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HEPATOPROTECTIVE EFFECT OF PROBIOTIC, CONTAINING LACTOBACILLUS BULGARICUS DWT1, IN ACUTE PARACETAMOL-INDUCED LIVER DAMAGE IN RATS

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ABSTRACT

Hepatic impairment is one of the most common organ damage and occurs asymptomatically until damage affects a significant part of the organ. The aim of the present study was to investigate a hepatoprotective activity of a new strain of Lactobacillus bulgaricus DWT1 against paracetamol-induced hepatic damage in Wistar rats. Laktera Nature, containing Lactobacillus bulgaricus DWT1, Lactobacillus helveticus DWT2, Lactobacillus lactis DWT3 and Streptococcus thermophilus DWT 4,5, 6, 7, 8 administered at oral doses of 800 mg/kg and 1600 mg/kg, showed significant hepatoprotective effects by decreasing the levels of serum marker enzymes such as alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), alkaline phosphatase (ALP), gama -glutamiltransferase (GGT), as compared to standard drug (silymarin) and negative control. Histopathological analysis showed that administration of the probiotic minimized liver damage, by reducing the level of morphological changes and necrosis. Our findings demonstrate the possible use of Laktera Nature, containing Lactobacillus bulgaricus DWT1, Lactobacillus helveticus DWT2, Lactobacillus lactis DWT3 and Streptococcus thermophilus DWT4,5, 6, 7, 8 for prevention of liver injury.

KEYWORDS: probiotic, paracetamol, liver damage, hepatoprotective effect.

INTRODUCTION

Hepatic drug damage is one of the most common side effects when using medicines that are constantly increasing as frequency. In the United States, drug-related hepatotoxicity is the leading cause of acute liver failure in patients indicated for hepatic transplantation, particularly in patients with unintentional or deliberate overdose of paracetamol. Acute overdose of paracetamol may lead to potentially fatal liver damage.[1, 2]

Probiotics are living microorganisms, which applied in sufficient quantities, provide a health benefit to the host and contribute to reducing the risk of disease. They are subject of increasing interest due to their proven immunostimulating, antioxidant and anti-cancer effect.[3, 4] The use of probiotics is considered as effective and safe alternative treatment for hepatotoxicity. Detailed studies of the hepatoprotective effect of probiotics on carbon tetrachloride model in rats were conducted by Georgieva M et al. 2002.[5] As a result of the systemic toxicological and clinical pharmacology studies carried out on Biostim LBS, containing *Lactobacillus bulgaricus*, its extremely hepatoprotective and antioxidant activity was established.[6] A comparable hepatoprotective effect with silymarin has been demonstrated.[7] *Lactobacillus bulgaricus DWT1* is a new original strain, isolated from spring water in Bulgaria. In 2015, Georgiev K et al., conducted a study on the antiproliferative effect of Laktera Nature, containing *Lactobacillus bulgaricus DWT1*, on a human coloncarcinoma cell line. The study shows that at high concentration, Laktera Nature inhibits the proliferation of the HT-29 carcinoma cell line.[8]

In the present study, we aimed to investigate a hepatoprotective effect of a new strain of *Lactobacillus bulgaricus DWT1* in experimental animal model of hepatic damage. We used the most common drug causing hepatotoxicity – paracetamol, to cause hepatic damage and for positive control - silymarin. Biochemical markers for hepatotoxicity and histopathology were included in the study.

MATERIALS AND METHODS

Chemicals

Laktera Nature® was kindly provided by the company Daflorn MLM 5 Ltd. (Sofia, Bulgaria). 1 mg of the substance contained 25 x 10^6 live and latent CFU *Lactobacillus bulgaricus DWT1*, Lactobacillus helveticus DWT2, Lactobacillus lactis DWT3 and Streptococcus thermophilus DWT 4,5, 6, 7, 8; Silymarin (Carsil®, Sopharma) was purchased commercially.

Before administration, the both substances were dissolved in water and administered via gastric probes.

Animals

Male adult Wistar rats (200 g ±20) received a standard rodent diet and were kept at 12 h light/dark cycle and constant temperature and humidity. The rats were housed in a controlled environment (temperature 23±5ºC, humidity 50±10%, and 12 h light/12 h dark cycle) with *ad libitum* access to food and water. All procedures concerning animal treatment and experimentation were conducted in compliance with the national laws and policies, in conformity with the international guidelines (European Economic Community EEC Council Directive 86/609, IL 358, 1, December 12, 1987).

Experimental design

The trial was conducted on 30 male Wistar rats which were divided into 5 groups of 6, as follows: saline-treated healthy controls; a control group treated with an overdose of paracetamol 1200 mg/kg to create the experimental models; a group of animals pretreated with Laktera Nature 800 mg/kg for 14 days, with an overdose of paracetamol being administered; group of animals pre-treated with Laktera Nature 1600 mg/kg for 14 days, with an overdose of paracetamol being administered; group of animals pretreated with Carsil® 45mg/kg for 14 days, with an overdose of paracetamol being administered. At the end of the experiment, all animals were sacrificed under diethyl ether anesthesia, blood samples were collected for biochemical analysis and livers were isolated for histopathological analysis.

Biochemical assays

Blood samples were centrifuged for 10 min at 7000 rpm using micro-centrifuge to separate the serum. The levels of enzymes, alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), alkaline phosphatase (ALP), gama-glutamiltransferase (GGT), were determined in a licensed hematological research laboratory in Varna/Bulgaria using Roche COBAS 6000 analyzer and methodologies described on their licensed site.[9]

Histopathology

The liver slides were fixed in 10% neutral formalin, embedded in paraffin, sectioned at a thickness of 5µm, stained with hematoxylin & eosin or Fouchet van Gieson's trichrome stain, according to the methods of Bio-Optica staining kits. The slides were evaluated for any structural changes under light microscope (Olympus BX43 microscope, XC30 software).

Statistical analysis

In all experiments, data were presented as means ±SD. One-way analysis of variance (ANOVA) was used to determine significance between the tested groups. Analysis was performed using SigmaPlot 11.0 software. A probability level of 0.05 or lower was considered as statistically significant.

RESULTS

The collected blood after the completion of the animal experiment was given for examination and the results were obtained.

The transaminase enzyme, alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT), were significantly elevated after acute overdose of paracetamol. ALAT levels were almost doubled, while those of ASAT were slightly increased. Pretreatment with Laktera Nature showed a dose-dependent decrease in transaminase levels (Figure 1 and 2).

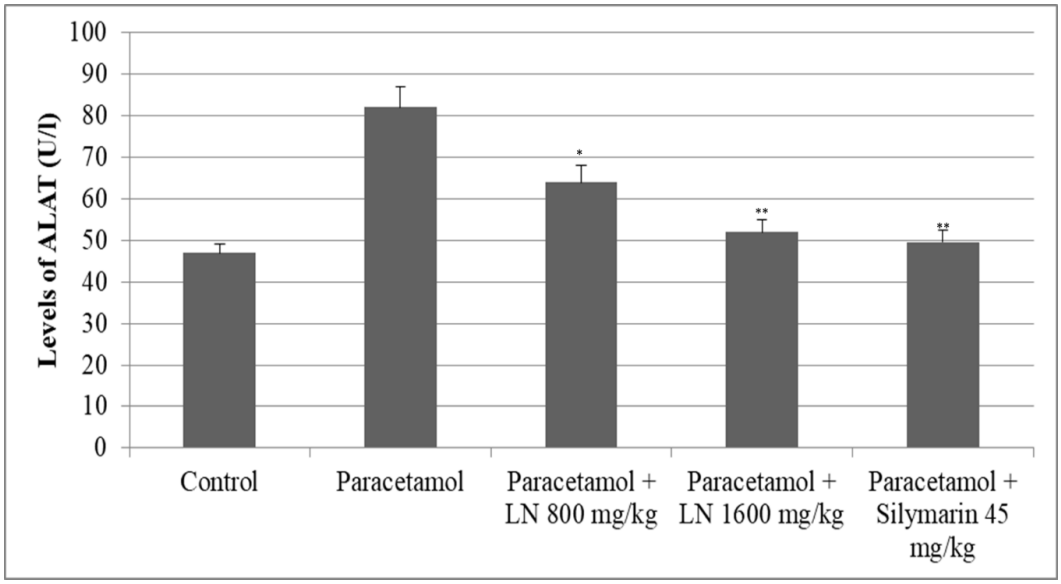

Figure 1: Changes of ALAT levels after acute overdose of paracetamol and after pretreatment with LN 800 mg/kg, 1600 mg/kg and Silymarin 45 mg/kg in rats. *p<0.05, **p<0.01, ***p<0,001

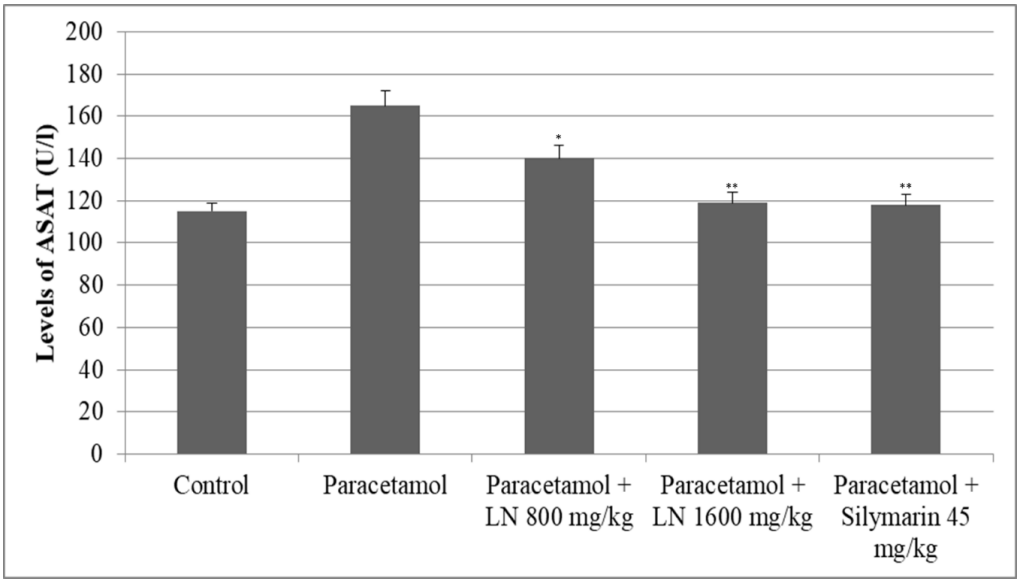

Figure 2: Changes of ASAT levels after acute overdose of paracetamol and after pretreatment with LN 800 mg/kg, 1600 mg/kg and Silymarin 45 mg/kg in rats. *p<0.05, **p<0.01, ***p<0,001.

The other two enzymes, alkaline phosphatase (ALP) and gama-glutamyltransferase (GGT), were elevated significantly as well. The levels of alkaline phosphatase (ALP) were doubled, while those of GGT were eight times higher than the control group (Figure 3 and 4).

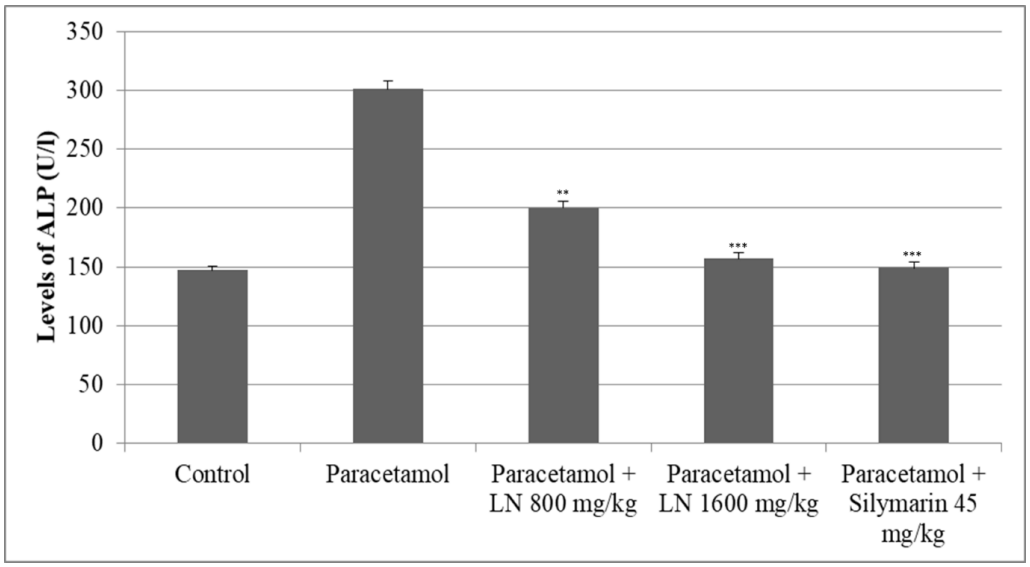

Figure 3. Changes of ALP levels after acute overdose of paracetamol and after pretreatment with LN 800 mg/kg, 1600 mg/kg and Silymarin 45 mg/kg in rats. *p<0.05, **p<0.01, ***p<0,001

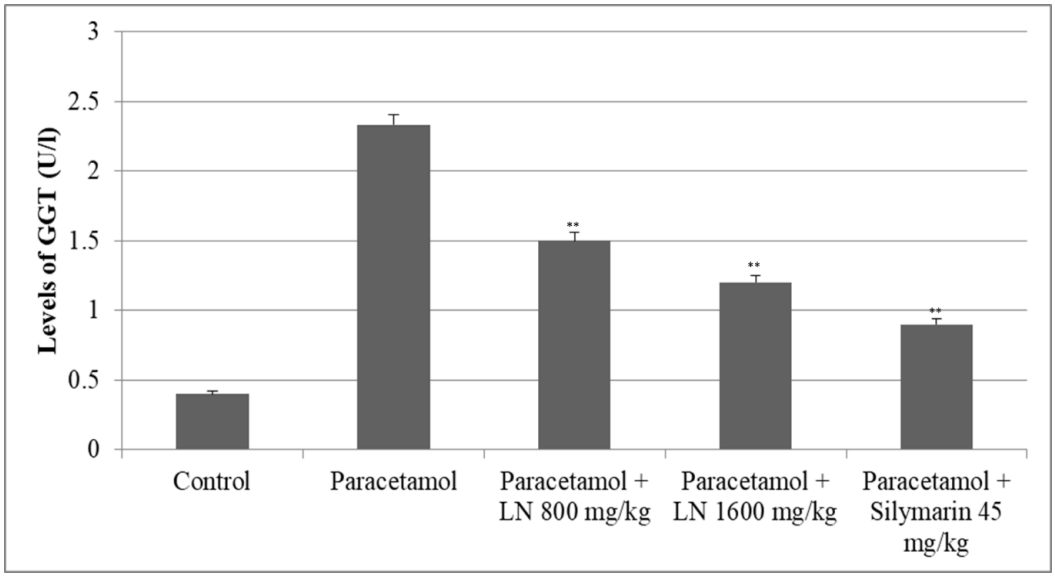

Figure 4: Changes of GGT levels after acute overdose of paracetamol and after pretreatment with LN 800 mg/kg, 1600 mg/kg and Silymarin 45 mg/kg in rats. *p<0.05, **p<0.01, ***p<0,001

The histopathological examination showed significant impairments in the use of paracetamol alone (e.g. necrosis), whereas pretreatment with the Laktera Nature 800 and 1600 mg/kg, showed normal hepatocellular architecture, similar to pretreatment with silymarin (results not showed).

DISCUSSION

In many liver diseases, the desired therapeutic effect of the standard therapy is not achieved. There is a significant percentage of treatment side effects and the treatment of patients with toxic liver damage is becoming an extremely challenging. The addition of the Bulgarian probiotic containing *Lactobacillus bulgaricus DWT1*, thanks to its proven hepatoprotective and anti-tumor effect, categorically provides a "powerful weapon" in both the therapeutic approach and the prevention of liver diseases. Paracetamol is one of the most commonly used analgesics-antipyretics worldwide. At therapeutic doses, paracetamol is safe, but if overdosed it can cause liver necrosis in both human and rat. The reason for this is the formation of a highly reactive metabolite under the action of cytochrome P450 enzymes.[10] The induction of these enzymes on the one hand and the depletion of hepatic glutathione on the other hand, are the basis of developing liver necrosis. Relating a hepatocyte lesion, a cellular leakage and a loss of cell integrate appear and multiple serum enzymes have been released.[11]

In the present study, paracetamol has caused significant elevation in the levels of ALAT, ASAT, ALP and GGT. Pretreatment with Laktera Nature in dosage 800 mg/kg and 1600 mg/kg, was found to be significantly reversing the changes induced by paracetamol. The hepatoprotective effect of lactobacilli is probably due to their elevated glutathione concentration in the liver, which is involved in the detoxification of endogenous and exogenous carcinogens and free radicals and modulates the immune function.[12, 13] The immune system plays an important role in the detoxification of the body, and the predominance of lactobacilli has an immunostimulating effect. Many studies have shown that the potential therapeutic effect of lactic acid bacteria, including their immunostimulating effect, is mainly due to their induced changes in gastrointestinal microeconomics. After entering the intestine, living or biologically active lactobacilli may activate the specific and non-specific immune response of the gastrointestinal lymphoid tissue and the systemic immune response. Reduction in the levels of ALAT and ASAT, in the larger dose 1600 mg/kg LN, is an indication of a possible regeneration process. From the histological analysis of the paracetamol overdosage group, extensive coagulation necrosis zones affecting hepatocytes from the central and intermedicinal areas of the liver pads were established. In many places, necrotic stretches merged into so-called "bridge necrosis." In the group pretreated with Laktera Nature at a dose of 800 mg/kg, there were significantly less pronounced necrotic liver changes. In the group pretreated with Laktera Nature at a dose of 1600mg/kg, the probiotic showed a significant hepatoprotective effect similar to that of silymarin, resulting in a reduction of affected liver fragments and lack of necrotic changes in hepatocytes. The use of probiotics minimizes hepatic impairment by reducing morphological changes and necrosis.

CONCLUSION

An extremely high hepato-prophylactic activity of Laktera Nature, containing *Lactobacillus bulgaricus DWT1,* Lactobacillus helveticus DWT2, Lactobacillus lactis DWT3 and Streptococcus thermophilus DWT 4, 5, 6, 7, 8 was observed for the first time in acute paracetamol-induced liver toxicity in rats. The Laktera Nature showed a pronounced hepatoprotective effect in paracetamol-induced acute liver toxicity in rats, expressed in preventing paracetamol-induced severe degenerative and necrotic liver changes, and decreasing paracetamol-elevated liver enzymes ASAT, ALT, ALP, GGT. The probiotic does not affect the serum transaminase values of healthy controls and can be used in the prevention of liver diseases.

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