

Effect of Vitamin D3 on Liver and Kidney Function of Diabetes Mellitus Male Rats

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Abstract: The present study was carried out in the animal house and laboratories of the College of Veterinary Medicine/ University of Basrah to investigate the role of the administration of vitamin D3 on liver and kidney function of alloxan induced toxic in mature male rats. For this purpose, 48 male rats were divided into 4 groups with 12 animals in each as the following; group (1), animals of were administered with D.W(0.2ml)/day by oral gavages for 6 weeks as control group, group(2) were injected intraperitoneally with 150 mg/kg b.w of alloxan as diabetic group, group (3) were injected with alloxan then administered with 500 UI/kg/day of alpha 25-hydroxy vitamin D3 by gavages for 6 weeks orally, group (4) were injected with alloxan then administer 1000 UI/kg/day of alpha 25-hydroxy vitamin D3 by gavages for 6 weeks orally. At the end of the experiment, animals were euthanized and blood samples were obtained for some biochemical assessment of liver and kidneys. In compares with control, alloxan treated rats recorded a significant increase ($p \leq 0.05$) in serum concentration of ALT, AST, ALP, urea and creatinine, while there was a significant decrease ($p \leq 0.05$) in serum total protein. On other hand, administration of vitamin D3 for diabetic rats induced a significant improvement ($P \leq 0.05$) of serum ALT, AST, ALP, total protein urea and creatinine.

Key words: Vitamin D, liver, kidney, Diabetes Mellitus, Male Rats.

I. Introduction:

Vitamin D is a one of the most important types of hormones that are characterized by their high ability to soluble in lipids, Vitamin D exists in a number of forms, where the major physiologically relevant forms are vitamin D2 is found in vegetable sources like mushrooms, and vitamin D3 (cholecalciferol), The most active form of vitamin D can be found in animal sources like fat fish, cod liver oil, egg yolk and fortified food like dairy products (Borradale and Kimlin, 2009).

However, a review with a global perspective found that 6 to 47% of Vit D intake may come from dietary supplements (Calvo and Whiting, 2005; Tripkovic et al., 2012). Importantly, the body itself has the ability to produce previtamin D3 through arrival of ultraviolet light (wavelength 290-315 nm) preformed 7-

dehydrocholesterol in the skin, which under normal temperature conditions isomers to form Vit D3. Vit D in all forms is transported in blood by vitamin D binding protein DBP (Elmubarak and zsoy, 2015).

Then it enters to the liver, where it is hydroxylated by one or more cytochrome P450 which results in the production of the inactive form 25-hydroxyvitamin D—25(OH)D. In the proximal renal tubule and some other tissues of the kidneys a second hydroxylation takes place by the enzyme 1 α -hydroxylase, which results in the biologically active form of Vit D, responsible for almost all of the biological actions of Vit D (Priest *et al.*, 2013; Mansuri, 2014).

Diabetes mellitus (DM) refers to a group of multifactorial metabolic disorders characterized by elevated blood glucose levels that result from defects in the body's ability to produce and/or insufficiency of insulin action (Chala and Ali, 2016).

Vitamin D supplementation early in life is a protective factor against the development of D1M (Takiishi *et al.*, 2010). For example, the Eurodiab study revealed a 33% reduced risk of developing D1M for children who received Vit D supplementation during their first year of life (Priest *et al.*, 2013). Studies by (Stene and Joner, 2003) demonstrated that use of cod liver oil from 7-12 months of life was associated with lowered risk of developing D1M in later life. Also, cod liver oil taken by pregnant mothers in the 3rd trimester of pregnancy was associated with decreased risk of D1M (Zipitis and Akobeng, 2008).

The aim of this study was to investigate the effects of Vitamin D3 on liver and kidney function of DM male rats.

II. Martial and methods:

1. Experimental design:

The present study was carried out at the animal house and laboratories of the College of Veterinary Medicine/ University of Basrah /Iraq, Forty eight adult male rats weighting (190-200 gm) were used for this study. The animals were kept in the animal house for acclimatization fifteen days before the beginning of the experiments. After the period of acclimation, 48 male rats were divided into 4 groups with 12 animals in each as the following:

Group1: (Control group) animals administered with D.W 0.2ml/kg/day by gavages for 6 weeks orally.

Group2: (Diabetic group)animals of this group injected intraperitoneally with (150mg/kg b.w of alloxan) (Al-Hilfy, 2013) .

Group3: diabetic rats treated with low dose vita- D3 group (the rats first were injected with alloxan then administer 500 IU/kg/day of alpha 25-hydroxy vitamin D3 by gavages for 6 weeks orally).

Group4: diabetic rats treated with high dose vita- D3 group (the rats first were injected with alloxan then administer 1000 IU/kg/day of alpha 25-hydroxy vitamin D3 by gavages for 6 weeks orally) (Alfawaz *et al.*, 2014).

At the end of the 6 weeks, animals of each group were anaesthetized by chloroform and sacrificed. The blood samples were collected by heart puncture of each rat and serum separated for biochemical tests.

2.Studied parameters:

Measurements of Aspartate Aminotransferase (AST) (U/I): Aspartate aminotransferase is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenyl-hydrazine (Schumann and Klauke, 2003).

Measurements Alanine Aminotransferase(ALT)(U/I):

Alanine aminotransferase is measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenyl-hydrazine(Schumann and Klauke, 2003).

Measurements Alkaline Phosphatase (ALP) (U/I):

This measurement was done by using the colorimetric determination of alkaline phosphatase activity (Biolabo-France) (Klin, 1980).

Measurements of Total Protein (g/L) :

The total protein was estimated by using a special chemical kit prepared by BIOLABO, SA, Maizy/France).Colorimetric method is described by (Tietz, 2006).

Measurements of Urea(mg/dl) :

Urea concentration was determined by using a special urea Kit (bioSystems, Spain) (Tietz, 1996).

Measurements of Creatinine(mg/dl) :

Creatinine concentration was determined by using a special creatinine Kit (BIO-LABO. SA, Maizy, France). (Tietz, 1999).

III. Results:

1. Effect of Vitamin D₃ on Serum ALT, AST and ALP Concentration in DM Male Rats.

The results of hepatic enzymes as shown in the table (1) below were showed a significant increase ($p\leq0.05$) in serum ALT, AST and ALP concentrations in DM group compared with control group and other groups but no a significant differences were observed in serum ALT , AST and ALP concentrations of two groups of Alloxn + vit D₃ and Control group.

Table (1): Effect of vitamin D₃ on Serum ALT, AST and ALP in DM Male Rats. (Mean $\pm$ SD) (n=12).

Group	Parameter	ALT (U/l)	AST (U/l)	ALP (U/l)
Control		31.40 $\pm$ 4.16 b	61.00 $\pm$ 4.66 b	25.10 $\pm$ 3.24 b
G1 (DM)		42.90 $\pm$ 6.06 a	93.70 $\pm$ 12.08 a	32.40 $\pm$ 5.54 a
G2 DM+ Low dose Vit-D		31.01 $\pm$ 3.96 b	64.90 $\pm$ 9.6 b	28.60 $\pm$ 4.29 ab
G3 DM+ High dose Vit-D		29.02 $\pm$ 3.45 b	59.80 $\pm$ 4.46 b	25.80 $\pm$ 3.85 b
LSD		11.5	28.8	6.6

Values expressed in the small letters mean significant differences at the ($P<0.05$).

2.Effect of vitamin D₃ on Serum Total Protein ,Urea and Creatinine Concentration in DM Male Rats.

In the table (2) the data revealed a significant ($p\leq0.05$) decreased in serum total protein of DM group compared with control and other groups , while no significant differences of serum total protein between DM+ high dose Vit D3 compare with control group .

There was a significant increase ($p\leq0.05$) of urea and creatinine in DM group and DM+Vit D3 group compare with control group (table 2).

e (2): Effect of vitamin D₃ on Serum Total Protein ,Urea and Creatinine in DM Male Rats (Mean $\pm$ SD) (n=12).

Group	Parameter	Total protein g/L	Urea mg/L	Creatinine mg/dl
Control		9.70 $\pm$ 0.46 a	61.02 $\pm$ 4.48 c	3.14 $\pm$ 0.56 c
G1 (DM)		6.52 $\pm$ 0.46 c	93.97 $\pm$ 1.56 a	7.96 $\pm$ 0.80 a
G2 DM+ Low dose Vit-D		7.98 $\pm$ 0.65 b	82.00 $\pm$ 5.37 b	6.88 $\pm$ 0.51 b
G3 DM+ High dose Vit-D		9.13 $\pm$ 0.76 a	78.45 $\pm$ 4.54 b	6.52 $\pm$ 0.67 b
LSD		0.57	11.97	1.08

Values expressed in the small letters mean significant differences at the ($P<0.05$).

IV. Discussion:

The present study showed a significant increase ($p\leq0.05$) in serum ALT, AST and ALP concentration in alloxan treated group compared with control while a significant improved in DM group treated with vitamin D3 compared with DM group.

The analysis of the activities of these enzymes in the serum was used to observe the condition of liver tissue and any damage might occur after being exposed to a certain pharmacological agent such as Alloxan , Liver as an insulin-dependent tissue plays a vital role in the metabolism of glucose and other substances. The damage of liver cells cause a leakage of the contents out of the tissue into the blood stream (Ozlem-Ozsoy *et al.*, 2006; Sarfraz *et al.*, 2017) reported that increased activities of serum AST, ALT and ALP level indicated

that hepatic dysfunction may be induced due to hyperglycemia in diabetic rats. The increase in the level of these enzymes in diabetes may be as a result of leakage from the tissues and migration into the bloodstream.

Diabetes mellitus is associated with the declined antioxidant capacity and increased production of ROS through increases of lipids, proteins and DNA oxidation products, Oxidative stress has a pivotal role in the pathogenesis of diabetes complications, The present results are in consistent with (Yuniarti and Lukiswanto, 2016; Mat Darus and Mohamad, 2017; Sarfraz *et al.*, 2017).

The improved of liver enzyme in animal treated with Vitamin D3 compare with allxon treated group may result from of anti-hepatotoxic effects of Vitamin D3 (Özerkan *et al.*, 2017; Liu *et al.*, 2016) reported that a therapeutic effects of vitamin D on diabetes-induced liver complications in a rat model by its anti-inflammatory activity.

The present study revealed that significant decreased in serum total protein and highest significant urea and creatinine of DM group compared with control while administration of Vitamin D3 induced improve of these parameters.

Kidneys are the major excretory organs and renal function tests are devised to detect possible renal damage. Increased serum levels of urea and creatinine are among the most sensitive indicators of kidney injury and hyperglycemia is known to induce elevations these parameters (Osigwe *et al.*, 2017) .

These result in agree with (Ogunmefun *et al.*, 2017; Osigwe *et al.*, 2017)all they reported that alloxan administration caused significant decrease in level of protein values and increase in serum urea and creatinine in diabetic animals as compared with control group.

A reduction in the serum protein levels in diabetic control rats may be as a result of possible damage to the hepatocytes induced by alloxan and chronic hyperglycemia (Osigwe *et al.*, 2017). It may also be due to increased rate of amino acid conversion to glucose and reduced ribosomal protein synthesis as a result of insulin deficiency (Narasimhanaidu and Ponnaian, 2006).

The high serum urea in diabetic control rats has been suggested to be due to the stimulation of gluconeogenesis as alternative glucose source as a result of insulin deficiency (Abdulazeez *et al.*, 2013).Gluconeogenesis is sustained by increased proteolysis which releases glucogenic amino acids that are subsequently deaminated in the liver resulting in high urea levels (Abdulazeez *et al.*, 2013).The primary metabolite derived from dietary protein and tissue protein turnover is urea while muscle creatinine catabolism results in production of creatinine (Thurman and Parikh, 2008).

Zeng *et al.*, (2017) indicated that 1, 25-(OH)₂ D₃ could protect kidney in T2DN rats. Developed a clinical trial that also indicated that vitamin D can protect diabetic

nephropathy independent of the control of blood glucose and blood pressure (Dong *et al.*, 2017)

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