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Effect of Ganoderma Fungi and Spirulina Alga in The Liver Tissue of Diabetic Mice Developed with Aloxane and Lead-Induced Anemia

ABSTRACT

The study was conducted on male mice (Albino mice), which ranged in age from (2-3) months and weights (25 - 30) g. The experiment was conducted in the General Company for the manufacture of medicines and medical supplies - Samarra. And for the period from the beginning of October 2017 to the end of March 2018, Used 42 animals were distributed in seven groups, each containing 6 animals, to determine the effects of both gannoderma and spirulina in laboratory mice induced by diabetes mellitus with a Aloxan at 150 mg / kg Bw. In laboratory mice with anemia with lead acetate of 50 mg / Kg body weight for 30 days (except control group) At the end of the 30-day feeding period.

1. control group on the regular diet.
2. control group infected diabetes and drugs on the regular diet.
3. The group of diabetic animals treated with ganoderma fungi powder (47 mg / kg body weight).
4. The group of diabetic animals and the treatment of spirulina algae powder (33 mg / kg body weight).
5. group of animals infected with anemia and spores on the regular diet and water containing lead acetate
6. Group of infected animals and treated with lead acetate + Ganoderma fungi powder (47 mg / kg body weight).
7. Group of infected animals and treated with lead acetate + Spirulina algae powder (33 mg / kg body weight).

Histological changes were observed in the Livre tissue: The results of the study of tissues in the group of animals treated with Alloxnesan damage to the overall liver tissue in the form of hepatocytes and hydrolysis and the expansion of the Sinusiod. In the treatment of diabetic animals with ganoderma and spirulina algae, there was improvement in the overall liver tissue in terms of normal hepatocytes, absence of congestion, and normal Sinusiod. the group treated with anemia and treated with lead acetate, we also notice significant damage to the liver tissue. When treated with lead acetate + gannoderma, and spirulina, we did not notice any improvement in liver tissue.

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INTRODUCTION:

Diabetes is a syndrome characterized by metabolic disorder and abnormally high blood sugar concentration due to insulin deficiency or low insulin sensitivity of insulin or both (Guyton and Hall, 2010)

Diabetes causes complications, serious complications, or even early death. This disorder is characterized by chronic metabolic disorders (Rodrigo, 2009). As a result of diabetes, glucose is not converted into energy, resulting in excess glucose in the blood while cells remain energy hungry. Over time, hyperglycemia develops (Hyperglycemia). This disease is very damaging to the nerves and large blood vessels and small vascular disease. It is characterized by retinopathy with a possible loss of vision and facilities of renal impairment leading to renal failure and thus lead to the incidence of diabetes mellitus and develop into the sore feet and pores (Poretsky, 2010)

Spirulina is one of the bluish green alga that live in salt lakes. It was discovered by the French scientist Clement in 1962 and is of high nutritional value through chemical analyzes to determine the proportions of its components. It was called by the Food and Agriculture Organization of the United Nations World Food (FAO) The name of the ideal food is super food (Ashraf, 2014). Spirulina algae has received increasing attention due to the fact that it represents one of the most promising sources of biologically active compounds that can be used as functional components, balanced chemical composition and its content of balanced good quality proteins, fatty acids, vitamins, antioxidants, minerals and its interesting properties Can be applied to the formulation of new food products (Spolaora et al., 2006).

Ganoderma lucidum a popular medicinal mushroom, has been used in traditional medicines in many Asian countries. It has been widely used as medicine to promote health and longevity in China for thousands of years (Mizuno, et al. 1994). Studies have shown that *Ganoderma lucidum* have different active constituents. Some of the chemical constituents of *G. lucidum* include polysaccharides, proteins, nucleosides, fatty acids, sterols and triterpenes (Yeung, et al, 2004). In East Asia, the fruiting body of the fungus *Ganoderma lucidum* has been used for centuries. It has long been used as a folk medicine to treat various human diseases such as cancer, hypertension, hepatitis, nephritis and so on (Mizuno, et al. 1994). Medical fungus containing biologically active compounds are sugars, triglycerides, nucleic acids and trace elements that are used in medical applications, usually have a solid texture and bitter taste (Ingo, 2013). Chi and Achron (2013) mentioned that the turbines in the *ganoderma* mushroom have an anti-inflammatory effect, and it undoubtedly contains many benefits and active ingredients, including organic germanium, which symbolizes its inorganic image in the chemical periodic table with code 32 Ge (Sing, 2003)

The liver is one of the largest glands in the body (The salivary glands and the pancreas), linked to the small intestine by the hepatic duct, which is opened by the bile duct in the duodenum (Sheikhly et al., 1988). Liver cells form 60% of the total cells of the liver tissue. Endothelial cells, which are located between the visceral cells, account for 30%. The remainder consists of angiogenesis, laryngeal tissue, and bile ducts (Guyton and Hall, 2010). The diameter of 2-1 mm lies between the liver cells, and are linked to each other in the liver density, and increase in size, forming a larger bile ducts and then a major channel called the bile Duct (Habel, 1999).

MATERIAL AND METHODS

1- Get dietary supplements

The nutritional supplement was obtained from the health wealth company located in Tikrit / Zohour Branch of the Malaysia company DXN.

- (a) - *Ganoderma lucidum*
- (b) – *Spirulina aglae*

2- Design Experience

six mice were isolated for standard treatment and 16 mice were injected with 150 mg / kg body weight (Saik *et al.*, 2008) by intravenous injection and left for a week. Thus, diabetes was examined for all mice injected with the Rosmax) Of the origin and ensure the incidence of disease, as the blood sugar rate of infected mice between (146_213), while the remaining mice (16) left until the start of treatment to develop anemia through giving lead acetate through drinking water at a concentration of 50 mg / kg bw (Ghazal *et al.*, 2012).

3- Division of groups

The male mice were divided into seven groups, each group had a different diet and the groups were left for 30 days.

1. The first group : A healthy control group that included 6 healthy mice and was fed on a regular diet and regular water.
2. The second group: A diabetic control group developed with aloxane and fed on the regular diet and normal water.
3. The third group: The group treated with diabetes and treated with fungus powder Ganoderma if given (47 mg / kg body weight) and according to the recommendations of the company DXN producer and mix with the regular diet and was given normal water.
4. The fourth group: The diabetic group treated with spirulina algae powder if given (33 mg / kg body weight) and according to DXN manufacturer recommendations and was mixed with the regular diet and given normal water (Chiricuța *et al.*, 1998).
5. The fifth group: A control group with anemia with lead-induced anemia (50 mg / kg body weight), fed on the regular diet and supplied with lead-containing water throughout the trial (Ghazal *et al.*, 2012).
6. The sixth group: A group with anemia and treated with a gannoderma powder if given (47 mg / kg body weight) and according to the manufacturer's recommendations, mixed with the regular diet and supplied with water containing lead acetate throughout the experiment.
7. The seventh group: A group with anemia and treated with spirulina algae powder if given (33 mg / kg body weight) and according to the recommendations of the DXN manufacturer and was mixed with the regular diet and supplied with water containing lead acetate throughout the experiment.

4- Isolation of organs: (dissection of animals)

After the experiment was completed, the animals were placed in the anatomy pelvis. The front and posterior limbs were stabilized by pins. The abdominal area was opened lengthwise using sharp scissors and scalpel, TheLivre member was removed and then placed in a petri dish containing the saline solution(NaCl 0.9%), for washing and then kept in the container of the solution solution (10% Formalin) for 24 hours (Joao *et al.*, 2006). To save it until it is used when studying this tissue.

5- Histological study:-

Preparation of histological section:-

Textile sections were prepared according to the following steps:-

1. **Fixation :-** Place the organ to be studied immediately after the dissection with the formalin stabilizer 10% for a whole day (Joao *et al.*, 2006).

2. **Washing :-** The following day the member was washed with running water for at least half an hour to remove the excess fixer.
3. **Dehydration :-** The samples were passed for two hours with elevated concentrations of ethyl alcohol from 70% -80% -90% -100% (half an hour at each concentration) to water extraction from samples.
4. **Clearing :-** Samples were put in xylene for a quarter of an hour to pull alcohol And to make it more transparent.
5. **Infiltration :-** The samples were placed in a mixture of xylene and paraffin wax 1: 1. The mixture was placed in the electric oven for a quarter of an hour at 60 ° C and samples were placed in two phases of molten wax for half an hour at each stage.
6. **Embedding :-** The eyes were immersed in the wax after pouring the wax in iron molds and passing hot needle near the sample to get rid of bubbles around the sample and then lifting the molds after confirmation of hardening.
7. **Trimming Sectioning :-** Using a sharp knife, place the molds with the Rotary microtome to obtain samples of thickness (7_5 micrometers) and then transfer them to a water bath at a temperature of 37-40 m for the cell brushes and prevent their accumulation. Then wipe with the albumin solution. With 50 mL of glycerin and several times and then add 1 g of thyme and keep it in the refrigerator until use). Remove the tissue on a chip marked with the name of the member and the treatment number, then transfer the slice to a thermal surface of 40 m for one minute and leave it to dry. Or tissue sections in the cellulose to get rid of the paraffin wax and then placed with the concessions Yeh% of ethyl alcohol 100-96% -90% -% 70.% 50-30% then in distilled water.
8. **Staining :-** Hematocycline Hares dye was used to color the sections for one minute and wash them with tap water for a quarter of an hour to distinguish the blue color in the blueing nucleus, the Eocene dye to color the sections for 50 seconds and wash the sections with alcohol 70% for 5 seconds to distinguish the purple color in the cytoplasm(Humason, 1979).
9. **Mounting :-**A drop of Canada balsam is placed on the tissue section and the slide cover is placed on the tissue sections and left to dry at the laboratory temperature.

6- Microscopic and Photographic Exanination of Histological Sectio:

The histological sections of the liver was examined using a Miotic optical microscope from the Japanese-made Olympus Company in the laboratory of the Faculty of Science / University of Tikrit. The required parts of the tissue sections were then measured using an ocular micrometer and imaging of tissue sections Different types of liver using digital camera Type Microscopy Camera Eyepiece for PC 0.3M Pixel DCE-PW1 for textile sections and attached to an electronic computer Type Laptop ASUS K401

RESULTS AND DISCUSSION:-

effect of *Ganoderma* and *Spirulina* supplement in liver tissue :-

The section tissue of (Figure 1). shows the hepato cyte of the liver in control group , The results showed that Hepato cyte is mostly normal, Sinusiod is also observed, The endothelial cells appear normal and clear within the central vein and the boundaries of the cells of the kufer cells.

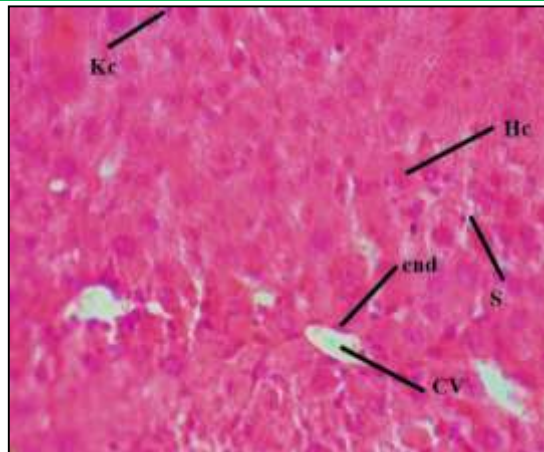


Figure 1: A section in the mice liver from the control show the normal shape of the central vein (CV), cofer cells (KC), liver cells (HC), Sinusiod (S). Endothelial network (end).40X

The section tissue of (Figure 2), show the hepatic cytotoxicity of the hepatic control group. The results showed that the incidence of diabetes led to the differentiation of hepatic cytotoxic cells in general by expansion of the sinusiod, due to weak venous flow at the level of the hepatic vein or hepatic vein The inferior vena cava, or the cause of the expansion of the blood vessels may result in hepatic portal vein (Karkar *et al.*, 2007), The hereditary arrangement of hepatic cells was observed, leading to damage to hepatic cells. The damage caused by hepatic cells is due to the immune or toxic effects of the toxin, The oxidative stress resulting from the free-radical aggregation leads to hepatic cell breakdown as well as lipid oxidation of the cell membrane or membranes (Majumdar *et al.*, 2008). The results show that some of the cells are in the stages of hydrolysis, which means that the cells absorb water and bloom because of the difference in the ammotic pressure through the cheat. Cell, leading to the large size of the cell and the large size of the kernel, which ends at the end of water degradation.

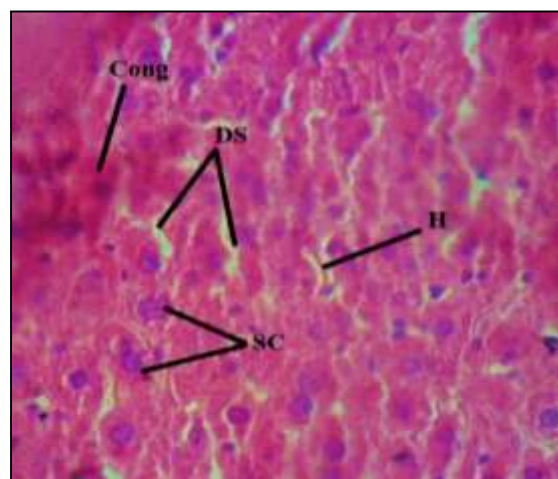


Figure 2 : A section in the liver tissue of the mice The diabetic group and the dioxin-based group shows the damage from the Cong, the expansion of the sinusiod (DS), the decomposition (H) and the large cell (SC), 40X

The histological section in(Figure 3). shows the liver of diabetic mice and treated with Ganoderma fungi powder. The results showed that hepatocellular cells were mostly the same size, indicating that the radiology of the cells was improved. Most of the jibians were normal among the hepatic cells, but there is still a clear hemorrhage in the central vein where the blood appears decomposed. The other part of the red blood cells is clear and next to the central vein, The bile duct, which appeared naturally in shape and the cells lining it, which are cubic-shaped and the cells of cofer that were swollen indicate the function of thrombocytopenia, which is found in the hepatic corpuscles, and there within the liver tissue infiltration of lymphocytes, which appear in some mother I am a group of lymph nodes that are either candid or out of the blood that goes through the central vein.

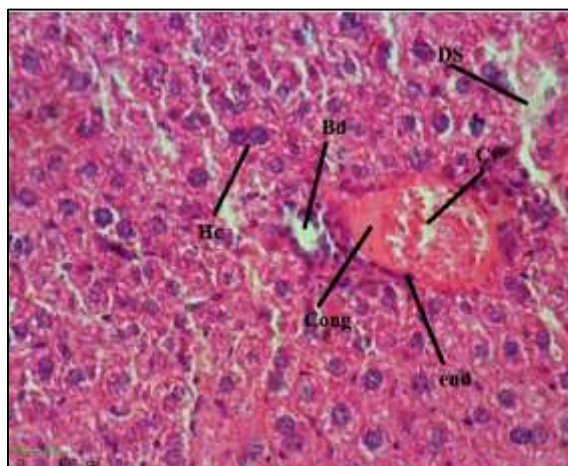


Figure 3 : A section in the liver tissue of the diabetic group and treated with Ganoderma, shows central venous prolapse (CV), bile duct (Bd), hepatocytes (HC), expansion of the sinusiod (DS), Cong and endothelial,40X

The histological section in (Figure 4) shows the liver of diabetic mice and the treatment with Spirulina algae powder, Liver cells are mostly normal, but parts of the liver cells remain unclear. There are sporadic but few seiz compared with the previous treatment and a fraction of the cells in which there is little programmed death.

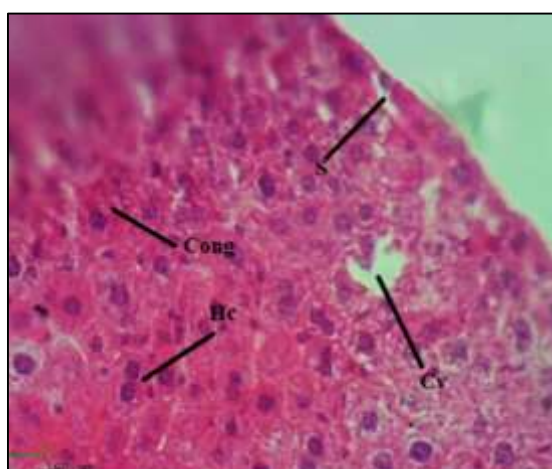


Figure 4 : A section in the liver tissue of mice of the diabetic group and treatment with spirulina algae, the central vein (CV), the cons (Cong), normal of the sinusiod (S), and hepatocytes normal (Hc),40X

The histological section in (Figure 5) .results showed that male mice were given lead acetate concentration (50 mg / kg body weight) for 30 days. The cells of the central vein of the central vein showed swollen and prominent and hepatic cells between The majority of the Sinusiod were in an abnormal state of decomposition. It was observed that there was congestion in the entire tissue of the liver with the lymphocytes. The bile ducts were enlarged due to the damage of the lining cells, She concurred with the findings of Jankeer and El-Nouri (2009), who found that injecting male mice with 2 and 4 mg / kg bodybuilders for four weeks resulted in necrosis, bleeding, inflammatory cell spasms, and central venous congestion in the rat liver, Lead acetate through changes in the liver tissue, which affects the work of liver cells and leads to increased liver enzymes or may have a direct effect on hepatic cells and the occurrence of toxicity (Gobor and Zoia, 1986), or lead acetate effect on liver calcification, DNA content and manufacturing Proteins of the amino acids (Ragab *et al.*, 2013).

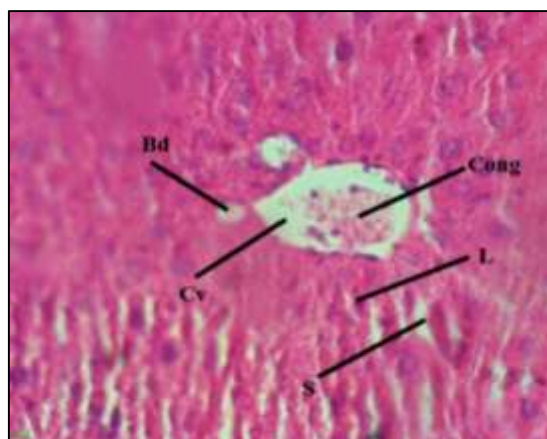


Figure 5 : A section in the liver tissue of mice exposed to lead acetate, Congestions (Cong), abnormal sinusiod (DS), central vein (Cv), and lymphocytic infiltration (L),40X

The histological section in (Figure 6). shows the liver of anemic mice through treatment with lead pellets + Ganoderma fungus. The results showed that most hepatic cells were abnormal and the radiographic arrangement was missing. The ligaments were enlarged with no congestion either in the central vein or in vain Liver.

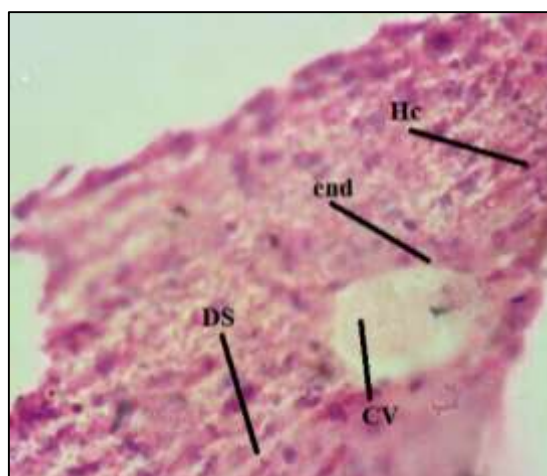


Figure 6: A section in the liver tissue of mice , treated with lead acetate and janoderma fungus. The central vein (CV), endothelial cells (end), abnormal hepato cytes (Hc), enlarged blood sinusiod (DS) and congestion (Cong).

The histological section in (Figure 7) shows the liver of anemia mice treated with lead cells + spirulina algae powder. The results showed that hepatocytes are missing radial order and cell boundaries are mostly unclear and cells divide them at different stages of decomposition and macrophages. Show mixed with cellular contents of hepatocytes, there is a leakage of lymphocytes

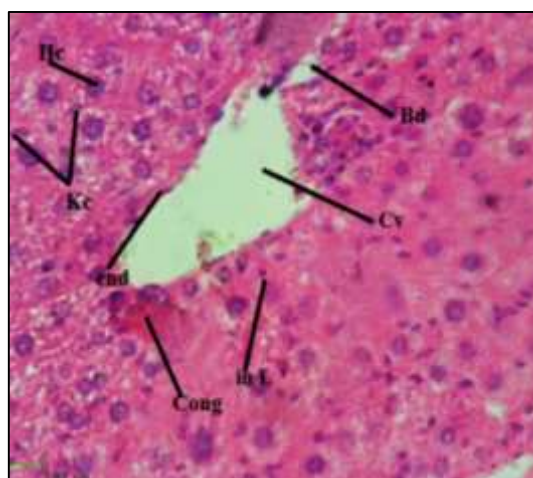


Figure 7: A section in the liver tissue of mice treated with lead acetate + Spirulina algae shows central vein expansion (CV), connective cells (KC), abnormal hepatocytes (HC), lymphocytic infiltration (inL), and bile duct (Bd), 40X

CONCLUSIONS

1. The development of experimental diabetes in male white mice has resulted in severe damage to the whole liver weave.
2. Results in animals with diabetes, Ganoderma and spirulina showed no positive effect on liver tissue.
3. The development of anemia using lead acetate in male white mice has led to many changes in the cruciferous tissue of the liver.
4. Results indicate that the administration of lead acetate + powder Ganoderma and lead acetate + Spirulina powder, no positive changes in liver tissue.

RECOMMENDATIONS

1. Study of other types of these supplements and the extent of their effect on liver tissue.
2. Conducting a study of the tissue of other members such as kidney, pancreas and spleen to know the impact of these diseases on them.
3. Study the components of these supplements to see how they affect the liver tissue Study the components of these supplements to see how they affect the liver tissue.

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تأثير فطر الجانوديرما *Ganoderma Lucidum* وطحالب السبيرولينا *Spirulina algae* في نسيج كبد الفئران المصابة بالسكري المستحدث بالآلوكسان وفقر الدم المستحدث بخلات الرصاص

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المستخلص

تمت الدراسة على ذكور الفئران (Albino mice) التي تراوحت أعمارها من (2-3) أشهر وأوزانها بين (25-30) غم وقد أجريت التجربة في الشركة العامة لصناعة الأدوية والمستلزمات الطبية - سامراء. لمدة، واستعمل منها 42 حيواناً وزعت بواقع سبع مجاميع تضمنت كل مجموعة 6 حيوانات لمعرفة تأثيرات كل من فطر الجانوديرما *Ganoderma lucidum* وطحالب السبيرولينا *Spirulina algae* في الفئران المختبرية المصابة بالسكري المستحدث بالآلوكسان بتركيز 150 ملغم / كغم من وزن الجسم ، في الفئران المختبرية المصابة بفقر الدم المستحدث بخلات الرصاص بتركيز 50 ملغم / كغم من وزن الجسم ، استمرت التجربة لمدة 30 يوماً:

1. مجموعة السيطرة السليمة والمغذات على العليقة الإعتيادية.
2. مجموعة السيطرة المصابة بالسكري والمغذات على العليقة الإعتيادية.
3. مجموعة الحيوانات المصابة بالسكري والمعالجة بمسحوق فطر الجانوديرما (47 ملغم/كغم من وزن الجسم).
4. مجموعة الحيوانات المصابة بالسكري والمعالجة بطحالب السبيرولينا (33 ملغم/كغم من وزن الجسم).
5. مجموعة الحيوانات المصابة بفقر الدم والمغذات على العليقة الإعتيادية والماء الحاوي خلات الرصاص.
6. مجموعة الحيوانات المصابة بفقر الدم والمعالجة بخلات الرصاص + مسحوق فطر الجانوديرما (47 ملغم/كغم من وزن الجسم).
7. مجموعة الحيوانات المصابة بفقر الدم والمعالجة بخلات الرصاص + مسحوق طحالب السبيرولينا (33 ملغم/كغم من وزن الجسم).

حددت بعدها التغيرات النسيجية لنسيج الكبد. أظهرت نتائج دراسة الأنسجة في مجموعة الحيوانات المعاملة بالآلوكسان حصول أضراراً بالغة في عموم نسيج الكبد من حيث شكل الخلايا الكبدية والتحلل المائي والتوسع في الجيبانيات ، أما في مجموعة الحيوانات المصابة بالسكري والمعالجة بفطر الجانوديرما وطحالب السبيرولينا، أدت إلى حدوث تحسن في عموم نسيج الكبد من حيث الخلايا الكبدية طبيعية، عدم وجود الاحتقانات ، والجيبانيات طبيعيه، وأما في المجموعة المصابة بفقر الدم والمعاملة بخلات الرصاص فقد لوحظ حصول اضراراً كبيرة في نسيج الكبد ، وأن معالجة الحيوانات بخلات الرصاص + فطر الجانوديرما، طحالب السبيرولينا لم يسبب حدوث أي تحسن يذكر في نسيج الكبد.

الكلمات المفتاحية: فطر الجانوديرما، طحالب السبيرولينا، داء السكري ، الآلوكسان، فقر دم، خلات رصاص، نسيج الكبد