Clinical Cancer Investigation Journal-ISSN: 2278-0513

Investigating the Metformin Effects on Liver Tissue and its Enzymes in Non-Diabetic Male Rats

Abstract

Metformin is a hypoglycemic factor and an oral antidiabetic drug from the biguanide class and is the first-line treatment for type 2 diabetes, particularly in obese people with normal kidney function. Metformin effectively improves insulin sensitivity and endothelial function, improving cardiovascular conditions in people with diabetes and controlling body weight. The current study examined the effects of metformin on tissue, the liver, and its enzymes in non-diabetic adult male rats. In the beginning, a metformin solution was prepared. After determining the appropriate dose of metformin, intraperitoneal injection was administered to 30 mice within 30 days with doses of 15 mg/kg.b.w (first group), 20 mg/kg.b.w (second group), mg/kg.b.w 25 (third group), control group (no injection) and sham (distilled water injection). The data was measured with $SPSS_{22}$ ANOVA, and Duncan's test with a significance level of ($P \leq 0.05$). In the macroscopic examinations, a significant weight decrease was observed in all three injection doses ($P \leq 0.001$). In the case of liver weight, a significant decrease ($P \leq 0.05$) was observed. A significant decrease was observed in AST and ALT liver enzymes ($P \leq 0.05$). In the liver tissue, changes in cell arrangement, reduction of sinusoids, and the number of cell nuclei were observed. Likewise, the vein diameter in the center of the liver lobule has increased significantly ($P \leq 0.05$). At large, it can be concluded that the consumption of different doses of metformin in mice has the same negative effect on their bodies because it causes a destructive effect on the liver tissue and also causes a decrease in the number of enzymes in this tissue below the normal level in the body. Consequently, its use should be done under the supervision of a doctor and consciously.

Keywords: *Metformin, Mice, Liver, Liver enzymes.*

Mina Jahanmir, Parvin Torabzadeh, *Mina Ramezani*
Biology - Animal Sciences, Developmental Cell Biology, Department of Biology, Faculty of Science, Karaj Branch, Islamic Azad University, Karaj, Iran.
MJahanmir92@gmail.com

Introduction

Metformin is an oral antidiabetic drug from the class of biguanides (derived from guanidine) and is the first-line treatment for patients who have non-insulin-dependent diabetes (type 2 diabetes) (1). The function of the drug is that it is sensitive to insulin and can limit the secretion of insulin and its increase as well as reduce the amount of androgen secreted from the ovary and adrenal gland (2). Unlike sulfonylureas, metformin does not cause hypoglycemia (a severe drop in blood sugar). This drug is sensitive to insulin and can limit insulin secretion and its increase. It also reduces the amount of androgen secreted from the ovary and adrenal gland. This drug does not stick to plasma proteins and is not metabolized (3).

The effect of this drug is achieved by a set of reactions in various organs and tissues, such as a decrease in gluconeogenesis in liver hepatocytes and an increase in glycogenesis and lipogenesis, a decrease in glucose absorption by intestinal lining cells, an increase in glucose absorption by muscle and fat cells, and an increase in the sensitivity of peripheral tissues to insulin (4, 5 & 6). Among its other uses, the treatment of metabolic disorders, the treatment of polycystic ovary syndrome, the treatment of lipodystrophy syndrome caused by HIV, and carbohydrates can be mentioned (7, 8 and 9).

The liver is the largest organ in the body that can make chemical agents and store and secrete many substances that play a role in metabolism. The location of the liver is effective in its function because the blood brings many nutrients directly from the gastrointestinal tract to this place, and the liver stores them or converts them into chemicals used elsewhere in the body for metabolism. The liver plays an important role in regulating protein and glucose metabolism. The liver produces and secretes bile, which plays an important role in the digestion and absorption of fat in the gastrointestinal tract. It also removes waste material from the bloodstream and secretes it into the bile produced by the liver is temporarily stored in the gallbladder until it is used for digestion, at which time the bile is emptied and enters the intestine (10). The liver is a large source of chemically active cells with a high metabolic rate, and its metabolic systems share in terms of substrates and energy, process many substances, transport them to other parts of the body, and do many types of other metabolic actions. The liver is best positioned in the circulatory system to collect and accumulate metabolites and neutralize and remove toxic substances. This deduction and elimination are done through bile (an exocrine secretion important in the digestion of fats). Hepatocytes are complex metabolic cells found in the liver that contain a high concentration of enzymes. These enzymes leak into the plasma due to liver damage and can be valuable for analyzing and determining liver damage (11). The key functions of the liver, including filtration and storage of blood, metabolism of carbohydrates, proteins, fat, hormones, and chemicals introduced into the body, are to make bile, store vitamins and iron, and make coagulation factors. The active chemical environment of the liver is known for its ability to

detoxify many drugs such as sulfonamides, penicillin, ampicillin, and erythromycin or expel them into the bile (12).

Numerous tests are used to assess liver damage, such as serum glutamate-Oxaloacetate transaminase (SGOT) or Aspartate aminotransferase (AST), serum glutamate pyruvate transaminase (SGPT) or Alanine aminotransferase (ALT), alkaline phosphatase γ (8- glutamyl transpeptidase), lebumin, and bilirubin. ALT is mainly found in the liver, but AST is also present in many other tissues. Consequently, it is less often mentioned as a specific symptom in liver diseases (13 and 14). The most significant of these tests is determining the activity of serum aminotransferases (ALT and AST), which indicate damage to hepatocytes. An increase in their activity always warns of the presence of active liver disease.

AST and ALT enzymes are also plentiful in the liver. AST exists in large amounts in other tissues such as the heart, kidneys, skeletal muscles, and red blood cells, but the concentration of ALT in skeletal muscles is small. The increased serum ALT and AST indicates the entry of muscle and liver enzymes into the blood circulation (15 and 16). An injury or disease that affects the liver parenchyma causes the release of the hepatocellular ALT enzyme into the bloodstream and causes an increase in ALT levels. Generally, the increase in ALT levels is a result of liver dysfunction.

Consequently, this enzyme is not only sensitive but also specific to hepatocellular disease. In hepatocellular diseases other than viral hepatitis, the ratio of ALT to AST will be less than one. SGPT is a liver-specific enzyme in the cytosol and is quantified as an indicator of liver cells (16, 17 and 18).

The amount of AST increased directly correlates with the number of damaged cells. Consequently, the increase depends on the time interval between injury and blood sampling. AST is cleared from the blood within a few days. Serum AST increases 8 hours after cell damage. AST is one of the enzymes measured in cardiac enzyme tests. This enzyme is not specific for myocardial damage, but along with the increase of creatine phosphokinase (CPK) and lactate hydrogenase (LDH), it is useful for diagnosing the time and severity of myocardial infarction (MI). Due to the presence of AST in liver cells, diseases affecting hepatocytes will increase the level of this enzyme. The serum AST level is frequently compared to the ALT level. Typically, AST/ALT is more than 1 in patients with alcoholic cirrhosis and metastatic liver tumors. In patients with acute hepatitis, infectious hepatitis, or infectious nonnucleosis, this ratio becomes less than 1. If AST increases to 10 times the normal value, the accuracy of this ratio will decrease.

Ziyai et al. (2013) compared the effects of metformin and pioglitazone on the hs-CRP level in patients with type 2 diabetes. In this study, the serum level (hs-CRP with high sensitivity) decreased significantly after three months of treatment in both the metformin and pioglitazone groups. The mean changes of hs-CRP in the metformin group were significantly higher than in the pioglutazone group. The average variables of HbAlC, FBS, Chol, ALT, and AST in both groups of the current study were significantly reduced compared to before the intervention (20). Feng et al. (2017) investigated a randomized trial comparing the effects of gliclazide, liraglutide, and metformin on people with diabetes and non-alcoholic fatty liver disease. The results indicated that both the content of HbAl levels and intrahepatic fat (IHF) decreased in all three groups. While the levels of HbAlc in the groups treated with liraglutide and metformin were lower compared to the group treated with gliclazide, and the reduction in IHF was greater with liraglutide than with gliclazide (21). Elattar et al. (2016) investigated the protective effects of 1alpha, 25dihydroxyvitamin D3 and metformin on the liver in rats with type 2 diabetes. Liver enzymes increased in diabetic rats, and histological results showed the harmful and negative effects of diabetes on the liver, 1α25 (OH) 2D3, metformin, and both drug treatments significantly improved liver enzymes compared to treated rats (22). Kayani Fard et al. (2011) did research on "histological, histomorphometrical, and histochemical changes of testicular tissue in streptotocin-induced diabetic rats treated with metformin." The results showed that body and testis weight decreased in untreated rats compared to control rats, and body weight decreased in rats treated with metformin compared to control rats. Likewise, in the histomorphological studies, a decrease in the diameter of the testicular capsule, ferruginous seminiferous tubules, and the diameter of the germinal epithelium, and an increase in the amorphous interstitial tissue, a decrease in the population and activity of cells, and a disorder in spermatogenesis were seen in diabetic rats that were not treated compared to the control group (23).

This research aims to examine the effects of metformin on liver tissue and liver aspartate aminotransferase and alanine aminotransferase enzymes in non-diabetic male rats.

Method

The current study examined the effects of metformin on liver tissue and hepatic aspartate aminotransferase and alanine aminotransferase enzymes in adult non-diabetic male rats. The similarity texture to human tissue and the ease of keeping and raising them are among the reasons for choosing this kind of animal. Adult non-diabetic male laboratory rats weighing 300-330 grams and with an age range of 2.5-3 months were purchased from Razi Serum and Vaccination Institute in Karaj. They were kept in the animal room of the Islamic Azad University, Karaj branch, under controlled conditions in terms of temperature and humidity. Using the automatic electric timer, 12 hours of light and 12 hours of darkness were established. The shelves were washed every 3 days to prevent contamination, and the straw was changed. The room

temperature was set at 22 degrees Celsius and the humidity was set to normal with a humidifier. The statistical population includes five groups: 1) the control group that does not receive injections. – 2) sham groups, which were injected with distilled water for 30 days and were killed the day after the last day of injection. 3) Experimental group 1, which was injected with metformin in the amount of 15 mg/kg for 30 days and were killed the day after the last day of injection. 4) experimental group 2, which was injected with metformin at the amount of 20 mg/kg for 30 days and was killed the day after the last injection, and 5) experimental group 3, which was injected with metformin at the amount of 25 mg/kg for 30 days and was killed the day after the last day of injection. Each group included six rats.

In order to find the $LD50$, doses of 25 mg/kg, 20 mg/kg, and 15 mg/kg of metformin were considered. The 500 mg metformin tablets were obtained from the pharmacy. The tablet was powdered, then 0.4 mg of metformin powder for a dose of 15 mg/kg, 0.6 mg of metformin powder for a dose of 20 mg/kg, and 0.7 mg of metformin powder for a dose of 25 mg/kg were measured by a new digital weight scale and were dissolved and diluted with 0.2 cc of distilled water. The solution was then passed through the Whatman filter paper to obtain a filtered solution. In all stages of metformin injection, the injection was done intraperitoneally in the groin area of the thigh with the help of a 1 mL insulin syringe. One day after the last injection of the animals, the mice were anesthetized with chloroform after weighing, and blood was taken from the heart by inserting a syringe into the chest and heart area. To prevent hemolysis, by removing the syringe head, the blood was slowly poured into 2 mL microtubes and placed at 32 degrees for one hour to clot and prepare the serum. Then the microtubes were centrifuged in a special microtube centrifuge for 5 minutes at 3000 revolutions per minute, the clot was settled, and the blood serum was separated by a sampler and placed in 1.5 mL microtubes to be stored in a freezer at -18°c for measuring liver enzymes. Then, at a suitable time, all serums were taken out of the freezing state by staying at room temperature for half an hour, and the concentration of each enzyme was measured by an auto analyzer and aspartate aminotransferase, alanine aminotransferase, and enzyme kits.

For the microscopic study, the liver of the mice was removed from the body after being killed, and then the weight of the liver was measured using a digital scale in the control, control and experimental groups. At this stage, the liver was placed in 10% formalin for 48 hours to be fixed for histological procedures. After weaving, the samples were painted. For a more detailed examination of the liver tissue, the slides prepared by the light microscope were examined, and the general arrangement of cells and the shape and number of cell nuclei were evaluated, as well as the diameter of the vein in the center of the lobule, using a square eye plate (Piece eyes) on the eyepiece of the light microscope. It was evaluated, and at the end of each experimental sample, it was compared with the control and control samples. Finally, photographs were taken of the desired sections. SPSS software, ANOVA, and Duncan's method were used to analyze the collected data. The significance limit of the tests was considered $P \leq 0.05$. Finally, the diagrams were drawn by SPSS software.

Research results

Body weight

In the investigations done on the difference in weight, before the first and after the last injection, it was found that the weight of the rats in the control and sham groups and all 3 experimental groups with the injection doses of 25 mg/kg and the secondary weight of the animals increased by 20 and 15 compared to their initial weight. Nonetheless, by examining the average secondary weight minus the primary weight in the groups, a significant decrease ($P<0.001$) was observed in the weighing of the experimental groups compared to the control and sham groups. Likewise, no significant difference was observed between the experimental groups, which means weight control in the experimental groups after taking metformin (Table 1 and Diagram 1).

Table 1: Weight difference before the first and after the last injection in terms of (g).

Studied groups	**SD**	**Mean**	**Significance compared to the control group, sham and between groups**
Control	±13.876	61.66	
Sham	±20.643	61.33	
Experimental dose 15 mg/kg.b.w (T1)	±25.277	46.66	aa $P < 0.001$
Experimental dose 20 mg/kg.b.w (T2)	±11.057	29.66	aa $P < 0.001$
Experimental dose 25 mg/kg.b.w (T3)	±22.713	29.50	aa $P < 0.001$

aa = significance of P <0.001 compared to the control and sham groups

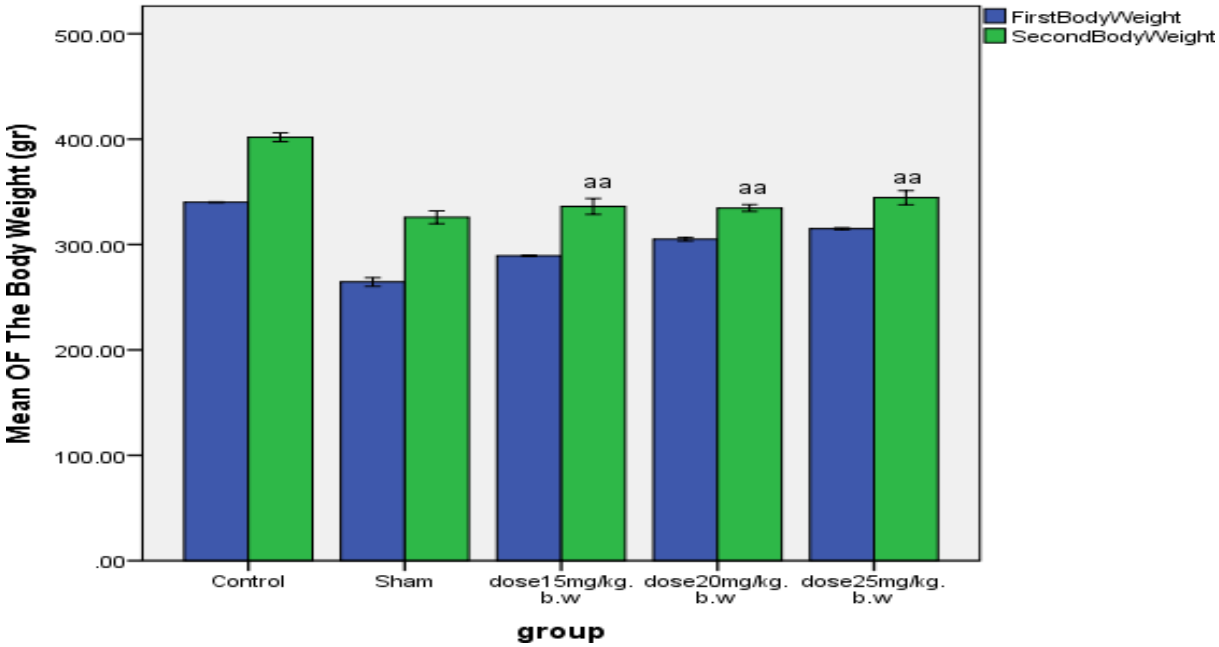

Diagram 1: Comparison of body weight of rats in the control, sham and experimental samples
Significance of <0.001= aa

Liver weight

In the studies conducted on the weight of the liver, it was found that the weight of the liver in the experimental groups 1 and 2 with doses of 20 mg/kg and 15 mg/kg decreased significantly compared to the control and sham groups (P<0.05) and in the experimental group 3, with a dose of 25 mg/kg, a significant reduction (P<0.001) was reported (Table 2 and Diagram 2).

Table 2: Liver weight in different doses (gr)

Studied groups	**SD**	**Mean**	**Significance compared to the control group, sham and between groups**
Control	±1.261	21.506	
Sham	±0.729	20.192	
Experimental dose 15 mg/kg.b.w (T1)	±2.478	17.403	a $P < 0.05$
Experimental dose 20 mg/kg.b.w (T2)	±1.672	17.108	a $P < 0.05$
Experimental dose 25 mg/kg.b.w (T3)	±1.265	15.812	aa $P < 0.001$

a = significance of <0.05 compared to the control and sham groups
aa = significance of P <0.001 compared to the control and sham groups

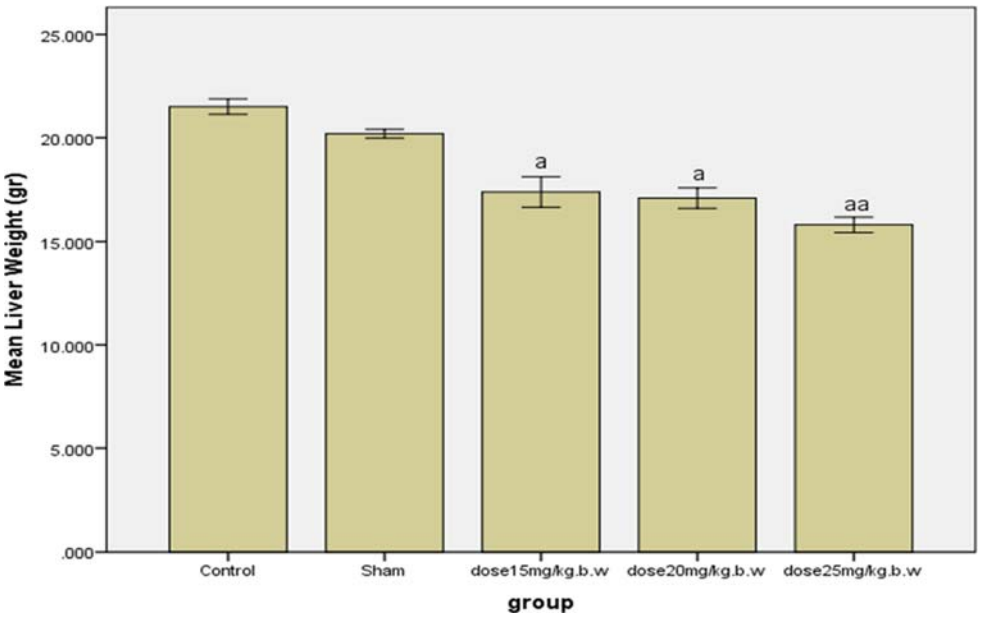

Diagram 2: Comparison of the liver weight of rats in the control, sham and experimental samples
aa = with significance of $P < 0.001$, a = with significance of $P < 0.05$

Results of liver aspartate aminotransferase (AST) or SGOT

Via the investigations, it was found that the amount of this enzyme decreased in experimental groups 1 and 3 with injection doses of 25 mg/kg and 15 mg/kg compared to the control and sham groups, but these changes were not significant. In experimental group 2, with an injection dose of 20 mg/kg compared to the control group and sham groups, a significant decrease was reported ($p < 0.05$) (Table 3 and Diagram 3).

Table 3: Results of liver aspartate aminotransferase enzyme (AST)(U/L)

Studied groups	**SD**	**Mean**	**Significance compared to the control group, sham and between groups**
Control	±20.3	151	
Sham	±35.3	141	
Experimental dose 15 mg/kg.b.w (T1)	±23.9	126	It is not significant
Experimental dose 20 mg/kg.b.w (T2)	±25.16	102	a $P < 0.05$
Experimental dose 25 mg/kg.b.w (T3)	±9.41	124	It is not significant

a = significance of <0.05 compared to the control and sham groups

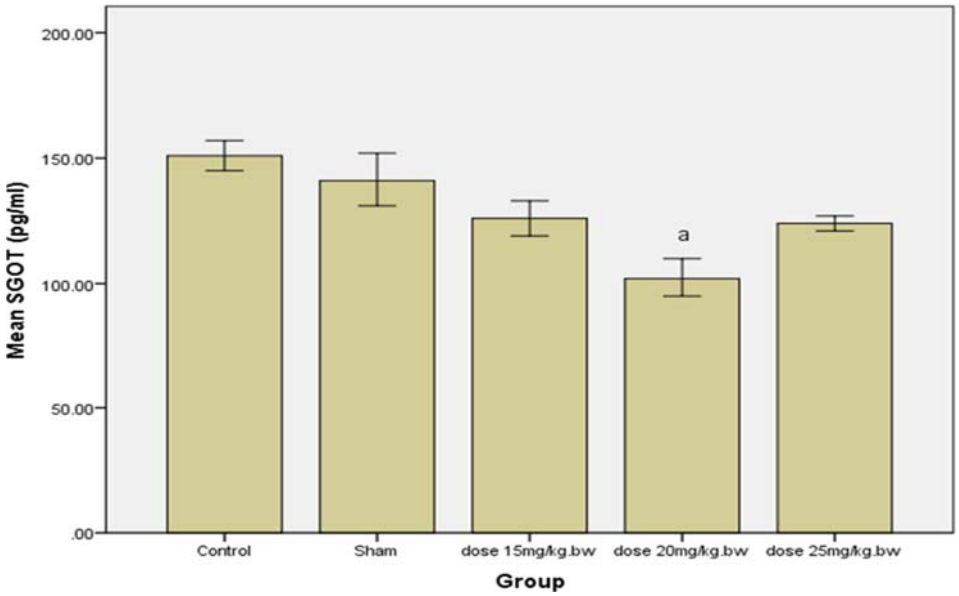

Diagram 3: Comparison of AST enzyme of rats in the control, sham and experimental samples
a = with significance of $P < 0.05$

Results of liver alanine aminotransferase (ALT) or SGPT

In the investigations, the amount of this enzyme decreased in experimental groups 1 and 3 with injection doses of 25 mg/kg and 15 mg/kg compared to the control and sham groups, but these changes were not significant, and in experimental group 2, with an injection dose of 20 mg/kg, it decreased compared to the control and sham groups. (P<0.05) (Table 4 and Diagram 4).

Table 4: Results of liver alanine aminotransferase enzyme (ALT) (U/L)

Studied groups	**SD**	**Mean**	**Significance compared to the control group, sham and between groups**
Control	±15.44	85.50	
Sham	±15.09	83.33	
Experimental dose 15 mg/kg.b.w (T1)	±17.47	80.16	It is not significant
Experimental dose 20 mg/kg.b.w (T2)	±11.52	58.16	a $P < 0.05$
Experimental dose 25 mg/kg.b.w (T3)	±8.74	68	It is not significant

a = significance of <0.05 compared to the control and sham groups

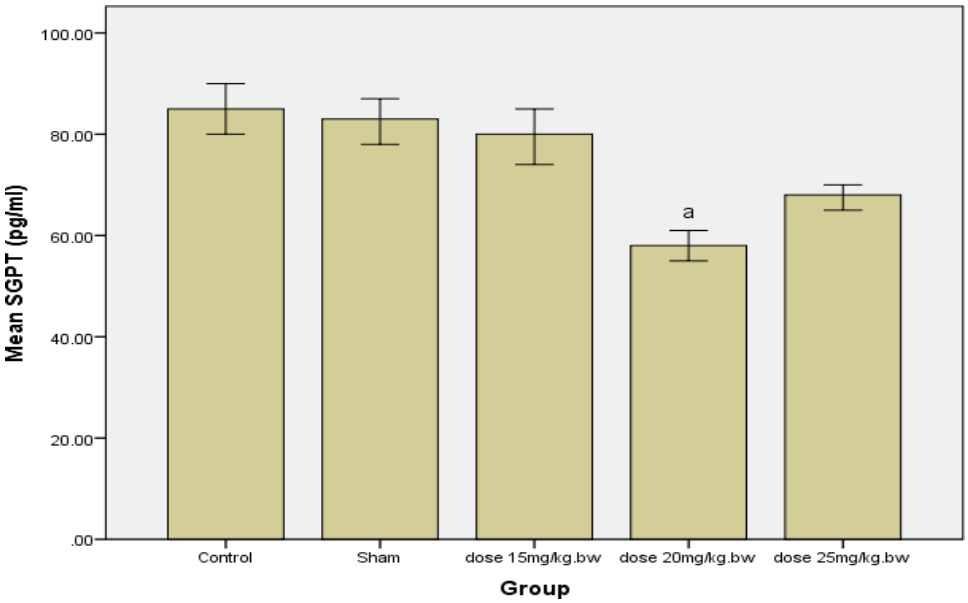

Diagram 4: Comparison of ALT enzyme of rats in the control, sham, and experimental samples
a = with the significance of $P<0.05$

Liver histological examination

In the control and sham groups, the vein of the center of the lobule, the hepatocysts, which have one or two granular nuclei and are placed in the form of cell strips radially from the center to the periphery and are called the Remak bundle, and sinusoids are between them, indicate the normal tissue of the liver.

The results of examining the changes in the diameter of the veins of the lobular liver center

In the investigations, it was found that the diameter of the interlobular veins in experimental groups 1 and 2 with injection doses of 20 and 15 mg/kg compared to the control and sham groups was significantly increased by 0.05 (p) and in experimental group 3, with injection dose of 25 mg/kg, increased compared to the control and sham groups. However, this increase was insignificant (Table 5 and Diagram 5).

Table 5: Results of changes in the diameter of the veins of the center of the liver lobule in different doses in terms of (mm)

Studied groups	**SD**	**Mean**	**Significance compared to the control group, sham and between groups**
Control	±0.008	0.06	
Sham	±0.006	0.06	
Experimental dose 15 mg/kg.b.w (T1)	±0.017	0.10	a $P < 0.05$
Experimental dose 20 mg/kg.b.w (T2)	±0.31	0.10	a $P < 0.05$
Experimental dose 25 mg/kg.b.w (T3)	±0.015	0.086	It is not significant

a = significance of <0.05 compared to the control and sham groups

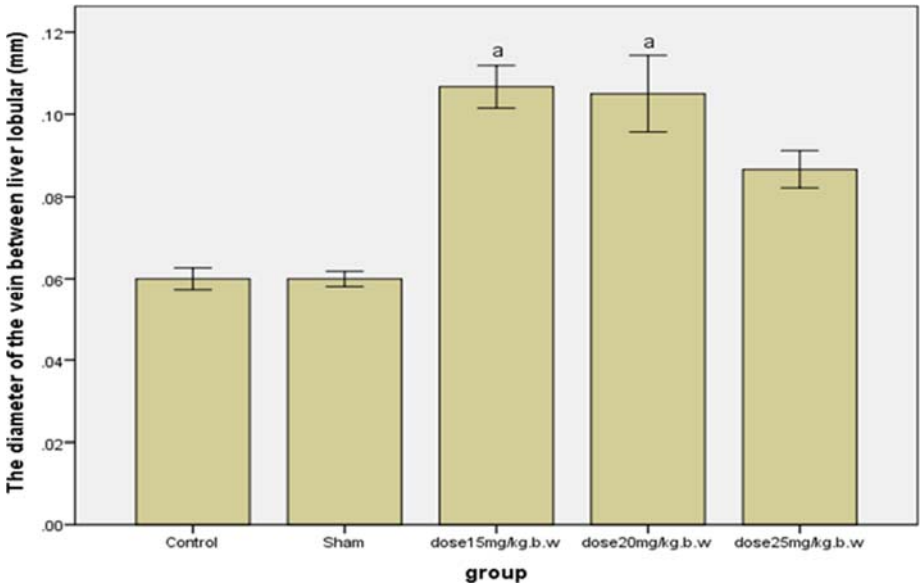

Diagram 5: Comparison of the diameter of the central lobular vein of mice liver in control, sham and experimental samples
a = with significance of P< 0.05

Discussion and conclusion

Metformin effectively improves insulin sensitivity, endothelial function, and cardiovascular conditions in people with diabetes and controls body weight. As well as the mentioned cases, widespread studies and research have shown that metformin has a promising and wide spectrum in the treatment of diseases such as polycystic ovary syndrome, gestational diabetes, fatty liver, lipodystrophy syndrome, breast cancer, lung cancer, prostate cancer, ovarian cancer, and pancreatic cancer. The current study investigated the effects of metformin on liver tissue and liver enzymes such as aspartate aminotransferase and lannin aminotransferase in adult non-diabetic male rats.

Tabatabai et al. (2005) indicated that metformin caused weight loss, BMI, and improved blood lipids, and among its common side effects were gastrointestinal side effects such as diarrhea (24). Yasuda et al. (2004) stated that incretin hormone and hormone (GLP-1 glucan-like peptide) decrease blood sugar through glucose-dependent insulin secretion and severely decrease appetite, and metformin causes a significant increase in this hormone. Consequently, metformin leads to a reduction in food consumption and prevents weight gain (25). In 2016, by investigating the effect of metformin on lipids and weight of diabetic patients, Afkhami et al. (2007) found a decrease in blood sugar and lipids and weight loss in patients (26). The current research results are consistent with previous researchers' results in that the injection of metformin drug in all three injection doses caused a significant decrease in the weight gain of mice, which is due to the fat-burning properties of metformin and the reduction of appetite that it causes. Likewise, we saw loose stools in the mice during the injection, all mentioned in the studies above.

Elattar et al. (2016) found that metformin alone and vitamin decrease liver enzymes and decreases apoptosis in liver cells in rats with type 2 diabetes (22). Ziaei et al.'s (2013) research indicates that the use of metformin and pepoglitazone in diabetic rats causes a decrease in ALT - AST - hs-CRP, FBS, Chol and HbAlC, which is more significant in the group receiving metformin (20). The study of Razvizadeh et al. (2013) indicated that the use of metformin and pepoglitazone in rats with non-alcoholic steatohepatitis causes a decrease in the levels of ALKP, ALT, and AST (27). The results of the current study disclosed that the use of metformin in all three injection doses of 15, 20 and 25 mg/kg causes a significant decrease in liver weight. This weight loss has been associated with microscopic changes in the liver tissue in the experimental groups compared to the sham and control groups. These changes include changing the shape of the cell arrangement, the loss of rhombic filaments, the reduction of sinusoids, the reduction of the number of cell nuclei, and the increase in the diameter of the central lobular vein.

The liver is an effective organ in metabolism and maintaining and preserving the blood glucose level within the normal range, and an increase in blood sugar leads to an imbalance in oxidation-reduction reactions within the hepatocytes. In this way, hyperglycemia through the balance of increased production (Advanced glycation end products, AGEs) facilitates the production of free radicals through disruption in the production of endogenous scavengers (Reactive oxygen species; ROS) such as superoxide desmutase (SOD), and it is catalyzed, which leads to cell damage. The level of lipid peroxidation caused by oxidative stress in cells is controlled by various defense mechanisms, including enzymatic and non-enzymatic free radical scavenging systems whose levels

change in diabetes (26 and 28). The liver is the primary organ of detoxification and purification of all substances entered into the body; many drugs detoxify the hormone secreted from the endocrine glands with chemical changes or expel it into the bile, which sometimes causes damage to the liver. The severity of these damages depends on several factors, including the type of chemical substance introduced into the body, the period of use, and the amount of the dose consumed. Harmful substances can change the shape and harden the liver due to the loss of tissue and its replacement by transplanted tissue. The created fibrous threads also destroy the natural structure of the liver by causing fibrosis, parenchymal damage, and changes in the diameter of the veins.

Metformin is one of the drugs used to control blood sugar. Metformin reduces hepatic glucose production by inhibiting gluconeogenesis and increases glucose absorption by stimulating insulin in muscle and fat tissue. Metformin reduces the flow of gluconeogenesis in the liver by stopping the absorption of hepatic lactate, and considering that adenosine triphosphate is an allosteric inhibitor of pyruvate kinase, metformin treatment also reduces the concentration of adenosine triphosphate in hepatocytes. Likewise, metformin reduces glucogenic activity by preventing the activity of pyruvate carboxylase and phosphoranol pyruvate carboxykinase and probably by increasing the conversion of pyruvate to alanine. Furthermore, metformin leads to the ease of stopping gluconeogenesis caused by insulin from several substances such as alanine, pyruvate, lactate, glycerol, and amino acids and resistance to the gluconeogenic activity of glucagon. The mechanism through which metformin reduces hepatic glucose production remains unknown.

Nonetheless, it seems that the main site of its activity is the mitochondria of hepatocytes, where the oxidation of the complex 1 respiratory chain is disrupted, preventing cellular respiration, causing a decrease in gluconeogenesis and can lead to the induction of glucose transporters and, as a result, glucose consumption (7 and 29). Metformin has a metabolic effect on insulin-sensitive tissues, which can be attributed to its glucose-lowering effects. Metformin can improve hyperglycemia by reducing the absorption of glucose after eating. According to the study, the increase in glucose consumption in the small intestine of patients treated with metformin can prevent the further transfer of glucose to the liver cycle. Consequently, this drug reduces hepatic glucose production by preventing gluconeogenesis and possibly glycogeolysis and increasing peripheral insulin sensitivity (30).

Based on the above information, the effect of metformin on the liver tissue of people with diabetes is beneficial, but in healthy people it causes tissue damage by disrupting the path of biochemical reactions. These damages were observed in the experimental groups, which included the change in the shape of the cell arrangement, the loss of Remak bundles, the reduction of sinusoids, and the reduction of the number of cell nuclei. So, the findings of the present study are inconsistent with previous findings. The mechanism of the change in the diameter of the veins in the center of the liver lobule can be seen as a result of the change, the loss of liver cells or the fibrosis of the vein endothelium because the arteries and veins of the body can change their diameter in response to hormonal, chemical, and nervous effects.

The most sensitive and widely used liver diagnostic enzymes are aminotransferases. These enzymes are usually located inside the liver cells. When the liver is damaged, the liver cells release enzymes into the bloodstream. An increase in the level of enzymes in the blood is a sign of liver damage. Aminotransferases catalyze chemical reactions in cells where the amine group is transferred from a donor molecule to a receptor molecule. ALT and AST are sensitive indicators of different types of liver disease. The highest levels of ALT and AST are caused by the extensive death of liver cells.

After conducting the current study, ALT and AST enzymes decreased in all three experimental groups compared to the control and sham groups. This reduction was not significant in the doses of 15 mg/kg and 25 mg/kg but was significant in the dose of 20 mg/kg. The use of metformin has caused a decrease in liver enzymes in diabetic samples, which has been done in previous studies. Similarly, the reduction of enzymes with metformin consumption was observed in healthy samples, so the observations of the current study are consistent with the previous findings in the reduction of liver enzymes.

Consequently, in this study, the injection of all three doses of 15 mg/kg, 20 and 25 mg/kg of metformin on non-diabetic male rats had the same effect on the weight loss of the rats, the weight loss of the liver tissue, and the reduction of liver ALT and AST enzymes. Because in none of the mentioned cases, according to the statistical analysis, there was no difference between the groups in the experimental groups. Likewise, the same tissue changes were observed in the experimental groups compared to the control and sham groups in the microscopic examinations. So, the results of the current study show the harmful effects of metformin on liver tissue and its enzymes in healthy people. Future studies are necessitated to gain a precise understanding of the place and mechanism of the molecular and cellular mechanisms effective in the pharmacological action of metformin.

Acknowledgments

None.

Conflict of interest

None.

Financial support

None.

Ethics statement

None

References

1. Kheybari, S., an attitude towards high-dose metformin, Razi journal, year 23, number 1, February 2011, pp. 238-242.
2. Safdarian, L., Motahari, N. The effect of metformin administration on ovulation and pregnancy in polycystic ovary syndrome resistant to clomiphene citrate of Shariati Hospital, Journal of Faculty of Medicine, Iran University of Medical Sciences, No. 5, Year 82, pp. 364-370.
3. Misbah, F., Bahri, A., Ghasemi, A. The effect of metformin on implantation and occurrence of pregnancy after inducing polycystic ovary syndrome in rats, Journal of Faculty of Medicine, 2011, Volume 69, Number 3, pp. 191-197
4. Parivar, K., Kochsefhani, H., Atlas of Experimental Embryology, Academic Jihad Publications, Tarbiat Moalem University, Tehran, 1993
5. Tavassoli, Sharifian, J., Therapeutic effects of metformin in polycystic ovary syndrome, Iranian Journal of Women, Infertility and Pregnancy, year 5, 10th and 11th issues, spring and summer 2010, pp. 89-98
6. Mahmoudi, H., Ghanizadeh, B., study on metformin and its use in polycystic ovary syndrome (summary and translation), Drug and Treatment Journal, No. 100, December 2012, pp. 40-44
7. Tabatabai, B., Mohibian, M. Menstrual cycle status in patients with polycystic ovary syndrome treated with metformin. Scientific Journal of Rafsanjan University of Medical Sciences, volume 2, numbers 3 and 4, summer and autumn 2003, pages 1.6-6
8. Bailey, C. J, Metformin-an update, Department of Pharmaceutical Sciences, Aston University, Birmingham, England General Pharmacology, 1993, 24(6), 1299-1309.
9. Parker, James, N, Philip, M, METFORMIN, ICON Health Publications, by ICON Group International Inc, 2004
10. Smelters, S., Translators, Dobirian, A., Shaban, M., Internal Nursing of Bruner and Sodart Gland and Liver Surgery, Andisher Rafi Publications, 2010
11. Hosseini Tehrani, S., Erfanian, M., Fundamentals of Cellular and Molecular Biology, Everest Publications, 1998, pages 529-537
12. Guyton, Hall, Medical Physiology, translator, Haeri Rouhani, 2010, Andisher-Rafi Publications, second edition, chapter 7
13. Larcan, A, Lambert, H, Laprevote-Heully, MC, Delorme, N, Light and electron microscopic study of hepatic lesions in the course of hyperlactatemia in diabetic patients, Diabetes Metab, 1979,5: 103-12.
14. Tokuyama, O, Nakamura, Y, Muso, A, Honda, K, Ishiko, O, Ogita, S, Expression and distribution of ocyclooxygenase-2 in human periovulatory ovary, Int J Mol Med, 2001,8(6):603 -6.
15. Naik, SR, Panda, VS, Hepatoprotective effect of GinkgoselectPhytosome in rifampicin induced liver injury in rats, Evidence of antioxidant activity, Fitoterapia, 2008, 79(6): 439-45.
16. Bablok W et al. A General Regression Procedure for Method Transformation. J ClinChemClinBiochem 1988;26:783-790.
17. Ajala, o, oyeume, m, oke, a, alakpa, c, 2000, hematological and biochemical, parameters inwestafriciandwarf (wad) bukes, Res, biomed, j, atr, vol 203.
18. DW, MOSS, AR, Henderson, 1999, clinical enzymology, Titez textbook of clinical chemistry, p; 617-721.
19. Bagana, translators, Khodam, R., Nejadi, N., Mousavi, R., Diagnostic and Laboratory Tests, Khosravi Publications, 2004, pp. 148-155
20. Ziaee A, Hashemipour S, Ghavam S, Javadi A, Ghavam R, Barikani A et al. Comparison of metformin and pioglitazone on hs-CRP levels in patients with Type II diabetes. sjimu. 2013; 21 (2) 28-36
21. FENG, W, GAO, C WU, M, Randomized trial comparing the effects of gliclazide, liraglutide, and metformin on diabetes with non-alcoholic fatty liver disease, Journal of Diabetes 9 (2017), 800-809.
22. Elattar, S, Estaphan, S, Mohamed, EA, Elzainy, A, Naguib, M, The protective effect of 1alpha, 25-dihydroxyvitamin d3 and metformin on the liver in type 2 diabetic rats, j.jsbmb.2016.11.012.
23. Kianifard, D, Sadrkhanlou, RA, Hasanzadeh, S, The Histological Histomorphometrical and Histochemical Changes of Testicular Tissue in the Metformin Treated and Untreated Streptozotocin-Induced Adult Diabetic Rats: Veterinary Research Forum, 2011;2(1):1324.
24. Tabatabai Malazi, A., Heshmat, R., Hijazi Moghadam, A. Comparison of the effects of generic metformin with metformin in type 2 diabetic patients. A double-blind, randomized clinical trial study, Iranian Journal of Diabetes and Lipid, Fall 2005, Volume 50, Number 1, Pages 49-58
25. Yasuda, N, Inoue, T, Nagakura, T. Metformin causes reduction of food intake and body weight gain and improvement of glucose intolerance in combination with dipeptidyl peptidase IV inhibitor in Zucker fa/fa rats, J Pharmacol Exp Ther. 2004 Aug;310(2):614-9.
26. Afkhami Ardakani, M., Motamedzadeh, A., Rashidi, M., Investigation of the effect of metformin on lipid status and weight in type 2 diabetic patients. Journal of Sabzevar Faculty of Medical Sciences and Health Services, Volume 14, Number 4, Pages: 205-210
27. Razvizadeh, F. Comparison of the therapeutic effect of pitoglitazone with metformin in patients with type 2 diabetes, Ilam University of Medical Sciences Scientific Quarter, 2013, 21, number 2, pages 28-36
28. Tavassoli, F., Sharifian, J., Tavassoli, S., comparison of endocrine and metabolic effects of metformin and Diane in women with polycystic ovary syndrome, Afog Danesh, Gonabad Faculty of Medical Sciences and Health Services Journal, Volume 10, Number 3, 2013, Pages 31-3
29. Lyons, M, Peterson, L, McGill, J, Herrero, P, Coggan, A, Impact of sex on the heart's metabolic and functional responses to diabetic therapies: J Physiol Heart Circ Physiol,2013;305:1584-1591.
30. Mohiti Ardakani, J., Didehdar, R., Afkhami Ardakani, M., Investigation of leptin hormone levels before and after treatment with metformin in type 2 diabetic patients, Journal of Shahid Sadougi University of Medical Sciences and Health Services, Yazd, Volume 13, Number 1, Spring. 2005, pp. 3-7.