Title: Protective effects of *Kleinhovia hospita* leaf extract against Triton X-100-induced hypercholesterolemia in rats

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ABSTRACT

Background: *Kleinhovia hospita* leaves have traditionally been used as herbal medicine to lower the blood cholesterol levels. However, scientific data about the antihypercholesterolemic properties of this plant are still lacking. This study was conducted to investigate the protective effects of *K. hospita* leaf extract against hypercholesterolemia induced by Triton X-100 in rats.

Methods: Twenty-four male Wistar albino rats were randomly assigned to four groups of six each. The treatment groups received *K. hospita* leaf extract at either 250 or 500mg/kg dosage for seven days. Sodium carboxymethyl cellulose (NaCMC, 0.5%) was given to the placebo group. This treatment was then followed by Triton X-100 administration at 400mg/kg orally on day-8 to induce hypercholesterolemia. Normal controls did not receive Triton X-100. After 48 hours, blood samples were collected and the rats' livers were dissected. The serum biomarkers were analyzed, including blood lipids and liver enzymes. The liver specimens were weighed to determine changes in the organ relative to the weight.

Results: Triton X-100 significantly increased the levels of total cholesterol (TC) and serum glutamic oxaloacetic transaminase (SGOT), but did not significantly elevate the triglycerides (TG), high density lipoprotein (HDL), or serum glutamic pyruvic transaminase (SGPT) levels. The administration of *K. hospita* leaf extract for seven days as a pretreatment, followed by Triton X-100, reduced the levels of TC and SGOT at 250 or 500 mg/kg.

Conclusions: The *K. hospita* leaf extract at 250 and 500 mg/kg protected against hypercholesterolemia and high SGOT levels in rats that had been treated with Triton X-100.

Keywords: Hypercholesterolemia; *Kleinhovia hospita;* Liver enzymes; Total Cholesterol; Triton X-100

INTRODUCTION

High blood cholesterol levels, or hypercholesterolemia, marks the beginning of atherosclerosis, which is the associated risk factor for cardiovascular disease. The American Heart Association states that the prevalence of hypercholesterolemia in the United States is as high as 11.9%, with around 28.5 million adults aged ≥ 20 years having high cholesterol levels [1, 2].

The etiology of hypercholesterolemia generally falls under two categories, i.e., primary and secondary [3]. In primary hypercholesterolemia, the low-density lipoprotein (LDL) receptors are dysfunctional or missing [4], while in secondary hypercholesterolemia, there is an abnormal lipid metabolism. This can be induced by certain diseases, hormonal imbalances, medications, and also unhealthy lifestyle, such as diets rich in carbohydrates and trans-fats. Trans-fats induce the synthesis of endogenous cholesterol by the liver and of apolipoprotein B (ApoB-100), which increases LDL cholesterol and atherogenic index, and decreases HDL-cholesterol synthesis [5].

Experimental animal models have been applied to induce hypercholesterolemia, including the use of chemicals, such as Triton X-100. This compound is a surfactant that accelerates hepatic cholesterol synthesis and enhances intestinal lipid absorption by the emulsification process. It suppresses the activity of lipoprotein lipase and blocks the uptake of lipoproteins from the circulation by extrahepatic tissues, resulting in increased blood lipid concentrations [6]. Besides lifestyle changes and the use of conventional medicines, alternative therapies are also used in patients with hypercholesterolemia. Herbal medicines are probably the most widely used and traditionally accepted ones due to their therapeutic efficacy and minimal adverse effects [7].

Kleinhovia hospita (*K. hospita*) leaves are commonly used as herbal medicine for their hepatoprotective effects [8-10]. Cycloartane triterpenoid alkaloids, the main active chemical compounds in *K. hospita*, have been shown to elicit antioxidant and hepatoprotective effects [8, 11]. Moreover, *K. hospita* leaf extract contains kaempferol and quercetin, both of which are flavonoids. These bioactive compounds regulate lipid metabolism, including cholesterol absorption, synthesis, transport, and excretion [12]. The extract of *K. hospita* leaves also contains saponins and tannins that have been shown to possess a variety of pharmacological activities, including antihypercholesterolemic effects [13].

Although the antioxidant and hepatoprotective activities of *K. hospita* leaf extract have been extensively reported [8, 9, 14], studies on its anti-hypercholesterolemic activity are still lacking. To date, the anti-hyperlipidemic effect of *K. hospita* extract was only reported from an *in vitro* study, showing the lipase inhibitory activity of this extract.

Aim of the Study: This study was conducted to investigate the protective effects of *K. hospita* leaf extract against hypercholesterolemia induced by Triton X-100 administration in rats.

MATERIALS and METHODS

Chemicals and Drugs: Triton X-100 was purchased from Sigma Chemical Co. (Germany). The other chemicals, such as 70% ethanol, sodium carboxymethyl cellulose (Na-CMC), and sodium chloride (0.9% NaCl) solution, were purchased from local distributors in Makassar, Indonesia.

Plant Materials and Preparation of the Extract: The *K. hospita* leaves were obtained in the areas surrounding Hasanuddin University in South Sulawesi, Indonesia. The leaves were washed in clean water and dried for 48 hours. The dried leaves without stems were cut into small pieces and immersed in 70% ethanol for three days with occasional stirring. The extract was concentrated, using a rotary evaporator until a thick solution was obtained. The thick extract was then lyophilized in a desiccator. The extract powder was suspended in 0.5% sodium carboxymethyl cellulose (NaCMC) and prepared at two concentrations of 250 and 500mg/kg of the rat body weight. The extract was administered to rats orally at a volume of one mL/100g of the rats' body weight.

Experimental Animals: Twenty-four male albino Wistar rats weighing 180-270 grams each were obtained from Makassar, Indonesia. The animals were housed at a temperature-controlled laboratory at 25°C and 50-55% humidity under natural light/dark cycles (12 hrs). The rats had free access to rat food and water. All study protocols were designed according to the standards of care for animal experiments, and were approved by the Institutional Ethics Committee of Hasanuddin University, Makassar, Indonesia (Registration #: 488/UN4.6.4.5.31/PP36/2023).

Experimental Groups: The rats were randomly divided into four groups of six animals each. The normal control rats received normal diet. The placebo group received 0.5% NaCMC for seven days before Triton X-100 was administered at 400mg/kg body weight on the 8th day. The groups treated with *K. hospita* extract received either 250 or 500 mg/kg of the extract for seven days, followed by the oral administration of 400mg/kg Triton X-100 on the 8th day. Prior to Triton X-100 administration, the rats were fasted for 18 hours. After 48 hours of Triton X-100 administration, a blood sample was collected from each rat by puncture in the retroorbital plexus, which was then centrifuged at 3,000 rpm for 20 minutes to obtain the serum followed by the analysis of the biochemical parameters of the blood samples from all rats.

Serum Biochemical Analyses: The blood samples were immediately centrifuged at 3,000 rpm for 20 minutes to obtain the sera. The sera were analyzed for total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), serum glutamic oxaloacetic transaminase

(SGOT), and serum glutamic pyruvate transaminase (SGPT) levels on a Humalyzer 3500 spectrophotometer (HUMAN; Wiesbaden, Germany).

Organ Weight: At the end of the study, the rats were anesthetized using ether via inhalation. The liver was harvested from each rat, cleansed in 0.9% NaCl solution, and dried with absorbent papers. Each rat's liver was immediately weighed to obtain the absolute weight, then the relative liver weights were calculated based on the following formula [15]:

Relative liver weight = $\frac{absolute\ organ\ weight}{body\ weight}$× 100%

Statistical Analyses: All experimental results were expressed as means ± standard errors. The data were analyzed for normality, using Shapiro–Wilk's test and one-way analysis of variance (ANOVA). The statistical significance of the differences among the means was evaluated by Fisher's least significant difference (LSD) post hoc test (P<0.05).

RESULTS

Effect of *K. hospita* Leaf Extract on the Serum Lipids: In the placebo group, there was a significant increase in the serum TC levels compared to the normal controls ($P<0.05$). With the extract pretreatment at 250 or 500 mg/kg, there was a dose-dependent reduction in the serum TC, where the higher dose (500 mg/kg) showed more significant ($P<0.01$) reductions than the 250 mg/kg dose ($P<0.05$) (Figure 1A). Meanwhile, the serum triglyceride levels in the placebo group did not increase following Triton X-100 administration compared to that of the rats in the normal control group. However, pretreatment with the extract at 250 mg/kg led to a reduction in triglyceride levels compared to that of the placebo group, but this did not reach the statistical significance (Figure 1B). Triton X-100-treated animals did not appear to experience a major change in their blood HDL levels (Figure 1C).

Effect of *K. hospita* Leaf Extract on Liver Enzymes: There was a significant elevation in the SGOT levels of the placebo group after administration of Triton X-100 compared to that of the normal controls ($P<0.01$). In contrast, the SGOT levels of both groups treated with the extract were markedly reduced compared to that of the placebo group (Figure 2A). Although there was a slight increase in the SGPT levels of the placebo group compared to the normal controls, the difference was not statistically significant. The rats treated with 250mg/kg of the extract had similar levels of SGPT compared to that of the normal rats. Interestingly, treatment of rats at 500mg/kg of the extract somewhat increased the SGPT levels of Triton X-treated rats, although the increase was insignificant (Figure 2B). In the placebo group, the relative liver weight was similar to that of the normal rats and those treated at 250 mg/kg of the extract. However, the relative liver weight showed a slight increase in rats treated with 500 mg/kg of the extract (Figure 2C).

Effect of *K. hospita* Leaf Extract on the Body and Liver Weight: During the seven-day extract treatment, all rats gained at least 4.73 grams in weight. However, post TritonX-100 treatment, all rats began to lose weight. In the placebo group, there was a 5.01% weight loss 48 hours post Triton X-100 administration. In the rat groups that were treated with the extract at 250 or 500 mg/kg, the weight loss was 4.62% and 3.59%, respectively (Figure 3).

DISCUSSION

Hypercholesterolemia is a condition characterized by the elevation of any or all of the lipids and/or lipoproteins in the blood. It is also referred to as hyperlipidemia or hyperlipoproteinemia. The term can also describe elevated levels of TC, TG, or LDL, or low levels of HDL [16].

In this study, we attempted to induce hypercholesterolemia, using the chemical, Triton X-100. This chemical has been widely used for its ability to increase the concentrations of blood lipids in experimental animals [17]. Our results showed that Triton X-100 induced a significant rise in total blood cholesterol and SGOT levels in the placebo group compared to those of the normal controls (P<0.05). The plant, *K. hospita,* has been used as a traditional, herbal medicine in Indonesia for a variety of health purposes. One of its applications is as an antihypercholesterolemic agent, although there is a lack of *in vivo* data to support its empirical use. The saponins, tannins, and flavonoids (quercetin and kaempferol) in the extract of *K. hospita* leaves are considered to have antihypercholesterolemic effects [9, 14]. Previous studies have demonstrated that saponins can increase the activity of lipoprotein lipase due to the rapid catabolism of LDL-cholesterol through its hepatic receptors during the final elimination in the form of bile acids [18-20]. This process could be a possible cause for the reduction in the blood lipids levels in the experimental rats in this study [13].

A previous study has demonstrated that tannins have hypocholesterolemic effects involving the inhibition of lipid absorption through increased fecal excretion of cholesterol as well as bile acids [21]. Lowering blood cholesterol levels can be achieved by the administration of flavonoids through multiple mechanisms. Firstly, flavonoids can increase the bile flow and enhance the excretion of cholesterol and bile acids. Secondly, flavonoids inhibit the absorption of cholesterol, speed up the catabolism of triglyceride-rich lipoproteins, and inhibit the enzyme 3-hydroxy-3-methylglutaryl-CoA reductase, hence reducing the cholesterol synthesis. Moreover, they can inhibit the enzyme acyl-CoA: cholesterol acyltransferase, resulting in low cholesterol esterification in both the intestine and liver. This leads to reduced absorption of cholesterol and its incorporation into lipoproteins [22].

In this study, the rat group that received *K. hospita* leaf extract had significantly lower total blood cholesterol levels than the placebo group. The extract at 500 mg/kg resulted in a significantly lower cholesterol synthesis than the one at 250 mg/kg dosage. The benefits of treatment with the *K. hospita* extract included a marked reduction in SGOT level, suggesting its protective effect on the liver and cardiac cells. This benefit of the extract has also been shown against isoproterenol-induced cardiotoxicity in rats [23]. However, since the SGPT levels were not significantly increased after treatment with Triton X-100, we had inadequate data necessary to confirm the evidence of liver injury arising from Triton X-100. There was a slight increase in the SGPT levels of rats that had been treated with both Triton X-100 and 500 mg/kg *K. hospita* extract. In this context, a slight increase in the relative liver weight was observed in this group. Hence, precautions are warranted when taking this extract at high dosages. The elevation of the serum enzyme markers and increased liver weight may have been suggestive of hepatotoxicity [24].

CONCLUSIONS

Triton X-100 induced a significant elevation of TC and SGOT levels in the serum. The administration of *K. hospita* leaf extract at 250 or 500mg/kg for seven days as pretreatment halted the elevation of TC levels significantly. The use of *K. hospita* leaf extract is considered safer at 250 mg/kg than at 500 mg/kg. This is because there was a slight increase in both the SGPT levels and the liver relative weight when using the extract at high doses. Further investigations in experimental animals are warranted to confirm the findings of the current study.

Conflict of interests: The authors had no conflict of interests with any internal or external entities in conducting this study.

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Ethical Guidelines: All of the experimental protocols involving animals received the institutional ethical clearance by the Ethics Committee, Faculty of Medicine, Hasanuddin University (Registration #: 488/UN4.6.4.5.31/PP36/2023).

Author's Contributions: Individual authors' contributions to this study were as follows:

AIS conducted the experiments and collected data for serum analyses, was involved in statistical analyses, and wrote the first draft of the manuscript. YYD designed the study, revised the final manuscript before submission for publication, and participated in all data analyses and interpretations. AR was involved in data analyses and interpretations, and contributed to manuscript revision before submission for publication. TM conducted the experiments and collected data for serum analyses. All authors reviewed and approved the final version of the manuscript prior to submission for publication.

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FIGURES

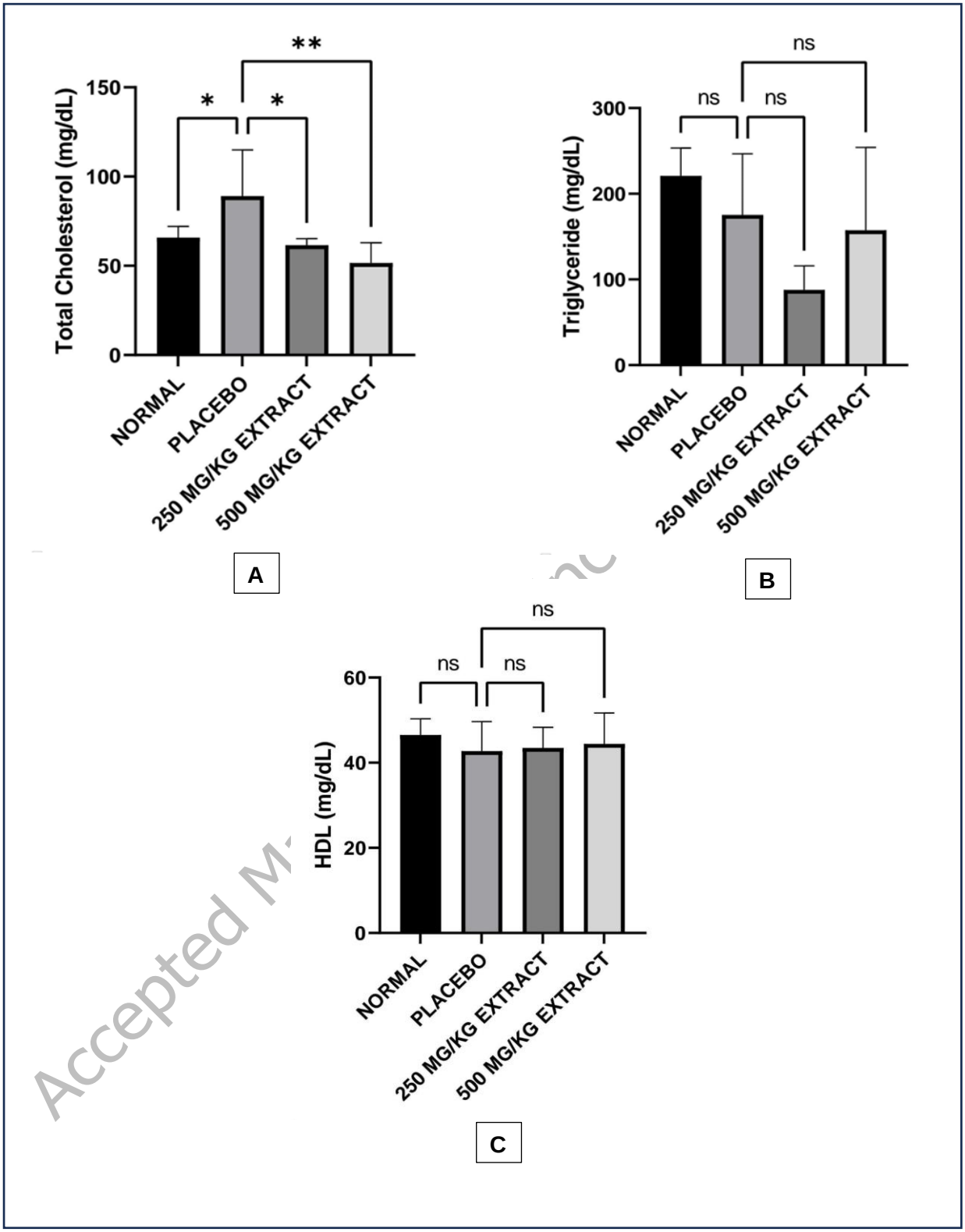

Figure 1: The levels of (A) Total Cholesterol (TC); (B) Triglycerides (TG); and (C) High Density Lipoprotein (HDL) in the experimental groups. *P<0.05, **P<0.01 compared with the placebo group.

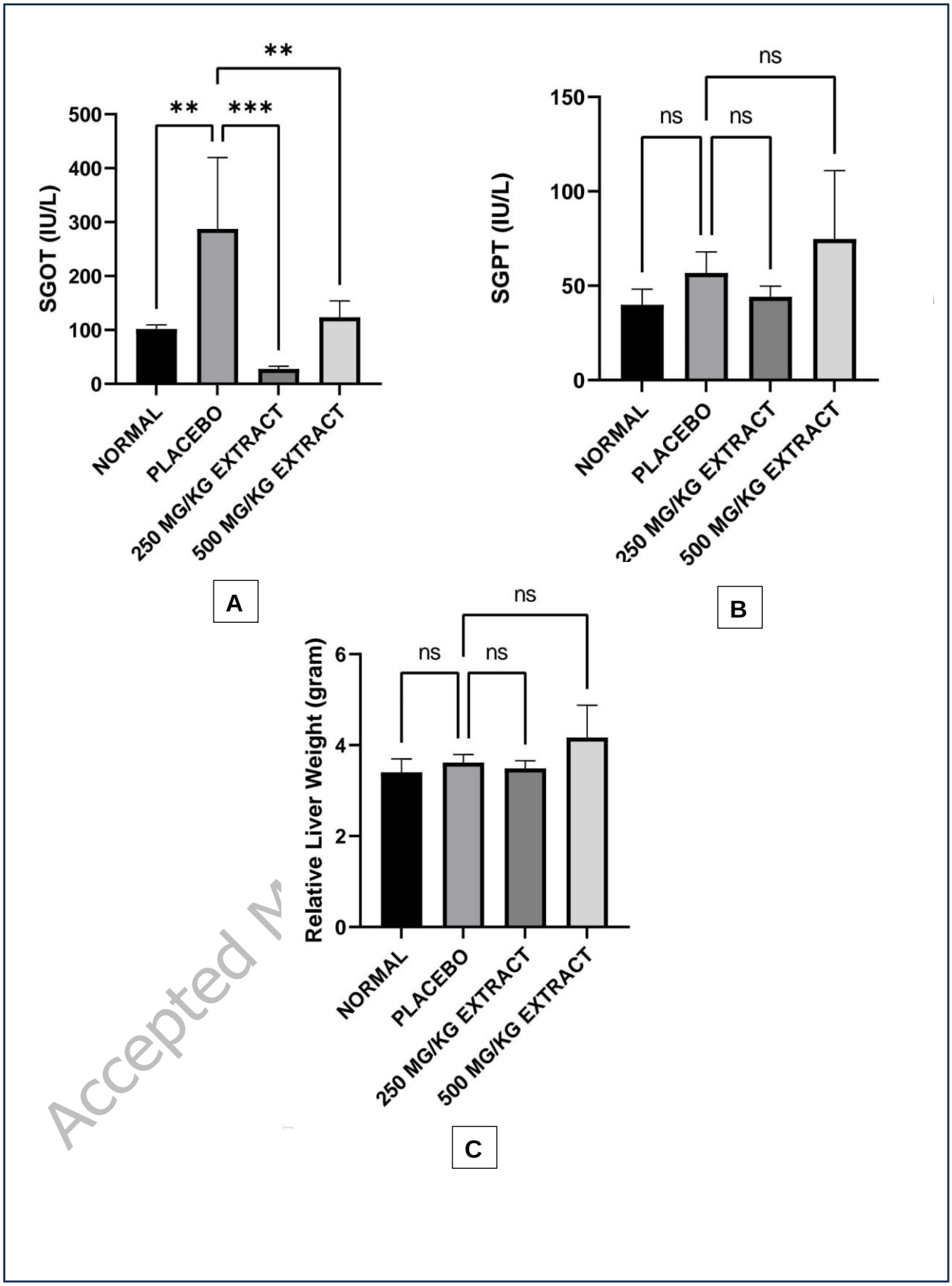

Figure 2: The levels of (A) Serum Glutamic Oxaloacetic Transaminase (SGOT), (B) Serum Glutamic Pyruvate Transaminase (SGPT), and (C) relative liver weight in the experimental groups.

***P*<0.05; ****P*<0.01; *****P<0.001 compared with the placebo group.**

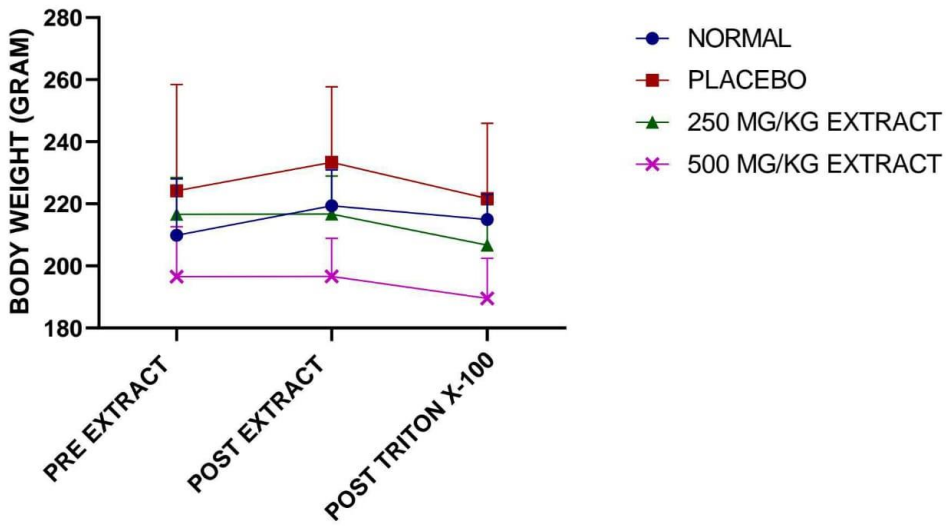

Figure 3: The rats' body weight before and after treatment with *K. hospita* extract, and after Triton X-100 administration.