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COMPARISON OF THE EFFECTIVENESS OF CURCUMA DOMESTICA EXTRACT AND CURCUMA XANTHORRIZA EXTRACT AGAINST LIVER FUNCTION AND HEPATIC CELL INFLAMMATION ON STREPTOZOTOCIN-INDUCED DIABETES MELLITUS MICE

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ABSTRACT

Background: Diabetes mellitus can cause complications including liver damage, which has an impact on increasing levels of SGOT and SGPT in the blood, as well as inflamation and hepatic steatosis. Turmeric (*Curcuma domestica*) and java turmeric (*Curcuma xanthorizza*) are known to have high levels of curcumin and xanthorizzol as an antioxidant and proven to improve liver function. Antioxidant therapy in patients with NAFLD is known to improve liver function and histopathological features. The effects of turmeric and java turmeric extract on liver function in streptozotocin-induced diabetes mellitus mice are still unknown. **Objective:** Comparing effects of administered turmeric and java turmeric extract on liver function and histopathologic features of streptozotocin-induced diabetes mellitus mice. **Method:** Research design Post Test Only Control Group Design are used. Male swiss mice around 25-30 gram are used. Streptozotocin-induced diabetes mellitus mice with a total samples (n = 20) divided into 4 groups, Control+PBS, Control+STZ, STZ+Turmeric, and STZ+Java Turmeric (^{a}n = 5). Blood glucose, weight, SGOT, SGPT levels and histopathologic features including percentage of inflamation and hepatic steatosis were examined at day 21. All the research data were analyzed using statistics program. **Result:** Turmeric and java turmeric extract cannot significantly reduce blood glucose level. Administration of java turmeric extract significantly reduce SGPT level against control+STZ group (84,12±17,53 vs. 36,3±27,4 u/L, *p*=0,018). Administration of turmeric extract significantly reduce hepatic cell inflamation against control+STZ group (30(26-68) vs. 20(15-30)%, *p*=0,035). **Conclusion:** Each turmeric and java turmeric extract can improve liver fuction and decreasing hepatic cell inflamation on streptozotocin-induced diabetes mellitus mice, but may not mediated by decreasing blood glucose level.

Keywords: Diabetes Mellitus, Turmeric Extract, Java Turmeric Extract, Streptozotoci

INTRODUCTION

Globally, the number of people with diabetes mellitus (DM) has increased four times in the last three decades, and diabetes mellitus ranks ninth in the leading cause of death. About 1 in 11 adults worldwide now has diabetes mellitus, 90% of whom are type 2 diabetes mellitus.[1]

Diabetes belongs to the group of metabolic diseases characterized by hyperglycemia caused by insulin resistance, failure of insulin secretion, or both, causing hyperglycemia.[2] Chronic hyperglycemia can cause a variety of damage, dysfunction, and failure of other organs such as eyes, kidneys, nerves, heart, blood vessels, and liver[3].

Research shows that DM is associated with liver disorders, such as non-alcoholic fatty liver disease (NAFLD), and causes an increase in abnormal liver function enzymes. NAFLD and the state of hyperglycemia can damage hepatocytes and also contribute to the increase of morbidity and mortality among diabetic patients[4].

One mechanism of fatty liver in diabetes caused by insulin levels in the

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blood increasing in response to the demand for glucose in peripheral tissues to overcome insulin resistance. Insulin should inhibit lipolysis in adipose tissue, but it does not occur in diabetic patients.[5] Insulin sensitivity in the liver is also affected, because insulin is an adipogenic hormone, causing triglyceride build-up. De novo lipogenesis (DNL) threefold increase in diabetes mellitus, worsening the accumulation of lipids in the liver[6].

Turmeric (Curcuma domestica) and java turmeric (Curcuma xanthorizza) are herbal plants native to Indonesia. Turmeric and ginger are known to have a high content of curcumin and xanthorizzol.[7] Curcumin has an antioxidant effect that can neutralize reactive oxygen species (ROS) including hydroxyl radicals (HO) and nitric oxide (NO) [8]. The content of xanthorrhizol in turmeric is known to function as an anticancer, antimicrobial, anti-inflammatory, antioxidant, antihyperglycemic, antihypertensive, antiplatelet, nephroprotective, hepatoprotective.[9] Antioxidant therapy in patients with NAFLD can improve liver function and histopathological features. Not all antioxidants provide satisfactory results,[10] also its effects on metabolic syndrome disorders, especially diabetes mellitus are not well known.

Previous studies testing routine extracts that are bioflavonoids found in apple peels, green tea and asparagus can reduce SGOT / SGPT levels, which are thought to improve liver function and reduce liver tissue damage in STZ-induced diabetic mice.[11] Researcher in China has doings some test for giving curcumin extract in mice with Acute Alcohol-Induced Liver Injury, SGOT / SGPT levels in the high-dose group decreased significantly[12].

Based on this background, diabetes mellitus can cause several complications including liver damage, which has an impact on increasing levels of SGOT and SGPT in the blood, as well as microscopic changes in the liver itself.[13] There have been no studies yet to compare the effectiveness of turmeric extract and java turmeric extract on mice. has been induced by streptozotocin, and see if there is a significant difference in the control group. Given the high antioxidant content both in turmeric and ginger, also consider that all the compounds that enter the human body will be metabolized in the liver which is expected to prevent further damage.

METHOD RESEARCH

This study used 20 male Swiss mice. The sample population was divided into 4 groups, namely groups K1, K2, P1, and P2, each group amounted to 5 mices. The study was conducted at the Animal Laboratory of the Faculty of Medicine, Diponegoro University, the study began with an acclimatization period of mice for 7 days all mice were given standard feed during the adaptation period.

Distribution of groups of mice by simple random sampling, K1 group of mice given ad libithum drinking water. K2 group of mice given streptozotocin, P1 group of mice given streptozotocin and turmeric extract, P2 group of mice given streptozotocin and java turmeric extract. Streptozotocin induction was carried out in groups K2, P1, and P2 then waited until the blood sugar of the mice rose or entered into a state of diabetes. The giving of turmeric extract in group P1 and temulawak extract in group P2 was carried out for 3 weeks.

Extraction Method

Turmeric and java turmeric are washed and sliced into small pieces. The sliced turmeric and java turmeric then dried at 50°C to remove water. The dried turmeric and java turmeric were grinded to become a powder. The powder are dissolved in 96% ethanol and heated at 50°C in water bath for

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1 hour. The solution then filtered and filtrate are put in rotary evaporator (at 90°C) to remove excess ethanol in the filtrate. Finally, curcumin gel were obtained.[12]

Blood Analysis

SGOT/SGPT Levels

1. Take 20 µl sample (blood of mice) in a test tube.
2. Add 1000 ml of SGOT/SGPT reagent.
3. Mix and incubate at 37°C for 1 minute.
4. Read absorbent on a spectrophotometer with wavelength of 340, factor 1745, K20 program.[13]

Histopathological Analysis

Immediately afer removal, liver were placed in formaline liquid fixed for 1 day, then preserved in 70% ethanol. Liver Specimens were dehydrated with a graded series of alcohol preparations and embedded in paraffin. Specimens were cut in 7 µm sections using a rotary microtome and mounted on 3-aminopropyltriethoxysilane-coated glass slides. Each section was deparaffinized in xylene, rehydrated in decreasing concentrations of alcohol in water, and stained with hematoxylin-eosin reagent.The slide then mounted with neutral balsam. The slides were analized by an anatomical pathologist. The slides analyzed were done randomly to avoid bias. Field of view in the form of a total magnification of 400x with a diameter of 0.5mm. Percentage of inflammation is the focal value of the inflammatory lesion per visual field. The assessment are carried out 6 fields of view on each preparation.[14]

RESULT

Blood sugar and body weight measurements were taken before and after the study. Measurement of SGOT and SGPT levels was carried out after termination, organ harvesting and preparation for histopathological readings were also carried out after termination. The results of measurements of blood sugar, weight, SGOT levels, SGPT and percentage of inflammation in the liver are attached in the following table.

Table 1. Weight Data, Blood Sugar, SGOT, SGPT, and Inflammation

	Control + PBS	Control + STZ	STZ + Turmeric	STZ + Java Turmeric
Weight (g) Pre	26,60±1,14	27,40±1,95	27,60±1,52	27,20±1,10
Post	30,40±1,67	32,60±1,95	30,60±1,14	30,00±2,00
Fasting Blood Sugar (mg/dL) Pre	163,60±53,47	150,40±17,05	180,60±17,90*	207,00±21,13*#
Post	111,20±72,49	193,62±32,07*	167,70±102,52*	216,76±27,51*
SGOT (u/L)	157,64(152,94-796,82)	208,60(163,21-226,46)	236,85(139,52-293,72)	292,62(147,83-338,81)
SGPT (u/L)	63,36±12,17	84,12±17,53	48,85±23,25	36,27±27,40#
% Inflammation	10,00(0,00-25,00)	30,00(26,00-68,00)*	20,00(15,00-30,00)#	22,00(19,00-29,00)*

PBS = Phosphate Buffer Saline, STZ = Streptozotocin
Data mean ± deviation standard, dan median (minimum value – maximum value)
*p<0.05 significant to control group + PBS
#p<0.05 significant to control group + STZ

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Weight

Normality test results obtained normal data distribution *p*=0,122. Statistical analysis showed that there was no significant difference in pre-intervention weight measurements with the One-Way ANOVA test with a value of *p*=0.732. The results of statistical analysis of post-intervention weight showed normal data distribution *p*=0.539.

Blood Sugar

Pre-intervention blood sugar normality test results obtained normal data distribution, showed that there was no significant difference in the measurement of pre blood sugar with the One-Way ANOVA test with a value of *p* = 0.059.

Results showed a significant difference in the Control + PBS group with STZ + Turmeric group *p*=0,004, the Control + PBS group with STZ + Java turmeric *p*=0,000, and the Control group + STZ with STZ + Java turmeric with values *p*=0,003.

Post-intervention blood sugar normality test results obtained normal data distribution *p* = 0.017.

The results showed a significant difference in the Control + PBS group with STZ + Temulawak *p*=0,007. Control + PBS with STZ + Turmeric *p*=0,015, and Control + PBS group with STZ + Java Turmeric *p*=0,013.

SGOT

Descriptive analysis was performed to see the distribution of research data in general, the normality test for SGOT levels was carried out with the Saphiro-Wilk. The test results obtained were abnormal data distribution with values *p*=0,000.

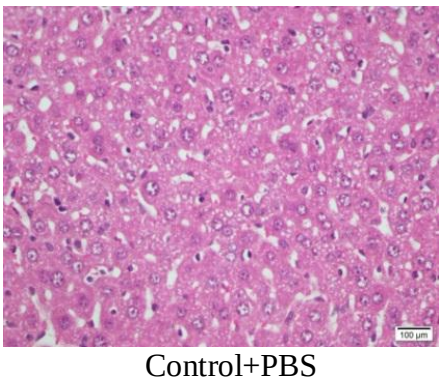
Kruskal-Wallis test was conducted to determine whether there are significant differences between groups. Kruskal-Wallis test results obtained values (*p* = 0.827) which indicate there are no significant differences between all the groups Control + PBS, Control+STZ, STZ + Turmeric, STZ + Java Turmeric.

Percentage of Inflammation

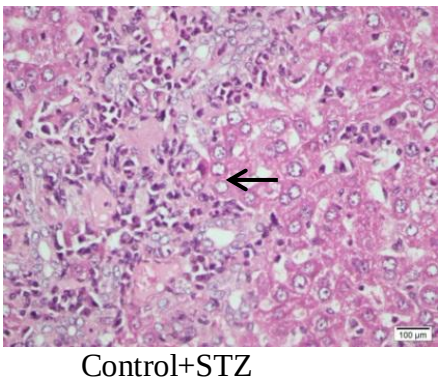
SGPT

The test results obtained were normal data distribution with values p>0,812 in all test. One Way Anova test was conducted to determine whether there are significant differences between groups. One Way Anova test results obtained value (p = 0.018) which indicates there is a significant difference. The level of difference in each group was seen through the Bonferoni Post-hoc test.

The table shows the test results from Bonferoni's Post-hoc analysis where the results were considered significant if the value (*p*<0.05). The results showed a significant difference in the Control group + STZ with STZ + Java Turmeric *p*=0,018.



Control+PBS



Control+STZ

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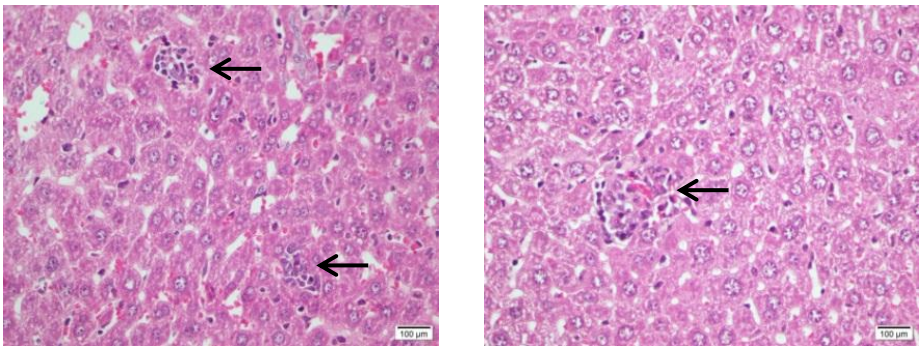

STZ+Turmeric STZ+Java turmeric

Figure 1. Histopathologic features of mice liver
Features of mice liver microscopically stained with Hematoxylin-Eosin with magnification of 400x.

The normality test results obtained abnormal data distribution (p=0.01) where the data is not spread normally. Data continued by Kruskal-Wallis test. Kruskal-Wallis test results obtained values (p=0.012) which means that there are significant differences in several groups.

Significant results were found in the Control + STZ group with Control + PBS p=0,009, the STZ + Turmeric group with Control + STZ p=0,035, and the STZ + Java Turmeric group with Control + STZ p=0,047.

DISCUSSION

Liver Function Test

Liver is one of the largest organs in a human body and has many important metabolic functions such as converting nutrients in food into substances that can be used by the body, besides the liver metabolizes toxic substances and turns them into harmless substances and ensures they can be released from the body[17]. The lever mechanism and its association with the gastrointestinal system make the liver as one of the organs that performs the first metabolism and if there is damage it will be shown in liver function tests (LFT)[18].

Enzyme levels of SGOT and SGPT can increase in almost all diseases. The highest levels are found in conditions that cause liver necrosis such as hepatitis, liver injury, and drugs intoxicity. When the liver cell is damaged, the enzyme will be released into the blood that the enzyme increasement can be measured, the amount of liver damage microscopically directly proportional to the increase in the levels of the SGOT and SGPT enzymes[16].

This study aims to determine the effect of turmeric extract and temulawak extract on levels of liver function (LFT) in the form of SGOT and SGPT.

Damaged blood samples cause lysis of erythrocyte which causes an increase in the value of SGOT / SGPT levels that the data obtained from this study can be said to be invalid.

Based on the results of the Post Hoc Bonferroni SGOT test, there were no significant differences in the form of decreasing SGOT levels in all groups Control + PBS, Control+STZ, STZ + Turmeric, STZ + Java Turmeric with a value of p=0,827.

SGPT test results obtained the value of the Control group + STZ with STZ + Java Turmeric group with a value of p = 0.018 where there are significant differences of decreasing SGPT levels. This result is in line with the research proposed by Oon et. al

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that xanthorrhizol obtained from Java Turmeric extract can reduce SGPT levels[7].

Based on the data obtained from the results of the study it can be seen that the giving of turmeric extract and ginger does not have a significant effect on changes in levels of liver function in the form of SGOT and SGPT in mice. These results are not in accordance with research conducted by Chuengsamarn et. al. in 2012 which proves that the giving of curcumin extract can give significant results. This can occur due to the time difference of research conducted in this study for 3 weeks, and research conducted by Chuengsamarn et. al. done for 9 months.

In addition, other variables affect the results, such as the extraction process. The process of extracting curcumin from turmeric and Java Turmeric is influenced by the amount of solvent, the length of time of extraction, and the amount of concentration that affects the collision and reaction rate, as well as the size of a powder-shaped material has more surface area compared to that in the form of slabs, that will react more quickly. Ramdja et. al. mention that the most curcumin results are using fine Java Turmeric because the surface area is larger[19].

Another study conducted by Sagala et. al also mentioned that the use of curcumin as an alternative drug has poor bioavailability due to poor absorption and rapid metabolism, researchers changed the extraction with encapsulation technology with nanoparticle methods to overcome water-soluble materials such as curcumin. The results showed that nano curcumin measuring 58.0 nm had the most significant effect on reducing blood glucose levels[20]. In this study the extraction results obtained in gel form and size from the unknown extraction results also made it possible that curcumin was not well absorbed by mice, thus giving insignificant results on changes in SGOT and SGPT levels in mice in this study.

Mishandling samples causing erythrocyte lysis also affect laboratory results, there can be a significant increase in SGOT and SGPT in lysis blood samples[21]. Erythrocyte lysis causes the release of hemoglobin and other intracellular components into the plasma, causing an increase in laboratory testing, readings using a spectrophotometer are also affected due to an increase in optical absorbance, where SGOT has a high absorbance level so it is more sensitive and provides more significant changes compared to SGPT or glucose[22].

Microscopic Features

Histopathological examination results were done by calculating the size of the focus of the lesion with percent inflammation per field of view, a total of 6 fields of view assessed on each mice. Olympus lenses with a magnification of 400x and a diameter of 0.5mm were used in this histopathological reading.

Mann-Whitney test results showed a significant difference in the Control + PBS group with Control + STZ with a value of p = 0.009, the Control group + STZ with STZ + Turmeric obtained a significant value that is p = 0.035, and the Control group + PBS against the STZ + Java Turmeric group with a value of p = 0.047.The focus of lesions in the form of inflammation can occur because streptozotocin can enter the intracellular through the GLUT 2 transporter so that it will eventually damage the organs, not focused only on the pancreas. The GLUT 2 transporter is also present in the kidneys and liver, which causes streptozotocin to damage the organ[15]. Histopathological features will change and show more obvious damage with a longer study duration, it takes at least 4 weeks of study to get a picture of necrosis in the portal area, kuppffer cell hyperplasia.

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A minimum study period of 6 weeks is required to obtain a features of steatosis[23].

Significant results obtained in the STZ + turmeric group against the control group + STZ revealed a difference in the two groups, where there was a repair process after damage caused by streptozotocin, this result is in line with studies that stated curcumin as a result of turmeric extract functioning as hepatoprotectant[14].

CONCLUSION AND SUGGESTION

Conclusion

Based on the results of research that has been done, giving turmeric extract and Java Turmeric extract obtained the following effects:

Each turmeric and java turmeric extract can improve liver fuction and decreasing hepatic cell inflamation on streptozotocin-induced diabetes mellitus mice, but may not mediated by decreasing blood glucose level. Data obtained from this study can be said to be invalid due to damage from blood samples.

Suggestion

1. Replacement of animal models, using rats.
2. Measurement of blood glucose before induction of streptozotocin.
3. Handling blood samples using a special box and the distance between the animal lab to the blood analysis laboratory needs to be considered that there is no damage to the blood sample.
4. The interpretation of histopathological slides with a minimum of 2 person to avoid measurement bias.

REFERENCES

1. Zheng Y, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. Nat Rev Endocrinol [Internet]. 2018;14(2):88–98. Available from: http://dx.doi.org/10.1038/nrendo.2017.151
2. A. J. Diagnosis and Classification of Diabetes Mellitus: New Criteria. Am Diabetes Assoc [Internet]. 2014;37(1):581–90. Available from: https://www.aafp.org/afp/1998/1015/p1355.html
3. Mohamed J, Nazratun Nafizah AH, Zariyantey AH, Budin SB. Mechanisms of diabetes-induced liver damage: The role of oxidative stress and inflammation. Sultan Qaboos Univ Med J. 2016;16(2):e132–41.
4. Saponaro C, Gaggini M, Gastaldelli A. Nonalcoholic Fatty Liver Disease and Type 2 Diabetes: Common Pathophysiologic Mechanisms. Curr Diab Rep. 2015;15(6):1–13.
5. Jantan I, Saputri FC, Qaisar MN, Buang F. Correlation between Chemical Composition of Curcuma domestica and Curcuma xanthorrhiza and Their Antioxidant Effect on Human Low-Density Lipoprotein Oxidation . Evidence-Based Complement Altern Med. 2012;2012(Ldl):1–10.
6. Ahangarpour A, Sayahi M, Sayahi M. The antidiabetic and antioxidant properties of some phenolic phytochemicals: A review study. Diabetes Metab Syndr Clin Res Rev [Internet]. 2019;13(1):854–7. Available from: https://doi.org/10.1016/j.dsx.2018.11.051
7. Oon SF, Nallappan M, Tee TT, Shohaimi S, Kassim NK, Sa'ariwijaya MSF, et al. Xanthorrhizol: A review of its pharmacological activities and anticancer properties. Cancer Cell Int. 2015;15(1):1–15.
8. Jurnalis YD, Sayoeti Y, Elfitrimelly.

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(Jurnal Kedokteran Diponegoro)
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E-ISSN : 2540-8844
Volume 9, Nomor 3, Mei 2020

Peran Antioksidan pada Non Alcoholic Fatty Liver Disease (NAFLD). J Kesehat Andalas. 2014;3(1):15–20.
9. Li C, Li Q, Guo L, Wu A, Hu L, Bai J. Effect of Rutin on Liver Function and Morphology in Type 1 Diabetes Mice Induced by Streptozotocin. Sichuan Da Xue Xue Bao Yi Xue Ban. 49(3):384–7.
10. Zeng Y, Liu J, Huang Z, Pan X, Zhang L. Effect of curcumin on antioxidant function in the mice with acute alcoholic liver injury. Wei Sheng Yan Jiu. 43(2):282–5.
11. Hyder M. Comparative Levels of ALT, AST, ALP and GGT in Liver associated Diseases. Eur J Exp Biol [Internet]. 2013;3(2):280–4. Available from: http://www.pelagiaresearchlibrary.com/european-journal-of-experimental-biology/vol3-iss2/EJEB-2013-3-2-280-284.pdf
12. McPhee SJ, Ganong WF. Patofisiologi Penyakit : Pengantar Menuju Kedokteran Klinis, Ed.5. Jakarta: Penerbit Buku Kedokteran EGC; 2017.
13. Victor P. E. Atlas Histologi diFiore dengan Korelasi Fungsional. 12th ed. dr. Y. Joko S et al., editor. Jakarta: Penerbit Buku Kedokteran EGC; 2016.
14. Devaraj S, Ismail S, Ramanathan S, Yam MF. Investigation of antioxidant and hepatoprotective activity of standardized curcuma xanthorrhiza rhizome in carbon tetrachloride-induced hepatic damaged rats. Sci World J. 2014;2014.
15. Radenković M, Stojanović M, Prostran M. Experimental diabetes induced by alloxan and streptozotocin: The current state of the art. J Pharmacol Toxicol Methods [Internet]. 2016;78:13–31. Available from: http://dx.doi.org/10.1016/j.vascn.2015.11.004
16. Hewlings SJ. Curcumin : A Review of Its ' Effects on Human Health. 2017;1–11.
17. How does the liver work? [Internet]. Institute for Quality and Efficiency in Health Care. 2016. Available from: https://www.ncbi.nlm.nih.gov/books/NBK279393/
18. Jaeschke H, Gores GJ, Cederbaum AI, Hinson JA, Pessayre D, Lemasters JJ. Mechanisms of Hepatotoxicity. 2002;176:166–76.
19. Ramdja AF, Aulia RMA, Mulya P, Teknik J, Fakultas K, Universitas T. MENGGUNAKAN ETANOL. 2009;16(3).
20. Saragih H, Ricky D. PADA TIKUS WISTAR DIABETES. 2016;(March).
21. Hur A, Atay A. Effects of hemolysis interference on routine biochemistry parameters. Biochem Medica. 2011;21(8):79–85.
22. Lippi G, Salvagno GL, Brocco G, Cesare G, Morfologico-biomediche DS. Influence of hemolysis on routine clinical chemistry testing. 2006;44(3):311–6.
23. Salih ND, Muslih RK, Hamoodi SR. Histological Liver Changes in Streptozotocin induced Diabetic Mice. 2009;8(1):8–11.