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INFLUENCE OF MILK THISTLE PRESSED PARTS ON RATS LIVER HISTOLOGY

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ABSTRACT

Milk thistle (*Silybum marianum*) is one of the best known and very often used herbs with positive effect on liver. The aim of this article was to study influence of addition of milk thistle pressed parts in feed ration on liver histology and weight increase of laboratory rats. The experiment was tested by 15 pieces of laboratory rats divided into 3 groups (A, B, C). The rats in first group (A) hade feed ration with addition of 10% milk thistle pressed parts, second group (B) hade feed ration with 20% and control group (C) hade feed ration without addition of milk thistle pressed parts. The silymarin content of pressed milk thistle seed was 26.2 mg/g. The aim of this article is to study influence of addition of milk thistle pressed parts in feed ration on liver histology and weight increase of laboratory rats. The hypothesis is that the feeding addition – milk thistle seed pressed parts has positive effect on weight performance and liver histology. Does the feeding addition have any effect on these health indicators? Does feeding of milk thistle seed pressed parts have any sense? In results, the groups with addition of milk thistle had significant bigger average gain increases than the control group. Histological results vary considerably among groups of rats. All rats in the control group had dystrophic liver with sinusoidal congestion. In most rats of the group A, the dystrophy was minimal without congestion. On the other hand, most of rats of the group B had liver dystrophy caused by large droplets steatosis with congestion. All rats in control group C were found to have significant dystrophy caused by steatosis. The results indicate rats receiving the addition of milk thistle pressed parts in their feed had a lower incidence of liver steatosis due to the hepatoprotective effects of silymarin.

Keywords: *Silybum marianum*; histology; growth performance; silymarin

INTRODUCTION

Effective agents of milk thistle stimulate regeneration of hepatocytes. Complex of milk thistle agents is called silymarin, this stabilises membranes of hepatocytes and thus silymarin increase hepatocytes resistance to toxins and oxidants. Effect of silymarin stimulates production of new hepatocytes. Extracts of milk thistle has been used in the treatment of acute and chronic liver disorders. Liver disorders are usually caused by toxins, drug, alcohol and hepatitis and gall bladder disorders **(Zhu et al., 2016)**. Silymarin appears to be safe and well tolerated **(Dhiman et al., 2005).** Pharmacological taking of milk thistle is known from history and has general dissemination.

Many studies have demonstrated that the active components of silymarin have many hepatoprotective properties **(Abenavoli et al., 2011)** and these studies are predominantly focused on human treatment however at present research get focused too on milk thistle (pressed milk thistle seed) like feed supplement for animals. Properties of production of *S. marianum* (such as oil, pressed seed or flour) markets too alternative uses **(Andrzejewska et al., 2015)**. **Jakubcova et al. (2015)** studied the antimicrobial and antioxidative effect of phytogenic additives, also phytobiotics have potential in animal feeding and it has effect on human nutrition in closing stage. Milk thistle constituents to the animal diet can promise for conventional methods of animal farming **(Kosina et al., 2017)** and seems to be a promising natural feed additive to improve the health condition **(Cullere et al., 2016)**. Results of different researches indicate positive effect of feeding *Silybum marianumon* growth performance but by way of contrast this exist some researches with different ambiguous results.

From point of influence of taking *S. marianum* on liver histology of view some studies state results, that using of *S. marianum* have not any significant effect on liver histology **(Blevins et al., 2010; Dhiman et al., 2005; Jacobs et al., 2002; Tedesco et al., 2004)** by contrast results **(Guo et al., 2016; Loguercio et al., 2012)** state association with improvement in liver histology. It should be noted that significant evaluation often is difficult and ambiguous, also next research is necessary and by reason this study can make a contribution to the issue of influence of *S. marianum* on liver histology.

Scientific hypothesis

The aim of this article is to study influence of addition of milk thistle pressed parts in feed ration on liver histology and weight increase of laboratory rats. The hypothesis is that the feeding addition – milk thistle seed pressed parts has positive effect on weight performance and liver histology. Does the feeding addition have any effect on these health indicators? Does feeding of milk thistle seed pressed parts have any sense?

MATERIAL AND METHODOLOGY

The experiment was established by 15 pieces of laboratory rats divided into 3 groups (A, B, C) in experimental facilities of Department of Animal Nutrition and Forage Production in Mendel University. The rats in first group (A) had feed ration with addition of 10% milk thistle pressed parts, second group (B) had feed ration with 20% and control group (C) had feed ration without addition of milk thistle pressed parts (only scraped barley). Served milk thistle pressed parts had flour-like structure and was mixed equally with scraped barley also the rats could not prefer any feeding component. Milk thistle variety MIREL was used. The feeding mixture and water were available *ad-libitum*. Water and feed ration were daily served and rests were removed.

The length of this experiment was 28 days. Starting average rat´s weight was 90 ±3 g. The rats were weighted in 0 – 7 – 14 – 21 – 28. The rats were sacrificed inhalational anaesthetic Isofuran way on 28th day and liver samples were taken to histological analyse. Histological analyse was performed at University of Veterinary and Pharmaceutical Sciences Brno.

Determination of silymarin

The content of silymarin was performed by HPLC method in Department of Chemistry and Biochemistry at Mendel University in Brno. Analysis of silymarin was performed on a HPLC-UV / VIS instrument (Dionex Ultimate 300). Chromatography column Hypersil GOLD Dim (150 x 4.6) was used for separation by temperature 30 °C. The sample (5 µL) was injected by autosampler. Flow rate was 1 mL.min^{-1}. Content of mobile phase was A: 0.1% formic acid, B: 100% methanol. The substances were leaved to infuse in an isocratic elution way (mobile phase A was 65% and mobile phase B was 35%). Detection of separated substances was in motion under circumstances of wavelength 288 nm.

Statisic analysis

The data were statistically processed using STATISTICA.CZ, version 10.0 (the Czech Republic). The results were expressed as average values (weight) with standard deviation (SD). Statistical significance was determined by the examining the basic differences between groups by ANOVA and Scheffé's test (one-way analysis). The differences with p <0.05 were considered to be significant.

RESULTS AND DISCUSSION

Growth Performance

The results show that the weight gain of the rats fed with mixtures of 10% the milk thistle was higher compared to rats of the control group. The highest average daily gains were found in rats fed by mixtures of 20% milk thistle. On the seventh day of the experiment, the difference between groups C and A was found to be statistically significant, same as the difference between groups C and B. Statistically significant difference was also observed between groups C and B on the 28th day. Rats receiving the addition of milk thistle in their feed dose grew more intensely than the control group. **Kosina et al. (2017)** describes the positive effect of the milk thistle on the growth potential of experimental animals (rabbits in this case). Therefore, the addition of the milk thistle in the feed dose could have a positive effect on the growth potential of animals. **Feng et al. (2016)** states that silymarin significantly affected weight gain in mice. This trend was also observed in our experiment.

In group C, all animals had wide-area liver dystrophy and congestive parenchyma according to histological images. Dilation of porto-biliary system and central veins were less common than in groups A, B.

In group A, liver parenchyma is showed like intact hardly any dystrophy with dilated porto-biliary system in the pictures LM 6, LM 7 and LM 8, whereas pictures LM 9 and LM 10 show distinct dystrophy caused by steatosis.

In group B, LM 11, LM 12, LM 14 and LM 15 show area-wide distinct liver dystrophy caused by steatosis.

In animals of all groups A, B, C, there was a more distinct dystrophy caused by steatosis. In groups B and C, the vast majority showed liver steatosis. In Group A, 40% of the animals were found with steatosis, 80% in group B, and 100% in group C. The results of **Guo et al. (2016)** suggest silymarin reduces inflammatory processes causing hepatic tissue damage. Also in our study, the incidence of hepatic dystrophy was lower in groups receiving the addition of milk thistle (especially group B). **Blevins et al. (2010)** and **Tedesco et al. (2004)** report histological examination of the liver found the occurrence of steatosis even in groups of animals fed by the addition of silymarin and did not confirm effect of silymarin reducing the incidence of steatosis.

Table 1 Average daily gain (g.day^{-1}) – Group C (Control group), Group A (rats fed with 10% part of milk thistle pressed parts), Group B (rats fed with 20% part of milk thistle pressed parts).

Groups	Average daily gains during the experiment				
	0.day (g.day^{-1} ±SD)	**7th.day (g.day^{-1} ±SD)**	**14th.day (g.day^{-1} ±SD)**	**21th.day (g.day^{-1} ±SD)**	**28th.day (g.day^{-1} ±SD)**
Group C	0.0	2.3 ±0,5^{a}	2.1 ±0.4^{a}	1.9 ±0.4^{a}	1.7 ±0.5^{a}
Group A	0.0	3.4 ±0.6^{b}	2.5 ±0.3^{a}	1.8 ±0.5^{a}	1.7 ±0.6^{b}
Group B	0.0	3.6 ±0.3^{b}	2.4 ±0.4^{a}	2.0 ±0.7^{a}	3.1 ±0.6^{a}

Note: Change in index ^{a},^{b} shows significant difference at the level (p <0.05)

Liver Histology

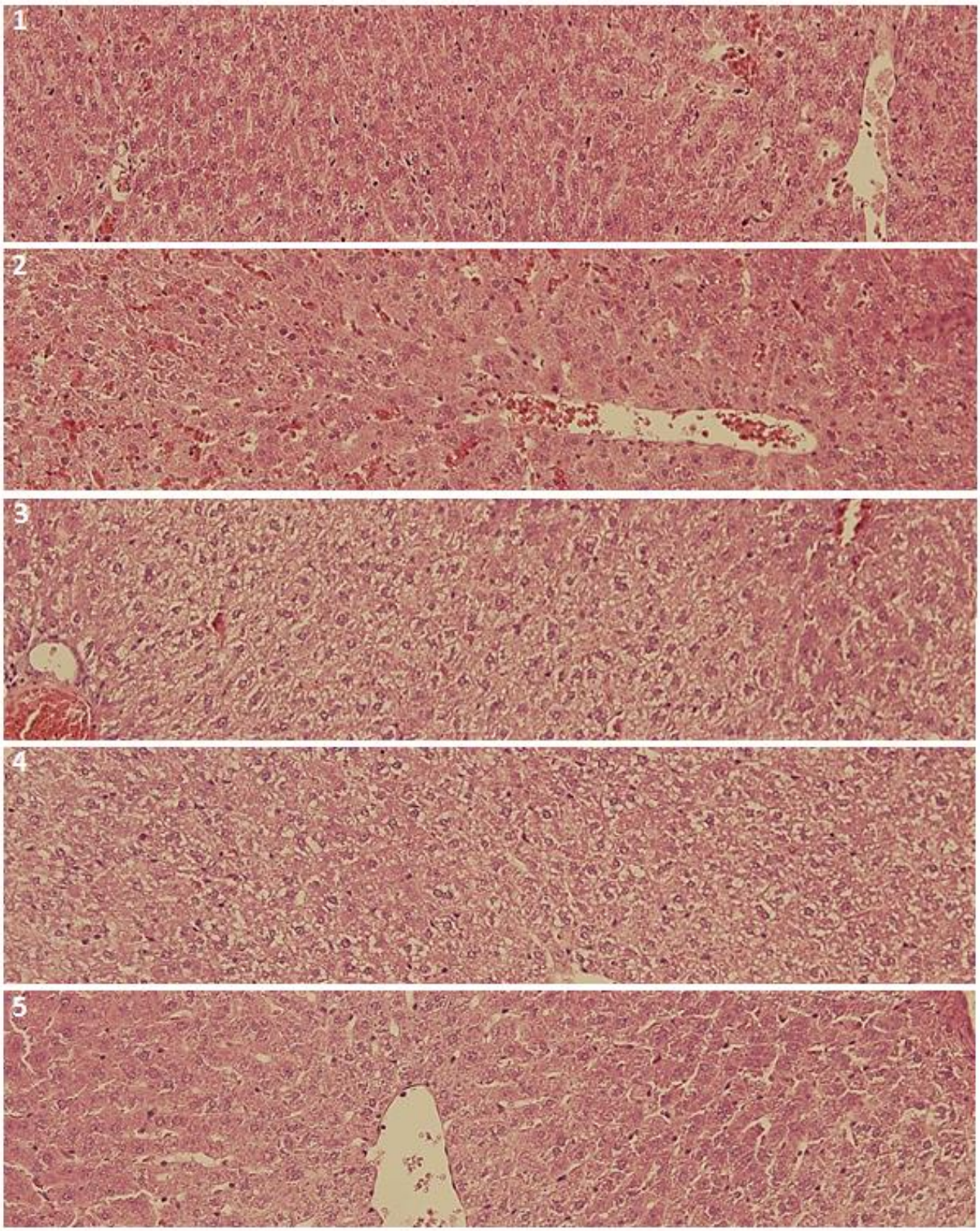

Figure 1 Liver Mash of rats in group C (control group). *Magnification 200x.*
Note: LM 1: Parenchyma is congestive, all-dystrophic, locally with trabecular system, without necrosis, with distinct dilation of porto-biliary system. LM 2: Locally distinct sinusoidal congestion, otherwise identical to LM 1. LM 3: Congestion, massive wide-area hepatodystrophy with structural destruction focuses. LM 4: Identical with LM 3. LM 5: Sinusoidal congestion, wide-area dystrophy but less distinct compared to the previous cases, trabecular structure in good condition.

Liver Histology

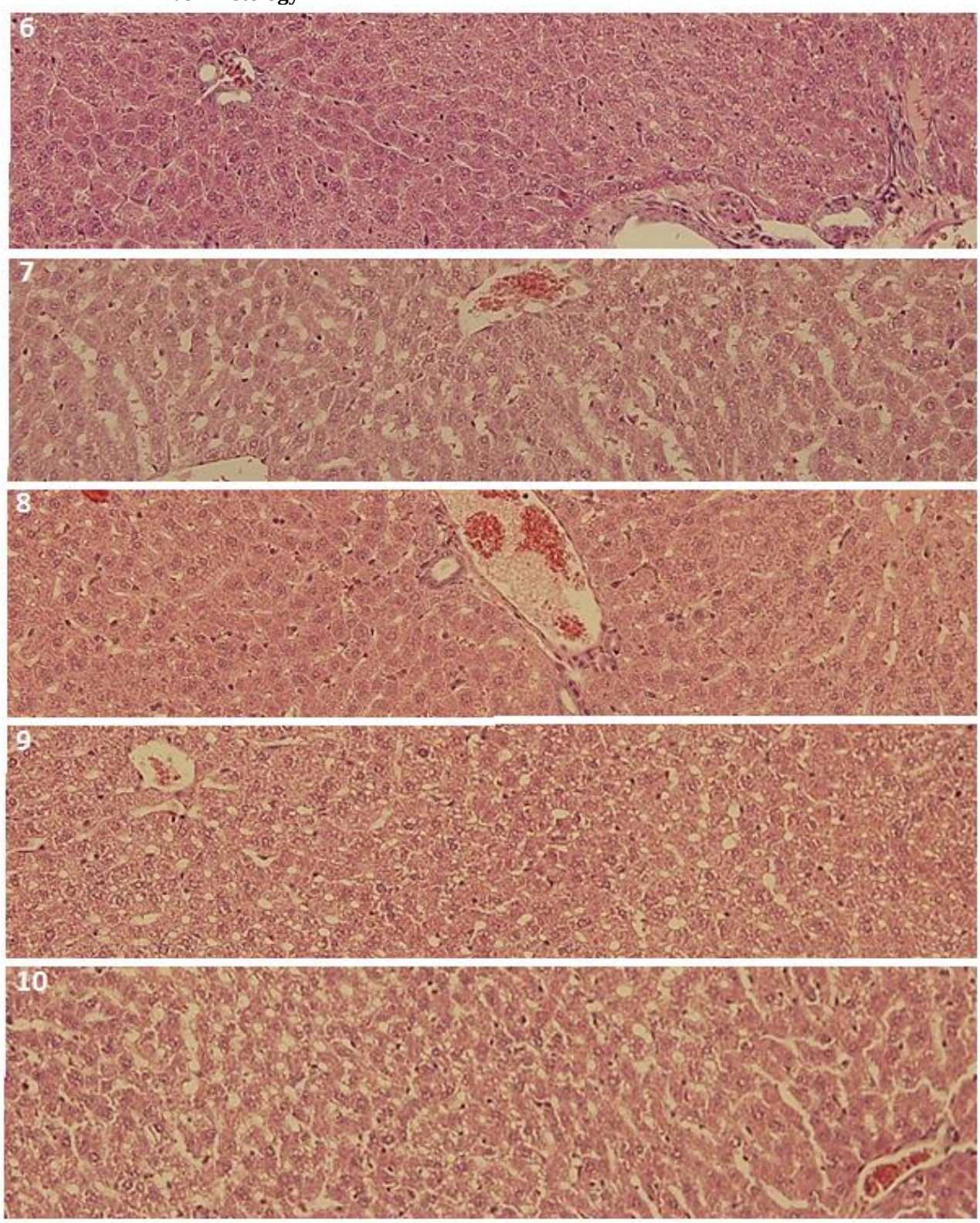

Figure 2 Liver Mash of rats in group A (10% part of milk thistle pressed parts). *Magnification 200x*.
Note: LM 6: Minimum dystrophy, dilatation of porto-biliary system, otherwise almost intact parenchyma.
LM 7: LM 7 is identical to LM 6. LM 8: LM 8 is identical to LM 6. LM 9: More distinct dystrophy caused by steatosis, trabeculary system in good condition, dilatation of central veins. LM 10: Distinct dystrophy caused by large droplets steatosis, moderate congestion, plates of hepatic cell with good structure.

Liver Histology

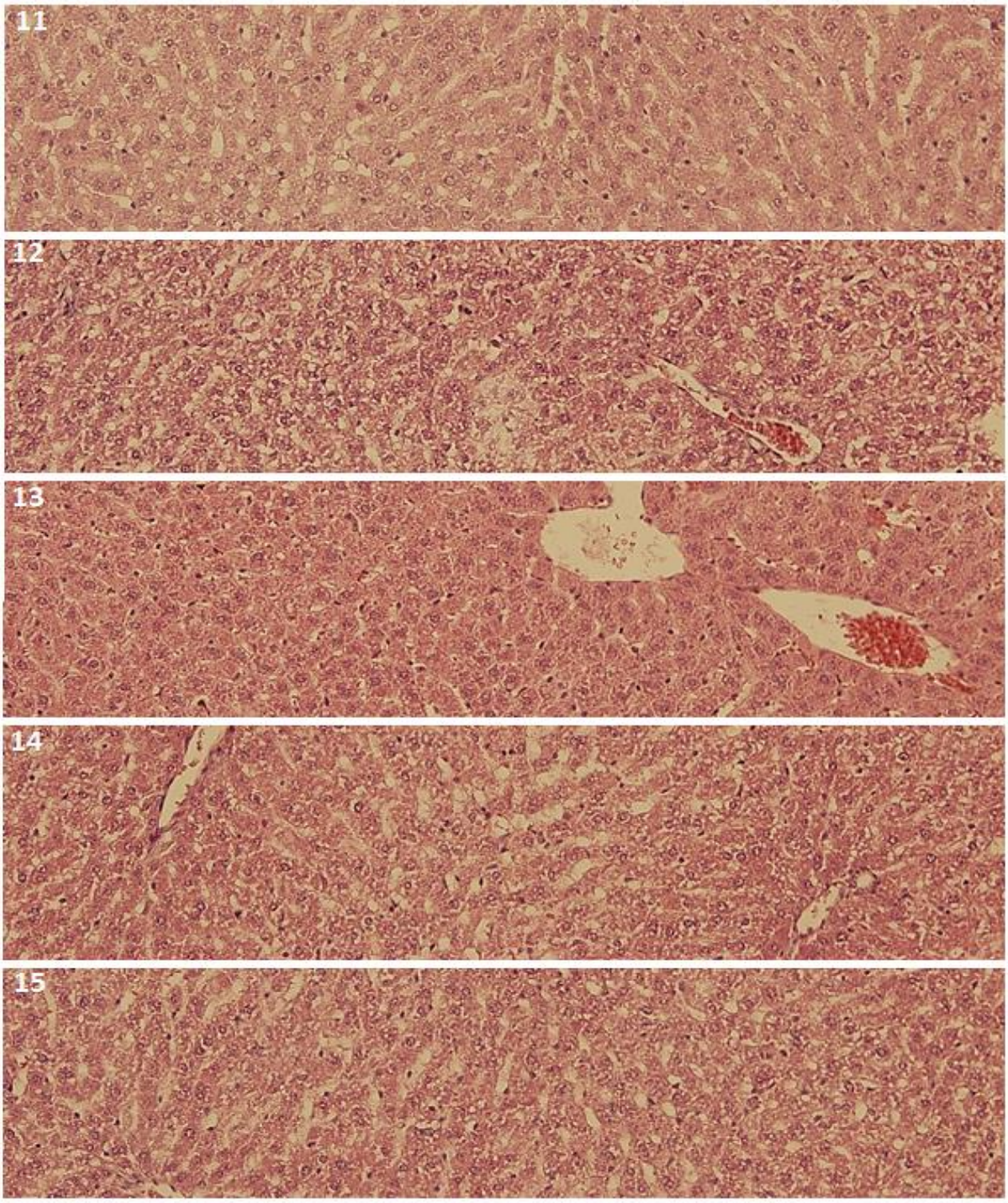

Figure 3 Liver Mash of rats in group B (20% part of milk thistle pressed parts). *Magnification 200x*.
Note: LM 11 is comparable with LM 10. LM 10: distinct dystrophy caused by large droplets steatosis, moderate congestion, plates of cells structure in good condition. LM 12: Wide-area massive dystrophy with persistence of trabeculary system, without necrosis, dilatation of porto-biliary system. LM 13: Compared to LM 12, significantly less dystrophic parenchyma, moderate congestion and dilatation of central veins, without alteration of plates of cells structure. LM 14: Congestion, wide-area dystrophy caused by large droplets steatosis. LM 15: Identical with LM 14. (Congestion, wide-area dystrophy caused by large droplets steatosis).

Dystrophy of the liver parenchyma caused by steatosis in rats of our experiment seems to be related to the barley monodiets – the animals received only barley and addition of milk thistle pressed parts in some groups. An unbalanced ratio of nutrients could be the cause of liver damage. Involvement of the health burden caused by inadequate nutrition was targeted in order to investigate hepatoprotective effects of silymarin as we do not anticipate the occurrence of histological findings in healthy animals with ideal nutrition. In animals receiving the addition of milk thistle pressed parts, the incidence of liver steatosis was lower which was probably related to the hepatoprotective effects of silymarin. From a histological point of view, the most convenient percentage of milk thistle pressed parts is 10% (group A) according to our results.

CONCLUSION

In the experiment, the effect of milk thistle was investigated on liver health in rats. In addition to liver histology, rats were also monitored for their continuous weight gains. Average daily weight gain of rats fed by mixtures with milk thistle compared to the control group increased, with the highest weight gain in the group B (20% addition of milk thistle). In animals in all groups A, B, C, there was more distinct dystrophy caused by steatosis. In groups B and C, the majority of rats were found with liver steatosis. According to histology findings, rats of group A were found with steatosis in 40%, 80% in Group B and 100% in group C. Dystrophy of liver parenchyma caused by steatosis in rats can be probably related to nutritional imbalance (barely monodiets). Induction of the health burden has been targeted in order to investigate the hepatoprotective effect of silymarin, as histologic findings are less probable in healthy animals with the ideal nutrition. In the animals fed with the addition of pressed parts of milk thistle, the incidence of liver steatosis was lower which may be related to hepatoprotective effect of silymarin. According to the results from the histological point of view, the most appropriate proportion of milk thistle pressed parts was 10% – group A. The content of silymarin in the pressed parts was 26.2 $mg.g^{-1}$.

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