Role of Hypovitaminosis D in the Incidence and Complications of Diabetes Mellitus in Rats

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ABSTRACT

Background: Diabetes mellitus continues to be a public health concern. Vitamin D had sparked widespread interest in the pathogenesis and prevention of diabetes. The aim of this study was to investigate the effect of vitamin D (deficiency & treatment) with alteration in fasting plasma glucose, insulin resistance in alloxan injected rat. **Materials and methods:** The experiment was carried out using 40 male albino rats (Sprague Dawley) weighing 150±10g. Animals were randomly divided into three groups; first group fed standard diet as a negative control group. Diabetic group injected subcutaneously by alloxan, and fed on standard diet. The third group fed standard diet without vitamin D for two weeks. After that glucose and insulin were determined in each rat of all groups to insure alteration in fasting plasma glucose, insulin resistance, Homeostasis model assessment insulin resistance (HOMA-IR) was calculated. Then the third group was divided to two subgroups. The first subgroup fed basil diet with required vitamin D; while the second subgroup fed standard diet with double dose vitamin D. At the end experiment (4 weeks), glucose, insulin, lipid profile, liver and renal functions were determined in blood and serum, while (HOMA-IR) and LDL were calculated for normal, diabetic group and both treatment subgroups. **Results:** Vitamin D deficiency group had the nearest results to the diabetic group injected with alloxan group in: insulin, glucose and HOMA-IR. Other groups had lower level than the other two groups in the same parameter. Our data explained the improvement in glucose level after feeding with vitamin D. Diabetic group injected with alloxan had increased in liver enzymes, renal function and lipid profile compared with other groups and showed variable changes in histopathological examination**. Conclusion:** Vitamin D deficiency status is associated with a higher risk of type 2 diabetes. Vitamin D improves glucose tolerance and insulin sensitivity. Vitamin D has also been shown to reduce the risk of diabetes associated complications.

Keywords: Alloxan – Diabetic- glucose tolerance- Insulin sensitivity .Vitamin D

INTRODUCTION

Diabetes mellitus is a metabolic disease that can affect nearly every system in the body .[1] Low vitamin D status can be caused by number of factors, including insufficient cutaneous synthesis (due to limited sunlight exposure or aging), inadequate intake and absorption of vitamin D, obesity or darker skin. Low blood levels of its main metabolite, 25(OH) D, have been linked to poor health outcomes such as fractures, poor physical function, diabetes, osteoporosis, cancer, cardiovascular, neurodegenerative, autoimmune and infectious diseases .[2]

Lips[3] explained that vitamin D and especially its activated metabolite 1, 25-dihydroxyvitamin D3 (1,25D3), are involved in controlling the normal function of the endocrine pancreas, and particularly insulin secretion. Whereas **Bourlon et al.,** [4] indicated that vitamin D deficiency inhibits rat pancreatic secretion and turnover of insulin, leading to impaired glucose tolerance, while replacement therapy with 1,25D3, and is able to reverse these abnormalities.

Vitamin D is the most important regulator of calcium homeostasis in the body by increasing absorption of calcium from food and reducing urinary calcium loss. It exerts important functions in skeletal development and bone mineralization elaborated that by **Holick** [5]. Yet, vitamin D has no hormone activity itself. Once it enters the blood circulation, either synthesized in the skin or ingested, it is bound by the vitamin D binding protein and transported to the liver for further metabolization. To become biologically active, vitamin D needs two successive hydroxylations in the liver (at carbon 25) and in the kidney (at a position of carbon 1). In the kidneys, 25-hydroxyvitamin D (25[OH] D) is converted to an activated (1, 25-dihydroxyvitamin D; 1, 25[OH] 2D) as well as an inactivated (24, 25-dihydroxyvitamin D; 1, 25[OH] 2D) form. The vitamin D hormone, 1, 25(OH2) D, exerts its effects mainly by activating the nuclear vitamin D receptor (VDR), a member of the nuclear receptor super-family of ligand activated

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transcription factors, and, when bound to this receptor, associates with specific recognition sequences called vitamin D-responsive elements, which are present in the promoter of target genes and are involved in regulating their own transcription explained this by **Haussler *et al.,*** [6]. In addition **Christakos *et al,*** [7] **and Vidal *et al.,*** [8] who were pointed out the mechanism of this transcriptional regulation is very complex and is only beginning to be unraveled. Classical vitamin D-responsive elements and other responsive sites are being discovered in genes with important functions in the pancreatic b-cells and in genes with key roles throughout the immune system (e.g. cytokines, transcription factors), making vitamin D an attractive molecule to investigate in the context of diabetes treatment.

The discovery of receptors for 1α,25-dihydroxyvitamin D3 (1,25(OH)2D3), the activated form of vitamin D, in tissues with no direct role in calcium and bone metabolism (e.g. pancreatic beta cells and cells of the immune system) has broadened our view of the physiological role of this molecule.[9,6] An increased prevalence of type 2 diabetes has been described in vitamin D-deficient individuals and insulin synthesis and secretion have been shown to be impaired in beta cells from vitamin D-deficient animals. Glucose tolerance is restored when vitamin D levels return to normal. [10]

The aim of this study is to investigate the role of hypovitaminosis D in the incidence and complications of diabetes mellitus in comparison with the effect of alloxan injection.

MATERIALS AND METHODS

Materials:

- Skimmed milk and corn starch were purchased from local market, Cairo, Egypt.
- ***Chemicals***: DL-methionine, choline chloride, vitamins, minerals, alloxan and kits required were obtained from El-Gomhorya Company for chemicals and Drugs, Cairo, Egypt.
- ***Animals***: Forty healthy adult male albino rats "Sprague Dawley strain" weighing (150±10g.) were obtained from vaccine and immunity organization Helwan Farm, Cairo, Egypt.
- ***Diets:*** The standard diet prepared as previously described by **Reeves *et al,*** [11] vitamin D was supplied by adding the required dose or double dose.

Methods:

Biological Experiment:-

Forty adult male albino rats (Sprague Dawley strain) weighing (150±10g) eight weeks old, were kept in wire cages. The diet was introduced to the rats in special food cups to avoid scattering of food. Also water was provided to the rats. Food and water were provided *ad-libitum* and checked daily.

Induction of diabetes

Alloxan monohydrate 150 mg/kg body weight was dissolved in normal saline (0.9 %) and injected subcutaneously after 18 hours fasting to induce hyperglycemia in experimental rats according to **Yanarday and Colak**.[12] The experimental animals were fasted for 18 hours before alloxan injection and the blood glucose level (BGL) was monitored after alloxanization in blood samples collected by tail tipping method using a Glucometer. Rats with blood glucose level greater than 150 mg/dl were considered diabetic according to **WHO.**[13] The control cohort was administered normal saline subcutaneously.

Experimental design

Forty healthy adult male albino rats were fed on standard diet for one week for adaptation. After this week, they were divided into three groups, each group with similar total body weight and were housed individually in wire cages. ***The first main group*** (10 rats) fed on basal diet (as a *negative control group*). ***The second main group*** (10 rats) fed on standard diet and injected with alloxan to induce diabetes in rats. After three days glucose was determined in each rat in the (first and second main group) to insure induction of disease. ***The third main group*** (20 rats) was fed standard diet without vitamin D for two week then glucose was determined in each rat to know the effect of deficiency of vitamin D then divided to two subgroups. ***The first subgroup*** (10 rats) fed on standard diet containing required vitamin D. ***The second subgroup*** **(**10 rats) fed on diet containing double dose of vitamin D. The blood glucose level (BGL) was monitored two time each week, blood samples collected by tail tipping method using a glucometer.

At the end of experiment (4 weeks), the animals were fasted overnight, and then sacrificed under anesthesia. Blood samples were taken in dry centrifuge tubes from the

hepatic portal vein. Serum was separated and kept in plastic vial at -20°c until analysis.

Biochemical analysis:

Fasting glucose level was determined by using a glucometer. Insulin was determined in serum by using the enzyme linked immunoassay (ELISA) method described by **Dhahir *et al,*** [14] Calculation of HOMA-IR as the value of fasting insulin (μIU/ml) X fasting glucose (mmol/L) divided by 22.5 according to **Pickavance *et al.*.**[15] Determination of ALT and AST were carried out according to the method of **Bergmeyer and Horder**.[16] Gamma – GT was determined according to ***Szasz*.** [17] Urea nitrogen was determined in the serum according to **Tabacco *et al.***[18] Uric acid was determined in the serum according to the method described by **Fossati *et al.*** [19] Creatinine forms colored complex when react with alkaline picrate. This reaction described by **Houot.**[20] The quantitative enzymatic – colorimetric determination of triglycerides in serum was used according to **Wahlefeld.**[21] Quantitative enzymatic colorimetric determination of total cholesterol and HDL in serum was determined according to **Stein.**[22] Low-density lipoprotein (LDL) cholesterol calculated according to **Friedewald *et al.***[23]

Histopathological examination:

Livers and pancreas of sacrificed rats were taken and immersed in 10% buffered neutral formalin solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. They were then cleared in xylol, embedded in paraffin, cut in sections of 4-6 microns thickness and stained with hematoxylin and eosin according to **Lambergton and Rothstein.**[24]

Statistical analysis:

Statistical analysis was carried out using the program of Statistical Package for the Social Sciences (SPSS), PC statistical software (Version 22; Untitled–SPSS Data Editor).

The results were expressed as mean ± standard error (mean ± S.E.). Data were analyzed using one way classification, analysis of variance (ANOVA). The differences between means were tested for significance using least significant difference (LSD) test at $p<0.05$.[25]

RESULTS

During experiment there was mortality of rat by 30% for injected alloxan group while the percentage was 20% for group fed standard diet without vitamin D (vitamin D deficiency group) and 37.5% for the group fed double of the required dose of vitamin D.

Relation of vitamin D, glucose and insulin sensitivity is available in table (1) which showed that all groups had nearly the same value of insulin except the group fed diet with two times required of vitamin D, which had increased insulin value with significant differences compared negative control. Vitamin D deficiency group had the nearest levels in glucose, and HOMA- IR level with alloxan injected group and higher than other groups with significant differences. Data in the table (1) explained the improvement in glucose and HOMA- IR level after feeding with vitamin D.

Liver function parameter showed in table (2), it showed an increase in ALT and AST activities in the group injected with alloxan compared to other groups. This difference was significant ($p<0.05$). Both subgroups fed vitamin D displayed decrease in ALT and AST activities than control or diabetic groups. GGT activity recorded insignificant change between all groups.

Kidney function parameters are present in table (3). It displayed boost of urea level in the alloxan group compared to other groups with significant differences. There was an increase in uric acid and creatinine level in group which injected with alloxan only.

Lipid profile parameters of this study are present in table (4). It showed elevation in the triglycerides, total cholesterol and LDL value in alloxan group compared to other groups. While HDL demonstrates a decrease in alloxan group, however there was an increase in group fed two times required vitamin D. While both group fed vitamin D required and control group were insignificant.

The data from table (5) showed that feed intake expressed a significant increase in rats of group fed VD two time required (second subgroup). There was a significant decrease in body weight gain in diabetic group. Feed efficiency ratio showed marked decrease in alloxan group and both groups fed with Vit.D.

Histological Results

Liver:

Table (6) and picture (1) demonstrates the liver of negative control group showing normal histological structure of hepatic lobules. Liver of rat injected with alloxan (positive control group) showed hydropic degeneration of hepatocytes, portal edema associated with

inflammatory cells infiltration. Liver of group fed diet supplemented with required vitamin D (after 2 weeks fed diet deficiency Vitamin D) (first subgroup), revealed cytoplasmic vacuolization and fatty change of hepatocytes (picture 3&4). The same group showed slight cytoplasmic vacuolization of hepatocytes (picture 5). Liver of group supplemented with double required vitamin D (after 2 weeks fed diet deficiency Vitamin D) (second subgroup), showed few perivascular inflammatory cell infiltration (picture 6).

Pancreas:

From table (7) picture (1) for pancreas of rat from group 1 as a negative group showed no histopathological changes. Pancreas of group injected with alloxan as positive control group showed vacuolation of pancreatic acini and vacillation, necrosis of islets of Langerhan's (photo 2&3). Pancreas of rat from first and second subgroups showed the same result as the normal control group "no histopathological changes" (photo 4&5).

DISCUSSION

Vitamin D deficiency inhibits pancreatic secretion and turnover of insulin, resulting in impaired glucose tolerance.[26] Alloxan and its reduction product dialuric acid establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. Thereafter, highly reactive hydroxyl radicals are formed by Fenton reaction. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of pancreatic beta cells.[27] There is accumulating evidences to suggest that poor vitamin D status is associated with a higher risk of type 2 diabetes [28,29, 30,] but little information exists on the association between vitamin D status and change in glycemic measures.[31]

Results showed **in table (1)** were matching with **Chiu *et al.*** [10] who found a positive correlation of 25-hydroxyvitamin D [25 (OH) D] concentrations with insulin sensitivity. Several studies have indicated a relationship between vitamin D status and the risk of diabetes or glucose intolerance. Vitamin D has been proposed to play an important role and to be a risk factor in the development of insulin resistance and the pathogenesis of type 2 DM by affecting either insulin sensitivity or β-cell function, or both.[10, 32, 33] The biological evidence implicating a potential influence of vitamin D on glucose homeostasis was summarized by **Pittas *et al.*** [34]. **Johnson** et al, [35] who gave a brief statement of the main points of the inferences for the manifold roles of vitamin D include the presence of specific vitamin D receptors (VDRs) on pancreatic β-cells; the expression of 1-α- hydroxylase enzyme in pancreatic β-cells which catalyzes the conversion of 25(OH) D to 1, 25-dihydroxyvitamin D (1, 25(OH) 2D), [36] the presence of a vitamin D response element in the human insulin gene promoter.[37] In addition, 1, 25(OH) 2D directly activates transcription of the human insulin receptor gene, 38 activates peroxisome proliferator activator receptor-δ, [39] stimulates the expression of insulin receptor, and enhances insulin-mediated glucose transport in vitro.[40] Few studies have examined the predictive value of 25 (OH) on future risks of type 2 diabetes mellitus.[30, 31, 41] **Forouhi *et al.*** [31] found baseline 25(OH) D to be inversely associated with fasting glucose, fasting insulin, and homeostasis model assessment-IR (HOMA-IR) at the 10-year follow up of the Medical Research Council Ely Prospective Study (European-origin adults), and independent of baseline outcome values. Also the results in this study agree with **Tuorkey and Abdul-Aziz** [42] who showed that vitamin D improves glucose tolerance. Vitamin D could also prevent type 2 diabetes through its role as an efficient antioxidant. Type 2 Diabetes is associated with systemic inflammation so that there was increase in liver enzymes showed in the same table for alloxan group. Systemic inflammation has been linked primarily to insulin resistance but elevated cytokines may also play a role in beta-cell dysfunction by triggering beta-cell apoptosis. Vitamin D may improve insulin sensitivity and promote beta-cell survival by directly modulating the generation and effects of cytokines.[43]

Data in **table (2)** illustrated the liver functions for different groups of rats injected with alloxan and other received diet added two levels with vitamin D. The most common liver function tests (LFTs) include the aminotransferases, such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), measure the concentration of intracellular hepatic enzymes that have leaked into the circulation and serve as a marker of hepatocyte injury. γ-glutamyl trans peptidase (GGT) act as markers of biliary function and cholestasis. Chronic mild

elevation of transaminases is frequently found in type 2 diabetic patients.[44] **Hamden *et al.*** [45] concluded that 1α, 25(OH) $_2$ VD_3 might be useful for the therapy and prevention of diabetes and the numerous side effects especially toxicity in liver. This protective effect showed when reduced toxicity in liver by significantly aspartate and lactate transaminase (AST and ALT) activities, in diabetic rats. The excess in free fatty acids found in the insulin-resistant state is known to be directly toxic to hepatocytes. Putative mechanisms include cell membrane disruption at high concentration, mitochondrial dysfunction, toxin formation, and activation and inhibition of key steps in the regulation of metabolism.[46] Other potential explanations for elevated transaminases in insulin-resistant states include oxidant stress from reactive lipid peroxidation, peroxisomal beta-oxidation, and recruited inflammatory cells.[47] All attribute elevated transaminases to direct hepatocyte injury. It is also hypothesized that elevation in ALT, a gluconeogenic enzyme whose gene transcription is suppressed by insulin, could indicate impairment in insulin signaling rather than purely hepatocyte injury**.**[48] Results obtained by **Ohlson *et al.*** [49] with similar results**,** concluded by **Vozaroza *et al.*** [50] that higher ALT is a risk factor for type 2 diabetes and indicates a potential role of increased hepatic gluconeogenesis and/or inflammation in the pathogenesis of type 2 diabetes. GGT is a nonspecific marker that is known to rise in patients with type 2 diabetes. GGT increases in diabetes, and increases in BMI, it has been proposed as another marker of insulin resistance.[51]

Table (3) displays kidney function for different groups, rat injected with alloxan and other received diet added two levels of vitamin D. The major long-term complications relate to damage to blood vessels. Diabetes doubles the risk of cardiovascular disease and about 75% of deaths in diabetics are due to coronary artery disease and kidney vessels. Other "macro vascular" diseases are stroke, and peripheral vascular disease.[52] Previous studies reported that Calcitriol, 1, 25-dihydroxyvitamin D3, and its analogs have been shown to have therapeutic potential in attenuating experimentally induced kidney disease. [53,54,55,56] Hamden ***et al.*** [45] concluded that 1α, 25(OH) 2VD3 might be useful for the therapy and prevention of diabetes and the numerous side effects especially kidney toxicity. This therapeutic effect occurred when reduced creatinine and urea levels in rats treated with vit. D.

The lipid abnormalities associated with type 2 diabetes is defined by a high concentration of TG and small dense LDL and a low concentration of HDL cholesterol. Plasma LDL cholesterol levels are generally normal. Insulin resistance is believed to contribute to this atherogenic dyslipidemia by increasing the hepatic secretion of VLDL and other apolipoprotein (apo) B-containing lipoprotein particles, as a result of increased free fatty acid flux to the liver.[57, 58] This may also be the result of a diminished suppressive effect of insulin on apoB secretion, either at the level of the regulation of apoB degradation, or inhibition of microsomal TG transfer protein activity.[59] These beforehand studies explained data obtained in the present study **(table 4).** In animal models, chronic hyperinsulinemia is found to predispose the liver to relative resistance to insulin. This is characterized by a failure of insulin to signal an increase in insulin receptor substrate-2. Upregulation of sterol regulatory element-binding protein 1c (SREBP-1c) also occurs, leading to increased lipogenesis.[60] Despite down-regulation of the insulin receptor substrate-2-mediated insulin signaling pathway in insulin-resistant states, the up-regulation of SREBP-1c and subsequent simulation of *de novo* lipogenesis in the liver leads to increased intracellular availability of triglycerides, promoting fatty liver. This also increases VLDL assembly and secretion.[61] Thus; hyperinsulinemia might directly lead to hepatic insulin resistance with associated fatty changes. Through the action of cholesterol ester transfer protein, TGs are transferred from VLDL to HDL, creating TG-rich HDL particles, which are hydrolyzed by hepatic lipase and rapidly cleared from plasma.[62]A similar cholesterol ester protein-mediated transfer of TGs from VLDL to LDL contributes to the formation of small dense LDL particles.[63] **Hamden *et al.***[45] concluded that 1α, 25(OH) 2VD3 might be useful for the therapy and prevention of diabetes and the numerous side effects especially toxicity by significantly triglycerides (TG), total cholesterol, in diabetic rats. Moreover, the plasmatic non-enzymatic antioxidant level of HDL-cholesterol, increased after 1α, 25 (OH) 2VD3 administrations.

Data in table (5) showed biological parameter **(**Feed intake, Body weight gain and Feed efficiency ratio) for different groups of rats received alloxan and vitamin D. Results illustrated significant decrease with biological parameter for group injected with alloxan and improved with VD. These results were in harmony with **Nyomba *et al.*** [64] who observed that the injection of streptozotocin reduced body weights and feed intake. Treatment with 1,25D3 to rats increased body weights.

Table (6) showed the liver histological structure**.** Liver histological picture showed degenerative changes in alloxan injection group. Other treatment group with vitamin D had little improvement in liver tissue. This results are in agreement with **Hamden *et al.*,** [45] who reported that 1α, 25(OH) 2VD3 might be useful for the therapy and prevention of diabetes and the numerous side effects especially toxicity in liver. The insulin-resistant state is also characterized by an increase in pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), which may also contribute to hepatocellular injury.[47]

Photos of **table (7)** showed the pancreas histological structure. When groups fed diet with both double dose of vitamin D, there were amelioration in histological picture of the pancreatic tissue. Pancreas of rat from first and second subgroups were given the same results as the normal control group "without abnormal histopathological changes". This result is confirmed by **Hamden *et al.*,** [45] who said the administration of 1α, 25 (OH) 2VD3 in diabetic rats protects against alloxan-induced histological changes in pancreas.

CONCLUSION

Based on findings obtained from the present study, vitamin D as a source to 1α, 25(OH) 2VD3 might be useful for amelioration and prevention of diabetes and to avoid the numerous side effects especially toxicity in some organs.

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Table (1) Insulin, Glucose and (HOMA- IR) for different rat groups injected with alloxan and received diet with or without vitamin D

Parameter	-ve Control group	Alloxan group (+ve control)	Vitamin D groups		
			Vitamin D Deficiency group	Group fed single dose of Vitamin D	Group fed double dose of Vitamin D
Insulin (µIU/ml)	5.8±0.1^{a}	5.7±0.2^{abcd}	5.9±0.2^{abcd}	5.8±0.1^{abcd}	6.6±0.1^{e}
Sig. with control	-	0.688	0.649	0.786	0.001
Glucose (mmol/L)	95.8±3.7^{a}	481.7±49.7^{bc}	424.6±46.6^{bc}	153.0±31.2^{ade}	114.0±28.1^{ade}
Sig. with control	-	0.001	0.001	0.946	0.943
HOMA-IR	24.7±1.0^{a}	122.0±9.1^{b}	111.3±2.7^{c}	39.4±5.0^{d}	33.4±8.9^{e}
Sig. with control	-	0.001	0.001	0.001	0.001

Mean values subscribed with different letters show significant differences between these values a calculated by ANOVA and LSD at P<0.05.

Table (2) Liver functions for different groups rat injected with alloxan and received diet with vitamin D

Parameter	-ve Control group	+ve Control group Alloxan injected	Vitamin D groups	
			Group fed single dose of Vitamin D	Group fed double dose of Vitamin D
ALT (U/L)	29±5.2^{a}	62±14.3^{b}	23±2.5^{acd}	20±0.1^{acd}
Sig. with control	-	0.006	0.676	0.329
AST (U/L)	55±9.8^{a}	125±18.9^{b}	41±2.7^{acd}	38.3±3.2^{acd}
Sig. with control	-	0.008	0.545	0.501
GGT(U/L)	28.6 ±0.9^{a}	30.5±1.2^{abc}	29.8±1.0^{abc}	26.3±0.5^{ad}
Sig. with control	-	0.186	0.321	0.150

Mean values subscribed with different letters show significant differences between these values a calculated by ANOVA and LSD at P<0.05.

Table (3) Kidney function for different groups rat injected with alloxan and received diet with vitamin D

Parameter	Control group	Alloxan group	Vitamin D groups	
			group fed single dose of Vitamin D	group fed double dose of Vitamin D
Creatinine mg/dl	1.02±0.15^{a}	1.65±0.79^{abcd}	1.1±0.24^{abcd}	0.8 ±0.06^{abcd}
Sig. with control	-	0.131	0.890	0.561
Uric Acid mg/dl	2.8±0.1^{a}	3.15±0.7^{abcd}	2.8±0.3^{abcd}	2.67±0.27^{abcd}
Sig. with control	-	0.511	1.000	0.817
Urea mg/dl	23.4 ±4.2^{a}	113.25±27.0^{b}	29.2±4.3^{acd}	23.3±3.3^{acd}
Sig. with control	-	0.001	0.770	0.997

Mean values subscribed with different letters show significant differences between these values a calculated by ANOVA and LSD at P<0.05.

Table (4) Lipid profile for different groups rat injected with alloxan and received diet with vitamin D

Parameter	Control group	Alloxan group	Vitamin D groups	
			Group fed single dose of Vitamin D	Group fed double dose of Vitamin D
Triglyceride (mg/dL)	61.6±3.0[a]	85.75±5.9[b]	70.75±2.8[acd]	72.0±6.1[acd]
Sig. with –ve control	-	0.000	0.770	0.997
T. Cholesterol (mg/dL)	132.4±4.6 [a]	170.5±16.9 [b]	125.8±5.0 [acd]	131±5.0 [acd]
Sig. with control	-	0.012	0.617	0.923
HDLc (mg/dL)	48.9±2.4 [a]	38.7±1.7 [b]	45.4±2.4 [acd]	54.9±0.37 [acd]
Sig. with control	-	0.015	0.603	0.248
LDLc (mg/dL)	75.8±3.2 [a]	106.4±13.6 [b]	70.1.4±7.1[acd]	61.6±2.64 [cd]
Sig. with control	-	0.001	0.060	0.001

Mean values subscribed with different letters show significant differences between these values a calculated by ANOVA and LSD at P<0.05.

Table (5): Feed intake, Body weight gain and Feed efficiency ratio for different groups rat injected with alloxan and received diet with vitamin D

Parameter	Control group	Alloxan group	Vitamin D deficiency group	
			Group fed single dose of Vitamin D	Group fed double dose of Vitamin D
Feed intake g/day	16.1±0.76[a]	12.2±0.13[b]	17.0±0.55[c]	25±0.85[d]
Sig. with control	-	0.001	0.001	0.001
Body weight gain g/period	38.2±12.1[a]	11.4±9.3[b]	24.0±2.5[c]	35.5±5.5[ad]
Sig. with control	-	0.001	0.003	0.334
Feed efficiency ratio	0.084[a]	0.033[b]	0.05[cd]	0.05[cd]
Sig. with control	-	0.001	0.001	0.001

Mean values subscribed with different letters show significant differences between these values a calculated by ANOVA and LSD at P<0.05.

Table (6): Photomicrographs of the Liver

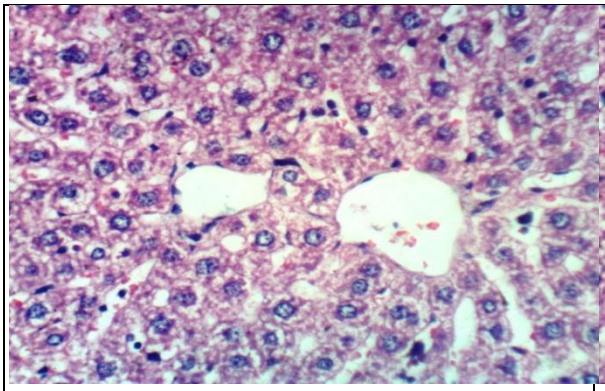
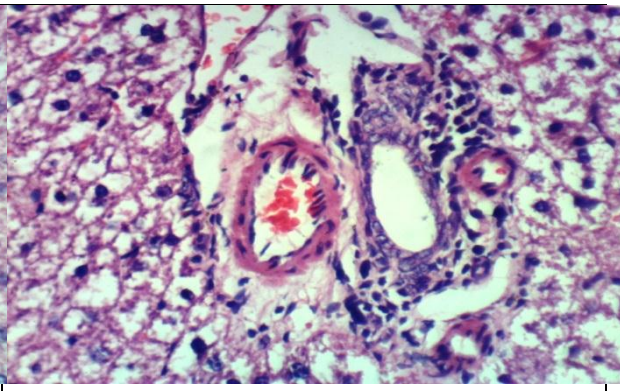
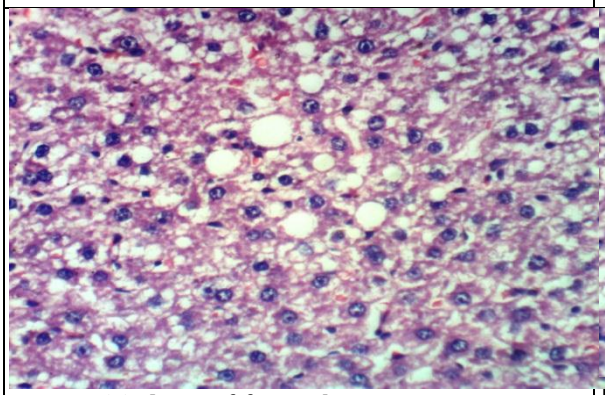
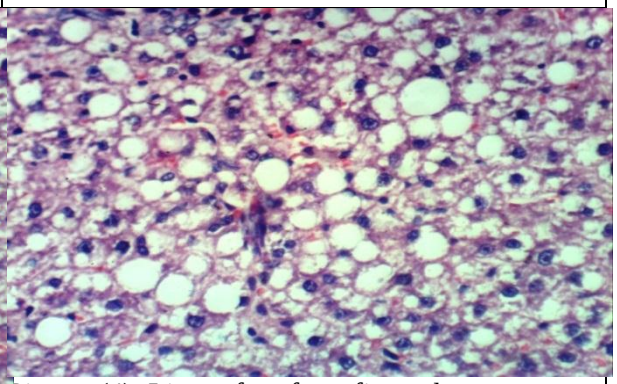
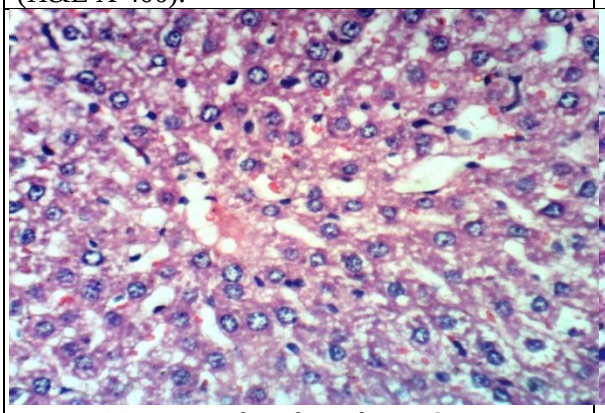
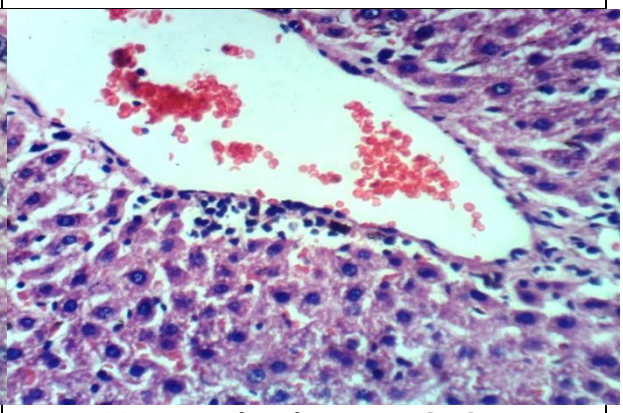
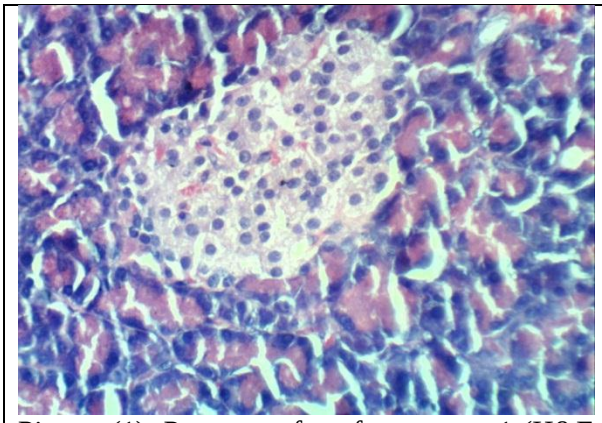
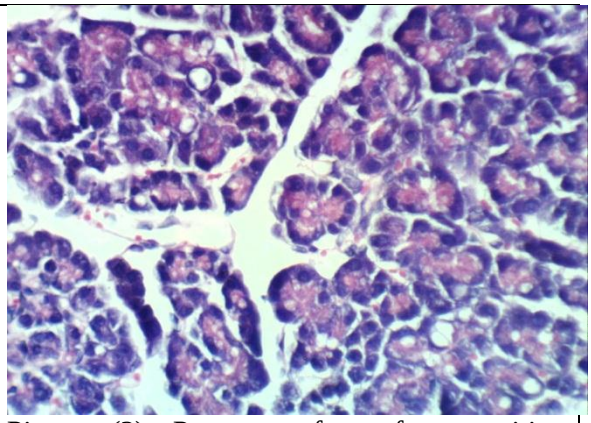
	
Picture (1) liver of negative control group (H & E X 400).	Picture (2): liver of rat injected with alloxan (H & E X 400).
	
Picture (3): liver of first subgroup, (H&E X 400).	Picture (4): Liver of rat from first subgroup (H&E X 400).
	
Picture (5): Liver of rat from first subgroup (H&E X 400).	Picture (6): Liver of rat from second subgroup (H&E X 400).

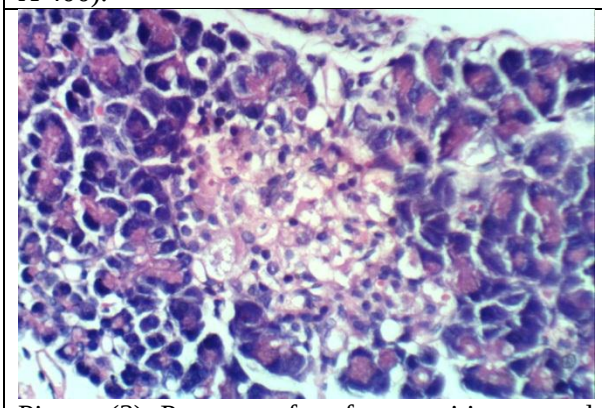
Table (7): Photomicrographs of the Pancreas



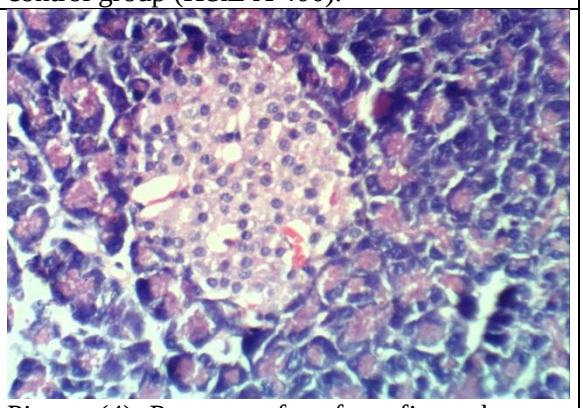
Picture (1): Pancreas of rat from group-1 (H&E X 400).



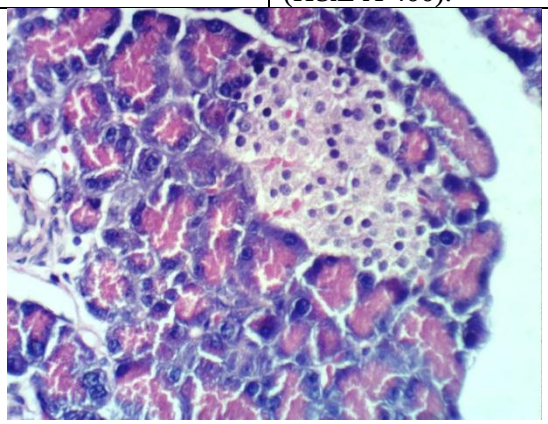
Picture (2): Pancreas of rat from positive control group (H&E X 400).



Picture (3): Pancreas of rat from positive control group (H&E X 400).



Picture (4): Pancreas of rat from first subgroup (H&E X 400).



Picture (5): Pancreas of rat from secound subgroup (H & E X 400).