

## Prevalence and pathological evaluation of hepatic fatty change in cattle slaughtered at Urmia abattoir, northwest Iran

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### Abstract

Three hundred and sixteen bovine livers were inspected during slaughtering at Urmia Abattoir in May 2016. Eighty-nine inspected livers have various gross and microscopic lesions. 89 hepatic tissue samples were processed routinely and stained by Harris Hematoxylin and Eosin (H&E), Periodic Acid- Schiff reagent (PAS) and Sudan IV for frozen sections. Hepatic lesions were observed in 89 samples (28.16%) of 316 liver samples. lesions in the inspected cattle were consisted 33/316 (10.44%) fatty change, 40/316 (12.65%) fascioliasis, 8/316 (2.53%) hydatid cyst, 6/316 (1.89%) focal coagulative necrosis and 2/316 (0.63%) hemorrhage. The results show that abundance of hepatic fatty change in male cattle is more than females. Indeed, abundance of mild type of hepatic fatty change 15/33 (45.45%) is more than moderate type 11/33 (33.33%) and severe type 7/33 (21.21%). According to presence of hepatic fatty change in most livers affected by fascioliasis, it seems fascioliasis may be a cause of hepatic fatty change in cattle slaughtered at Urmia Abattoir.

**Keywords:** Cattle liver, Fatty change, Pathology, Urmia abattoir  
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### الانتشار والتقييم المرضي للتغير الدهني الكبدي في الأبقار المذبوحة في مجزرة أورميا، شمال غرب إيران

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### الخلاصة

تم فحص ٣١٦ كبد أبقار أثناء الذبح في مجزرة أورما - إيران في شهر أيار ٢٠١٦. ظهر ان ٨٩ كبد من الأكبادة التي خضعت للفحص كانت تحوي على آفات عيانية ومجهريّة مختلفة. أخذت ٨٩ عينة نسجية من الكبد ومررت نسجياً بشكل روتيني ولونت بملون هاريس هيماتوكسيلين والإيوسين وملون حامض البريوديك كاشف شيف وملون سودان الرابع للعينات المجمدة. وكانت الآفات الكبدية في ٨٩ عينة المفحوصة وبنسبة (٢٨,١٦%) من أصل ٣١٦ عينة كبدية مفحوصة. توزعت الآفات في الأبقار كما يلي التغير الدهني ٣١٦/٣٣ وبنسبة (١٠,٤٤%)، داء المورقات ٣١٦/٤٠ وبنسبة (١٢,٦٥%)، الكيس العدري ٣١٦/١٨ وبنسبة (٥,٥٣%)، النخر التجلطي ٣١٦/٦ وبنسبة (١,٨٩%) والنزف الكبدي ٣١٦/٢ وبنسبة (٠,٦٣%). أظهرت النتائج أن التغير الدهني الكبدي في الأبقار شكل أكثر الإصابات وكانت الإصابات في الذكور أكثر من الإناث. كما أن الإصابات البسيطة من التغير الدهني الكبدي ٣٣/١٥ وبنسبة (٤٥,٤٥%) كانت أكثر من الإصابات المتوسطة حيث شكلت ٣٣/١١ وبنسبة (٣٣,٣٣%) في حين شكل النوع الشديد ٣٣/٧ وبنسبة (٢١,٢١%). وكانت معظم الإصابات بالتغير الدهني مترافقة مع الإصابة بالدودة الكبدية. وعلى ما يبدو ان الإصابة بالديدان الكبدية تترافق مع التغير الدهني.

## **Introduction**

Information about meat inspection considered a potential source of data, which plays an important role in epidemiology and preventive veterinary medicine (1). The pathologic lesions of the liver, produced by different pathologic living agents like bacteria, viruses, fungi and others can be resulted in a direct economic loss as confiscation of the injured livers of slaughtered animals or indirectly by the decrease of animal production and reproduction (2). Fatty change (Fatty degeneration) is found in the different cells including liver cells, endothelium cells and muscle fibers and its occurrence reasons are intoxications and infections. In some intoxication and infectious diseases, degeneration and fatty infiltration are seen simultaneously and this status is called fatty metamorphosis (3). Liver function can be severely impaired by fatty change and loss of the amount of serum albumin is one of these problems (4). Hypoalbuminemia is a feature of chronic hepatic disease which occurs when the functional liver's mass is 20% or lesser (4). Bovine hepatic lipidosis is related to ketosis, abomasum displacement, bronchopneumonia and mastitis (5). Fatty change (fatty liver) is an important metabolic disorder in cows which affects almost half of the herd especially obese periparturient cows (6,7). Fatty change reduces the productions, reproduction and immune function, and may lead to hepatic failure and premature death (8,9). The results of a study on the prevalence of liver diseases in slaughtered cattle, sheep, and goat at Arusha Municipal slaughterhouse of Tanzania revealed that the cattle have some hepatic diseases especially fascioliasis which is the most important cause of liver diseases in these animals (10). In addition, Hydatid cyst, *C. tenuicollis* cysts, and hepatic fascioliasis were found as the main liver diseases of cattle, sheep, and goat (10). Hepatic fascioliasis was 8.6%, 3.1% and 3.1% in cattle, sheep and goat, respectively (10). In the other pathological study at Kirkuk abattoir in Iraq, liver fluke infestation of slaughtered cattle has been reported 3.34% (213/6211) (11). Moreover, prevalence of bovine fascioliasis has been reported in Tanzania (14.04%), Ethiopia (24.3%) and Zambia (41.3%) (10). The aim of the present research was study of the prevalence and differential histopathologic diagnosis of the hepatic fatty change in the inspected livers.

## **Materials and methods**

The study was conducted on cattle slaughtered at west Azerbaijan province of Iran. In this study, 316 cattle liver (199 male and 117 female) were inspected within 1 month in May 2016 at Urmia Abattoir. The inspected cattle divided into three groups of age: less than two years,

between 2-4 years and older than 4 years. The current study was carried out within 1 month and the livers of bovine carcasses were inspected in the slaughterhouse. In addition, tissue sampling of the livers that showed gross lesions was performed and placed in 10% buffered formalin solution for fixation (12). After fixation, stages of tissue processing including dehydration, clearing, infiltration, and embedding were performed by an automatic tissue processor machine. Then preparing of paraffin- embedded tissues were cut by microtome with 5  $\mu$ m thick (12). Tissue sections were stained by three different staining methods in pathology laboratory of Islamic Azad University, Urmia branch. Harris Hematoxylin and Eosin (H&E) stain, Periodic Acid Schiff (PAS) and Sudan IV stain (for frozen sections) were performed for differential diagnosis of fat vacuoles from glycogen in hepatocytes. Grading of fatty change in bovine livers was considered based on fat contents of hepatocytes as normal (<5%), mild (5%-33%), moderate (>33%-66%) and severe (>66%) (13).

In this study, data has been presented as Mean  $\pm$  SE and analyzed by SPSS (VERSION 19) statistical software. One-way ANOVA test was used for the statistical analysis and followed by Duncan's post hoc test.

## **Results**

Among 316 inspected cattle's liver, 89 (28.16%) livers had various lesions including: Fascioliasis 40 (12.65%), fatty change 33 (10.44%), hydatidosis 8 (2.53%), hemorrhage 2 (0.63%), focal necrosis 6 (1.89%) (Tables 1). Although, in some livers multiple lesions were observed simultaneously (such as presence of both hemorrhage and fascioliasis in a liver). Abundance of mild type of fatty change 15/33 (45.45%) was higher than moderate type 11/33 (33.33%) and severe type 7/33 (21.21%) in bovine livers (Table 2). In the gross lesions of bovine livers, there were thick white lines with different sizes on the surfaces of livers affected by fascioliasis that they are distended and calcified bile ducts (Figure 1C). In hepatic fatty change, the liver was partly bigger than normal size and pale colored in addition to that the edges of the liver have lost their sharpness (Figure 1A). Focal necrosis areas, calcified areas and hydatid cyst were observed in the hepatic gross lesions (Figure 1B, D). In histopathologic evaluation of the microscopic sections, there were many clear fat vacuoles within hepatocytes in fatty livers. These vacuoles had compressed the nucleus of hepatocytes to the cell walls that these hepatocytes seem as fat cells (Figure 2). Bile duct hyperplasia, increased connective tissues, inflammatory lymphocytic cells infiltrations and chronic cholangitis were seen in fascioliasis affected livers (Figure 3A). Moreover, coagulative necrosis and hemorrhage were observed in bovine livers (Figure 3B, C).

Table 1: Frequency of liver lesions according to age, sex and breed

Hepatic lesions	Age (year)			Breed			Sex		Mean ±SE
	<2	2-4	>4	H	C	N	Male	Female	
Fascioliasis	6	13	21	13	17	10	21	19	13.33±6.12
Fatty change	13	9	11	8	14	11	19	14	11.00±1.15
Hydatid cyst	2	4	2	1	4	3	5	3	2.66±0.66
Focal coagulative necrosis	1	3	2	2	1	3	2	4	2.00±0.57
Hemorrhage	1	0	1	0	2	0	1	1	0.66±0.33

H (Holstein), C (Cross-breed), N (Native).

Table 2: Frequency and type of fatty change in the liver of slaughtered cattle according to age, sex, and breed

		Age (year)			Sex		Breed		
		<2	2-4	>4	Male	Female	Holstein	Cross-breeds	Native
fatty change severity	Mild	7	4	4	9	6	3	8	4
	Moderate	6	3	2	6	5	3	3	5
	Severe	0	2	5	4	3	1	4	2
Total number of cattle with fatty change		13	9	11	19	14	7	15	11

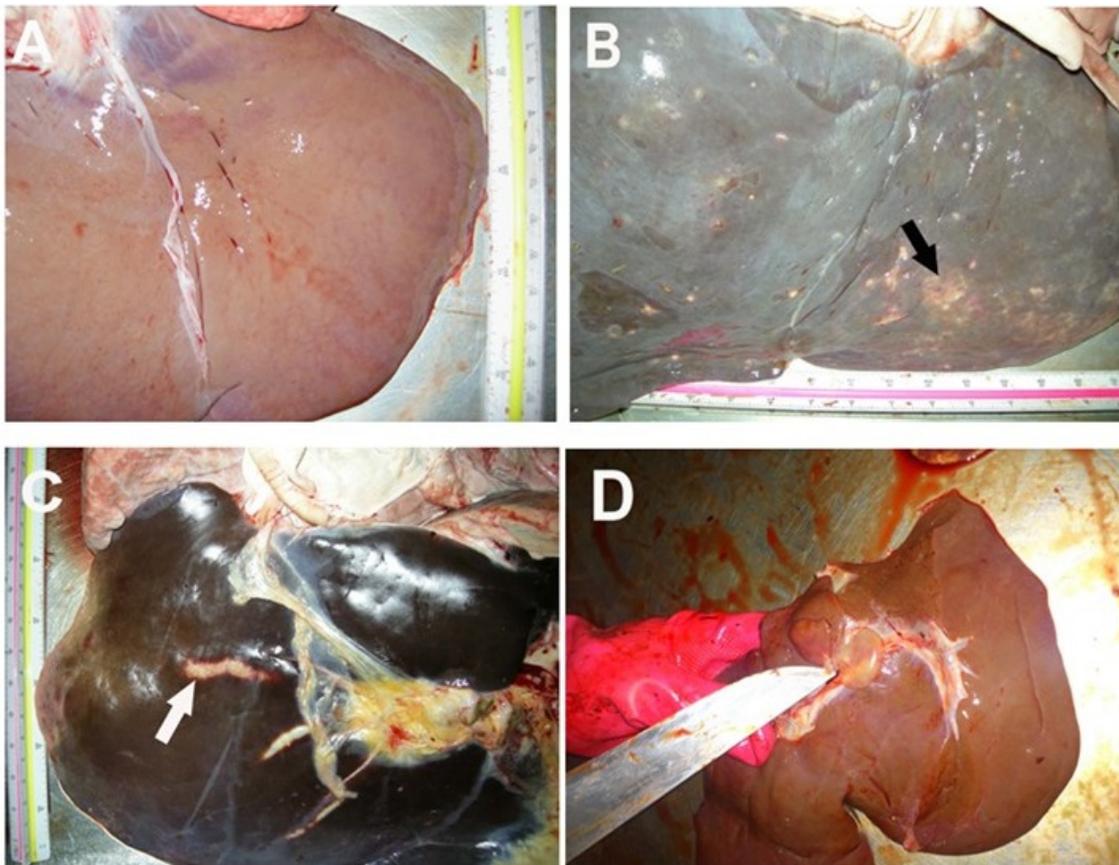


Figure 1: Some observed lesions in the liver of slaughtered cattle in Urmia Abattoir grossly. (A): cattle liver with fatty change; pale discoloration is grossly visible. (B): White spots which show the calcified areas (arrowhead) and the focal necrosis areas (arrow). (C): Liver with fascioliasis that the white arrow shows distended and calcified bile ducts. (D): Hepatic hydatidosis.

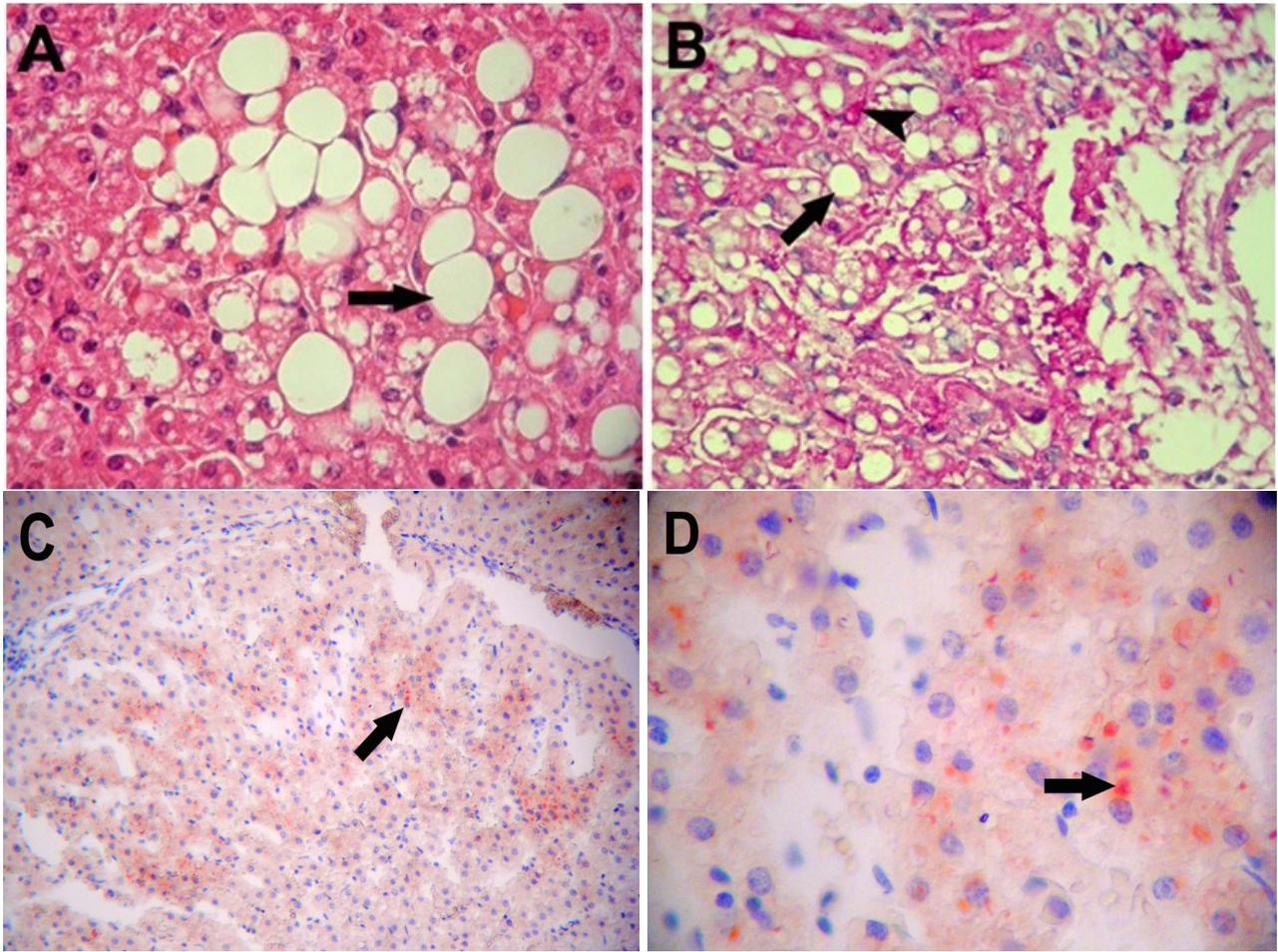


Figure 2: Microscopic evaluation of hepatic fatty change. (A): Clear fat vacuoles (arrow) in hepatocytes which cause nucleus compressed to the cell wall (H&E stain, X100) (B): White colored fat vacuoles (arrow) and red colored glycogen vacuoles (arrowhead) in the liver (PAS stain, X100) (C, D): Lipid droplets in orange color (arrow) in hepatocytes (Sudan IV stain; c: X40, d: X400).

## Discussion

Hepatic lesions in animals have been investigated by many researchers in the world and Iran. The results of current study showed that the prevalence of hepatic fascioliasis (12.56%) and fatty change were (10.44%). Prevalence of hepatic fatty change in the present study was 10.44% that according to table 1, this lesion was found in male cross-breeds cattle more than others. In addition, the most prevalence of hepatic fatty change in our study was in cattle group aged under 2 years old. Although prevalence of bovine hepatic fatty change occurs in high-producing dairy cows generally however, in this research prevalence of hepatic fatty change was in male animals more than females. Fatty liver may happen due to other factors such as displaced abomasum and immune suppression in cattle (14). Of course, we studied bovine livers for evaluation of

fatty liver based on gross lesions and microscopic findings in abattoir. So, to find definitive cause of fatty change more study is needed. In a study has been conducted in southeast Iran, the prevalence of bovine fatty liver has been reported 11.7% (11/94) (15) that was higher prevalence rate in comparison with our result (10.44%). Another research has reported the prevalence of acute fatty liver of dairy cows 15% (8). In the current study, the inspected bovine livers affected by fatty change were diagnosed accidentally and without clinical signs. On the other hand, the most cattle with hepatic fatty change were male and were not related to the fatty liver syndrome in dairy cows around parturition time. A fatty liver is pale, yellow colored, and greater than a normal size that its edges have been rounded and lose their normal architecture (shape) grossly. Based on the recent report, the clear fat vacuoles can be diagnosed, are orange- red colored in Sudan IV stained tissue sections

(15). In the parasitic diseases of livers like fascioliasis, accumulation of reactive oxygen species (ROS) in the hepatocytes causes damage to biomolecules especially lipids (16). On the other hand, changes in oxidation and metabolic conversion of lipid and lipoprotein fractions in ovine fascioliasis may have an effect to increasing of intensity of hepatic lipidosis (17). Since our microscopic evaluations of the histopathologic sections showed that there was fatty change (mild or moderate) in most livers affected by fascioliasis, so fascioliasis may be considered as one of the possible agents for hepatic fatty change. In our research, the prevalence rate of hepatic fascioliasis was 12.65% that was higher than the result of other study that has reported its prevalence rate (fluke infestation) 3.34% in Iraq (11). Moreover, in Iraq the prevalence of hydatid cyst (3.12%) and hemorrhage with congestion (1.03%) that have been reported (11) that were higher than our results for hydatid cyst (2.53%) and hemorrhage (0.63%) in livers of slaughtered cattle. The prevalence of hydatid cyst in cattle, sheep, and goat has been reported as 3.2%, 3.5% and 4.5% respectively (10). In similar research prevalence of cystic echinococcosis has been reported as cattle (19.4%), sheep (3.6%) and goats (4.5%) (18). Although, different incidence rate of hydatid cyst in the liver of cattle, sheep and, goat has been reported in Iraq (11), Sudan (19), Morocco (20) and Ethiopia (21). The difference in the prevalence of hydatid cyst may be due to the differences in environmental conditions including the persistence of the parasite, the frequency of the final host, animal husbandry, pasture type and the pattern of animal grazing (10). Stray dogs around the slaughterhouses, which feed the confiscated organs that it has an important role on the transmission to human and increase rate of prevalence (10). The prevalence of hydatid cyst in liver of slaughtered cattle in Shiraz between years 1999 to 2004 has been reported at 1.27% (22). The prevalence of hydatid cyst in Urmia city is higher than Shiraz, it seems that higher rate prevalence of hydatidosis may be due to more availability of stray dogs which feed on livers infected by hydatid cysts in Urmia city (10). The prevalence of hydatid cyst in other countries such as Tanzania, Ethiopia and Iraq have been reported 19.7%, 32.1% and 1.1%, respectively (10,23,24). In the present study, hepatic congestion was observed in some of the livers with different injuries, but it is not considered as an independent lesion in this research because it can be produced by the physical agent (handling by the abattoir's workers) during and after slaughter (25). Another lesion that was found in our research was hepatic focal coagulative necrosis with a prevalence rate of 1.89%. This lesion was seen as the white spots on the liver surface. Focal necrosis has been reported in cattle livers affected by mercury toxicosis (26).

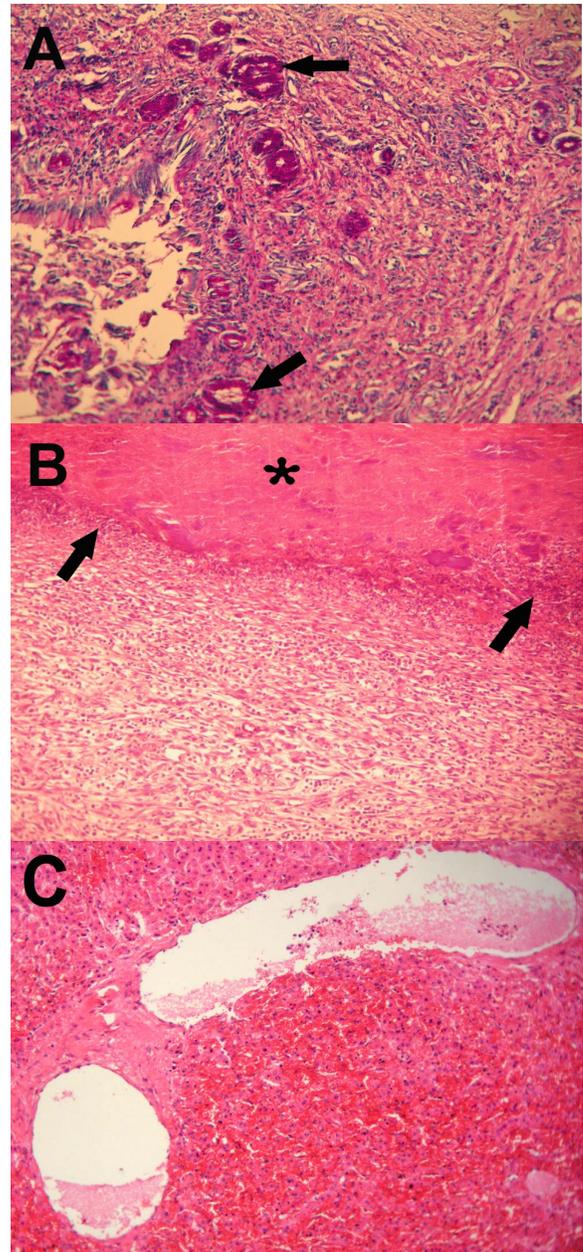


Figure 3: Microscopic findings in the hepatic lesions in addition to fatty change. (A): Bile duct hyperplasia (arrow) in fascioliasis affected liver with increased connective tissue in hepatic parenchyma (PAS stain, X100). (B): Hepatic necrotic area (asterisk) and increased connective tissue around the necrotic area with accumulation of lymphocytic inflammatory cells in the surrounding of coagulative necrosis area (arrow) (H&E stain, X100). (C): Hemorrhage in liver parenchyma (H&E stain, X100).

## Conclusion

The results of present study showed that prevalence rate of hepatic fatty change was 10.44%. This lesion was the second important hepatic injury after fascioliasis with the prevalence of 12.65% in the slaughtered cattle in the Urmia Abattoir. Microscopic examination of fatty change was performed by H&E, PAS, and Sudan IV staining methods in the affected livers. Other injuries such as hydatid cyst, focal coagulative necrosis, and hemorrhage were also observed in the inspected livers. According to our microscopic results, presence of fatty change in most bovine livers affected by fascioliasis shows that fascioliasis could be considered as a possible agent of hepatic fatty change. The confirmation of definitive factors of hepatic fatty change requires further research.

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