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Original Article

Effect of Vitamin D Supplementation with or without Metformin on Diabetes Mellitus in Adult Male Albino Rats

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ABSTRACT

Background: Diabetes mellitus is characterized by hyperglycaemia and a variety of consequences. Vitamin D was an essential element in reducing the possibility of diabetes. Metformin has powerful antioxidant abilities in addition to its potential to treat diabetes.

Aim of the work: This study aimed to look into the role of vitamin D supplementation with or without metformin in diabetes mellitus in adult male albino rats.

Patients and methods: The current study examined the effect of vitamin D supplementation with or without metformin on diabetes mellitus in adult male albino rats of the local strain, weighing between 130 and 150 g. Rats were split into six equal groups at Random. The first group negative control group, where rats received regular diet every day for 4 weeks. The second was the positive control, where it received vehicle. Group III was the alloxan induced diabetic group, while group IV was the diabetic group with vitamin supplementation, and the fifth group was the diabetic plus metformin-treated group. The last group was the diabetic group with vitamin D and metformin treatment. Rats were given ether anaesthesia at the conclusion of the trial and blood samples were taken before the following metrics were assessed: blood glucose, vitamin D, alanine aminotransferase [ALT], aspartate aminotransferase [AST], alkaline phosphatase [ALP], homeostatic model assessment for insulin resistance [HOMA-IR] and malondialdehyde [MDA].

Results: Blood glucose, ALT, AST, alkaline phosphatase, HOMA-IR, and MDA were significantly reduced when vitamin D, metformin or both were supplemented. The prior metrics improved more when metformin and vitamin D were administered together.

Conclusion: Vitamin-D is promising for treatment of diabetes-related issues [e.g., hyperglycaemia and insulin resistance]. Also, metformin has been shown to improve blood glucose and when addition vitamin D with metformin it showed better results that led to near normal levels in diabetes.

Keywords: Insulin Resistance; Vitamin D; Antidiabetic Drugs; Metformin.

INTRODUCTION

Diabetes mellitus [DM] is a long-term condition characterized by a persistently high blood sugar level caused by deficiencies in the action, secretion or both of insulin. Additionally, it is linked to varied degrees of impairment in the metabolism of carbohydrates, lipids and proteins [1].

The World Health Organization [WHO] has divided diabetes mellitus [DM] into two primary types: Type 1 diabetes is also known as juvenile diabetes or insulin dependent diabetes mellitus [IDDM], while type 2 diabetes mellitus [T2DM] is also known as non-insulin dependent diabetic mellitus. Type 1 diabetes usually starts during childhood or before the age of forty. It is brought on by the full or partial degeneration of the beta cells in the pancreas, which leads to insufficient insulin [2]. However, type 2 diabetes mellitus that develops beyond the age of 40 is typically not insulin-dependent and is brought on by either a malfunctioning insulin receptor [insulin resistance] or insufficient insulin synthesis. This disease is less severe than juvenile forms and is easily treated with diet and oral hypoglycemic drugs [3]. About 460 million people globally are calculated to have type 2 diabetes [4].

Gestational diabetes mellitus [GDM] is a third kind of diabetes that is similar to type 2 diabetes in many ways, including relatively low insulin secretion and reactivity. Pregnancy-related DM, most commonly T2DM, affects 5–10% of women with GDM [5]. One or more single-gene mutations that affect insulin synthesis are the cause of maturity onset diabetes of the young [MODY], an autosomal type of diabetes. It is much less common than the three main kinds. MODY has at least thirteen subtypes. Insulin is not necessary for patients with MODY to manage their condition [6]. Secondary diabetes mellitus is defined as diabetes that arises as a result of an endocrine condition, an infection, a genetic illness, or a side effect of another medication [7].

Cushing's syndrome and acromegaly are common endocrine illnesses that cause secondary diabetes mellitus; they primarily cause insulin resistance in the early stages, though advanced stages can also show signs of insulin insufficiency [8]. Steroids are the most common drug-induced secondary DM cause, with thyroid hormone, β-adrenergic agonists, statins, and steroids are other potential causes [9].

Diabetes is characterized by weight loss, polyuria, polydipsia and polyphagia. Symptoms of type 2 diabetes usually show up considerably later and can be mild or nonexistent, whereas those of type 1 diabetes can show up weeks or months before [10].

A sedentary lifestyle, physical inactivity, smoking and alcohol consumption are among the many lifestyle factors that have a substantial impact on the development of type 2 diabetes [11].

The majority of diabetes-related problems are either macrovascular or microvascular. However, new problems are becoming more prevalent than those that are typically recognized and researched due to advancements in life-saving treatment and innovative therapies. The traditional complications of diabetes mellitus include peripheral neuropathy, nephropathy, heart failure, stroke, coronary heart disease and peripheral vascular disease. Rather, connections between diabetes mellitus and infections, cancer, liver disease, mental disorders and cognitive and functional impairment are beginning to emerge [12].

Unlike other vitamins, vitamin D is a hormone since it is produced in the body. Currently, T2DM patients frequently have vitamin D insufficiency, it is still unclear, though, if this is a chance or if low vitamin D levels may have played a role in the disease's beginnings. Vitamin D deficiency may have a significant role in the pathophysiology of type 2 diabetes [T2DM] by changing numerous important pathways involved in the occurrence of diabetes and its complications, including peripheral insulin resistance, systemic inflammation, immunological activation, downregulation of the insulin receptor gene and pancreatic insulin production [13].

Compared to other antidiabetic medications like insulin, glibenclamide or chlorpropamide, metformin has a higher potential to lower diabetes-related outcomes [14].

According to **Sliwinska *et al.*** [15], Metformin can lower adenosine triphosphate [ATP] levels and increase adenosine monophosphate [AMP] levels in hepatocytes by inhibiting complex I of the mitochondrial electron transport chain. AMP-activated protein kinase [AMPK] is subsequently activated through the traditional adenine-nucleotide-dependent route [16]. In order to prevent gluconeogenesis, elevated AMP also inhibits adenylate cyclase and fructose-1,6-bisphosphatase-1 [17].

Another very rare side effect of metformin is lactic acidosis, which almost always occurs in those who are at high risk of getting the condition even if they are not taking the medication [18]. Additionally, patients with hepatic impairment and those who abuse alcohol should not take metformin [19]. Progressive vitamin B12 insufficiency occurs in certain metformin-using patients [20].

An imbalance between the body's antioxidant capacity and oxygen-derived radicals results in oxidative stress. The natural cellular balance between defense and radical generation is upset by this. Nucleic acids, lipids, and proteins are oxidatively damaged as a result. According to several studies, metformin's main action is to block mitochondrial complex I [21].

According to **Vinothkumar *et al.*** [22], mitochondrial complex I may play a significant role in the generation of ROS within cells. It is commonly known that when this complex is blocked, less electrons are transported from NADH plus H+, which lowers the generation of reactive species [23]. Thus, there is evidence that metformin lowers mitochondrial levels of endogenous ROS [24].

As time went on, new applications for metformin were found and its advantages for a number of illnesses and even aging were confirmed. Malignancies, obesity, liver, cardiovascular and renal disorders are among these illnesses [25]. The current study was an experiment to look into the role of vitamin D supplementation with or without metformin in diabetes mellitus in adult male albino rats.

MATERIALS AND METHODS

60 adult male albino rats weighing 130–150 g were used in the investigation; they were purchased from Nile Pharmaceuticals Company. Each of the five rats was housed in a cage that measured 30 x 32 x 30 cm and was kept at a comfortable temperature with an ordinary light-dark cycle. Rats received rat chow and had unrestricted access to water during the four-week experiment. Before the experiment began, the rats were housed for two weeks to allow them to acclimatize to their new surroundings.

Rats were split into six equal groups at random:

1. Group I [**normal control group 1]:** received a regular diet every day for four weeks.

2.Group II [**normal control group 2]:** As a vehicle group, participants were given 0.3 mL/kg BW of olive oil by oral route.

3. Group III [diabetic group]: When alloxan was used to induce diabetes, it was dissolved in 0.9% NaCl solution and given intraperitoneally [IP] in a single dose of 90 mg/kg BW [26] and it was preceded by nicotinamide in a single dose of 110mg/kg [27].

4.Group IV [diabetic-vitamin D treated group]: Following this group's implementation of diabetes, each rat received vitamin D dissolved in 0.3 mL olive oil in a dose of 500 IU [12.5 µg] /kg BW/day for 4 weeks[28].

5.Group V [diabetic-metformin treated group]: After induction of diabetes in this group, rats were administrated 100 mg/kg BW/day of metformin by oral gavage for 4 weeks [29].

6.Group VI [diabetic-vitamin D & metformin treated group]: Following diabetes induction, rats were given 500 IU/kg BW/day of vitamin D and 100 mg/kg BW/day of metformin by oral gavage for four weeks. To avoid coprophagia, rats were housed in specially designed boxes with permeable floors for a full day. The next day, nicotinamide was mixed in 0.9% sodium chloride. Each rat was weighed, and then given 110 mg/kg BW of nicotinamide intraperitoneally [27].

Within a few minutes of preparation, alloxan was dispersed in 0.9% NaCl and given intraperitoneally in a dose of 90 mg/kg body weight. This took around 20 minutes [26]. Before receiving the alloxan injection, two millilitres of glucose [5%] were taken orally. Blood samples were extracted from the tail vein after 48 hours in order to use a consumable glucometer to measure the glucose levels. Diabetic rats were defined as having blood glucose levels more than 250 mg/dL [30].

Collection of blood samples:

To sustain the ether vapour used to anaesthetise the rat using ethyl ether, a piece of cotton wool at the bottom of the anaesthetic box was periodically soaked with liquid ether. When the rat achieved the surgical stage of anaesthesia, as evidenced by the elimination of the withdrawal reflex, it was removed and placed on a table. A heparinised capillary tube with an inner diameter of 0.75 to 1.0 mm was inserted into the medial canthus medial to the eye globe in order to collect three millilitres of blood from each rat's retro-orbital plexus [31].

Blood was drawn into a graduated glass centrifuge tube that had been cleaned and dried, and it was quickly placed in a centrifuge set to 5000 rotations per minute for approximately 15 minutes in order to extract serum. The majority of the serum was extracted into Eppendorf tubes and kept frozen at -200C until it was needed to determine fasting blood glucose level, fasting insulin, Serum vitamin D level, Liver function tests [alanine transaminase [ALT], aspartate transaminase [AST] & alkaline phosphatase], Insulin resistance by homeostasis model assessment [HOMA-IR] and Serum malondialdehyde level [MDA]. All analysis was performed according to analysis kit manufacturer structures. HOMA-IR was calculated from the equation [HOMA-IR = insulin [µU/mL] x glucose [mmol/L] / 22.5].

Statistical Analysis:

The statistical package of the social sciences [SPSS] version "24" was the computer program used to conduct the statistical analysis [32]and the mean and standard deviation [SD] of the results were shown. The ANOVA [Analysis of Variance] test was used to measure differences between groups. Multiple comparisons between groups are performed using the Bonferroni post hoc multiple comparison test. Statistical significance was defined as a P-value of less than 0.05.

RESULTS

Effect of different treatments on Fasting Blood Glucose Level [mg/dl]: The current study's findings [Fig 1] showed a significantly higher level of fasting blood glucose in the diabetic group [Group III] from [85,67± 3.51] and [93.33 ± 8.96 mg/dl] to [395.83 ± 32.24 mg/dl] as compared to the control groups [p<0.05]. Administration of vitamin D [Group IV] showed a significantly lower level of blood glucose from [395.83±32.24 mg/dl] to [262.67±19.53 mg/dl] as compared to the diabetic group [Group III] [p<0.05]. Treatment with metformin [Group V] led to a significantly lower level of blood glucose from [395.83±32.24 mg/dl] to [242±15.31 mg/dl] as compared to the diabetic group [Group III] [p<0.05]. Treatment with a combination of metformin and vitamin D [Group VI] showed a significantly lower level of blood glucose from [395.83±32.24 mg/dl] to [227.76±12.24 mg/dl] as compared to the diabetic group [Group III] [p<0.05]. Combination therapy using vitamin D and metformin [Group VI] led to a significantly lower level of blood glucose from [262.67±19.53 mg/dl] to [227.67±12.24 mg/dl] as compared to the diabetic treated with vitamin D [Group IV] [p<0.05].

Effect of different treatments on serum vitamin D level [ng/ml]: Results of the present study [Figure 2] showed a significantly lower values of vitamin D levels in the diabetic group [Group III] from [30.13±2.79 ng/ml] and [31.47±2.26 ng/ml] to [27.80±2.80 ng/ml] as comparison to the control groups [Group I and Group II] [p<0.05]. Administration of vitamin D [Group IV] led to significantly higher levels of serum vitamin D levels from [27.80 ± 2.80 ng/ml] to [37.55 ± 3.40 ng/ml] as compared to the diabetic group [Group III] [p<0.05]. On the other hand, treatment with metformin [Group V] led to non-significant change of serum vitamin D levels from [27.80 ± 2.80 ng/ml] to [28.50 ±1.70 ng/ml] as compared to the diabetic group [Group III] [p>0.05]. Combination therapy using vitamin D and metformin [Group VI] showed a significantly higher levels of serum vitamin D from [27.80 ± 2.80 ng/ml] to [39.63 ±3.80 ng/ml] in comparison to the diabetic group [Group III] [p<0.05].

Effect of different treatments on serum alanine aminotransferase [ALT] level [U/L]: The current study's findings [Figure 3] showed a significantly higher levels of ALT in the diabetic group [Group III] from [34.67±5.13 U/L] and [39.30±3.79 U/L] to [81.17±15.64 U/L] when compared to the control groups [Group I and Group II] [p<0.05]. Administration of vitamin D [Group IV] led to a significantly lower value of ALT level from [81.17 ± 15.64 U/L] to [56.33 ± 3.61 U/L] as compared to the diabetic group [Group III] [p<0.05]. In the same way, treatment with metformin [Group V] led to a significantly lower value of ALT levels from [81.17± 15.64 U/L] to [50.83 ±5.85 U/L] as compared to the diabetic group [Group III] [p<0.05]. Combination therapy using vitamin D and metformin [Group VI] showed a significantly lower level of ALT from [81.17 ± 15.64 U/L] to [47.83 ±3.87 U/L] in comparison to the diabetic group [Group III] [p<0.05].

Effect of different treatments on serum aspartate amino-transferase [AST] level [U/L]: The current study's findings [Figure 4] showed a significantly higher levels of AST in the diabetic group [Group III] from [92.67±9.50 U/L] and [94.67±6.60 U/L] to [142±11.24 U/L] as compared to the control groups [Group I and Group II] [p<0.05]. Administration of vitamin D [Group IV] led to a significantly lower value of AST level from [142 ± 11.24 U/L] to [113.33 ± 9.75U/L] as compared to the diabetic group [Group III] [p<0.05].On the other hand, treatment with metformin [Group V] led to a significantly lower value of AST level from [142± 11.24 U/L] to [110.60 ±11.31 U/L] as compared to the diabetic group [Group III] [p<0.05]. Combination therapy using vitamin D and metformin [Group VI] showed a significantly lower levels of AST from [142 ± 11.24 U/L] to [98.33 ±9.95 U/L] in comparison to the diabetic group [Group III] [p<0.05].

Effect of different treatments on alkaline phosphatase level [U/L]: The current study's findings [Figure 5] showed a significantly higher levels of alkaline phosphatase in the diabetic group [Group III] from [74±7.0 and 97.33 ±4.51U/L] to [105.67±8.19 U/L] as compared to the control group [Group I and Group II] [p<0.05]. Administration with vitamin D [Group IV] led to a significantly lower value of alkaline phosphatase levels from [105.67 ± 8.19 U/L] to [92.67 ± 3.67 U/L] as compared to the diabetic group [Group III] [p<0.05]. In the same way, treatment with metformin [Group V] led to a significantly lower value of alkaline phosphatase level from [105.67± 8.19 U/L] to [90.17 ±4.49 U/L] as compared to the diabetic group [Group III] [p<0.05]. Combination therapy using vitamin D and metformin [Group VI] showed a significantly lower level of alkaline phosphatase from [105.67 ± 8.19 U/L] to [83.0 ±5.40 U/L] as compared to the diabetic group [Group III] [p<0.05].

Effect of different treatments on fasting blood HOMA-IR level: The current study's findings [Figure 6] showed a significantly higher levels of fasting blood HOMA-IR level in the diabetic group [Group III] from [0.39±0.05] and [0.45±0.08] to [0.82±0.76] as comparison to the control groups [Group I and Group II] [p<0.05]. Administration of vitamin D [Group IV] led to a significantly lower levels of fasting blood HOMA-IR from [0.82 ± 0.76] to [0.74 ± 0.77] as compared to the diabetic group [Group III] [P<0.05]. In the same way, treatment with metformin [Group V] led to a significantly lower level of fasting blood HOMA-IR from [0.82 ± 0.76] to [0.69 ±0.28] as compared to the diabetic group [Group III] [p<0.05]. Combination therapy using vitamin D and metformin [Group VI] showed a significantly lower levels of fasting blood HOMA-IR from [0.82 ± 0.76] to [0.68±0.52] as compared to the diabetic group [Group III] [p<0.05].

Effects of different treatments on serum malondialdehyde level [MDA] [µmol/L]: The current study's findings [Figure 7] showed a significantly higher levels of malondialdehyde in the diabetic group [Group III] from [6.95±0.85µmol/L] and [6.29 ± 0.85µmol/L] to [19.28±1.62 µmol/L] as compared to the control groups [Group I and Group II] [p<0.05]. Administration of vitamin D [Group IV] led to a significantly lower value of malondialdehyde level from [19.28±1.62 µmol/L] to [13.17±1.03µmol/L] as compared to diabetic group [Group III] [p<0.05]. In the same way, treatment with metformin [Group V] led to a significantly lower value of malondialdehyde level from [19.28±1.62 µmol/L] to [12.94±0.95µmol/L] as compared to the diabetic group [Group III] [p<0.05]. Combination therapy using vitamin D and metformin [Group VI] showed a significantly lower value of malondialdehyde level from [19.28±1.62 µmol/L] to [12.12±1.15µmol/L] as compared to the diabetic group [Group III] [p<0.05].

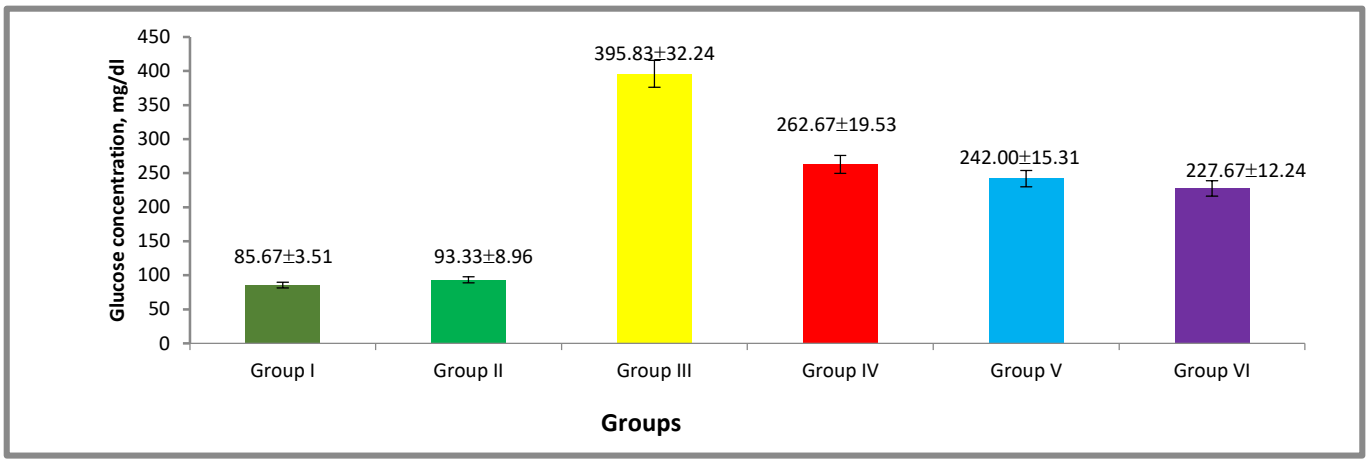

Figure [1]: Effect of different treatments on fasting blood glucose level

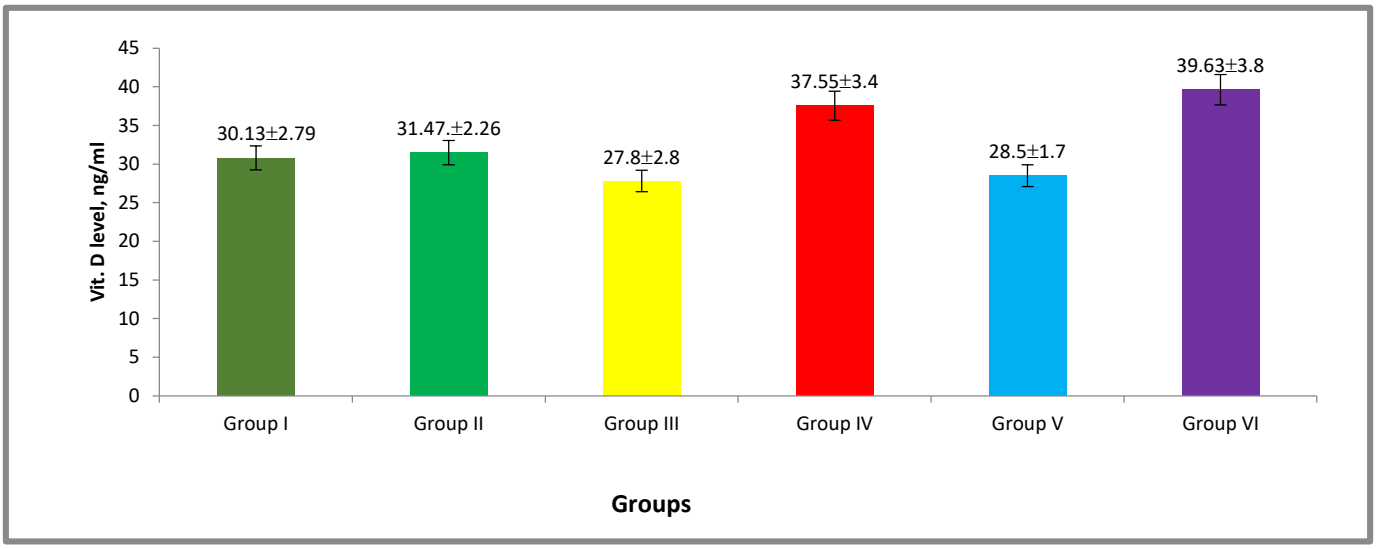

Figure [2]: Effect of different treatments on serum vitamin D level

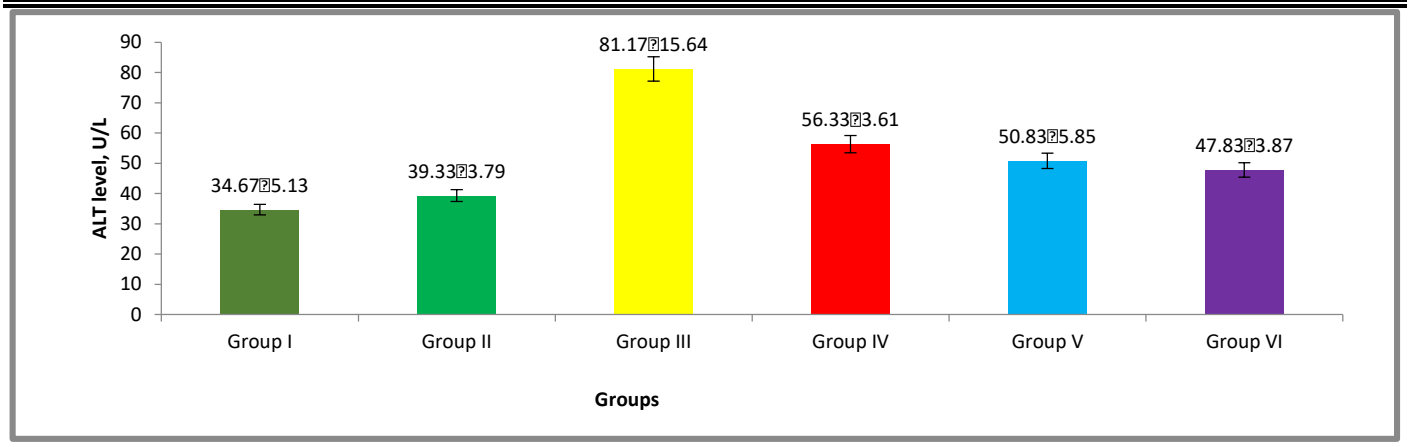

Figure [3]: Effect of different treatments on ALT level [U/L]

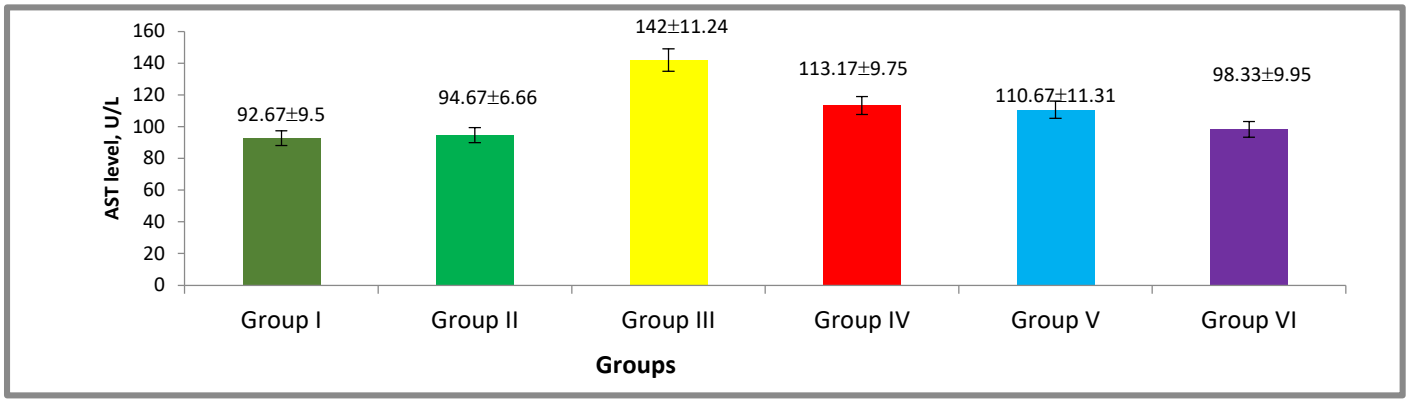

Figure [4]: Effect of different treatments on AST level

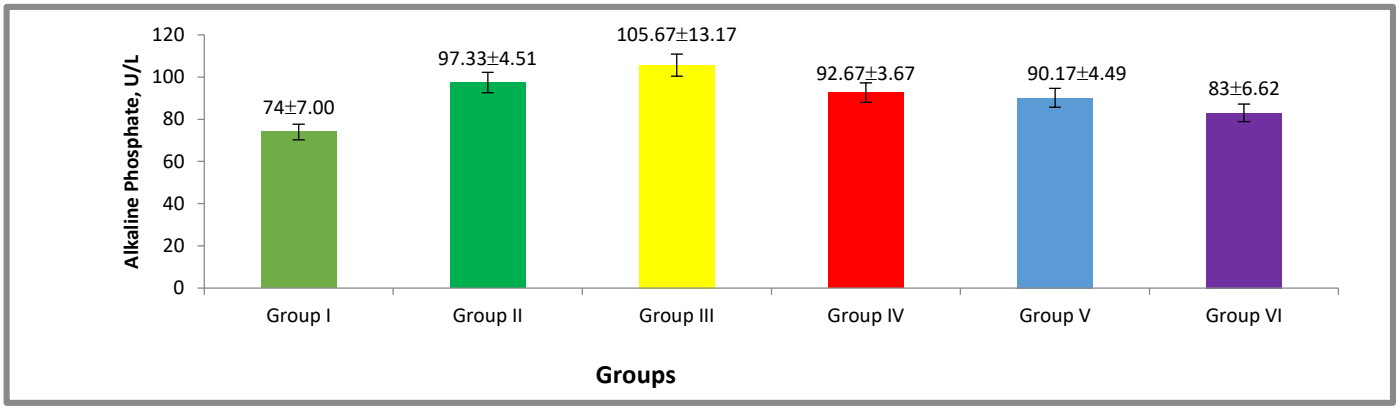

Figure [5]: Effect of different treatments on alkaline phosphatase level [U/L]

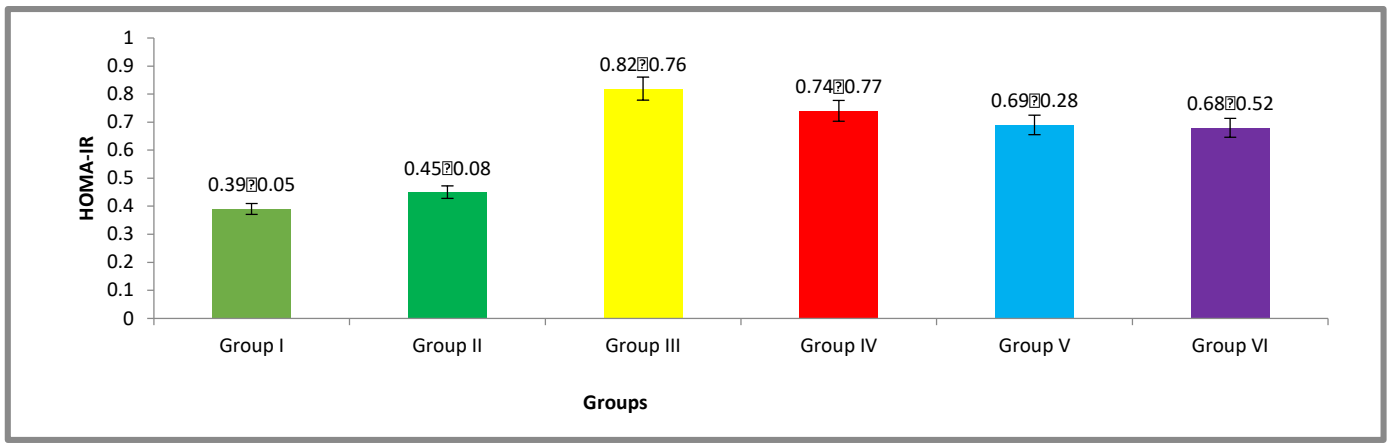

Figure [6]: Effect of different treatments on fasting blood HOMA-IR level

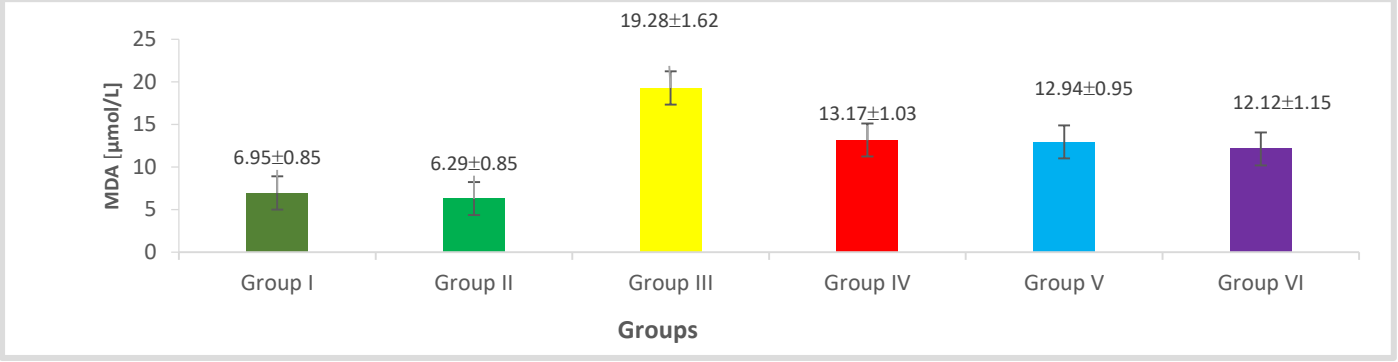

Figure [7]: Effects of different treatments on serum malondialdehyde level [MDA] [µmol/L]

DISCUSSION

Diabetes mellitus [DM] is a chronic inflammatory and metabolic condition marked by hyperglycemia. According to latest World Health Organisation [WHO] statistics, diabetes is the leading worldwide health concern. An estimated 463 million people globally, or 9.3% of the adult population, suffer from diabetes [33]. Vitamin D promotes cell proliferation and differentiation, protects several bodily organs from oxidative damage and serves as a transcriptional regulator for numerous genes [34].

Metformin is an anti-diabetes medication that seeks to lower blood glucose levels close to normal [35]. Metformin has been found to have substantial antioxidant capabilities in addition to its therapeutic efficacy against DM [36]. Therefore, the present study investigated the effect of vitamin D supplementation with or without metformin throughout measuring blood glucose, vitamin D, serum calcium, serum phosphate, liver enzymes [ALT, AST& alkaline phosphatase], insulin resistance by homeostasis model assessment [HOMA-IR] and serum MDA level on diabetes mellitus in adult male albino rats.

In the present study, supplementation of vitamin D alone, metformin alone or a combination of vitamin D and metformin showed a significant decrease of blood sugar levels. Vitamin D's effect on beta cell insulin receptors is one conceivable mechanism linking it to diabetes. It has been demonstrated that vitamin D increases the transport of glucose from the intestine and stimulates the production of the insulin receptor gene. Another way is that 1,25 [OH]2D3 helps the stomach absorb calcium, which is necessary for beta cells to release insulin [37].

Metformin's anti-hyperglycemic effect is primarily due to a reduction in hepatic glucose output, which suppresses hepatic gluconeogenesis, intestinal glucose absorption, improves insulin sensitivity and increasing glucose uptake and utilisation [38].

The current findings are consistent with a study by **Dray *et al.*** [39] in which glucose levels were reduced while absorption and utilisation by tissues were increased.

In this study, it was observed that serum vitamin D level dropped down in diabetic rats Supplementation with vitamin D resulted in significantly greater serum vitamin D levels compared to diabetic rats. However, when compared to diabetic rats, metformin treatment by itself did not significantly raise serum vitamin D levels; however, when metformin and vitamin D were combined, serum vitamin D levels were much greater.

This supports the findings of **Out M, *et al.*** [40] who discovered that metformin has no effect on vitamin D levels in type 2 diabetic patients.

Considering that vitamin D is absorbed in the duodenum and proximal gut, this is biologically plausible. According to studies using fluorodeoxy-glucose positron emission tomography [FDG-PET], metformin is absorbed more widely in the ileum and colon but less frequently in the duodenum [41].

Serum sample biochemical examination revealed that, in comparison to normal control groups, levels of ALT and AST activities rose following the induction of diabetes mellitus. These liver enzymes are helpful indicators of damage to the hepatocellular membrane. Therefore, increased levels in diabetics who are not receiving treatment may be a sign of liver damage or inflammation. Rats with diabetes exhibit noticeably higher levels of enzyme activity. When compared to the diabetes control, all therapies significantly decreased the serum activity of these liver enzymes. It's interesting to note that metformin and vitamin D together reduced these liver enzymes more than each drug did by itself. By lessening liver damage, vitamin D by itself can treat hepatic dysfunction brought on by hyperglycemia.

Our results are in agreement with **Lim C, *et al.*** [42] who imply that vitamin D improves increased liver enzymes in diabetic rats, and with **Uchendu *et al.*** [43] who show that metformin improves raised liver enzymes in diabetic rats.

Furthermore, the current study agrees with **Abdel-Rehim *et al.*** [44] who said that vitamin D, either alone or in combination with metformin, has a protective effect on hepatic cells as seen by decreased blood ALT and AST activity.

The reported beneficial benefits of vitamin D on liver function tests may be attributed to its anti-diabetic properties via enhancing hepatic metabolism, as metformin avoided liver damage [45]. Vitamin D may protect the liver by regulating the expression of PPAR-α and inflammatory cytokines [46].

Rats with diabetes had significantly higher levels of HOMA-IR, a biomarker for measuring insulin resistance, than the control group. On the other hand, when compared to the diabetic group, supplementation with vitamin D alone, metformin alone or both statistically significantly decreased this surge. By decreasing HOMA-IR, metformin was found to lessen T2DM-induced insulin resistance; however, when metformin and vitamin D were taken together, the reduction in HOMA-IR was more effective than when either drug was taken alone.

This is in an agreement with **Abdel-Rehim *et al.*** [44] who mentioned that diabetic rats had significantly higher levels of glucose and HOMA-IR than control rats.

Rats treated with alfacalcidol alone or in combination with metformin

showed a significant reduction in HOMA-IR compared to untreated diabetic rats. Metformin reduces insulin resistance in diabetic rats by lowering HOMA-IR levels via many pathways. Metformin works predominantly in the liver to reduce hepatic glucose production [47].

By preventing the liver from synthesising glucose, metformin lowers blood glucose levels and improves insulin sensitivity. Additionally, compared to a placebo, long-term metformin treatment stabilises body mass index [BMI] and improves body composition in obese, insulin-resistant teenagers [48]. Obesity reduction improves insulin sensitivity.

The extremely reactive compound malondialdehyde [MDA] is a sign of lipid peroxidation and oxidative stress. It can damage proteins and DNA and is created when polyunsaturated fatty acids break down. MDA levels are frequently used to evaluate oxidative stress in a number of diseases, such as diabetes, cancer, and neurological issues. According to our research, vitamin D administration led to a much lower level of malondialdehyde than the diabetic group, while diabetic rats had significantly higher levels of MDA than control rats.

This is consistent with **Alatawi *et al.*** [49] who stated that vitamin D supplementation can lower malondialdehyde levels via the following mechanisms: Antioxidants included in vitamin D can directly shield cells from oxidative damage. While boosting the activity of antioxidant enzymes such glutathione peroxidase and superoxide dismutase [SOD], vitamin D prevents the production of reactive oxygen species [ROS].

In addition, Vitamin-D helps in the control of hyperglycemia by improving insulin sensitivity and secretion. Uncontrolled hyperglycemia significantly contributes to oxidative stress and lipid peroxidation in diabetes [50].

Vitamin D has anti-inflammatory properties, decreasing the production of proinflammatory cytokines such as interleukin-6 [IL-6], which can contribute to oxidative stress [51]. Vitamin D prevents death and malfunction in pancreatic beta cells, retaining their ability to make insulin and control glucose metabolism [52]. Similarly, metformin administration to diabetic rats resulted in significantly decreased serum malondialdehyde levels, which is consistent with **Ibrahim *et al.*** [53] findings. Metformin's ability to reduce MDA shows that it has antioxidant benefits in DM. Metformin reduces oxidative stress through several mechanisms, which include: inhibition of the respiratory chain complex in the mitochondria. It activates AMPK, which has immune-modulatory and anti-inflammatory properties and somewhat reduces the production of ATP [54]. In addition, inhibiting mitochondrial glycerol 3 phosphate dehydrogenase [mGPD] reduces hepatic glucose synthesis [55].

Conclusion: Vitamin D showed a potential in treating diabetes mellitus-related issues like insulin resistance and hyperglycaemia. Oxidative stress associated with DM is improved by vitamin D by decreasing MDA level. Also, metformin improves blood glucose by different mechanisms. Adding vitamin D to metformin showed better results than each drug alone.

Disclosure: None to be disclosed.

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