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The Metabolic Impact of Vitamin B12 in The Context of Metabolic Syndrome

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Abstract

Metabolic syndrome (Mets) refers to a group of symptoms that increasing the risk of heart disease, stroke and type 2 diabetes (T2DM). One of the most difficult health issues facing the world today is diabetes mellitus (DM). In diabetes, chronic hyperglycemia can cause both immediate and delayed consequences. Cobalamin, or vitamin B12, is a water-soluble vitamin essential for proper neuronal and vascular function, normal hemopoiesis, and DNA synthesis. The aim of this study was to determine the impact of vitamin B12 in the streptozotocin (STZ)-induced diabetic rats. In this study, 30 males' rats were divided into three groups, for a period of 9 weeks, the rats were injected with vitamin B12. Serum lipid levels, some biochemical, molecular parameters and histopathology of liver and brain tissues were determined. Our results demonstrated that compared to rats in the diabetic groups, the vitamin B12 reduced glucose, insulin, HOMA-IR, glycated haemoglobin (HbA1c), cholesterol, triacylglycerol (TAG), high density lipoprotein-cholesterol (HDL-c), low-density lipoprotein-cholesterol (LDL-c) levels, and Malondialdehyde (MDA), while vitamin B12 increased vitamin 12, glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) levels ($P < 0.05$). An upregulation was found in the gene expression in the homogenate of hepatocyte growth factor (HGF), leptin receptor (LEPR), and glucose transporter -2 (GLUT-2). On the other hand, there was a significant downregulation in the mRNA expression of Janus kinase3 (JAK3), signal transducer and activator of transcription3 (STAT3), Transforming growth factor–β (TGF-β), and protein tyrosine phosphatase 1B (PTPN1). In conclusion our findings suggested that vitamin B12 supplementation can mitigate the Impact of an STZ in diabetic rats. This new research provides further evidence that vitamin B12 may be useful as a treatment for diabetes.

KEYWORDS
Vitamin B12, Metabolic syndrome, Type 2 diabetes, Streptozotocin.

INTRODUCTION

Metabolic syndrome (Mets) is a cluster of metabolic abnormalities that includes insulin resistance (IR), abdominal or central obesity, abnormal lipid profiles, and high blood pressure (Elnagar *et al*., 2018; Khalil *et al*., 2018a). Its prevalence is increasing at an exponential rate, and it now affects massive populations all over the world. Mets can arise from any number of factors. Mets has numerous proposed causes, from an increase in very low-density lipoprotein and fatty acid production to an increase in visceral adiposity (Ashok *et al*., 2021; El-Dawy, 2022; Ahmed *et al*., 2023).

Vitamin B12 requires methionine synthase and L-methylmalonyl-CoA mutase, two enzymes found in higher mammals, can't function without. As a byproduct of microbial synthesis, it requires complex systems of absorption and transport to ensure its availability. Dietary vitamin B12 binds to intrinsic factor (IF) and is taken up by the body through the cubam receptor in the distal small intestine. When people don't eat enough meat or IF, they get a B12 deficit. Megaloblastic anaemia, demyelinating neurologic disorders, and elevated methylmalonic acid and homocysteine levels are all symptoms of a deficiency in humans (Abdel-aleem *et al*., 1998; Reddy *et al*., 2020).

The methylation activities of DNA and RNA require activated coenzyme B12, which is also crucial for the production of proteins and lipids. Lack of it has been linked to problems with DNA synthesis, inflammation, abnormal fat storage, and elevated levels of homocysteine and lipogenesis. Increased homocysteine levels and adipose tissue alterations associated with B12 deficiency had a role in IR and, by extension, glucose intolerance (Kouroglou *et al*., 2019).

Studies into the impact of micronutrient deficiencies on the development of metabolic chronic diseases is a major focus in global health, particularly in the Middle East. Several observational studies have found a negative correlation between vitamin B12 consumption and metabolic problems such obesity, abnormal lipid profiles, and cardiovascular disease. Studies in animals have shown a possible causative relationship between low maternal B12 levels and poor lipid profiles in their offspring (Boachie *et al*., 2020).

In most prevalent IR situations, including obesity, hepatocyte growth factor (HGF) levels are elevated, making it a significant component of the pathogenesis of IR. In addition to its role in β-cell homeostasis and its ability to modulate the inflammatory response, HGF also contributes to the metabolic flow of glucose in a variety of insulin-sensitive cell types (Oliveira *et al*., 2018).

Important metabolic hormones and cytokines that require

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signal transduction include growth hormone, leptin, erythropoietin, interleukin (IL-4, IL6), and IFNγ. The Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway is essential for this process. Both clinical and basic science studies have shown evidence that the JAK/STAT pathway has a role in the etiology of metabolic illness (Dodington *et al*., 2018; Khalil *et al*., 2018b).

By stimulating the leptin receptor (LEPR), leptin plays a role in mediating inflammatory and energy homeostasis processes. Leptin is a precursor to adipokines, basal metabolism and insulin secretion are all regulated by leptin after it has been secreted into the circulation and has reached the central and peripheral nervous systems. Glucose, blood pressure, and lipid metabolism can all be controlled by LEPR. Increased hunger and fat accumulation occur when LEPR is not present in the body. Transforming growth factor–β (TGF-β) signalling pathways are two potential biological processes that contribute to DM development and its consequences, such as cardiomyopathy, retinopathy, and nephropathy (Heydarpour *et al*., 2020). In the insulin-PI3K/Akt signalling pathway, protein tyrosine phosphatase 1B (PTPN1), can dephosphorylate insulin receptor substrate 1 (IRS-1) to impact downstream cascade processes and suppress signal transduction (Du *et al*., 2022). Furthermore, LEPR is localized in pancreatic β-cells, suggesting a potential role for this cell type in the development of persistent hyperglycemia and uncontrolled T2DM (Zhang *et al*., 2018). The beta cells of the pancreas and the liver both rely on glucose transporter -2 (GLUT-2) as their primary glucose transporter. It's crucial for -cell insulin secretion and hepatocyte glucose metabolism. Therefore, this study aimed to determine the possible impact of vitamin B12 in the streptozotocin (STZ)-induced diabetic rats.

MATERIALS AND METHODS

Drugs

Vitamin B12 ampules obtained as DEPOVIT B12 AMP (1000 mcg/1mL) produced by Amriya for Pharmaceutical Industries S.A.E, Alexandria, Egypt. Streptozotocin (STZ) was purchased from Sigma Chemical Co (USA).

Animals and study protocol

The Experimental animal house of the Faculty of Veterinary Medicine, Zagazig University provided 30 adult male Sprague-Dawley rats (150-200 g) approved by committee of ZU-IACUC with approval number (ZU-IACUC/ / 2F/142/2023).

All animals fasted overnight, and then a single dosage of STZ (35 mg/kg body weight) diluted in citrate buffer was injected intraperitoneally. After 7 days of STZ administration, tail vein blood samples were taken and the concentration of glucose in the blood was determined, the rats was considered diabetic if fasting blood glucose levels >250 mg/dL (Lekshmi *et al*., 2015).

The animals were divided into three groups (n=10), Control group: rats were fed a regular diet of food during the study. Diabetic group: rats received no drug and served as the STZ-induced diabetic group. Diabetic+ B12 group: diabetic rats were received IP injection of vitamin B12 at doses of 25 µg kg^{-1}, respectively, twice a week for 9 weeks (Tamaddonfard *et al*., 2013).

Measuring body weight

Body weight was measured at day zero, third week and at the end of the experiment.

Collection of blood and tissue samples

All the rats were anaesthetized with pentobarbital sodium at the end of the trial, after they had fasted for 12 hours. The liver and brain were removed surgically, and blood was drawn from the orbital venous plexus and a complete blood sample was taken on EDTA for the purpose of calculating haemoglobin A1c, the serum samples spun at 3000 rpm for 15 minutes to remove any clots. lipid, glucose, and insulin levels were measured in the serum samples.

To preserve tissue for histological analysis, parts of the liver and brain were placed in 10% neutral buffer formalin, while the rest parts were frozen in liquid nitrogen, the other tissue parts were collected in1 ml of thiazole and stored at -80°C for analysis of the gene expression and oxidant stress markers.

Biochemical and oxidant stress markers assay

Following the manufacturer's instructions, commercial kits were used to help in measuring serum vitamin B12 levels (MyBioSource, USA). Glucose (MyBioSource, USA) and insulin (MyBioSource, USA). In order to determine the homeostasis model assessment for IR (HOMA-IR) index, which is calculated by multiplying blood glucose level (mg/dl) by insulin level (ng/ml) and then dividing on 405. Cholesterol (HDL and LDL/VLDL, USA), tria-

Table 1. Primers sequences for the real time PCR.

Gene	Sequence	pb	Accession number
HGF	F 5′- TGATCCCCCATGAACACAGC-3' R 5'- CCCCTCGAGGATTTCGACAG-3'	84	NM_017017.2
JAK3	F 5′- CGCCTCCATCTCTGGAGTTT-3' R 5'- GAGAGGTGTCTCCTCGCTTG-3'	70	NM_012855.2
STAT3	F 5′- CTGAGGTACAATCCCGCTCG-3' R 5'- CACTGTCTCTGGGGCTGAAG-3'	132	NM_012747.2
TGF-1β	F 5′- AGGGCTACCATGCCAACTTC-3' R 5'- CCACGTAGTAGACGATGGGC-3'	168	NM_021578.2
PTPN1	F 5′- CGATAAGGCTGGGAACTGGG-3' R 5'- TCCGACTGTGGTCAAAAGGG-3'	140	NM_012637.2
LEPR	F 5′- TATGCTGGGATGTGCCTTGG-3' R 5'- GTGGCGCACAAAACAGCTTA -3'	168	NM_012596.2
GLUT-2	F 5′- CTCTGTGCTGCTTGTGGAGA-3' R 5'- CGGCACAGAAAAACATGCCA-3'	77	NM_012879.2
GADPH	F 5′-GCATCTTCTTGTGCAGTGCC-3' R 5'-TACGGCCAAATCCGTTCACA-3'	74	NM_017008.4

cylglycerol (TAG) (Abcam, USA), High-density lipoprotein-cholesterol (HDL-c) (Abcam, USA), LDL-c (Crystal Chem's Rat LDL, USA). While in tissue homogenate the MDA (MyBioSource, USA), CAT (MyBioSource, USA), GPx (Biodiagnostic, USA), and SOD (Abcam, USA) were evaluated.

Measurement of glycated haemoglobin (HbA1c)

HbA1c levels were measured using a DCA-2000 system, the glycated Hb beta chain has a unique amino acid sequence that the used monoclonal antibody recognized. Glucose and this specific amino acid sequence were necessary for antibody binding. In the absence of HbAlc, the second reagent agglutinated monoclonal antibody attached to latex beads. Glycated Hb prevented agglutination by competing for antibody binding sites with the agglutinating agent. Because of the agglutination reaction, the absorbance at 531 nm was measured to be greater than expected. The proportion of HbAlc in the total Hb was used to represent the HbAlc concentration. The percentage value for HbA1c was calculated by dividing its concentration by the total amount of haemoglobin in the blood (Hsieh *et al*., 2013).

Gene expression

Following the protocol provided by the manufacturer, total RNA was isolated using TRIZOL reagent (Invitrogen, Germany). The extracted RNA concentration was measured with a Nano-drop 2000 spectrophotometer. The eluted RNA was frozen at -80 °C for later use. Second, using the TaqManTM Small RNA Assays according to the manufacturer's instructions. The real-time PCR was performed using the recommended Maxima SYBR Green/Rox qPCR Master Mix (2X) and protocols. After normalization to housekeeping GADPH (Livak and Schmittgen, 2001), fold change in gene expression was reported as $2^{-\Delta\Delta CT}$ relative to control. The primer sequences of the studied genes were illustrated in Table 1.

Histopathological investigations

Tissue samples from the liver and brain were fixed in 10% neutral buffered formalin for 48 hours, rinsed overnight in running water, and then examined under the microscope. After being washed, the samples were dehydrated using ethyl alcohol at concentrations ranging from 70% to 100%. After being submerged in ethyl alcohol for 12 hours, the samples were given a 2-hour xylol rinse. The samples were then placed in molten paraffin in a crucible. Sections 5-micron-thick tissue were used for microscopic analysis. Histopathological sections stained with H&E.

Statistical analysis

The results were reported using the mean±standard error. The impact of the groups on the several biochemical indicators was analyzed using one-way analysis of variance (ANOVA) with Duncan's multiple test for post hoc comparisons. The significance level was below 0.05. Statistical analyses and graphs were made using SPSS 25 and GraphPad Prism 8.

RESULTS

Impact of vitamin B12 on body weight in STZ-induced rat

Figure 1 revealed that rats in the all groups demonstrated non-significant change in the body weight at day zero, third week and at the end of the experiment.

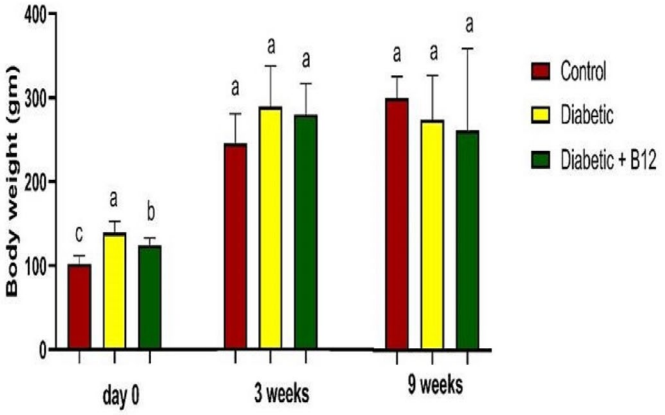

Fig. 1. Impact of vitamin B12 on body weight (gm) in STZ-induced rat.

Impact of vitamin B12 in some biochemical parameters in STZ-induced rat

The level of vitamin B12 in Figure 2 showed a significant decrease in the diabetic group compared with the diabetic+B12 group and control group. While the level of vitamin B12 was significantly increased in diabetic+B12 group compared with the diabetic group and control group.

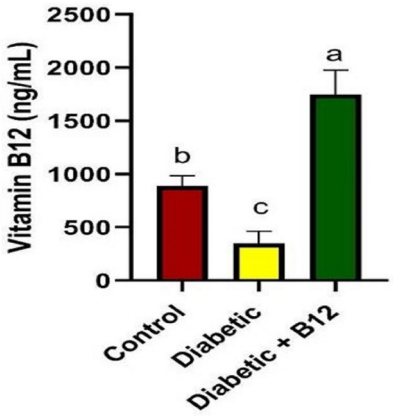

Fig. 2. Impact of vitamin B12 in the level of vitamin B12 in STZ-induced rat.

The data represented in Figure 3 showed that glucose, insulin, HOMA-IR and HbA1c levels were the highest ($p < 0.05$) in diabetic group compared with diabetic+B12 group and control group. The diabetic+B12 group showed significant improvement compared with diabetic group. While their levels were non-significantly changed in between diabetic+B12 group and control group.

Impact of vitamin B12 in lipid profile in STZ-induced rat

Figure 4 showed that cholesterol, TAG, HDL-c and LDL-c levels were the highest in diabetic group compared with diabetic+B12 group and control group. While their levels were significantly ($p < 0.05$) improved in the diabetic+B12 group and control group compared with diabetic group. The cholesterol, TAG, and LDL-c levels were significantly differing in diabetic+B12 group and control group, while the HDL-c in diabetic+B12 group and control group were non-significantly differ in between each other.

Impact of vitamin B12 in the levels of oxidant stress markers in STZ-induced rat

Data illustrated in Figure 5 demonstrated that GPx, CAT, and

SOD activities were the lowest ($p < 0.05$) in diabetic group compared with diabetic+B12 group and control group. While their levels were significantly ($p < 0.05$) improved in the diabetic+B12 group and control group compared with diabetic group.

Impact of vitamin B12 in the expression levels of genes in STZ-induced rat in tissue homogenate

The levels of mRNA expression of HGF, LEPR, and GLUT-2 in Figure 6 demonstrated their activities were the lowest levels in diabetic group compared with diabetic+B12 group and control group and significantly upregulated in diabetic+B12 group and control group compared with diabetic group ($p < 0.05$). on the other hand, the mRNA expression levels of JAK3, STAT3, TGF-1β, and PTPN1 were the highest in diabetic group compared with diabetic+B12 group and control group and significantly down-regulated in diabetic+B12 group and control group compared with diabetic group.

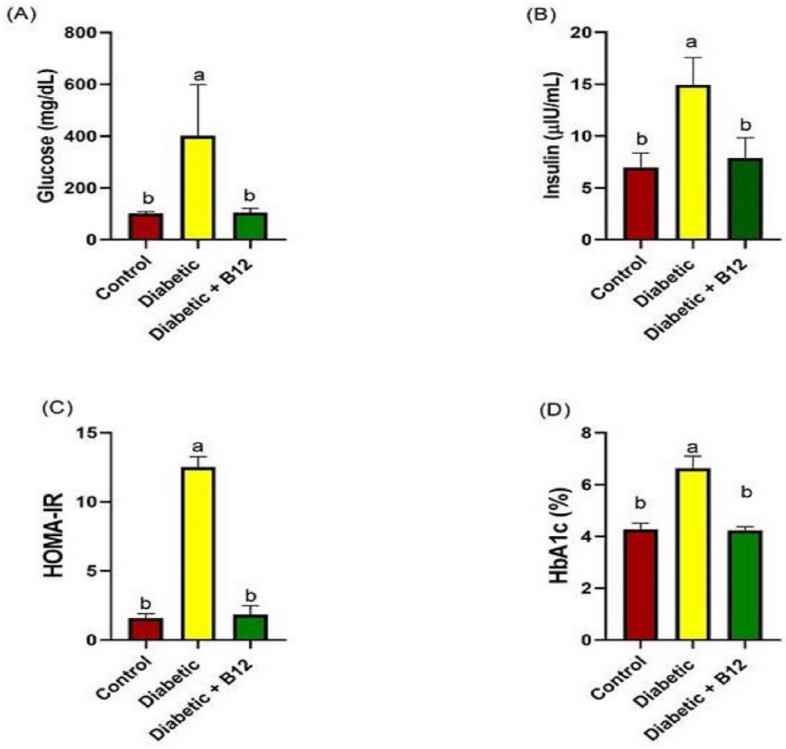

Fig. 3. Impact of vitamin B12 in the levels of some biochemical parameters in STZ-induced rat. (A) Glucose (mg/dL), (B) insulin (µIU/mL), (C) Homa-IR, (D) HbA1c (%).

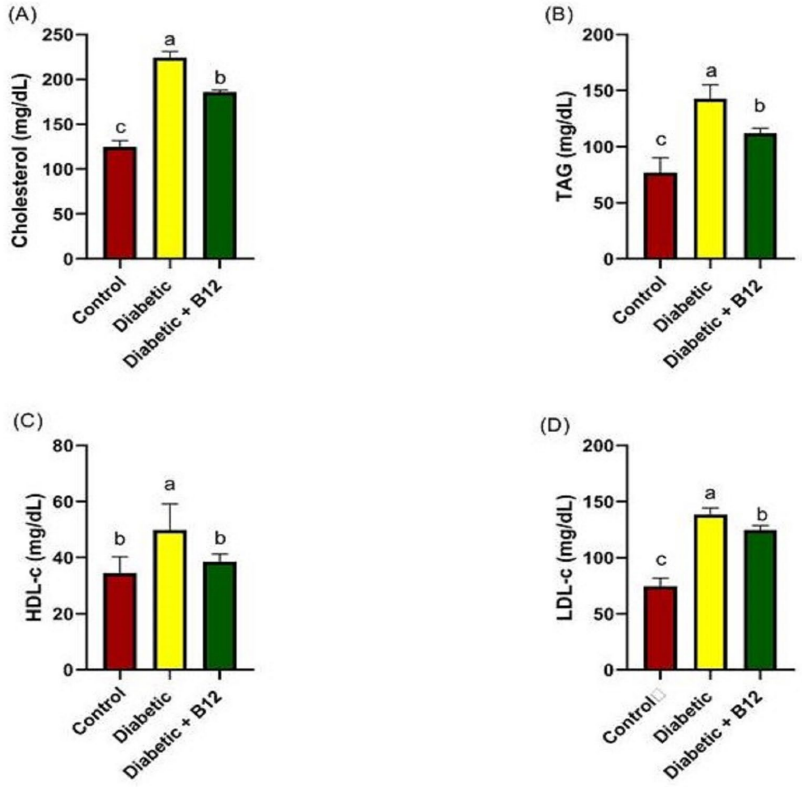

Fig. 4. Impact of vitamin B12 in lipid profile in STZ-induced rat. (A) cholesterol (mg/dL), (B) triacylglycerol (mg/dL), (C) HDL-c (mg/dL), (D) LDL-c (mg/dL).

Histopathological results

Control group of liver (figure 7A) exhibited normal histology of hepatic cords, central veins, portal areas, and stromal structures. But, Diabetic group (figure 7B, C, D) showed multifocal areas of hepatic necrosis which invaded and encircled by inflammatory cells. Further, centrolobular degenerative changes mostly hydropic degeneration and fatty changes were also noticed. The treated group (figure 7E, F) showed few scattered lymphocytes infiltrates and mild degenerative changes primarily fatty change in some examined sections.

Brain tissue of control group showed preserved histological structures of meningeal layers, neurons, glia cells and neuropil (figure 8A). While, diabetic group (figure 8B, C, D) revealed meningeal edema with round cells infiltrates and engorged cerebral vasculatures. As well, degenerated and necrotic neurons were de-

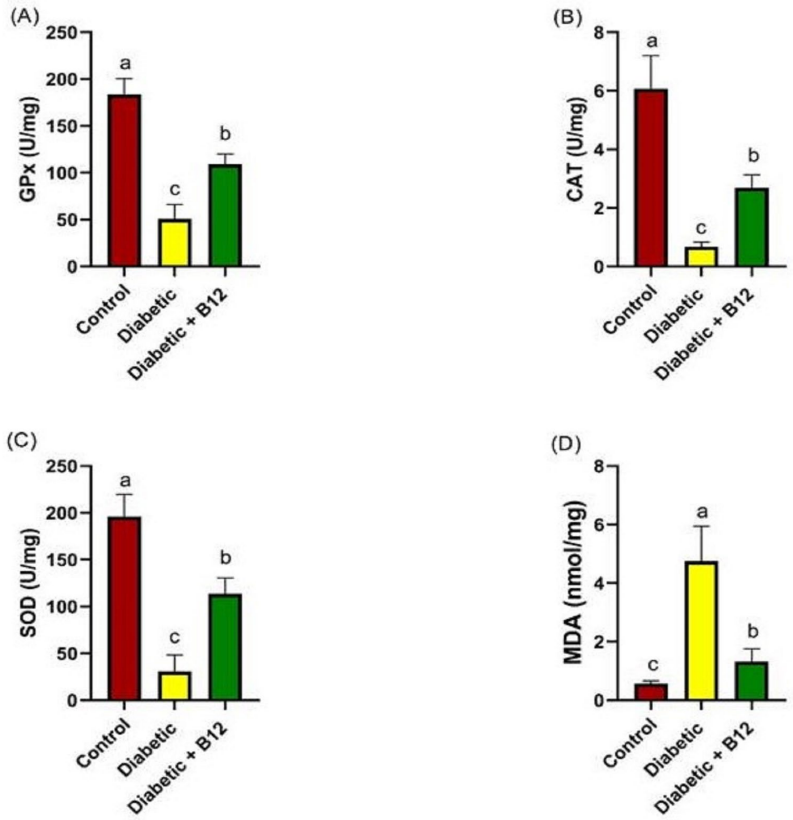

Fig. 5. Impact of vitamin B12 in the levels of oxidant stress markers in STZ-induced rat. (A) GPx (U/mg), (B) CAT (ng/mg), (C) SOD (U/mg), (D) MDA (nmol/mg).

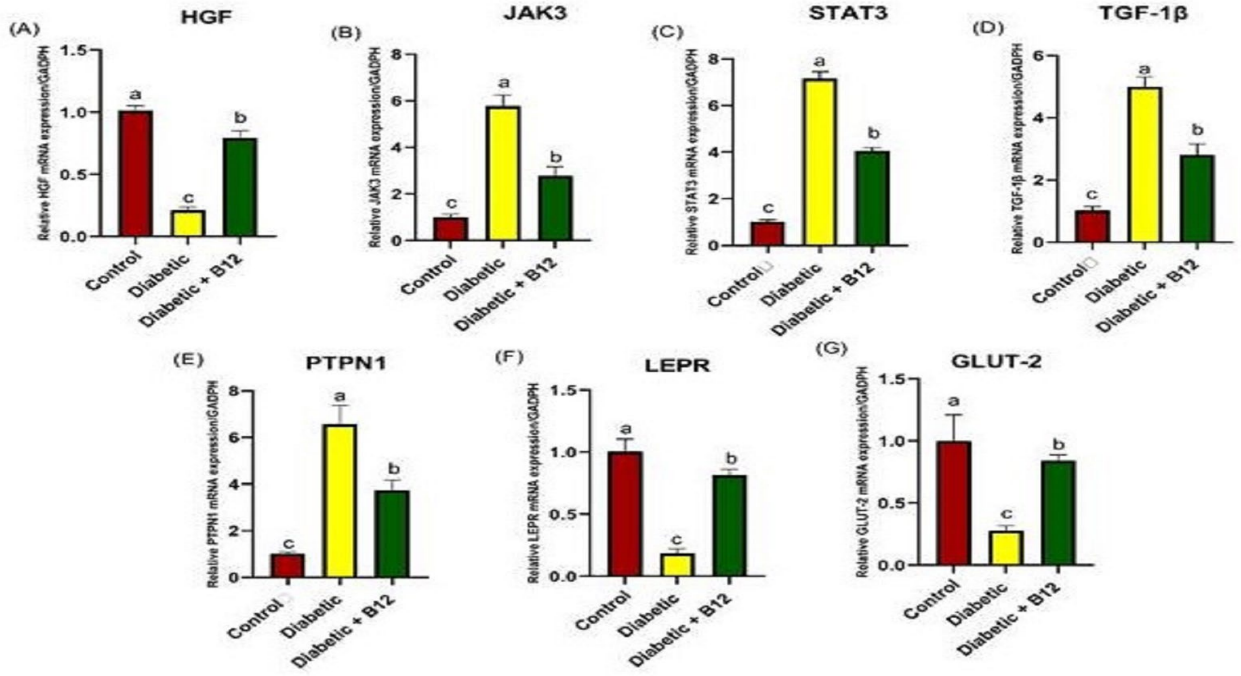

Fig. 6. Impact of vitamin B12 in the mRNA expression of some genes in STZ-induced rat. (A) HGF, (B) JAK3, (C) STAT3, (D) TGF-1β, (E) PTPN1, (F) LEPR, (G) GLUT-2.

tected, which were replaced by glia cell aggregates. In the other hand, in treated group (figure 8E, F), there are improvement in brain architectures with presence of few number of degenerated neurons.

DISCUSSION

Diabetes mellitus is a syndrome characterized by chronic hyperglycemia caused by an absence or inadequate production of insulin (El-Dawy *et al*., 2019; Sukar *et al*., 2020). IR in peripheral tissues or decreased insulin production in the pancreas are the root causes of DM's elevated blood glucose levels (El-naggar & El-Dawy, 2019). Abnormalities in lipid and protein metabolism accompany the impairment in glucose and carbohydrate metabolism brought on by the disease. Oxidative damage occurs in multiple organs due to elevated blood sugar, but the liver, kidneys, and pancreas are hit the worst. Therefore, we investigated the Impact of vitamin B12 supplementation on the STZ-induced rat model (Ahmed *et al*., 2019).

The STZ produced from the Streptomyces achromogenes antibiotic is cytotoxic to pancreatic β-cells. DM can be induced by STZ in many different species of animals. β-cells in the pancreas die off and degenerate when exposed to STZ. In our study glucose, insulin, Homa-IR, HbA1c, and lipid profile levels were significantly ($P<0.05$) higher in the diabetic group compared to the diabetic+B12 group and control group, demonstrating that STZ at a dose of 45 mg/kg was able to provoke a sustained hyperglycemia in both groups, and vitamin B12 improved these adultera-

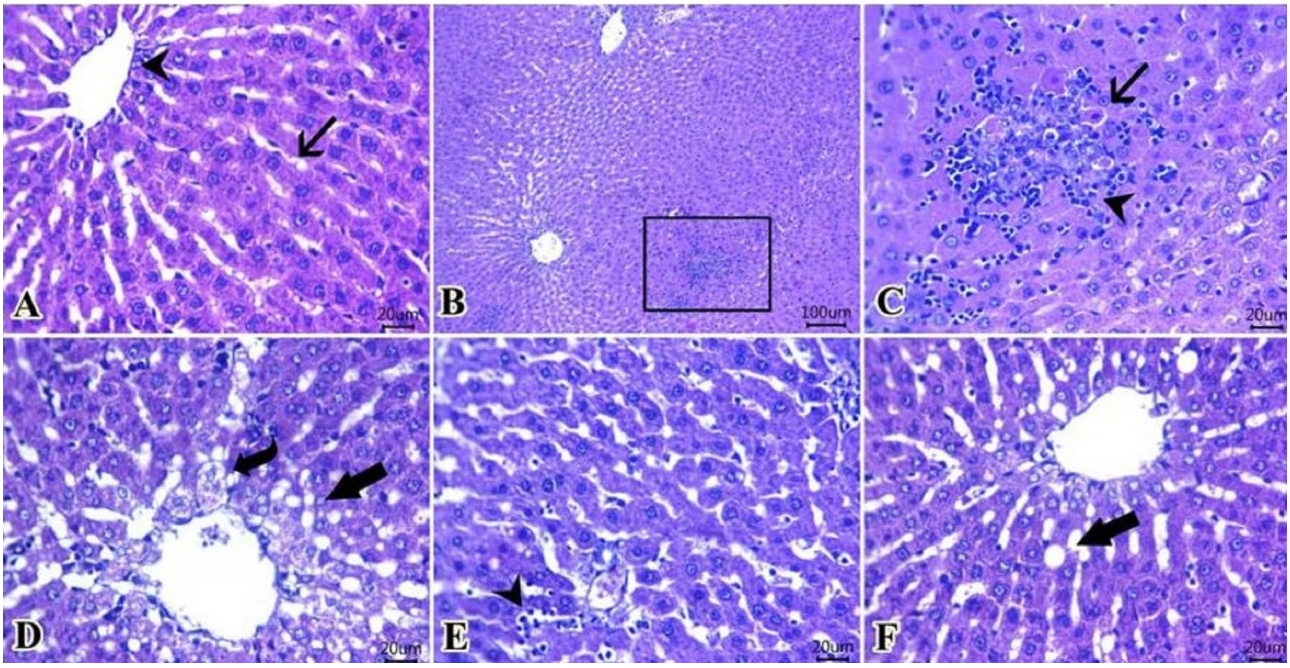

Fig. 7. Representative photomicrograph of H&E stained sections from liver showing: normal histology of hepatic cords (arrow), central veins (arrowhead), and stromal structures in control group (A). Multifocal areas of hepatic necrosis (thin arrow) invaded by inflammatory cells (arrowhead), centrolobular hydropic degeneration (curved arrow) and fatty changes (thick arrow) in diabetic group (B, C, D). Few number of interstitial lymphocytes aggregates (arrowhead) and mild degenerative changes primarily fatty change (thick arrow) in treated diabetic group (E, F). Scale bar A, C, D, E, F 20 μm, B 100 μm.

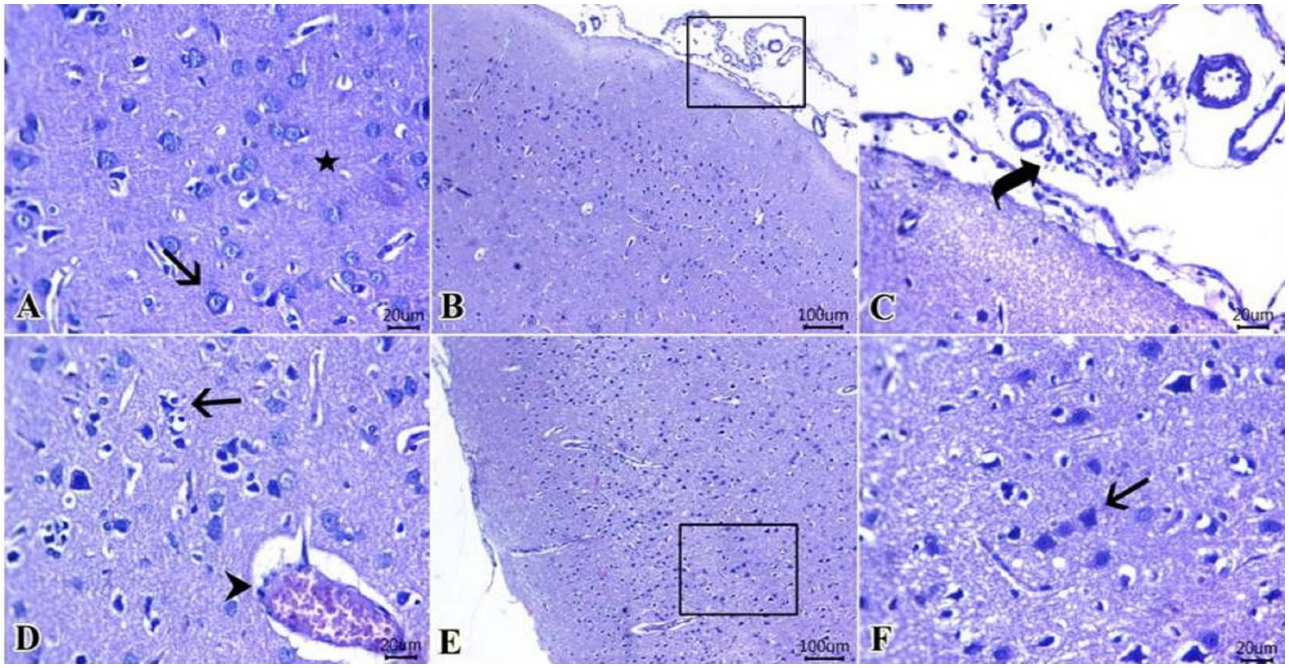

Fig. 8. Representative photomicrograph of H&E stained sections from brain showing: preserved histological structures of neurons (arrow), glia cells and neuropil (star) in control group (A). Meningeal edema with round cells infiltrates (curved arrow), engorged cerebral vasculatures (arrowhead), degenerated and necrotic neurons replaced by glia cell aggregates (arrow) in diabetic group (B, C, D). Apparent normal architectures in the majority of nerve cell bodies (arrow) with presence few numbers of degenerated neurons in treated diabetic group (E, F) Scale bar A, C, D, F 20 μm, B, E 100 μm.

tions induced by STZ.

The levels of oxidant stress markers (GPx, CAT, and, SOD) were low in diabetic group, while MDA was elevated and vitamin B12 ameliorated these effects. The following studies are in line with following studies.

Tiwari *et al*. (2013) believed that the reactive oxygen species (ROS) formation causes oxidative stress is a common mechanism in the pathophysiology of diabetes illnesses. Researches along many years have seen a rise in the ROS production and its part in the onset of diabetes problems. Persistent hyperglycemia has been shown by Giacco and Brownlee (2010) to increase oxidative stress that considered as a key player in the development and worsening of diabetes and associated consequences.

The GPx, SOD, CAT, glutathione reductase, and vitamins are all part of the cellular antioxidant defense mechanism under normal physiological conditions. HbA1c is a commonly used diagnostic marker for diabetes that is modified by ROS, and glutathionylation of this protein appears to be increased in diabetic individuals. Lipid peroxidation (oxidative damage/degradation of lipids) also occurs, influencing membrane fluidity and permeability. Studies with T2DM have been shown to have elevated levels of several lipid peroxidation markers, including 8-isoprostane, MDA, and thiobarbituric acid reactive substances. Protein and lipid oxidative stress indicators are also elevated in T2DM (Bhatti *et al*., 2022).

The mRNA expressions of HGF, LEPR, and GLUT-2 were the lowest levels in diabetic group compared with diabetic+B12 group and control group and significantly upregulated in diabetic+B12 group and control group, while the levels of JAK3, STAT3, TGF-1β, and PTPN1 were the highest in diabetic group compared with diabetic+B12 group and control group and significantly downregulated in control group and diabetic+B12, scanty studies in this point were found in relation to vitamin B12.

Chen *et al*. (2021), defined that JAK, and STAT1 are the fundamental components of the JAK/STAT signalling pathway. Kidney function can be improved, renal inflammation and fibrotic lesions can be reduced, and the course of diabetes can be slowed by negative control of JAK/STAT. Extensive research has been conducted on HGF, a protein that interacts to the hepatocyte growth factor receptor and controls cell proliferation, cell motility, and tissue regeneration in diabetes and diabetic complications. Exogenous HGF has been shown to reduce proteinuria and tubulointerstitial fibrogenesis in mouse models by suppressing TGF-β expression and speeding up kidney repair (Tang *et al*., 2020). Our findings corroborate those of prior research showing a reduction in HGF mRNA in diabetic rat, which would indicate a protective function for HGF in diabetes.

Our findings were in line with Du *et al*. (2022), illustrated that PTPN1, a significant negative regulator for insulin receptor signalling, has been identified in multiple studies as a promising therapeutic target for the treatment of type 2 diabetes (T2DM) and obesity. Insulin receptor phosphorylation in liver and muscle is reduced and glucose homeostasis is maintained with about half the quantity of circulating insulin when PTPN1 is deleted genetically or inhibited pharmacologically. Studies on T2DM with leptin therapy have been shown to experience improvements in metabolic, glucose, and lipid abnormalities. Researchers have shown a link between the LEPR gene and several indicators of MetS, including IR, cardiovascular illness, and hypertension. Disruptions in energy balance, excess weight gain, and increased risk of T2DM have all been linked to variations in leptin and LEPR expression, which may have both hereditary and environmental causes (Perumalsamy *et al*., 2023).

Glucose is transported and sensed in rodent islets via a transmembrane carrier protein called GLUT-2. The GLUT-2 is expressed on pancreatic beta cells, liver, kidney, and the intestine. The degree of vulnerability to STZ in several animal models is characterized by GLUT-2 gene expression levels in the pancreas. Prediction of diabetes development from reduced glucose tolerance by single nucleotide polymorphisms in the GLUT-2 gene (Marghani *et al*., 2019).

Cobalamin, or vitamin B12, is essential for normal nerve and blood cell development and DNA synthesis. Deficiencies in vitamin B12 are linked to severe haematological, mental, and neurological diseases, as well as higher possibility of permanent nerve damage. Vitamin B12 plays an essential role in deoxyribonucleic acid synthesis, erythropoiesis, and neural function. Surrogates for IR, like HOMA-IR has been shown to correlate negatively with cobalamin concentrations in obese teenage populations. Beta-cell function may be further enhanced by vitamin B12 due to their ability to mitigate oxidative damage (Kanti *et al*., 2020).

In line with our study Adaikalakoteswari *et al*. (2014), declared that TAG and HDL cholesterol levels were found to be independently linked to vitamin B12 insufficiency in this research of people with T2DM. In atherosclerosis related studies a detected was found between B12 levels and total cholesterol and TAG. However, this association was lost in regression analysis. Methylmalonyl-CoA (MM-CoA) is converted to succinyl-CoA with the help of vitamin B12. Because of the absence of vitamin B12, MM-CoA builds up and inhibits the rate-limiting enzyme for fatty acid oxidation, which lead to lipogenesis. This may explain why B12 insufficiency is associated with unfavourable lipid characteristics.

Neurodegeneration, the main symptom of vitamin B12 insufficiency, has been related to oxidative stress, despite the fact that the underlying disease mechanisms underlying vitamin B12 deficiency are not fully known. In response to vitamin B12 deprivation, Caenorhabditis elegans showed large increases in cellular H_2O_2 and NO content, whereas antioxidant enzyme (SOD, and CAT) activity and glutathione and L-ascorbic acid levels were significantly decreased. Treatment of vitamin B12-deficient worms with antioxidants was able to restore lipid peroxidation (MDA) to normal levels (Bito *et al*., 2017).

In agreement with our study Ramadan (2013), examined how much vitamin B-sub 12 pregnant women with diabetes take in and how that affects their risk for radiation-induced harm. Malformation rates were found to be higher in diabetes pregnancies where there was an excess of free oxygen radicals in the embryos. Vitamin B12 supplementation during pregnancy protected pregnant rats and their offspring from radiation-induced damage by lowering maternal glucose and insulin levels and increasing liver glycogen and decreasing glutathione levels.

The strength of the present research is that it is the first publication we're aware of that has evaluated the effects of vitamin B12 on HGF, TGF-1β, PTPN1, LEPR, GLUT-2, and JAK3/STAT3 pathways.

CONCLUSION

This study concluded that vitamin B12 induces potential hypoglycemic, hypolipidemic, and antioxidant activity in the diabetic rat model and has an impact on JAK3/STAT3 pathways, HGF, TGF-1β, PTPN1, LEPR, and GLUT-2 levels, further studies are advised.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

REFERENCES

Abdel-aleem, S., St. Louis, J., Hendrickson, S.C., El-Shewy, H.M., El-Dawy, K., Taylor, D.A., Lowe, J.E., 1998. Regulation of carbohydrate and fatty acid utilization by L-carnitine during cardiac development and hypoxia. Mol. Cell. Biochem. 180, 95-103.

Adaikalakoteswari, A., Jayashri, R., Sukumar, N., Venkataraman, H., Pradeepa, R., Gokulakrishnan, K., Anjana, R.M., McTernan, P.G., Tripathi, G., Patel, V., Saravanan, P., 2014. Vitamin B12 deficiency is associated with adverse lipid profile in Europeans and Indians with type 2 diabetes. Cardiovasc. Diabetol. 13, 1-7.

Ahmed, A.I., Nasr, N.Y., Alattar, R.H., El-Dawy, K., 2023. Carnosine-loaded oral niosomes ameliorate high-fructose-induced metabolic syndrome in rats via modulation of SIRT1, a metabolic master switch. Slov. Vet. Zb. 60, 75-85.

Ahmed, A.K., Nihat, M., Handan, M., 2019. Investigation of the Antidiabetic Effects of Mistletoe (*Viscum album* L.) Extract in Experimental Diabetes in Rats. Vet. J. Vet. J. 30, 121-125.

Ashok, T., Puttam, H., Tarnate, V.C.A., Jhaveri, S., Avanthika, C., Treviño, A.G.T., Sandeep, S.L., Ahmed, N.T., 2021. Role of vitamin B12 and folate in metabolic syndrome. Cureus, 13.

Bhatti, J.S., Sehrawat, A., Mishra, J., Sidhu, I.S., Navik, U., Khullar, N., Kumar, S., Bhatti, G.K., Reddy, P.H., 2022. Oxidative stress in the pathophysiology of type 2 diabetes and related complications: Current therapeutics strategies and future perspectives. Free Radic. Biol. Med. 184, 114-134.

Bito, T., Misaki, T., Yabuta, Y., Ishikawa, T., Kawano, T., Watanabe, F., 2017. Vitamin B12 deficiency results in severe oxidative stress, leading to memory retention impairment in Caenorhabditis elegans. Redox boil. 11, 21-29.

Boachie, J., Adaikalakoteswari, A., Samavat, J., Saravanan, P., 2020. Low vitamin B12 and lipid metabolism: evidence from pre-clinical and clinical studies. Nutrients 12, 1925.

Chen, D., Liu, Y., Chen, J., Lin, H., Guo, H., Wu, Y., Xu, Y., Zhou, Y., Zhou, W., Lu, R., Wu, J., 2021. JAK/STAT pathway promotes the progression of diabetic kidney disease via autophagy in podocytes. Eur. J. Pharmacol. 902, 174121.

Dodington, D.W., Desai, H.R., Woo, M., 2018. JAK/STAT–emerging players in metabolism. Trends Endocrinol. Metab. 29, 55-65.

Du, R., Wu, N., Bai, Y., Tang, L., Li, L., 2022. circMAP3K4 regulates insulin resistance in trophoblast cells during gestational diabetes mellitus by modulating the miR-6795-5p/PTPN1 axis. J. Transl. Med. 20, 180.

El-Dawy, K., 2022. Prophylactic Effects of Syzygium aromaticum L. and Cynara Cardunculus L. Extracts against d 7, 12-dimethylbenz (a) anthracene (DMBA)-Induced Colorectal Cancer in Male Albino Rats. TRS 8, 1218-1242.

El-Dawy, K., El-Sayed, A.S., El-Hefnawy, S.A., El-Kahky, N.M., 2019. Biochemical and metabolic implications of tricarboxylic acids and their transporters. JPAM 13, 757-766.

Elnagar, A., El-Belbasi, H.I., Rehan, I.F., El-Dawy, K., 2018. Asprosin: a novel biomarker of type 2 diabetes mellitus. VMHE 55, 333-347.

El-naggar, A., El-Dawy, K., 2019. Role of micro RNAs and its associated genes in prediction of obesity and diabetes mellitus (Type II). Zagazig Vet. J. 47, 57-67.

Giacco, F., Brownlee, M., 2010. Oxidative stress and diabetic complications. Circ. Res. 107, 1058-1070.

Heydarpour, F., Sajadimajd, S., Mirzarazi, E., Haratipour, P., Joshi, T., Farzaei, M.H., Khan, H., Echeverría, J., 2020. Involvement of TGF-β and autophagy pathways in pathogenesis of diabetes: a comprehensive review on biological and pharmacological insights. Front. pharmacol. 11, 498758.

Hsieh, F.-C., Lee, C.-L., Chai, C.-Y., Chen, W.-T., Lu, Y.-C., Wu, C.-S., 2013. Oral administration of Lactobacillus reuteri GMNL-263 improves insulin resistance and ameliorates hepatic steatosis in high fructose-fed rats. Nutr. Metab. 10, 35.

Kanti, G., Anadol-Schmitz, E., Bobrov, P., Strassburger, K., Kahl, S., Zaharia, O.P., Sarabhai, T., Karusheva, Y., Burkart, V., Markgraf, D.F., GDS Group, 2020. Vitamin B12 and folate concentrations in recent-onset type 2 diabetes and the Impact of metformin treatment. J. Clin. Endocrinol. Metab.105, e2222-e2231.

Khalil, S.S., Ahmed, A.I., Mohammed, N.A., El-Naggar, A.A., Ali, H.A., Al-Saadawy, H.R., 2018a. A review on metabolic syndome: Biochemical investigations. VMHE 55, 427-443.

Khalil, S.S., Ali, H.A., Al-Saadawy, H.A., El-Dawy, K., 2018b. Combination of gliclazide drug and lupin seeds powder alleviate hyperglycemia on induced-diabetic rats receiving high-fat high fructose/sucrose diet. Slov. Vet. Zb. 55, 273-281

Kouroglou, E., Anagnostis, P., Daponte, A., Bargiota, A., 2019. Vitamin B12 insufficiency is associated with increased risk of gestational diabetes mellitus: a systematic review and meta-analysis. Endocr. J., 66, 149-156.

Lekshmi, R. K., Rajesh, R., Mini, S., 2015. Ethyl acetate fraction of Cissus quadrangularis stem ameliorates hyperglycaemia-mediated oxidative stress and suppresses inflammatory response in nicotinamide/streptozotocin induced type 2 diabetic rats. Phytomedicine 22, 952-960.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25, 402-408.

Marghani, B., Ateya, A., Saleh, R., Eltaysh, R., 2019. Assessing of antidiabetic and ameliorative Impact of Lupin seed aqueous extract on hyperglycemia, hyperlipidemia and effect on pdx1, Nkx6. 1, Insulin-1, GLUT-2 and Glucokinase genes expression in streptozotocin-induced diabetic rats. J. Food Nutr. Res. 7, 333-341.

Oliveira, A.G., Araújo, T.G., Carvalho, B.D.M., Rocha, G.Z., Santos, A., Saad, M.J., 2018. The role of hepatocyte growth factor (HGF) in insulin resistance and diabetes. Front. Endocrinol. 9, 503.

Perumalsamy, S., Huri, H.Z., Abdullah, B.M., Mazlan, O., Wan Ahmad, W.A., Vethakkan, S.R.D., 2023. Genetic Markers of Insulin Resistance and Atherosclerosis in Type 2 Diabetes Mellitus Patients with Coronary Artery Disease. Metabolites 13, 427.

Ramadan, F.L., 2013. Cobalamin (Vitamin B) Role on the Biochemical, Histological and Teratological Changes Induced in Diabetic Irradiated Pregnant Rats. Journal of Nuclear Technology in Applied Science 1.

Reddy, S.S., Prabhakar, Y.K., Kumar, C.U., Reddy, P.Y., Reddy, G.B., 2020. Impact of vitamin B12 supplementation on retinal lesions in diabetic rats. Mol. Vis. 26, 311.

Sukar, K.A.O., Abdalla, R.I., Humeda, H.S., Alameen, A.O., Mubarak, E.I., 2020. Impact of pearl millet on glycaemic control and lipid profile in streptozocin induced diabetic wistar rat model. Asian J. Med. Health 18, 40-51.

Tamaddonfard, E., Samadi, F., Egdami, K., 2013. The effects of vitamin B12 and diclofenac and their combination on cold and mechanical allodynia in a neuropathic pain model in rats. Vet. Res. Forum 4, 19-24.

Tang, Y.L., Dong, X.Y., Zeng, Z.G., Feng, Z., 2020. Gene expression-based analysis identified NTNG1 and HGF as biomarkers for diabetic kidney disease. Medicine 99, e18596.

Tiwari, B.K., Pandey, K.B., Abidi, A.B., Rizvi, S.I., 2013. Markers of oxidative stress during diabetes mellitus. J. Biomark. 2013, 378790.

Zhang, L., Qin, Y., Liang, D., Li, L., Liang, Y., Chen, L., Tong, L., Zhou, J., Li, H., Zhang, H., 2018. Association of polymorphisms in LEPR with type 2 diabetes and related metabolic traits in a Chinese population. Lipids Health Dis. 17, 1-8.