HDL. Apo A1 can also suppress LDL multiplication, so that LDL oxidation does not occur[17].

Inflammation that occurs due to cholesterol induction was also seen in the histopathology of the liver where the liver cells were seen to be fatty and cell infiltration occurred in the Chol group (Figure 1). Inflammatory cell infiltration is caused by the oxidation of LDL. Oxidation of LDL initiates an acute inflammatory process that causes vasodilation, so that monocytes in the blood enter through the gap between the endothelium and fat degeneration also occurs. This damage is caused by the induction of hypercholesterolemia which triggers free radicals and results in an inflammatory reaction and causes fattening of the hepatocyte cells[18]. Excessive cholesterol disrupts the metabolic process, so that cholesterol accumulates in the liver. Cholesterol that enters the liver cannot be transported entirely by lipoproteins to the liver from the bloodstream throughout the body[19]. This situation increases total cholesterol and LDL cholesterol levels, increased LDL will trigger free radicals and cause an inflammatory process.

I. reptans extract therapy which has a strong antioxidant content shows a good effect on the histopathological picture of the liver. It can be seen that there was no infiltration in the IE200 group but there was still fat. The best results were obtained when treated with *I.reptans* extract at a dose of 400 mg/kg where it was seen that there was a reduction in cells that were experiencing fat and a reduction in areas of inflammation. The presence of antioxidant action from *I.reptans* extract in the form of quercetin, tannins, and saponins, can inhibit LDL oxidation by reducing ROS and increase HDL by increasing Apoprotein A1, so that the inflammatory process and LDL oxidation are reduced resulting in improvements in the histopathology of the liver of hypercholesterolemic rats.

From histopathology results indicate that induction of cholesterol for 21 days with quail egg yolk and animal oil causes hypercholesterolemia in rats which causes histopathological damage to the rat's liver where there is fattening to fatty degeneration in hepatocyte cells, necrosis and inflammation (Figure 2). This results in line with previous research that high cholesterol leads to hepatocellular degeneration[18,20,21]. The irregular location of the sinusoids may also be caused by lipid peroxidation and oxidative stress which then increases the production of hydroxyneale (HNE) and malondialdehyde (MDA) which increases liver fibrosis through activation by stellate cells which causes an increase in the production of transforming growth factor-beta (TGFβ)[22–24]. Therapy

using *I.reptans* extract at a dose of 200 mg/kgBW was not enough to improve the fat degeneration that occurred, but at a dose of 400 mg/kgBW the fat degeneration could improve but was not optimal (there was still inflammatory infiltration of inflammatory cells). This may be due to the process or duration of therapy which is only carried out for 14 days, and a longer duration of therapy is needed to obtain more optimal results in further research.

CONCLUSION

Based on the results and discussion above, it can be concluded that *I.reptans* extract therapy at doses of 300 mg/kgBW and 400 mg/kgBW significantly reduced LDL levels and increased HDL levels in the blood. Improvement in liver histopathology as indicated by reduced inflammatory and fatty cells was obtained after therapy with *I.reptans* extract at a dose of 400 mg/kg BW only.

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