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Histopathological study in the spleen of Albino Mice treated with glucosamine sulfate drug

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Abstract:
The current study designed to determine the effect of Glucosamine sulfate in the spleen organ of Albino mice .the study included (24) mice divided in to 3groups (control group had distilled water orally).The other groups treated with (1500, 2500) ml/kg .respectively for (2, 3) months .the spleen have been taken from dissected animal for microscopic preparation to study. Statistical results showed that the average weight of the spleen gradually decreased as the concentration and duration of the drug increased ,The histopathologicale changes appeared in the spleen of the exposure groups during (2,3) month ,the effects were Congestion,hyperplasia,atrophy, amyloid and necrosis.

Key words: Glucosamine Sulfate, Spleen, Pathology, Amyloid, Hyperplasia, Atrophy.

I. Introduction:

In recent years there have been many drugs and a variety used by humans to treat diseases, although the use of these drugs to benefit but they may have many side effects symptoms, diseases, disabilities and deformities result for violating the correct treatment assets.

Glucosamine is a drug which began to use it increasingly to treat the disease of friction Joints, several studies have been conducted looking at the range the effectiveness of this modern drug compared to conventional drugs. the results of most studies were in the direction of confirmation on the utility of this treatment and its usefulness in alleviating the symptoms of the disease, and a greater understanding of the mechanism of action of this drug in the treatment of the disease; however, it has not been clearly seen whether it has side effects in the long term [1]

The drug glucosamine sulfate is a combination of glutamine and glucose with sulfate is used to treat tendonitis and osteoarthritis .It is derived from chitin which there is in shrimp, lobsters and crabs crust. It has been recommended by practitioners of complementary medicine For arthritis treatment tests have shown that use of glucosamine sulfate orally (2000) mg per day for a period of (6) weeks for patients with arthritis in the knee gives positive results including a significant reduction in Joint pain is improved in its functions as well glucosamine sulfate appears to be effective in reducing loss Cartilage in the knee joint for three years It is therefore normal to recommend glucosamine sulfate For the treatment of chronic arthritis.[2]

Glucosamine sulfate is one of the drugs that has many diseases causes for human and animal such as chronic hepatitis (inflammation of the liver) use of Patients with rheumatoid arthritis rheumatism, back pain and other diseases that are treated by this drug[3] , induced diabetes because of excessive glucosamine sulfate has an effect on insulin resistance and weakens the secretion of insulin through its effect in the beta cells found in the Islands of Langerhans [4], cause of acute renal dysfunction by drugs [5].

This study is designed to determine the effects which are caused by this drug in the spleen tissue. The spleen is also the largest secondary lymphoid organ containing about one-fourth of the body's lymphocytes and initiates immune responses to blood-borne antigens, the functions of the spleen are centered on the systemic circulation. The red pulp is a blood filter that removes foreign material and damaged and effete erythrocytes. It is also a storage site for iron, erythrocytes, and platelets. In rodents, it is a site of hematopoiesis, this function is charged to the white pulp which surrounds the central arterioles. [6]

II. Material and method:

Experimental design

(24) Male adult mice were randomly divided to (3) groups (8 animals each) treated daily orally for (2, 3 months) according to the following design:

Group I: Control: distal water orally administered.

Group II: treated orally administered of GS with (1500) mg/kg for 2, 3 month.

GroupIII: treated orally administered of GS with (2500) mg/kg for (2, 3 month)

Histopathology: preserved tissue samples were routinely processed and embedded in paraffin, after which thin Sections (5 µm) were cut and stained with hematoxylin and eosin for light microscope examination. [7]

Statistical Analysis

In order to determine the impact of three levels of concentrations (Control, 1500, 2500) mg/kg in each case of spleen carried out in complete randomized design (CRD). Analysis of variance (ANOVA), F-test, t-test and least significant differences (LSD) was used to explain the differences between means at ($p < 0.05$), express that as (mean± SEM). Further that using t-test to comparison between two spleen cases. Capital and small letters indicate to comparison between columns and rows respectively, and the similar letters are non-significantly different

Using SSPS program 2010 and excel application to find the result and draw the figures with some effect. [8, 9]

Weight changes:

The results showed there was a difference in the mean weight of the spleen of the groups treated with glucosamine sulfate compared to the control group. After (2) month of the dosage, the statistical results showed a significant increase in the mean weight in concentrations (1500) mg/ kg the increase value for spleen weight (0.252± 0.013) mg / kg compared to the control group as shown in (Table 1 and fig.1). The results showed that after period of (3) months of the dosage, there was significant decrease in the spleen weight of the treated groups in all concentration. The minimum value of the spleen weight was (1500) mg / kg (0.197 ± 0.007) mg / kg, compared to the Control group as shown in (Table 1 and fig.1)

Table 1: Effect of glucosamine sulfate in the mean weight of spleen after (2,3)months .

Type Concentration	Spleen weight after two month	Spleen weight after three month	P-Value
Control	0.244 Aab	0.261 Aa	
	±	±	**0.207**
	0.011	0.003	
1500 (mg/kg)	0.252 Aa	0.197 Bb	
	±	±	**0.019**
	0.013	0.007	
2500 (mg/kg)	0.209 Ab	0.236 Aab	
	±	±	**0.357**
	0.012	0.023	
LSD P ≤ 0.05	**0.042**	**0.048**	

Capital and small letter s indicate to comparison in row and column respectively, similar letters are non-significantly differences between means at ($p \leq 0.05$), using (LSD test).

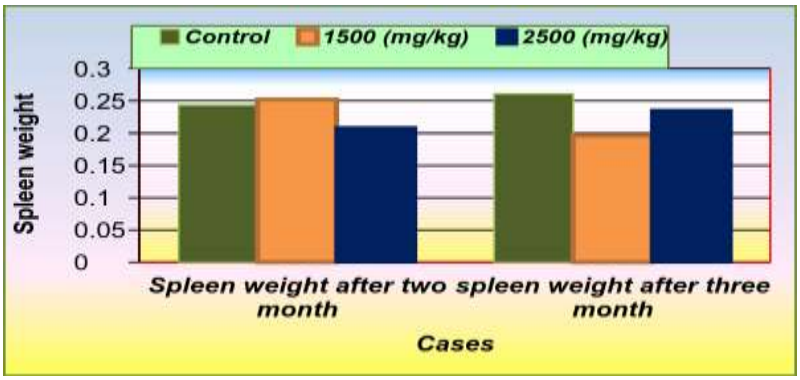

Figure 1: Histogram showing effect of treatment with glucosamine sulfate (1500, 2500) mg/kg For (2, 3) months with control groups

III. Histopathological changes:

The light microscopic study of the control group revealed that the splenic tissue consist of discrete while nodules called white pulp, embedded in a red matrix called the red pulp (fig.2), A sectional view of (1500,2500) mg/kg of GS treated mice after (2 month) spleen showed dilated as congestion in the central vein as (fig.3), hyperplasia of the lymphoid tissue and hyperplasia of the white pulp as (fig.4,5).while in (fig. 6),in showed the megakaryocyte**.** We can see Necrosis and hemosiderin pigment as (fig.7). A sectional view of (1500, 2500) mg/kg of GS treated mice after (2, 3 month) spleen showed amyloid deposited in the red pulp and adjacent to the white pulp as (fig.8).after (3months) we can see increase number of megakaryocytes In (Fig.9). The splenic tissue readily undergo the atrophy after treatment period with GS (Fig.10), degeneration in the spleen and necrotic tissue in (fig.11), we can see the pyknosis in nucleus of spleen cells in (fig.12).

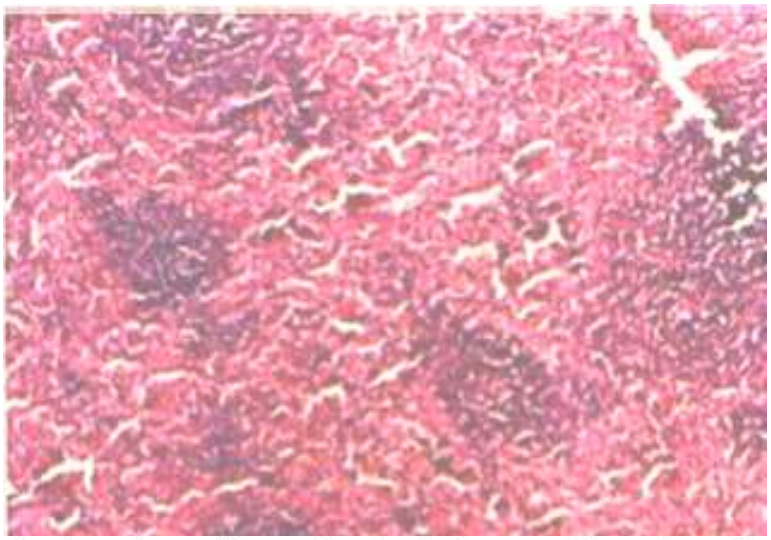

Fig. 2: a section in the spleen of control animal shows: white pulp and normal red pulp . (H&E, 100X)

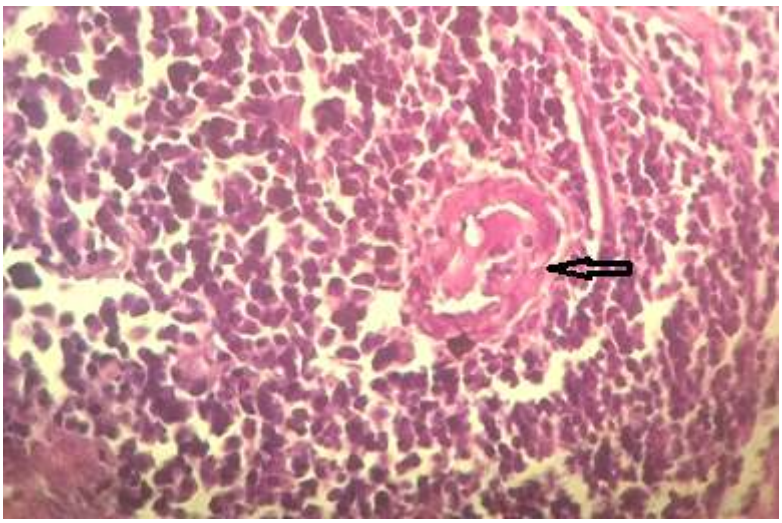

Fig. 3: Cross section in the spleen tissue of animal treated with 1500 mg / kg concentration for (2 month) from GS shows: hemosiderin pigment with congestion (➔), (H&E, 400X).

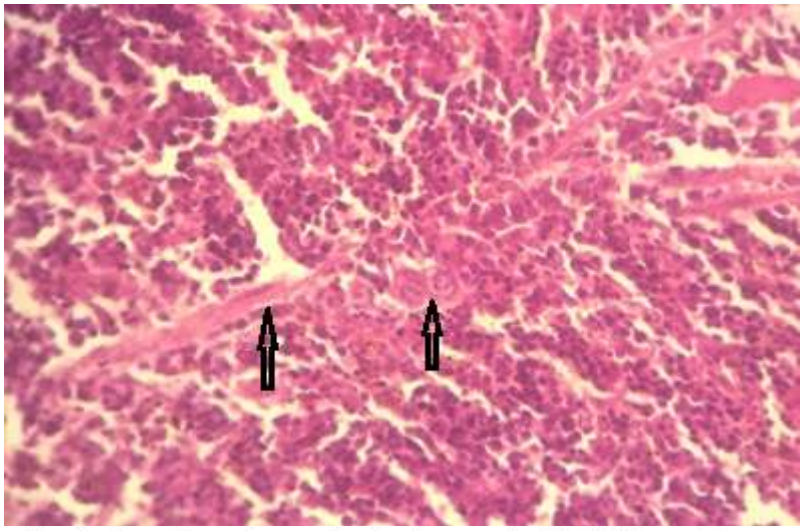

Fig. 4: Cross section in the spleen tissue of animal treated with 1500 mg / kg concentration for (3 month) from GS shows: hyperplasia of the lymphoid tissue in the peri-arterial sheath (↗), (H&E, 400X)

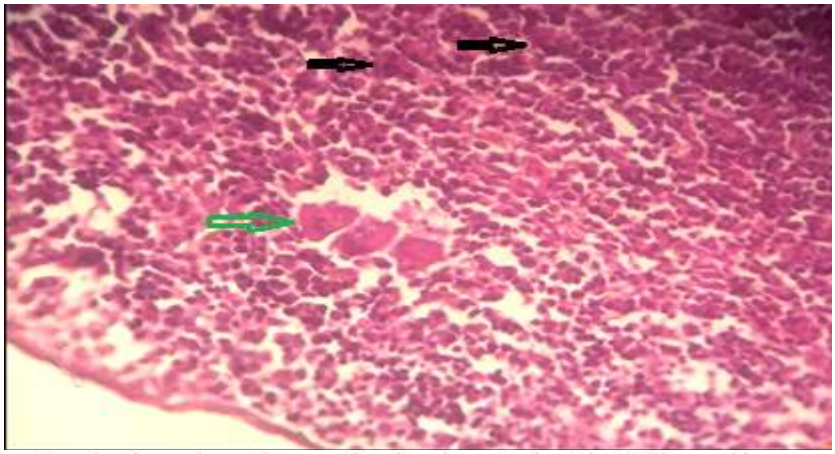

Fig. 5: Cross section in the spleen tissue of animal treated with 1500 mg / kg concentration for (2 month) from GS shows: hyperplasia of the white pulp (→), Megakaryocytes (→), (H&E, 400X)

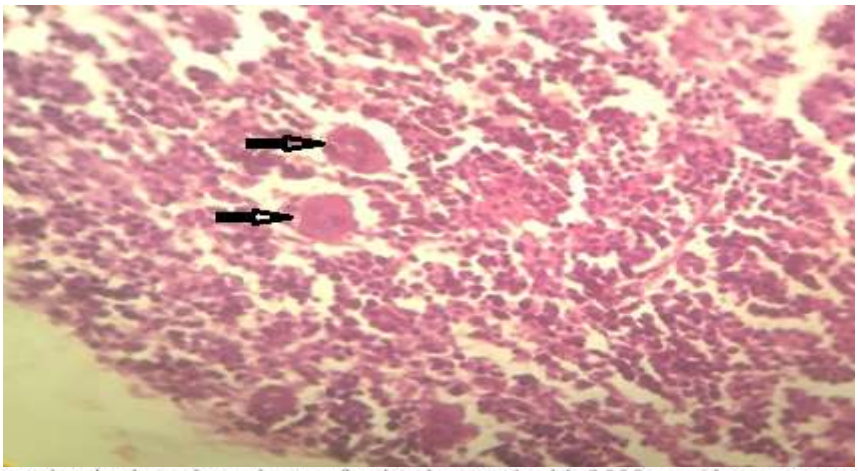

Fig. 6: Cross section in the spleen tissue of animal treated with 2500 mg / kg concentration for (2 month) from GS shows: Megakaryocyte (→), (H&E, 400X)

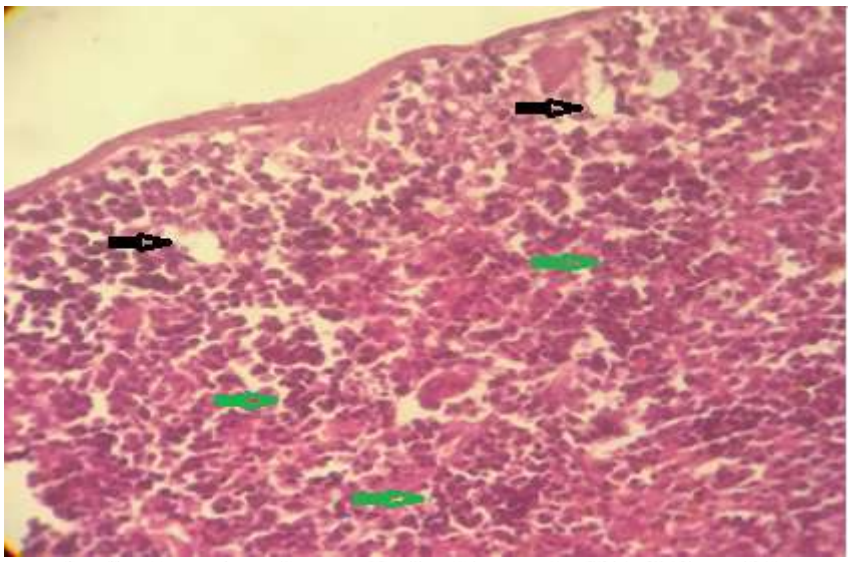

Fig. 7: Cross section in the spleen tissue of animal treated with 2500 mg / kg concentration for (2 month) from GS shows: Necrosis (➔), hemosiderin pigment (➔), (H&E, 400X).

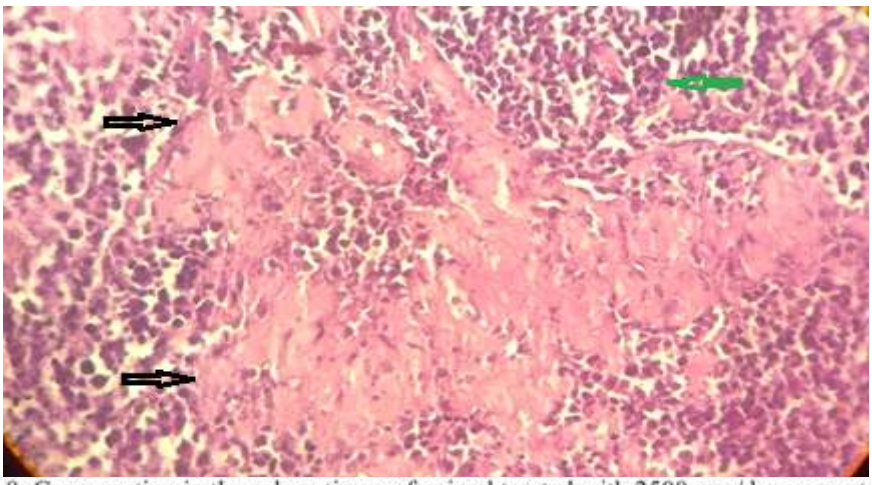

Fig. 8: Cross section in the spleen tissue of animal treated with 2500 mg / kg concentration for (3 month) from GS shows: deposition of amyloid (➔). Hemosiderin pigment (⟵), (H&E, 400X)

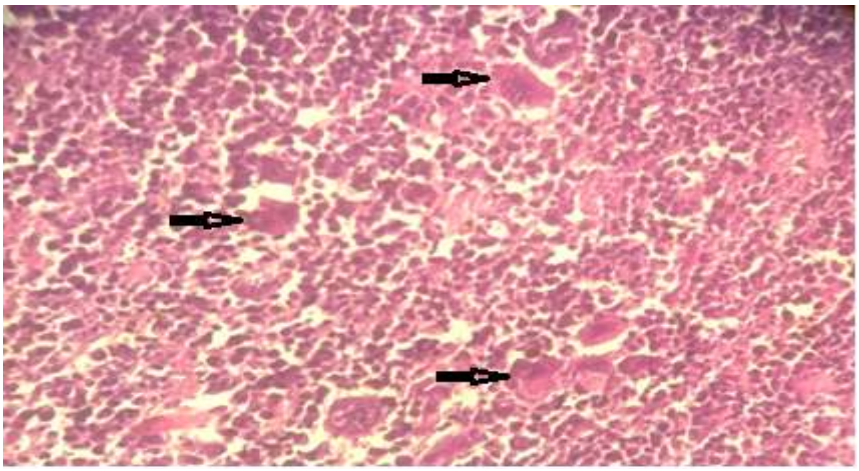

Fig .9 :Cross section in the spleen tissue of animal treated with 2500 mg / kg concentration for (3 month) from GS shows: Megakaryocyte (➔), (H&E, 400X)

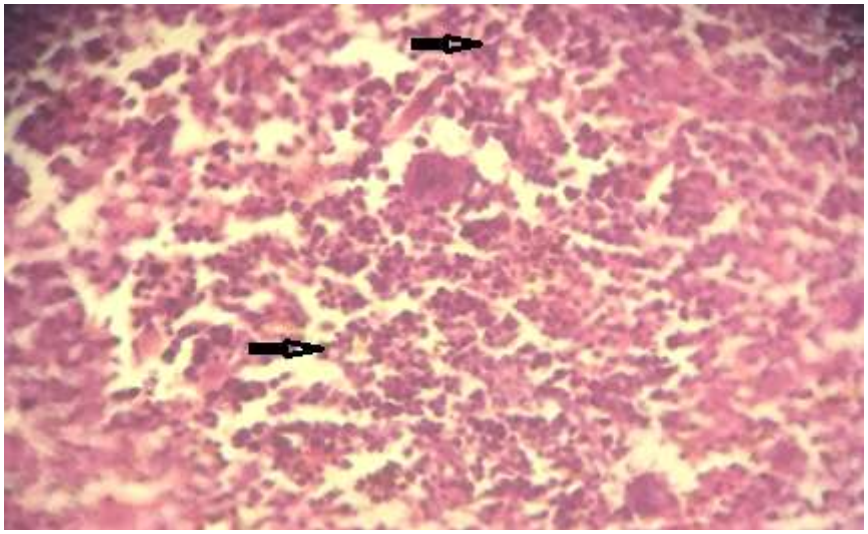

Fig. 10: Cross section in the spleen tissue of animal treated with 1500 mg / kg concentration for (3 month) from GS shows: the atrophy of splenic tissue (➔), (H&E, 400X)

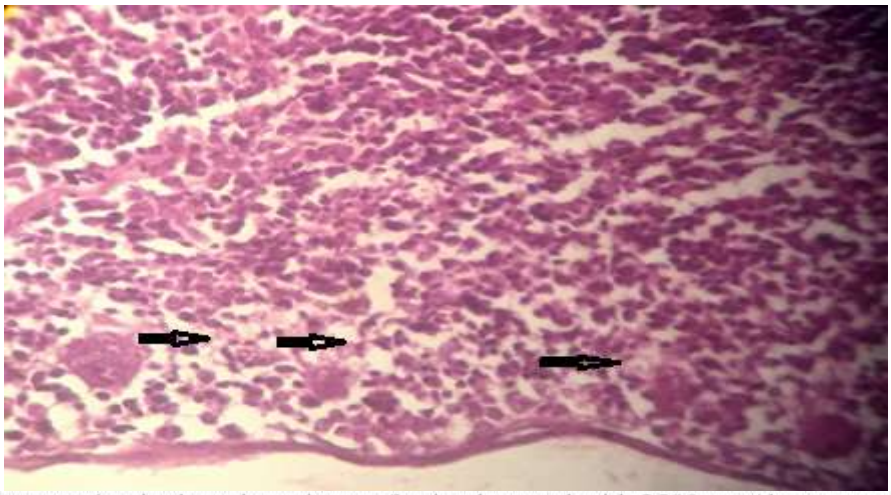

Fig. 11: Cross section in the spleen tissue of animal treated with 2500 mg / kg concentration for (3 month) from GS shows: lysis in the spleen and necrotic tissue, (➔), (H&E, 400X)

IV. Discussion:

The results of the statistical analyzes in the spleen after two months showed a significant increase in the weight, specifically the concentration of (1500) mg / kg. Therefore, any presentation in the spleen may lead to dysfunction As it works to break down the old red blood cells as well as abnormal cells and the transfer of waste, such as Balrolubin and iron to the liver during the splenic and portal vein, while the rest of the other substances in the blood is subjected to the process of phagocytosis as well as blood storage, and if any neurological induction of the device Sympathetic nervous system can restore all the quantity Stored in the bloodstream[10]. The spleen contains T and B lymphocytes, which are effective in the presence of antibodies, such as bacterial or other infections, and then lymphocytes will multiply and the cells of repeated infections and temporary infections, the spleen becomes splenomegaly [11].while after (3 month) showed a significant decrease in the weight of the spleen in all concentration compared to the control group with increased concentration and lengthening of the duration of the dosage, a reduction in weight may be observed due to disease caused by the drug such as amyloid, atrophy , necrosis and degeneration with lysis of cells, which acquired large areas of spleen tissue.

The present study showed some histopathologicl effects in spleen tissues, include congestion in several area of splenic tissue Which was the most frequent finding as a result from weaken of blood vessel wall due to accumulation of red blood cells in the vessels[12] .

The splenic tissue readily undergo hyperplasia after treatment period with GS which is the meant an increase in the cell number of a part and it gradually merges into the process of neoplasia or tumor formation[13] .we can

see cell lysis that resulted by damage to its plasma membrane which caused by chemical agent in the present study. On the other hand the increase in number of megakaryocytes, which produce platelets that have antibody receptors, paralleled the increase in antibodies [14].

The presence of cells that produce platelets and multi-nucleated (Megakaryocytic) is evidence of the inflammation that led to bone loss in the function of the generation of platelets. The cell that produces the enlarged blood platelets in the spleen instead of the bone form platelets and this is knows Extramedullary Hematopoiesis (EMH). [15].

Showed deposition of amyloid like substance around the white pulp which leads to depletion of white pulp Amyloid is a febrile glycoprotein material which lay down in the tissue usually extracellular. Amyloidosis is occurring as result to disturbance in protein metabolism and causative factors are still unknown [16], while (curren and croker) mention this condition associated with chronic inflammation like rheumatoid arthritis and by toxic compounds [17], and when severe can result in atrophy of the white pulp. Hemosiderin pigment can be increased in treatment-induced hemolytic anemia or methemoglobinemia. Focal deposits of hemosiderin may be associated with areas of hemorrhage secondary to malignant neoplasms. [18]

the results of our current study showed the adaptation of the spleen tissue through the atrophy of these cells as a means of defense against the toxicity of the drug and the expansion of cavities , Since cell atrophy is a way for the cell to stay alive as much as possible. The life-chemical mechanism of this condition may lie in the process of synthesis and non-synthesis of proteins as there is a balance of protein synthesis and analysis within the cell. Therefore, the increase in the process of protein degradation leads to the state of destruction Catabolism and the occurrence of atrophy, and that the release of these enzymes, the situation inside the cells is not easy, but increases in cases of disorder that occurs inside the cells [19]. Showed necrotic area is dead cells in live tissue which refers to harmful effects of high intensity of GS on spleen tissue [20, 21].

V. Conclusions:

From the result of this study it can be conclude that the high concentration of glucosamine sulfate (GS) effect on histopathological in spleen of mice that induced with decrease in the weight of the spleen in all concentration compared to the control group with increased concentration and lengthening of the duration of the dosage.

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