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Histopathological Studies on Fatty Liver Disease

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Abstract

The present study was conducted to evaluate the efficiency of natural *Spirulina* preparation in on fatty liver. The histopathological examinations were done in rats at the start, with normal diet, after induction of fatty liver (naturally and chemically) and after using different *Spirulina* concentration in the diet after 1, 2 and 3 weeks. This study proved that *Spirulina* has effect for regulation of fat metabolism of fatty liver.

1. Introduction

Fatty liver, also known as fatty liver disease (FLD), is a common cause of chronic liver disease and refers to accumulation of excess fat in the liver. It is diagnosed that if fat exceeds 5% of the total weight of normal liver or when more than 30% of the hepatocytes in a liver lobule have lipid deposits, most of the fat that accumulates in the liver is triacylglycerols and fatty acids; other forms of fat, such as cholesterol, cholesterol ester, and phospholipids, are also present [1].

Fatty liver is often associated with alcoholic liver disease, hyperinsulinemia, and insulin-resistance. Accordingly, it is most often observed in alcoholics, obese persons, and diabetic patients. It is also frequently caused by pregnancy [2], malnutrition [3], chemical intoxication [4], drugs [5], viral hepatitis [6], and intestinal bypass surgery [7], morphologically it is difficult to distinguish alcoholic FLD from non alcoholic FLD and both show micro-vesicular and macrovesicular fatty changes at different stages [8]. As reported elsewhere, *Spirulina* prevents the formation of fatty liver in animal models and in humans [9]. The effectiveness of *Spirulina* against fatty liver may follow from its antioxidants, which include GLA, selenium, phycobilins, vitamins and carotenoids (β -carotene). In addition, essential fatty acids like GLA can prevent the accumulation of cholesterol in the body [10].

In an animal model, fatty liver has been reported to be induced by a high cholesterol diet, a 60% fructose diet, carbon tetrachloride, and alloxan induced experimental diabetes [11].

The high fructose diet induces fatty liver because the rapid conversion of fructose to acyl-CoA or α -glycerophosphoric acid elevates plasma lipid level. Fructose has been reported to have less effect on lipoprotein lipase (LPL) activation and to promote the activities of fatty acid synthesis related enzymes such as acetyl-CoA carboxylase, fatty acid synthetase, and malic enzyme [12].

The effectiveness of administering *Spirulina* to an animal with high fructose diet-induced hyperlipidemia appears to be demonstrated in hypolipidemic effect, reduced

liver triacylglycerol, and hypocholesterolemia. The beneficial effect of *Spirulina* may derive from the activated LPL activities, which are determined using post-heparin serum [13].

Carbon tetrachloride-induced hepatocyte injury and fatty liver have been suggested to be caused by an increase in the synthesis of liver fatty acids, elevated lipoperoxidation and altered release of hepatic lipoprotein [14]. The prevention by *S. maxima* of carbon tetrachloride-induced fatty liver is evidenced by restored lipoprotein levels and hypocholesterolemic, and hypotriacylglycerolemic effects. It has a similar protective effect on a high fructose and hypercholesterolemic diet induced fatty liver.

In an animal study of fatty liver induced by the administering of simvastatin (75 mg/kg body weight), ethanol (20%) and a hypercholesterolemic diet (1% cholesterol) to male CD-1 mice for 5 days, significant measured liver total lipids (40%), liver triacylglycerols (50%), serum high-density lipoprotein (HDL) (45%), and serum triacylglycerols (50%) all markedly decreased when animals received *Spirulina* treatment (10% of diet) 2 weeks prior to the onset of the fatty liver [15].

The fatty liver is commonly associated with Type II diabetes, which is related to the variation in insulin resistance and hyperinsulinemia. One study indicated that the dietary administering of 5% *S. maxima* (SM) dried powder for 4 weeks to alloxan induced (250 mg/kg body weight, intraperitoneal) diabetes in CD-1 mice prevented the formation of fatty liver in male and female animals [16].

Spirulina also attenuates alcohol-induced fatty liver through ALDH activity, which is inactive in subjects intoxicated by alcohol, especially in Asian populations [17]. In an experimental model, the Km value of ALDH decreased from 0.91 to 0.70 mM after treating with *Spirulina*. However, the activity of alcohol dehydrogenase (ADH) did not change. Apparently, *Spirulina* facilitates alcohol metabolism through enhanced clearance of accumulated aldehyde, which may increase susceptibility to alcoholic liver disease (ALD), such as fatty liver and fibrosis [18].

Studies of the preventive effect of *Spirulina maxima* on fatty liver development induced by carbon tetrachloride, in the rat by prevention of fatty liver development, induced in rats by a single intraperitoneal dose of carbon tetrachloride (CCl₄). Liver and serum lipids were evaluated 4 days after treatment with this agent [14].

Some preliminary evidence suggests that *Spirulina* may help protect against liver damage and cirrhosis (liver failure) in those with chronic hepatitis. Without more research, however, it is impossible to say whether *Spirulina* offers any real benefit [19].

2. Materials and Methods

2.1. Application on Experimental Animals

Adult male rats weighing (99-108) gram obtained from

Faculty of Science, Zoology Department, Tanta University.

The animals were housed according to the time needed for each experiment.

2.2. Experimental Design

1- Natural induction of hyperlipidemia and fatty liver [20]

Group (A)

This group divided into two subgroups: Sub group (1) and Sub group (2)

Subgroup (1): This group feeded on high rich oily diet, the oil percentage about (25%) of total diet and housed for 15 days.

Sub group (2): This group feeded on high rich Butter percentage about (25%) of total diet and housed for 15 days.

2- Chemical Induction of hyperlipidemia and fatty liver [14]:

Group (B):

In this group, hyperlipidemia and fatty liver induced by using two chemical inducers; Carbon Tetra Chloride (CCl₄) and triton x100 for 4 days. This group divided also into two subgroups:

Subgroup (1): This group using carbon tetrachloride (CCl₄) to induce hyperlipidemia and fatty liver by injection of (CCl₄) intraperitoneal one single dose equal 1.0 ml (1kg).

Subgroup (2): By using triton x100 to induce hyperlipidemia and fatty liver but this experiment was adapted according to (150 mg /kg) by single intraperitoneal injection of fresh prepared solution of triton x100 in physiological saline [21]. 15 rats were treated with the following doses of triton x100: 0, 50, 100, 150 and 200.

3-Healthy rats (control):

Group (C): This group feed normal standard diet.

2.3. Histological Examination

Preparation of biopsies for histopathology examination:

After the liver samples were collected from the rats, they were preserved in 10% formalin for one hour. The large biopsies were cut to be smaller and thinner. Thin biopsies were immersed in 99% ethyl alcohol for one hour. The dehydrated samples were rinsed in xylene for one hour. In liquid paraffin wax, the biopsies were kept for two hours at 65°C and transferred to clean liquid paraffin wax and blocks were kept at 0°C for one hour. Using microtome, the paraffin blocks were sectioned into very thin section in worm water bath, and prepared for staining [22].

Staining method:

For examination of the liver biopsy, the biopsy samples were blocked in paraffin blokes. Using microtome and worm water bath, the paraffin blokes were cut into thin section. On glass slides, thin films from different paraffin blokes were adhesive on to the slides. The different slides were put in an oven at 65 for 15 minutes to remove the excess of paraffin wax. The slides were kept in pure xylene for one hour and then the tissues were washed twice by xylene and ethyl alcohol and finally with water. The samples were transferred

to haematoxylin for 10 minutes and then washed with water, dilute HCL and concentrated HCL, respectively, and finally with water, and then transferred to eosin blue for 2.0 minutes. The samples on slides were washed with water, ethyl alcohol and finally xylene. A cover slides were fixed on stained tissues and examined by light microscope. The cytoplasm of the cell stained with red colour and nuclei are observed with violet colour [23].

3. Results

As regard to the histopathological examination on rats; Figure 1 show normal liver biopsy slides; the cytoplasm of the cells stained with red color and nuclei are observed with violet color.

Figure 2: Show the liver of rat after 15 days of treatment with soybean oil. The biopsy show a fatty liver with white areas of fat tissues.

The histopathological examination of rat liver induced naturally by butter after 15 days of treatment also show white area represent deposition of fats (Figure 3).

Figure 4: Show liver biopsy of rat after 4 days of treatment with carbon tetrachloride (CCl₄). The slide show deposition of fat tissues and also liver cell fibrosis. The same results were obtained when the fatty liver in rat was induced chemically by Triton x100. (Figure 5)

When the rats which induced naturally for fat liver were treated by 5% and 10% *spirulina*, there was reduction of fat tissues as compared with control (Figure 6 and 7).

The histopathological examination of rat with fatty liver induced chemically revealed that treatment with 5% and 10% *spirulina* reduced the fat tissues as compared with control, (Figure 8 and 9).

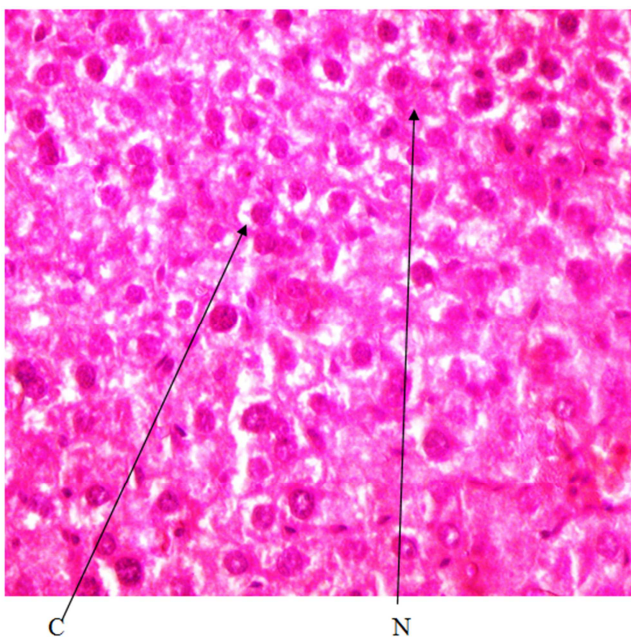


Figure 1. The histopathological examination of normal liver biopsy of rat showed red cytoplasm (C) and violet nuclei (N).

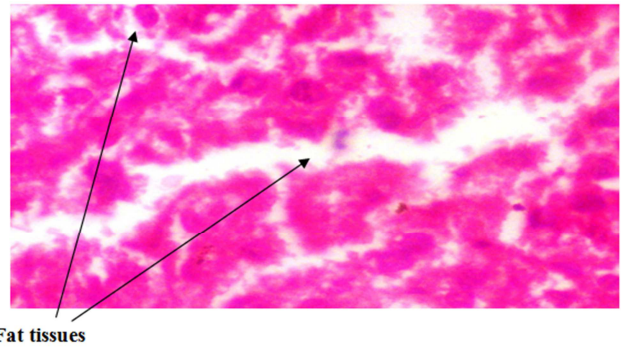


Figure 2. The histopathological examination of fatty liver of rat induced naturally by soya bean oil.

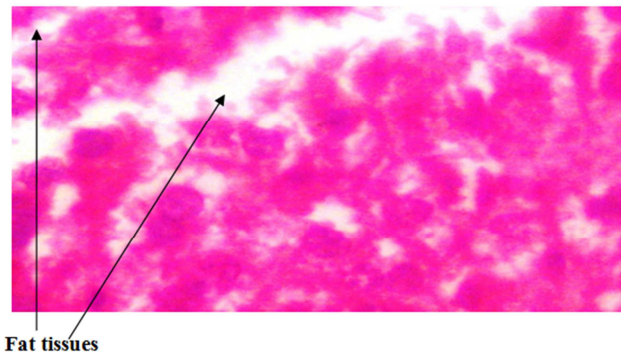


Figure 3. The histopathological examination of fatty liver of rat induced naturally by butter.

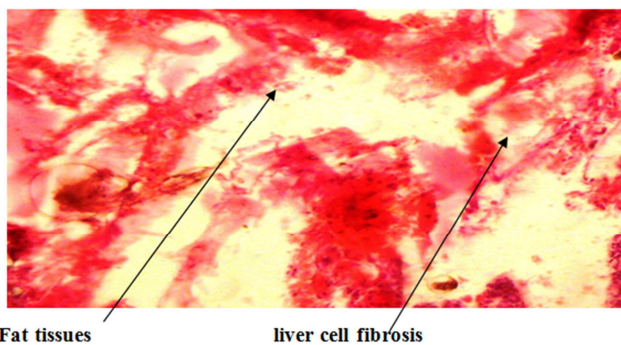


Figure 4. The liver biopsy of rat after 4 days of treating by carbon tetrachloride (CCl₄) demonstrating liver fibrosis.

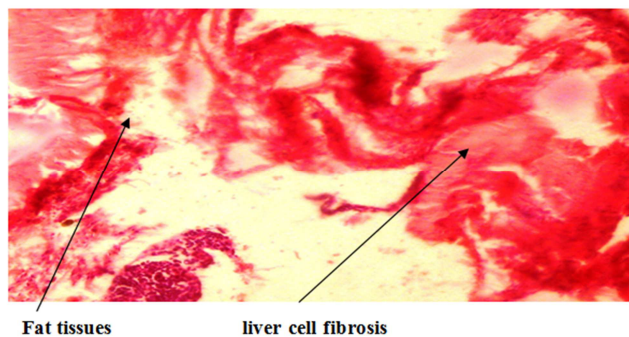


Figure 5. The liver biopsy of rat after 4 days of treating by Triton x100 demonstrating liver fibrosis.

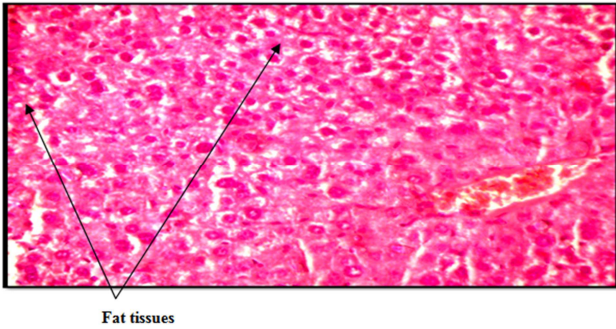


Figure 6. The histopathological examination of rat with fatty liver (naturally induced) after treatment with 5% *Spirulina* demonstrating reduction of fat tissues

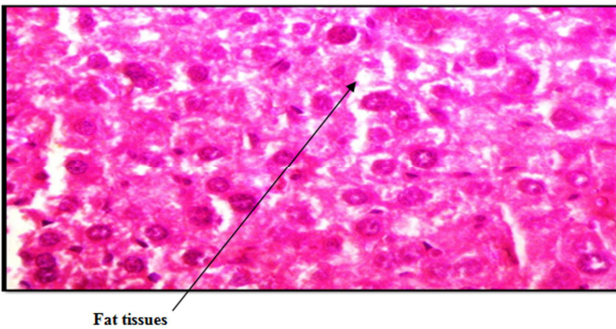


Figure 7. The histopathological examination of rat with fatty liver (naturally induced) after treatment with 10% *Spirulina* demonstrating reduction of fat tissues

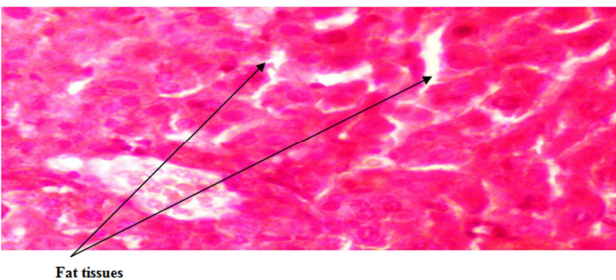


Figure 8. The histopathological examination of rat with fatty liver (chemically induced) after treatment with 5% *Spirulina* demonstrating

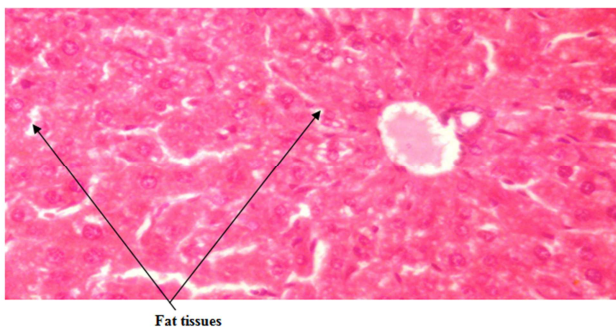


Figure 9. The histopathological examination of rat with fatty liver (chemically induced) after treatment with 10% *Spirulina* demonstrating reduction of fat tissues.

4. Discussion

For all groups, the histopathological examination were done at the start, with normal diet, after induction of fatty liver (naturally and chemically) and after using different *Spirulina* concentration in the diet after 1, 2 and 3 weeks. In the present study, the experiments proved that *Spirulina* has effect for regulation of fat metabolism of fatty liver

Fatty liver, also known as fatty liver disease (FLD), steatorrheic hepatitis, steatosis hepatitis and hepatosteatorrheic, is a reversible condition where large vacuoles of triglyceride fat accumulate in liver cells via the process of steatosis. Despite having multiple causes, fatty liver can be considered a single disease that occurs worldwide in those with excessive alcohol intake and those who are obese (with or without effects of insulin resistance). The condition is also associated with other diseases that influence fat metabolism. Morphologically it is difficult to distinguish alcoholic FLD from non alcoholic FLD and both show micro-vesicular and macrovesicular fatty changes at different stages [8].

The prevalence of FLD in the general population ranges from 10% to 24% in various countries. However, the condition is observed in up to 75% of obese people, 35% of whom will progress to non-alcoholic FLD, despite no evidence of excessive alcohol consumption. FLD is the most common cause of abnormal liver function test in the US [24].

In agreement with these results, a study reported that *Spirulina* prevents the formation of fatty liver in animal models and in humans [16]. The effectiveness of *Spirulina* against fatty liver may follow from its antioxidants, which include GLA, selenium, phycobilins, vitamins and carotenoids (β -carotene). In addition, essential fatty acids like GLA can prevent the accumulation of cholesterol in the body [10].

In the same direction a results of a study proved that *Spirulina* may be considered an alternative treatment for patients with non-alcoholic fatty liver diseases and dyslipidemic disorder [25].

5. Conclusion

The thrust of this study to evaluate histopathological changes in fatty liver after treatment with *Spirulina*. This study proved that *Spirulina* has effect to prevent the formation of fatty liver disease and also helps in the regulation of fat metabolism of fatty liver.

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