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Comparison between immunoassay technique and a manual laboratory method for the measurement of sorbitol dehydrogenase (SDH) activity for different types of diabetes in Nineveh governorate.

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Abstract

In this research ,the efficacy of the sorbitol dehydrogenase (SDH) enzyme was compared with immunoassay technology and laboratory method for diabetic patients in Mosul city. Types of diabetic patient were divided into four groups whose ages range from (8-62) years of both sexes (64 males and 56 females).The first group of patients who depend on their treatment on insulin injection only known as type1 diabetes classified T1DM and the second group was classified for patients who depend in their treatment on pills only to treat diabetes, which is known type 2 diabetes T2DM, while the third group of gestational diabetes was classified exclusively T3DM. In the four group of patients who depend on pills and injections in their treatment T4DM. The activity of (SDH) enzyme was estimated in the serum of healthy people without any mentioned disease, and they were considered a control group. The results showed a high significant in glucose concentration in serum of patient diabetes when compared with control group, also it was observed that there was a significant increase in the activity of the (SDH) enzyme in the serum of person with diabetes in the four group when compared with the control group which measured by the immunoassay technique and by the laboratory method. The results showed a significant increase in sorbitol dehydrogenase activity have been compared with the control group. The relationship was a study between (SDH) activity and glucose concentration of patient and control groups by finding the linear correlation coefficient. The positive significant correlation have found between the (SDH) activity of enzyme and the glucose concentration in control and patient groups. The aim of this research is to find the difference in the activity of (SDH) when measured by immunoassay technique and a manual laboratory method.

Keyword: Sorbitol dehydrogenase ,immunoassay , diabetes mellitus

1. Introduction

Sorbitol dehydrogenase (SDH), also known as glucitol, polyol, or L-iditol dehydrogenase (EC1:1.1.14), catalyzes the oxidation of sorbitol to fructose with NAD+ as a cofactor. It has been detected in a wide variety of animals, plants, and microorganisms [1]. The (SDH) is the second enzyme in the polyol pathway in concert with NAD+, also reduces NAD+ to NADH. Excess flux through (SDH), as would be prevailing under diabetic conditions, creates an imbalance in the cytoplasmic NAD+/ NADH ratio [2]. The (SDH) isolated from microorganisms is characterized by its high substrate specificity and it degrades D-sorbitol, used as a substrate, into fructose [3]. It is believed that diabetic complications are caused by sorbitol accumulation. The conventional enzymes in the market react with sugars other than sorbitol; however, (SDH) with high substrate specificity enables accurate quantification of sorbitol [4].When reacted with typical sugars as substrates including glucose, mannitol, and galactose (at 0.5mol/L concentration) independently or in a mixture, the absorbance did not change, proving high substrate specificity of (SDH). It is useful not only in research on aggravation mechanism of complications but also in sorbitol quantification in foods. Because these early functional changes may contribute to eventual pathology in affected diabetic tissues, research has been targeted towards investigating the role of (SDH) in the field of diabetic complications [5,6]. It is classified within the family of dehydrogenases which is one of the oxidative enzymes [7], so the increase in the activity of the enzyme (SDH) leads to an increase in the formation of fructose, which

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is the main cause of complications of diabetes. Recent studies have shown that increasing the effectiveness of (SDH) can be observed in patients with diabetes and chronic ischemia. Inhibition of (SDH) plays an important role in reducing chronic ischemia which is associated with decreased levels of cytosol secretion of NAD^+/NADH, and with increased glucose uptake and oxidation process. It will lead to metabolic complications such as depletion of NADPH which is very necessary for the regeneration process of glutathione and which is an antioxidant [8]. The major metabolic changes caused by hyperglycemia are increased glucose influx into the polyol pathway and elevated formation of oxygen-free radicals [9]. The (SDH) is found in highest catalytic activity in the cytosol of mammalian liver cells,while myocardial and skeletal muscles have only 7.9% and 15.6%, respectively of the liver concentration [10,11]. The (SDH),was shown to be structurally, mechanistically, and ancestrally related to alcohol dehydrogenase [12]. The substrates of (SDH), fructose and sorbitol, are considered important in special organs, such as male sexual organs [13], or special disease state, such as hyperglycemic cataract formation [14] and possibly diabetic neuropathy [15]. The polyol route may be related to diabetic complications that damage the tiny blood vessels in the retina as well as the kidneys. Researchers have confirmed that glucose is a highly reactive compound, and if it is not metabolized, it will affect body tissues [16], as the increase in sugar levels, such as that occurring in diabetes, activates alternative biochemical pathways, thus leading to a decrease in glutathione and an increase in free oxygen molecules. Inhibitors of this enzyme have shown efficacy in model animals to prevent the development of neuropathy, while most body cells depend on insulin to regulate the entry of glucose into the cell, but the cells of the retina,

kidneys and nervous system do not depend on the hormone insulin, and therefore there is a free exchange of glucose from inside and outside the cell, regardless of the action [17]. A group of researchers noticed that any amount of glucose not used to obtain energy will enter the urea pathway and converted to sorbitol, and this exchange does not cause any problems with the normal levels of glucose in the blood, because the rate of increase in the normal aldose reductase enzyme inhibition of glucose is slight [18], however, increases the inhibition of the aldose reductase enzyme of glucose when the level of glucose in the blood increases, which means higher levels of sorbitol and lower levels of NADPH, a compound used when the polyol pathway is required for restoration reduced glutathione [19].

2. Material and Methods :

Patients blood was collected from hospitals in Mosul city, ages range from (8-62) years. (5) milliliters of blood sample were collected from each patients, allowed to alot and serum separated by centrifugation. Serum was kept frozen at (-20˚C) until used [20]. The patients were divided into four groups : the first group included patients who were T1DM and their treatment was with insulin injection only, and the second group who had T2DM and were treated with pills only, and the third group were patients with gestational diabetes T3DM, and the fourth group included those who were treated with pills and injection together T4DM. Sorbitol dehydrogenase (SDH) was measured by Elisa using a kit from "CUSABIO BIOTECH,LTD" and by the manual method [21].

3. Statistical analysis :

SPSS software version 10.0 was used. The mean ± standard deviation of data was recorded. Comparison between patients and control group which measured by the immunoassay technique and by a laboratory method were carried out using the independent.

4. Results and Discussion :

After conducting a statistical analysis of the results, they are explained in the following tables. Data are shown as mean± S.D

Table (1): Glucose concentration in patient groups compared to the control group.

Types of diabetes	Number	Glucose concentration (FBS test) (mg/100ml)
Control group	41	6.1±0.8
First group	21	13.2±0.7
Second group	31	13.1±0.5
Third group	15	9.7±0.3
Fourth group	11	12.6±0.5

It was observed that there was a significant increase in the concentration of glucose in the patients in Table (1) when it compared to the control group, when the level of glucose in blood is heard, it is signals to the pancreatic insulin section , insulin is turn on the opening of cells to enter the glucose to it. To provide energy to the cells to work correctly. The insulin hormone helps the glucose that we get from food to enter the different cells of the body, and failure to manufacture the insulin hormone as required or the body faces problems in using it causes a high level of glucose in the blood, and high blood sugar may be a sign of disease diabetes, especially if the problem of high blood sugar persists, and it is worth noting that diabetes is classified into two types: The first type express cases in which the body does not produce insulin, and the second type express the inability of the body's cells to respond to the insulin hormone secreted, and it should be noted that leaving the problem of high blood glucose untreated causes severe

health complications that may lead to the life of the injured [22].

As shown in Table (2), it was observed that there was a significant increase in the activity of the (SDH) enzyme in patients compared to the control group. The increase in glucose metabolism through the polyol pathway is probably the most important mechanism, as the rise in blood sugar leads to an increase in cytosol (NADH / NAD^+) in red blood cells. The rate of the reduction of glucose to sorbitol will increase with increasing levels of glucose in tissues that do not need insulin to absorb excess glucose [23,24].The defect in pathway levels in diabetics is the result of weakening of the blood vessels and nerves in the retina, peripheral nerves and aorta. It can be concluded that vascular and nerve impairment are the most vulnerable to infection as a result of the increased oxidation of sorbitol to fructose from the state of osmotic stress [25,26,27].
The results shown in tables (1 and 2) were analyzed statistically and a comparison was made between the patient groups with the control groups, and as shown in the following figures.

Table (2): (SDH) activity in patient groups compared to the control group which measured by immunoassay technique and by laboratory method. Data are expressed as mean± S.D.

Types of diabetes	Number	SDH activity by laboratory method (U/ml) mean± S.D	SDH activity by immunoassay technical (U/ml) mean± S.D
Control group	41	0.78±0.22	0.69±0.03
First group	21	1.39±0.01	1.98±0.71
Second group	31	1.29±0.01	1.75±0.24
Third group	15	1.44±0.03	1.20±0.14
Fourth group	11	1.09±0.07	1.6±0.02

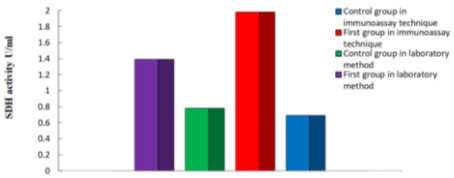

Fig (1): SDH activity which measured by immunoassay technique compared with laboratory method in T1DM and control group.

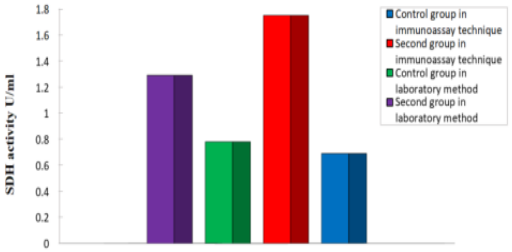

Fig (2): SDH activity which measured by immunoassay technique compared with laboratory method in T2DM and control group.

When observing Figure (1) and comparing the efficacy of the (SDH) enzyme measured by the immunoassay technique and by the laboratory method in T1DM, since there was a significant increase in the activity of the (SDH) enzyme in the serum of people with diabetes in the first group when compared with the control group.The reason for this may be due to an increase in the flow of glucose sugar to the polyol pathway in diabetic patients, which leads to an increase in the activity of the (SDH), which converts sorbitol into fructose, the results were found converging.

When observing the Figure (2) and comparing the efficacy of the (SDH) enzyme measured by the immunoassay technique and by the laboratory method in T2DM, there was a significant increase in the effectiveness of (SDH) enzyme. The increase in the effectiveness of (SDH) in the serum of people with diabetes in the second group, when compared with the control group, may be due to a decrease in the secretion of the insulin hormone, resulting in high blood sugar and increased activity of the polyol pathway in diabetics, which leads to an increase in the activity of the (SDH) enzyme, which converts sorbitol into fructose, and the results are similar in both methods.

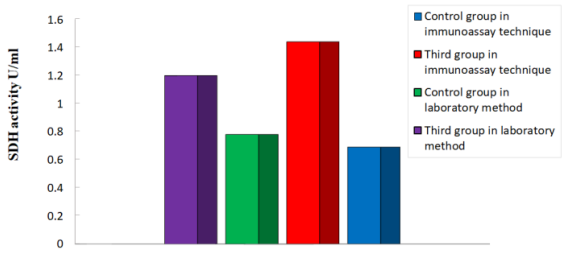

Fig (3): SDH activity which measured by immunoassay technique compared with laboratory method in T3DM and control group.

When observing Figure (3) and comparing the activity of (SDH) enzyme measured by the immunoassay technique and the laboratory method in T3DM, the results indicated a significant increase in the activity of the enzyme when compared with the control group,

in gestational diabetes is the level of sugar in the blood is high enough to diagnose a woman's diabetes during pregnancy, and this type of health problem usually disappears after the woman's birth, and it is worth knowing that diabetes pregnancy can appear at any stage of pregnancy, but it is more common in the second or third trimester and the results were somewhat similar in the two methods.

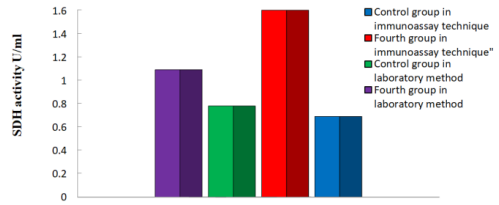

Fig (4): SDH activity which measured by immunoassay technique compared with laboratory method in T4DM and the control group.

When observing Figure (4) and comparing the efficacy of (SDH) enzyme measured by the immunoassay technique and by the laboratory method in T4DM, the results indicated a significant increase in the activity of the enzyme when compared with the control group, Diabetes is an imbalance in the body's ability to secrete or use the hormone insulin (secreted by the pancreas), which leads to high levels of sugar in the blood, and to treat this increase, injections and pills can be used in some cases, in order to maintain a normal blood sugar level, the results indicated a convergence between the two methods.

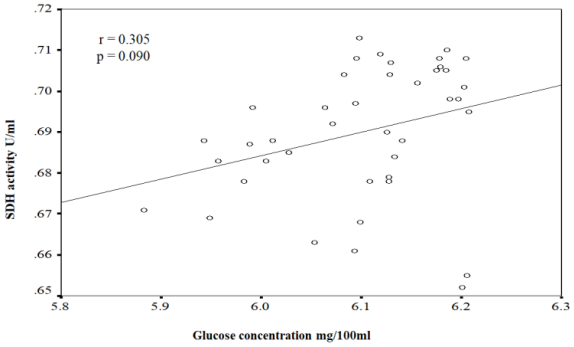

Figure (5):Correlation between SDH activity in immunoassay technique and glucose level in the control group.

When the results were observed in Figures(5,6,7,8,9),a positive correlation was observed at the level of (P≤0.001) between the glucose concentration and (SDH) activity of the enzyme which measured by immunoassay technique in the patients group.

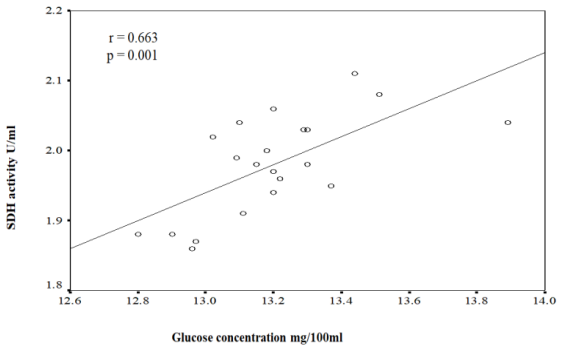

Figure (6): Correlation between SDH activity immunoassay technique and glucose level in T1DMpatients.

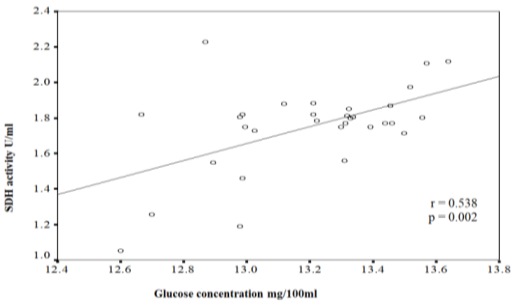

Figure (7):Correlation between SDH activity immunoassay technique and glucose level in T2DM patients.

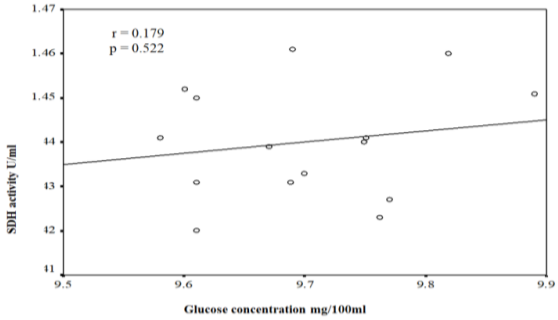

Figure (8):Correlation between SDH activity immunoassay technique and glucose level in T3DM patients.

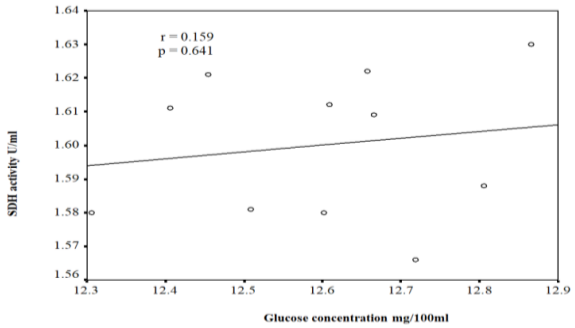

Figure (9): Correlation between SDH activity immunoassay technique and glucose level in T4DM patients.

In Figures (10,11,12,13,14), also positive correlation was observed at the level of (P≤0.001) between the

(SDH) activity of the enzyme and glucose concentration which measured by laboratory method in the group of patients, glucose metabolism may be increase through the polyol pathway is probably which most important mechanism, as the rise in blood sugar leads to an increase in cytosol (NADH / NAD^+) in red blood cells.

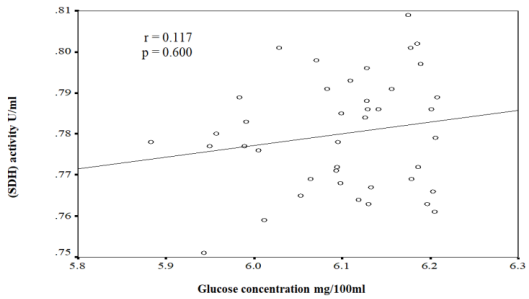

Figure (10): Correlation SDH activity in laboratory method and glucose level in the control group.

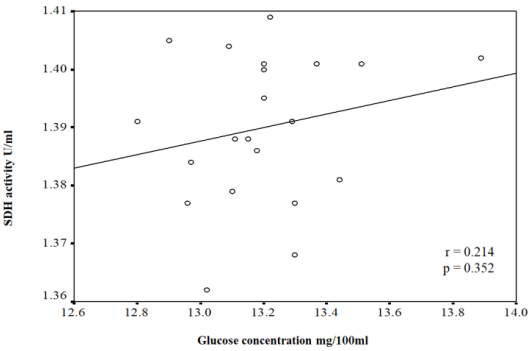

Figure (11): Correlation between SDH activity in laboratory method and glucose level inT1DM patients.

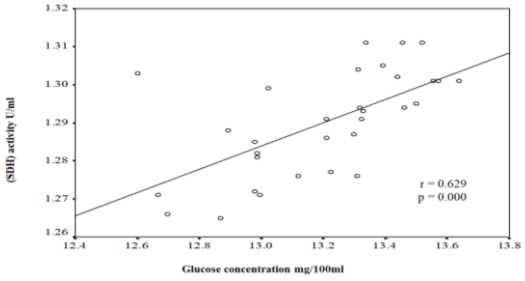

Figure (12): Correlation between SDH activity in laboratory method and glucose level in T2DM patients.

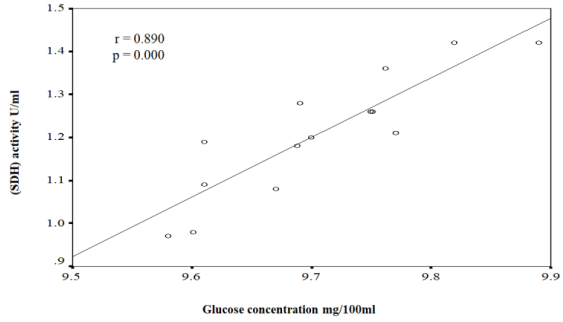

Figure (13): Correlation between SDH activity in laboratory method and glucose level in T3DM patients.

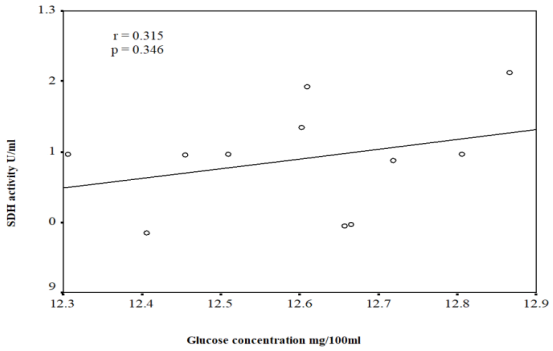

Figure (14): Correlation between SDH activity in laboratory method and glucose level in T4DM patients.

Any defect in pathway levels in diabetics is the result of weakening of the blood vessels and nerves in the retina. In addition to the above glucose reacts with proteins in a non-enzymatic way which leads to the formation of advanced products, and through these products and the several steps, types of free radicals such as reactive oxygen species (ROS) are formed. In diabetics, the metabolism of glucose is enhanced through the polyol pathway (sorbitol), which also leads to the production of super oxides ($O_2^{\bullet -}$), and this is consistent with what researchers have found for experiments on saliva of diabetic patients [28,29,30,31]. Measuring the effectiveness of (SDH) using the immunoassay technique requires the purchase of an analysis kit, and it may not be available in the country and need to be imported from outside the country, so we will take effort and time, while measuring the effectiveness of (SDH) by the laboratory method, its materials are available and cheap compared to that of immunoassay technique and with very close results (the same efficiency on the one hand).

Reference:

1. Thorley ,A., Jeffery, J., and Jornvall, **H.** (2019)SDH: Past, Present, Future***Ado.*** *PracticalEnzymol.* 61,47-106.
2. Williamson, J. R.; Chang, K.; Frangos, M.; Hasan, K. S.; Ido,Y.; Kawamura, T.; Nyengaard, J. R.; Van Den Enden,M.; Kilo, C.; Tilton, R. G.(2019)Diabetes Book Reviews, 42, 801.
3. Arun, N. &Nalini, N. (2002). Efficacy of turmeric on blood sugar and polyol pathway in diabetic albino rats, Plant Foods for Human Nutrition 57:41–52.
4. Berg, J. M.; Tymoczko, J. L.; Stryer, L. (2007). Biochemistry. W. H. Freeman and

Company. New York, USA. pp.138, 139, 145, 146.

5. Cameron, N. E.; Cotter, M. A.; Basso, M.; Hohman, T. C,(2019)Metabolic and vascular factors in the pathogenesis of diabetic nephropathy.Diabetalogia, 40,271.
6. Ido, Y.; Chang, K.; Oates, P. J.; Mylari, B. L.; Williamson, J. R.(2019)Metabolic and vascular factors in the pathogenesis of diabetic nephropathyDiabetes,July. 48, A150.
7. Ng, T. F.; Lee, F. K.; Song, Z. T.; Calcutt, N. A.; Lee,A. Y.; Chung, S. S.; Chung, S.K.; Ng, D. T.; Lee, L.W.(2020),An overview of diabetes types and treatments,Updated on June 17, 47, 961.
8. Oates, P. J.Tomlinson, D. R.,.(2002) The Neurobiology of Diabetic Neuropathy.; International Review of Neurobiology, Vol 50; Academic: London,; p325.
9. Latha M. and Paril L. Brazil.(2004).Diabetes, J. of Medi and Biol. Research. 37. 577-586 .
10. Asada, M. &Galambos, J. T. (1963). Sorbitol dehydrogenase and hepatocellular injury: an experimental and clinical study, Gastroenterology. 44, 578–587.
11. Gerlach, U. &Hiby, W. (1974), Sorbitol Dehydrogenase, In: Methods of Enzymatic Analysis, 2, pp. 569 - 573 (Bergmeyer,H. U., ed.) VCH, Weinheim, FRG.
12. Silveira MM, Jonas R (2002) The biotechnological production of sorbitol. ApplMicrobiolBiotechnol 59:400–408.
13. Jornvall, H., Persson, M. & Jeffery, J. (1984)Sorbitol dehydrogenase the primary structure of the sheep-liver enzyme, Proc. Natl Acad. Sci. USA 78,4226-4230.
14. Mann, T. (2016) in Biochemistry of Semen and of the Male Reproductive Tract (Methuen, London), pp. 237-307.
15. Chylack, L. T. & Kinoshita, J. H. (2017) Refractive change in hyperglycaemia: hyperopia, not myopia. Invest. Ophthalmol. 8, 401-412.
16. Carroll, M. F.&Schade, D. S. (2003). A practical approach to hypercalcemia. Amer. Fam. Phys. 67 (9): 1959–1966.
17. Takazakura, E., Nakamoto, Y., Hayakawa, H., Kawai, K., Muramoto, S.,Yoshida, K., Shimizua, M., Shinoda, A. &Takeuchi,J. (2013)Metabolic and vascular factors in the pathogenesis of diabetic nephropathy Diabetes 24, 1-9.
18. ImsamranW, Leelawat K, Leelawat T, Subwongcharoen et al. (2013),Simple technique in the measurement of liver volume. J Med Assoc Thai.;86:151–156.
19. D. Dodda and V. Ciddi,(2014),Plants used in the management of diabetic complications, Indian J Pharm Sci;76(2):97-106
20. Fehey, J. L.Rose, N. R. ; friedman , H. (2014) ; Manual of Clinical Laboratory immunology. 3rd ed., Mieele East Edition. p. 226–235.
21. Khalil, A. ;Tessier, D. ; Fulop, T. (2016) Liver volume in patients with or without chronic liver diseases. J.Gerontol. Ser. A.Biol. Sc. Vol.54, No 11, pp.541–545.
22. Holme, D. J. & Peck, H. (2016). Analytical biochemistry. John Wiley and Sons, Inc., New York, p: 86.
23. Gad, S. C, and Weil, C. S. (2014). Statistics for toxicologists. In Principles and Methods of Toxicology (A.W. Hayes Ed.), 2nd ed., pp. 435- 48. Raven, New York.
24. Dvornik, D.(2013). Aldose Reductase Inhibition: an Approach to the prevention of diabetic Complications. McGraw Hill, New York, p 368.
25. Tilton, RG. ; Baier, LD. ; Harlow, JE. ; Smith, SR. ; Ostrow, E. ; Williamson, JR.(2017). Diabetes– induced glomerular dysfunction: links to a more reduced.
26. Liang-J..Baeza J, Smallegan MJ, Denu JM. (2018). Redox imbalance stress in diabetes mellitus: Role of the polyol pathway Animal Model Exp Med. Mar; 1(1): 7–13.
27. Dixon ,D. G. ; Hodson, P. V.; Kaiser, K. L. E. (2009).Serum sorbitol dehydrogenase activity as an indicator of chemically induced liver damage in rainbow trout.Environmental Toxicology and Chemistry. p 685–696.
28. Vanderjagt, D.J. ; Harrison, J.M. ; Rattif, D.M. ; Hunsaker, L.A. (2001). Oxidative stress Indices in IDDM. Subjects with and without Long–Term Diabetic Complication.ClinicalBiochemistry,Vol. 34,No.4, pp. 265–270.
29. Tessier, D. ; Khalil, A. ; Fulop, T.(1999). Effect of An Oral Glucose Challenge on Free radicals / Antioxidant Balance in An Older

Population with Type Diabetes. J.Gerontol. Ser. A.Biol. Sc. Vol.54, No 11, pp.541–545.

30. WaleedB.M.AskarM. Ahmad F. et al.,(2014),Role of aldose reductase and arginase inhibitors in diabetic vascular and behavioral complications. Journal of Diabetes and its Complications, 17: 337-342.
31. Feifei S., Chunyan D. Jiansheng X., and Xun H., (2017).Biochemical Issues in Estimation of Cytosolic Free NAD/NADH RatioAdva. J. list 41: 778–88