Egypt. J. Chem. **Vol. 65,** No. SI:13B pp. 219 - 223 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Comparative Biochemical Effects Between Natural And Synthetic Insulin in Induced STZ Rats

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Abstract

Diabetes mellitus (DM) is a multifactorial disease which is characterized by hyperglycemia, lipoprotein abnormalities and altered intermediary metabolism. The first insulin preparation can regulate glucose levels in the bloodstream and induces glucose storage in the liver, muscles, and adipose tissue, resulting in overall weight gain. Insulin therapy has the longest history in the treatment of DM among other antidiabetic agents. The current study was followed the comparison of biochemical effects between Mixtard 30® (human natural insulin) and Novomix 30® (synthetic insulin) after two weeks. eighty male albino rats (200-220 gm). They were randomly divided into four groups. First group conserved as control group. The other groups received STZ (100 mg/kg body weight) to become diabetic. The second group conserved as diabetic, the third group treated with a Mixtard 30® (55 IU/kg body weight/day). The fourth group treated with Novomix 30® (55 IU/kg body weight/day) for two weeks. The results indicated that STZ diabetes caused significant increases in blood glucose, HbA1c, hematological parameters (WBCs, lymphocyte and PLT), ALT, CRP, Creatinine, total Cholesterol and TG and decreased in RBCs, Hb, HCT, MCV and MCHC and body weight when compared to control -ve group. The Mixtard 30® and Novomix 30® improved these results, there was no statistical difference in mean biochemical results between Mixtard 30® and Novomix 30® as treatments of diabetes.

Keywords: Diabetes mellitus; Mixtard 30®; Novomix 30®; biochemical effects.

1. Introduction:

Diabetes mellitus (DM) is a multifactorial disease which is characterized by hyperglycemia, lipoprotein abnormalities and altered intermediary metabolism[1]. DM is a common disease and also a prevalent one affecting the citizens of both developed and developing countries. According to Karalliedde and Gnudi [2], DM is a heterogeneous condition which occurs when there is defect in insulin production by the pancreas, insulin resistance/action or a combination of both, which leads to an increased concentration of glucose in the blood (hyperglycemia).

Insulin therapy has the longest history in the treatment of DM among other antidiabetic agents. DM is a progressive disease and is characterized by progressive loss of pancreatic β-cell function. Majority of patients with DM require some sort of insulin therapy to maintain glycemic control eventually. Over the past several decades, insulin has undergone innovative reengineering in its molecules, administration, and delivery method. There are two types of insulin, i.e., prandial and basal insulin. Soluble insulin and neutral protamine Hagedorn (NPH) insulin are modified human insulin used worldwide. Soluble insulin is a short-acting insulin and is known as prandial insulin to control postprandial hyperglycemia[3]. Modern insulin or insulin analog involves modification to the human insulin molecule that alters the rate of dissociation of insulin once injected to mimic the physiological action of insulin. Ultrashort-acting insulin, i.e., insulin Aspart, Lispro, and Gluilisine, can be injected just before a meal for post prandial hyperglycemia control. Premixed formulation of short-acting soluble insulin with intermediate insulin (e.g., Mixtard 30/70) or ultrashort-acting insulin with intermediate insulin (e.g., Novomix 30/70) is available for once-, twice-, or thrice-daily injection [4]. The aim of this work has been carried out to investigate the comparison between Novomix 30 and Mixtard 30 as treatments after two weeks on biochemical effects in diabetic rats with STZ.

2. Materials and methods

2.1. Materials:

Streptozotocin (STZ) Was Purchased from Sigma-Aldrich, USA. Mixtard 30® and Novomix 30® were

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Receive Date: 02 April 2022, Revise Date: 19 May 2022, Accept Date: 24 June 2022

DOI: 10.21608/EJCHEM.2022.131075.5767

Procured from a local pharmacy provided from Novo Nordisc Company.

2.2. Animals, experimental design and blood sampling:

A total of 80 male albino male rats weighing 200-220 g were used for the study. The rats were housed in plastic cages at a controlled temperature of 22 ± 3°C with a 12-hour light/dark cycle. Animals were fed with standard chow and supplied with drinking water ad libitum. They were acclimatized for one week before the beginning of experiments and were purchased from the laboratory animal house, faculty of veterinary medicine, Ismailia, Egypt.

The rats were divided into 4 groups of 20 each: controls, diabetic rats, diabetic rats treated with Mixtard 30® Natural human insulin 55 mg/kg/day, diabetic rats Novomix 30® treated with Synthetic insulin 55 mg/kg/day. All treatments and procedures were approved by the animal use ethical committee of Suez Canal university, faculty of veterinary medicine (certificate 2021/39).

After treatment period of two weeks, blood samples were collected (from retro-orbital sinus) for biochemical tests, each blood sample was collected into two tubes, the first one containing EDTA for CBC analysis and the second one was free from EDTA for serum collection (rest of biochemical analysis). Serum samples were centrifuged under cooling (4ºC) for 30 min at 3500 rpm for serum separation.

2. Induction of diabetes mellitus:

After the period of acclimatization, test rats were given a single intraperitoneal injection of freshly prepared streptozotocin was dissolved in 0.1 M sodium citrate buffer (pH 4.5) not longer than 5 minutes before its use, at a dose of 100 mg streptozotocin/kg body weight. The diabetic state suffered from loss of body weight and high blood glucose levels.

2.4. Biochemical analysis:

1. Blood

Assaying of hematological parameters, hemoglobin content [5], RBCs and total WBCs count using CBC counter according to the method of Sood[6] and differential leukocytes count was carried out from Giemsa stained blood smears of all Rats.

2. Serum

Glycosylated and Glucose levels in the plasma were measured using the glucose oxidase method as stated in the leaflet of Randox laboratory kits. Plasma insulin concentrations were measured using a rat-specific radioimmunoassay kit procedure. Serum aminotransferases (ALT)) enzyme activity was measured by the method described by Reitman and Frankel[7], the activities of the enzymes were extrapolated from a standard curve and expressed as units/ml. Total cholesterol and triglycerides (TG) of serum were estimated by using standard methods Zlatkis, Zak & Boyle[8], The C-reactive protein (CRP) was determined in serum samples using ready-made kits produced by Diamond by the method of. Byun JY et al.[9], Serum creatinine was assayed using ready-made kit produced by Diamond by the method of Murray (a, b)[10,11]. Serum creatine kinase isoenzyme MB (CK MB): was determined by using ready-made kit produced by Diamond by the method of Foreback CC[12].

2.5 Statistical analysis:

Data were statistically analyzed using Graphpad Prism® software. All of the data of control and treated groups were expressed as mean values ± standard errors. One-way ANOVA followed by post-Fisher's test was carried out to find if there was any significant difference between groups.

3. Results

3.1. Effect of studied insulins on hematological parameters:

After two weeks of treatments, control -ve group, Mixtard and Novomix treated groups showed significant increase ($P<0.05$) in RBCs, Hb, HCT, MCV, MCH and MCHC ($5.32 *10^6$ /ul, 15.1g/dl, 39.86%, 86.5fl, 29.04pg. and $33.32*10^3$/ul), ($4.48*10^6$ /ul, 12.54g/dl, 32.02%, 79.34fl, 26.48pg. and $32.6*10^3$/ul) and ($4.52*10^6$ /ul, 13.06g/dl, 33.56%, 81.7fl, 27.4pg. and $32.8*10^3$/ul) respectively, when compared to non-treated control +ve group. Non-treated control +ve group showed significant decrease ($P>0.05$) in RBCs, Hb, HCT, MCV and MCHC ($2.96*10^6$ /ul, 9.72g/dl, 26.34%, 70.50fl, 22.66pg. and $26.26*10^3$/ul) when compared to control -ve group. While all these parameters were significant ($P>0.05$) increased when compared with control +ve (diabetic non-treated) group. Treated animals with Mixtard and Novomix showed significant increase ($P<0.05$) in lymphocyte and PLT and insignificant change in WBCs count ($10.02*10^3$/ul, 39.84% and $369*10^3$/ul) and ($10.08*10^3$/ul, 38.36% and $371.2*10^3$/ul) when compared to control -ve group. Non-treated animals control +ve group showed significant increase ($P<0.05$) in table (1).

Table (1): Effect of studied insulins on hematological parameter

Groups of experiment	RBCs (10^6)/ul	HGB (g/dl)	HCT %	MCV (fl)	MCH (pg)	MCHC (g/dl)	WBC (10^3)/ul	lymphocyte %	PLT (10^3)/ul
Control –ve	5.32±0.30^{a}	15.1±0.31^{a}	39.86±0.96^{a}	86.5±2.67^{a}	29.04±0.48^{b}	33.32±0.73^{a}	9.90±0.33^{b}	32.28±0.65^{c}	287.4±26.9^{c}
Control +ve	2.96±0.17^{c}	9.72±0.40^{c}	26.34±0.73^{c}	70.50±0.97^{c}	22.66±0.23^{ab}	26.26±0.40^{b}	11.62±0.39^{a}	44.8±0.86^{a}	469.6±14.9^{a}
Mixtard	4.48±0.21^{b}	12.54±0.41^{b}	32.02±0.79^{b}	79.34±1.46^{b}	26.48±0.88^{a}	32.6±0.88^{a}	10.02±0.19^{b}	39.84±0.65^{b}	369±32.9^{b}
Novomix	4.52±0.18^{b}	13.06±0.26^{b}	33.56±1.00^{b}	81.7±0.57^{b}	27.4±0.93^{a}	32.8±0.93^{a}	10.08±0.27^{b}	38.36±0.28^{b}	371.2±29.7^{b}

All values are expressed as mean ± SE. Different alphabetic letters within the same column indicate significant difference at (P<0.05).

3.2. Effect of insulins on body weight, blood glucose and glycosylated hemoglobin parameters:

Treated rats with Mixtard and Novomix showed significantly increase (P<0.05) in the body weights (225.60g and 223.20g) when compared to normal control -ve group (206.80g). On the other hand, the blood glucose and HbA1c were insignificantly increased (P<0.05), (126.40mg/dl and 5.57%) and (121.80mg/dl and 5.25%) when compared to normal control -ve group (95.80mg/dl and 4.96%). Diabetic non-treated animals (control +ve group) showed significant decrease (P>0.05) in body weight (165.80g), but significant increase (P<0.05) in blood glucose and HbA1c (445.20mg/dl and 6.60%) when compared to treated group and control –ve group. Effect of two weeks of treatment with Mixtard and Novomix on body weight, blood glucose and glycosylated hemoglobin in normal and STZ-induced diabetic male rats was shown in table (2).

3.3. Effect of insulins on C-Reactive protein, liver and kidney functions:

Rats treated with Mixtard and Novomix showed significantly increase (P<0.05) in the level of CRP, ALT, and creatinine (3.16mg/l, 28.00u/l and 1.30mg/dl) and (2.96mg/l, 27.40u/l and 1.37mg/dl) when compared to normal –ve control group (2.12mg/l, 13.02u/l and 1.07mg/dl). Non-treated +ve group showed a significant increase (P<0.05) in CRP, ALT and creatinine levels (7.92mg/l, 48.10u/l and 2.46mg/dl) when compared to treated group and normal –ve control group. Treatment with Mixtard and Novomix decreased the elevated CRP and ALT but not significantly enough to normal values, while both insulin treatments significantly decreased the elevated creatinine to normal values elevated creatinine to normal values was shown in the table (2).

3.4. Effect of insulins on lipid profile and creatine kinase test:

Non-treated diabetic rats showed a significant increase (P<0.05) in serum cholesterol, TG and CK MB levels (155.80mg/dl, 192.60mg/dl and 31.4iu/l) compared to normal -ve group. While, treated animals with Mixtard and Novomix showed non-significant increase (P<0.05) in the level of serum cholesterol and TG (131.60mg/dl and 144.20mg/dl) and (128.20mg/dl and 142.20mg/dl), and significant increase in CK MB levels (23.4iu/l and 24.9iu/l) when compared to normal -ve control group (115.60 mg/dl, 132.00 mg/dl and 13.2 iu/l) was shown in the table (2).

Table (2): Effect of insulins on Serum (body weight, blood glucose, glycosylated hemoglobin C-Reactive protein, liver and kidney functions, lipid profile and creatine kinase tests).

Groups of experiment	Body weight (g)	Blood glucose (mg/dl)	HbA1c %	CRP (mg/l)	ALT (u/l)	Creatinine (mg/dl)	Serum cholesterol (mg/dl)	TG (mg/dl)	CK Mb (iu/l)
Control –ve	206.80±3.82^{b}	95.80±3.97^{b}	4.96±0.12^{a}	2.12±0.17^{c}	13.02±1.10^{c}	1.07±0.10^{b}	115.60±15.83^{b}	132.00±5.40^{b}	13.2±2.6^{c}
Control +ve	165.80±9.20^{c}	445.20±36.55^{a}	6.60±0.23^{b}	7.92±0.37^{a}	48.10±2.59^{a}	2.46±0.38^{a}	155.80±21.46^{a}	192.60±14.43^{a}	31.4±3.2^{a}
Mixtard	225.60±5.67^{a}	126.40±6.32^{b}	5.57±0.10^{a}	3.16±0.14^{b}	28.00±1.47^{b}	1.30±0.09^{b}	131.60±4.84^{ab}	144.20±7.52^{b}	23.4±2.2^{b}
Novomix	223.20±5.74^{a}	121.80±5.45^{b}	5.25±0.52^{a}	2.96±0.17^{b}	27.40±1.66^{b}	1.37±0.10^{b}	128.20±6.06^{ab}	142.20±3.38^{b}	24.9±1.9^{b}

All values are expressed as mean ± SE. Different alphabetic letters within the same column indicate significant difference at (P<0.05).

4. Discussion:

Diabetic group with complications are characterized with a decrease in RBCs, Hb, HCT, MCV and hemoglobin concentration (MCH, and MCHC) ($2.96*10^{6}$ /ul, 9.72g/dl, 26.34%, 70.50fl, 22.66pg. and $26.26*10^{3}$/ul) paramount in erythrocyte function and these changes can cause decreasing the oxygen-carrying capacity due to increase in hemolysis

rate and thus decreasing their lifespan; a condition resulting. However, the treated groups with Mixtard and Novomix were seen to have increased levels of RBCs, Hb, HCT, MCV and hemoglobin concentration (MCH, MCHC) (5.32 *10^6 /ul, 15.1g/dl, 39.86 %, 86.5fl, 29.04pg. and 33.32*10^3/ul), (4.48*10^6 /ul, 12.54g/dl, 32.02%, 79.34fl, 26.48pg. and 32.6*10^3/ul) and (4.52*10^6 /ul, 13.06g/dl, 33.56%, 81.7fl, 27.4pg. and 32.8*10^3/ul). The reason could be the antidiabetic effect of insulins. Furthermore, the increase in the levels of WBC, lymphocyte and PLT count (11.62*10^3/ul, 44.8% and 469.6*10^3/ul) of this study could be as a result of the damaging effects of STZ This result agreed with Fagbohun et al.[13]. Mixtard and Novomix treated groups improved this results (10.02*10^3/ul, 39.84% and 369*10^3/ul) and (10.08*10^3/ul, 38.36% and 371.2*10^3/ul).

In the present study, STZ-induced diabetic group showed developed progressive increase in blood glucose level and HbA1c (445.20mg/dl and 6.60%) which seems to be explained by deficient insulin secretion as a result of β-cell destruction by STZ. This result agreed with salama et al.[14], The results from this study also revealed a drastic loss of weight of untreated diabetic rats (165.80g) compared to non-diabetic (206.80g) and treated animals (225.60g and 223.20g). Similar loss in weight has been observed in STZ-diabetic group by Mestry et al.[15], Mixtard and Novomix treated groups showed increase in body weight compared to control non diabetic group and improved blood glucose and HbA1c (126.40mg/dl and 5.57%) and (121.80mg/dl and 5.25%).

The level of plasma creatinine in the experimental rats was assessed in the present study. The increased level of plasma creatinine in diabetic control rats implies impaired renal function in the STZ-diabetic group (2.46mg/dl). This result agreed with Amartey et al.[16], Mixtard and Novomix treated groups improved creatinine level (1.30mg/dl and 1.37mg/dl).

Insulin can affect the adipocytes by inhibiting lipolysis and promoting storage of triglycerides in adipocytes. Thus, insulin lack in diabetes enhances hydrolysis of triglycerides into diglycerides, unesterified fatty acids and free glycerol. In this study total cholesterol and TG levels increased in STZ-diabetic rats (131.60mg/dl and 144.20mg/dl) and (128.20mg/dl and 142.20mg/dl), this result agreed with Sakuludomkan et al.[17], Mixtard and Novomix treated groups improved total cholesterol and TG (131.60mg/dl and 144.20mg/dl) and (128.20mg/dl and 142.20mg/dl) compared to diabetic group.

Plasma alanine aminotransferase (ALT) was also monitored in both treated and STZ-diabetic and non-diabetic rats. Tissue damage is usually associated with the release of enzymes specific to the affected tissue or organ which could result in the increase in the activity of such enzyme showed in the blood STZ-diabetic rats (48.10u/l). This result agreed with Zhao et al.[18]. ALT is a cytoplasmic enzyme found in high amounts in the liver and an increase in ALT in the blood indicates liver damage so CRP activity increased (7.92mg/l) due to this result agreed with Parmer et al.[19], Mixtard and Novomix treated groups improved in ALT level (28.00u/l and 27.40u/l) and CRP activity decreased (3.16mg/l and 2.96mg/l) compared to STZ-diabetic rats.

Elevated plasma CK MB level activity is a reliable clinical indicator of organ injuries, especially those adversely affected by chemical toxicity, namely, the heart, liver, and muscle Ahmed et al.[20]. The relatively raised level of CK MB activity was an obvious indication of systemic toxicity in STZ-induced DM rats (31.4iu/l). Mixtard and Novomix treated groups improved in CK MB level (23.4iu/l and 24.9iu/l) compared to STZ-diabetic rats.

5. Conclusion

All mammalian cells use similar molecular mechanism, structure and function between species. Rats and human are good example of this metabolic homogeneity, they have the same organs, systemic physiology and great similarity in disease pathogenesis. This study supports the concept that (natural and synthetic) insulins used as anti-hyperglycemic, anti-inflammatory effects and consequently improved liver, kidney and heart function caused by STZ-induced diabetes, there is no significant differences between Mixtard 30® and Novomix 30® as therapies for diabetes, this type of therapies represents a good remedy for treatment of diabetes mellitus.

Abbreviations

DM, Diabetes mellitus; ANOVA, analysis of variance; B.W., body weight; CBC, complete blood count; °C, degree Celsius; gm, gram; H&E, hematoxylin and eosin; Hb, hemoglobin; Hct, hematocrit; kg, kilogram; L, Lymphocyte; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; mg, milligram; mL, milliliter; RBCs, red blood cells; PLT, platelet count; SE, standard error of the mean; STZ, streptozotocin; PK/PD, pharmacokinetic /pharmaco-dynamics; RHI, regular human insulin; ALT, Alanine aminotransferase; CRP, C-reactive protein; CK MB, creatine kinase isoenzyme MB; PAS, periodic acid Schiff; HbA1C, Glycosylated hemoglobin; TG, Triglyceride; M, molar; %, percent; mL, milliliter; pg, pictogram; µL, microliter; mL, milliliter; fl, femtoliter; dl, deciliter.

6. References

1- Kim H. W. (2021). Metabolomic Approaches to Investigate the Effect of Metformin: An Overview. *International journal of molecular sciences*, *22*(19), 10275. https://doi.org/10.3390/ijms221910275 .

2- Ricciardi, C. A., & Gnudi, L. (2021). Kidney disease in diabetes: From mechanisms to clinical presentation and treatment strategies. *Metabolism: clinical and experimental*, *124*, 154890. https://doi.org/10.1016/j.metabol.2021.154890

3- Vargas-Uricoechea H. (2022). Current State and Principles of Basal Insulin Therapy in Type 2 Diabetes. *Journal of clinical medicine research*, *14*(1), 8–21. https://doi.org/10.14740/jocmr4660.

4- Hemmingsen, B., Metzendorf, M. I., & Richter, B. (2021). (Ultra-)long-acting insulin analogues for people with type 1 diabetes mellitus. *The Cochrane database of systematic reviews*, *3*(3), CD013498. https://doi.org/10.1002/14651858.CD013498.pub2 .

5-Drabkin DL. The standardization of hemoglobin measurement. Am J Med Sci (1949): 217(6): https://doi.org/10.1097/00000441-194801000-00017.

6-Sood S. (2017): Neutropenia with Multiple Antipsychotics Including Dose Dependent Neutropenia with Lurasidone. *Clinical psychopharmacology and neuroscience: the official scientific journal of the Korean College of Neuropsychopharmacology*, *15*(4), 413–415. https://doi.org/10.9758/cpn.2017.15.4.413.

7-Reitman, S., & Frankel, S. (1957): A colorimetric method for the determination of serum glutamic-oxaloacetic and glutamic-pyruvate transaminases. American Journal of Clinical Pathololgy, 28, 56–63 https://doi.org/10.1093/ajcp/28.1.56.

8-Zlatkis, A., Zak, B., & Boyle, A. J. (1953): A new method for the direct determination of serum cholesterol. Journal oF Laboratory and Clinical Medicine, 41, 486–492. https://doi.org/10.1016/0009-8981(61)90038-9.

9-Byun JY, Shin YB, Kim DM, Kim MG. (2013): A colorimetric homogeneous immunoassay system for the C-reactive protein. Analyst. 7;138(5):1538-43. https://doi.org/10.1039/c3an36592a.

10-Murray R. (1984a): Creatinine. Clinical Chemistry theory, analysis, and correlation. Kaplan LA, Pesce AJ, eds. second ed. St. Louis: The CV Mosby Company,1247-3. https://doi.org/10.1073/pnas.81.11.3486.

11-Murray R. (1984b): Creatinine. Clinical Chemistry second ed, 1247-3 https://doi.org/10.1073/pnas.81.11.3486

12-Foreback CC, Chu JW. (1981): Creatine kinase isoenzymes: electrophoretic and quantitative measurements. CRC Crit Rev Clin Lab Sci.15:187–230 https://doi.org/10.3109/10408368109105871.

13- Fagbohun OF, Awoniran PO, Babalola OO, Agboola FK, Msagati TAM. (2020): Changes in the biochemical, hematological and histopathological parameters in STZ-Induced diabetic rats and the ameliorative effect of *Kigelia africana* fruit extract. Heliyon. 21;6(5): e03989. http://doi.org/10.1016/j.heliyon.2020.e03989.

14- Salama, A., Asaad, G. F., & Shaheen, A. (2021). Chrysin ameliorates STZ-induced diabetes in rats: possible impact of modulation of TLR4/NF-κβ pathway. *Research in pharmaceutical sciences*, *17*(1), 1–11. https://doi.org/10.4103/1735-5362.329921.

15-Mestry, S. N., Dhodi, J. B., Kumbhar, S. B., & Juvekar, A. R. (2017): Attenuation of diabetic nephropathy in streptozotocin-induced diabetic rats by Punicagranatum Linn. Leaves extract. Journal of Traditional and ComplementaryMedicine, 7(3), 273–280 https://doi.org/10.1016/j.jtcme.2016.06.008

16-Amartey, N. A., Nsiah, K., & Mensah, F. O. (2015): Plasma Levels of Uric Acid, Urea and Creatinine in Diabetics Who Visit the Clinical Analysis Laboratory (CAn-Lab) at Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. *Journal of clinical and diagnostic research : JCDR*, *9*(2),BC05–BC9. https://doi.org/10.7860/JCDR/2015/10905.5530

17- Sakuludomkan, W., Yeewa, R., Subhawa, S., Khanaree, C., Bonness, A. I., & Chewonarin, T. (2021): Effects of Fermented *Houttuynia cordata* Thunb. on Diabetic Rats Induced by a High-Fat Diet with Streptozotocin and on InsulinResistance in 3T3-L1 Adipocytes. *Journal of nutrition and metabolism*, *2021*, 6936025. https://doi.org/10.1155/2021/6936025

18- Zhao, M., Qin, J., Shen, W., & Wu, A. (2021): Bilobalide Enhances AMPK Activity to Improve Liver Injury and Metabolic Disorders in STZ-Induced Diabetes in Immature Rats via Regulating HMGB1/TLR4/NF-κB Signaling Pathway. *BioMed research international*, *2021*, 8835408. https://doi.org/10.1155/2021/8835408

19-Parmar, K. S., Singh, G. K., Gupta, G. P., Pathak, T., & Nayak, S. (2020): Evaluation of De Ritis ratio in liver-associated diseases. International Journal of Medical Science and Public Health, 5(9), 1782–1788 https://dx.doi.org/10.5114%2Famsad.2020.103028

20- Ahmed, O. M., Abd El-Twab, S. M., Al-Muzafar, H. M., Adel Amin, K., Abdel Aziz, S. M., & Abdel-Gabbar, M. (2021): Musa paradisiaca L. leaf and fruit peel hydroethanolic extracts improved the lipid profile, glycemic index and oxidative stress in nicotinamide/streptozotocin-induced diabetic rats. *Veterinary medicine and science*, *7*(2), 500–511. https://doi.org/10.1002/vms3.389