ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Modulatory Role Of Vitamin D And Coconut Oil On The Disorders Of The Thyroid Hormones And Cytoskeletal Intermediate Filaments Of Diabetic Mice.

Nabila I. El-Desouki*, Mohamed L. Salem, Dalia F. Afifi, Nasef M, and Faten M. Abdallah.

Department of Zoology, Faculty of Science, Tanta University, Tanta 31527, Egypt.

ABSTRACT

The purpose of the present study is to evaluate the modulatory role of either vitamin D or coconut oil or both together on the thyroid hormones and intermediate filaments, cytokeratin and vimentin filaments, by using immunohistochemistry (IHC) of diabetic adult male mice (*Mus musculus*) induced by streptozotocin (STZ).The mice were divided into 7 equal groups (10 mice/each). The duration of the experiment was 4 weeks. Gp.I: normal control mice group without any treatments. Gp.II&Gp.III: non-diabetic mice groups received vitamin D orally in a daily dose of 6.25 ml/kg b.w/d or coconut oil at dose of 7.5 ml /kg b.w/d. for 4 weeks separately. Gp.IV: diabetic mice group injected i.p. with a single dose of STZ dissolved in saline solution in a dose of 200 mg/kg b.w to induce diabetes. Gps.V, VI & VII: diabetic mice groups administered orally with vitamin D or coconut oil or both together at the same previous doses. The results recorded non- significant changes in the blood glucose, insulin, TSH, T3 & T4 levels of non-diabetic mice groups as compared to normal control group. A high significant increase in blood glucose and significant increase in T3 & T4levelsand a significant decrease in insulin &TSH levels of diabetic mice group as compared to the normal control ones were recorded. Diabetic mice group received vitamin D only recorded significant increase in blood glucose, T3 & T4 levels, and non-significant decrease in insulin level and significant decrease in TSH level; while the diabetic mice received coconut oil alone or co-administered with vitamin D recorded non-significant increase in blood glucose, T3 & T4 levels and non-significant decrease in insulin, while diabetic mice administered with vitamin D and coconut oil together recorded a slight increase in TSH as compared to normal control group.IHC observations in the thyroid glands of normal control or non-diabetic mice group that received either vitamin D or coconut oil expressed normal moderate immunoreactivity to vimentin and cytokeratin. Vimentin filaments are expressed at the basal part of thyrocytes and in blood vessel walls, while cytokeratin immunoreactivity is seen at the apical surface of follicular cells and in the endothelia of the blood vessels.STZ diabetic mice group revealed markedly intense vimentin and cytokeratin filaments immunoreactivity, while after administration of vitamin D or/and coconut oil daily for 4 weeks to diabetic mice expressed a marked improvement of vimentin and cytokeratin immunoreactivity but more obviously recovery to approximately normal expression with coconut oil alone or co-given with vitamin D together more than that seen with vitamin D only. In conclusion, the present results indicated that coconut oil alone or co-administered with vitamin D play an important role in the modulation and recovery of the thyroid hormones and intermediate filaments of diabetic mice to normal status more than that taken vitamin D alone.

Keywords: Hyperglycemia, Glucose, Insulin, TSH, T3, T4, Thyroid gland, Immunohistochemistry, Intermediate filaments, Image analysis, Mice.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Diabetes mellitus (DM) is the most common serious metabolic disease in human with a hall mark of an elevated blood glucose concentration caused by many physiological alterations**.** Symptoms of high blood sugar include frequent urination, increased thirst, and increased hunger. If left untreated, DM can cause many complications. Acute complications can include diabetic ketoacidosis, non-ketotic hyperosmolar coma, or death. Serious long term complications include heart disease, stroke, chronic kidney failure, foot ulcers, and damage to the eyes (1). DM develops when the body doesn't make enough insulin or is not able to use insulin effectively, or both (2).

There are links between elevated glucose turnover, increased intestinal glucose absorption, elevated hepatic glucose output, increased free fatty acid concentrations, increased fasting and/or postprandial insulin and pro-insulin levels, and increased peripheral glucose transport accompanied by glucose utilization. Further, other symptoms such as increased insulin degradation, increased glucagon secretion, increased hepatic glucose production (3), enhanced catecholamines and insulin resistance are also included (4).

In 2015, the prevalence of type 2 diabetes in Egypt was found in around 15.6% of all adults aged 20 to 79. The World Bank reported an even higher percentage (16.7%) (5). In Egypt, there were more than eight million cases of diabetes in 2017 and this number is expected to double by 2035 (6).

Thyroid dysfunction is a common endocrine disorder with variable prevalence. There is a deep underlying relation between DM and thyroid dysfunction, thyroid hormones directly control insulin secretion from beta pancreatic cells; as it can produce significant metabolic disturbances (7,8).

The thyroid gland controls rate of use of energy sources, protein synthesis, and controls the body's sensitivity to other hormones. It participates in these processes by producing thyroid hormones, the principal ones being thyroxine (T_4) and triiodothyronine (T_3**)** which is more active. These hormones are synthesized from iodine and tyrosine, and regulate the growth and rate of function of many other systems in the body. Hormonal output from the thyroid is regulated by thyroid stimulating hormone (TSH) produced by the anterior pituitary which itself is regulated by thyrotropin – releasing hormone (TRH) produced by the hypothalamus (9).

Cytoskeleton consists of three kinds of protein filaments (microfilaments, intermediate filaments (IFs) and microtubules). IFs contain cytokeratin, vimentin, desmin, glial fibrillary acidic proteins, neurofilament proteins, nuclear lamins and nestin. Cytoskeletal filaments play a major role in maintenance of cell shape, cell motility and division, organelles transport and participate in cell-cell & cell-matrix junctions, protection from environmental stresses, intracellular organelles anchorage and muscle contraction (10).

Vimentin is widely distributed in the cells of mesenchymal nature and in stroma of the mesenchymal cells. It is proposed to conistitute a regulatory structure at the receptor enabling efficient signal transmission (11). It is found in almost sarcomas and melanomas but is variable in lymphomas and even some carcinomas (12). It may be co-expressed with cytokeratins in a wide range of carcinomas and other tumors.Increased vimentin expression has been reported in various epithelial cancers including prostate cancer, gastrointestinal tumors, CNS tumors, breast cancer, malignant melanoma, lung cancer and other types of cancers(10,13).

Vimentin plays a significant role in supporting and anchoring the position of the organelles in the cytosol. It is attached to the endoplasmic reticulum and mitochondria, either laterally or terminally (14). Vimentin has been shown to eliminate toxic proteins in juxta nuclear quality control compartment and insoluble protein deposit inclusion bodies in asymmetric division of mammalian cell lines (15).

Cytokeratins (CK) are protein filaments found in intra-cytoplasmic cytoskeleton of epithelial tissue and participate in epithelial cell protection from mechanical and non-mechanical stressors. Keratins are used as diagnostic tumor markers as in epithelial malignancies (16). CK intermediate filaments help cells resist mechanical stress. Expression of CK within epithelial cells is used as diagnostic markers in tumor pathology and clinically to identify the origin of various human tumors (17,18).

The expression of intermediate filament proteins in thyroid tumor by immunohistochemical method was studied by Miettinen *et al*. (19), and they found the intermediate filament proteins of cytokeratin and vimentin were evaluated in non-neoplastic thyroid glands and in different types of thyroid neoplasms. Also, follicular epithelia of both normal and goitrous thyroids showed a strong reaction with anti-cytokeratin antibodies that widely cross react with various simple epithelia, and only the stromal and interstitial cells reacted with antibodies to vimentin.

Vitamin D refers to a group of fat-soluble steroids responsible for increasing intestinal absorption of calcium, magnesium, phosphate, and zinc (20).There are several forms of vitamin D (vitamers). The two major forms are vitamin D2 (ergo-calciferol) and vitamin D3 (cholecalciferol). Vitamin D without a subscript refers to either D2 or D3 or both. These are known collectively as calciferol (21). Vitamin D from the diet or dermal synthesis from sunlight is biologically inactive; activation requires enzymatic conversion (hydroxylation) in the liver and kidney. It is estimated that 80% - 90% of vitamin D in the body are produced through skin synthesis and the remaining by the ingestion of foods and supplements of this vitamin (22).

Vitamin D is necessary for normal insulin secretion, and may play a functional role on glucose tolerance through its effects on insulin secretion and insulin sensitivity. The identification of 1,25 (OH)2D receptors and the 1 α hydroxylase expression in pancreatic beta cells support the possibility of vitamin D role in the pathogenesis of DMT2 (23). In animals, it has been demonstrated that the secretion of pancreatic insulin is inhibited by vitamin D deficiency, and that in humans, vitamin D deficiency is related to glucose intolerance and DMT2(24).

Coconut oil contains specific fats that support the body's natural hormone production, and it includes lauric acid, capric acid and caprylic acid which have antibacterial, antifungal and antiviral properties, thus coconut oil is beneficial for immune support (25).The fatty acids of coconut oil metabolizes fast and raises metabolic rate that may assist in preventing obesity and stimulate weight loss in diabetic obese patients and increased production of needed insulin and increases absorption of glucose into cells, thus helping diabetes ones(26).Also, it is very important during pregnancy as it can provide baby with necessary fats for development. When it used in food, it may support healthy thyroid function and governs brain development including myelination (27, 28).

Coconut oil antioxidant may also enhance the sensitivity to insulin or otherwise may also reduce insulin resistance and injury to pancreatic beta cells by scavenging reactive oxygen species in diabetic patients (29) and may help to improve insulin levels when consumed regularly and increase absorption of calcium and magnesium (30).

The aim of the present study is to evaluate the beneficial role of vitamin D or / and coconut oil on the blood glucose, insulin, TSH, T3 & T4 levels as well as immunohistochemical changes of intermediate filaments (vimentin and cytokeratin) of the thyroid glands of STZ diabetic adult male albino mice.

MATERIALS AND METHODS

Animals' selection and care:

Seventy adult male albino mice *(Mus musculus)*, weighing 25±2g and aged 6-8 weeks were obtained from Vacsera, Cairo. Animals were housed in plastic cage (10 per cage) for one week acclimatization under the same natural dark- light cycle and temperature. Tap water and food were freely available to the animals throughout the experiment. All care and procedures adopted for the present investigation were in accordance with the approval of the Institutional Animal Ethics Committee of Tanta University and in accordance with recommendation of the proper care and use of laboratory animals.

Induction of Diabetes mellitus (DM):

DM was induced in adult mice by intraperitoneal (i.p.) injection with a single dose of STZ dissolved in saline solution in a dose of 200 mg/kg of b.w according to Deeds *et al.* (31). STZ was obtained from "Sigma Chemicals Co., St. Louis, Mo., USA".

Treatment:

Vitamin D was obtained from "Egyptian Group for Pharmaceutical Industries Co., Egypt, and coconut oil was received from local Pharmacy (Al-badawia Company for Herbal and Oil Extraction, Mansoura, Egypt). Both were administered orally by a gastric tube.

Experimental design:

The mice were divided into 7 equal groups (10 mice for each), and the duration of the present experiment was four weeks; Gp.I: normal control mice group with no treatment. Gp. II: non-diabetic mice group received vitamin D orally in a dose of 500 international units (6.25 ml)/kg of bw/d; Gp.III: non-diabetic mice group received coconut oil daily in a dose of 7.5 ml /kg of bw/d. Gp.IV: diabetic mice group injected i.p. with a single dose of STZ to induce diabetes as discussed above. Gps.V, VI & VII: diabetic mice administered orally with either vitamin D or coconut oil or both together at the same previous doses daily for 4 weeks, respectively.

Samples collection and sera separation:

At the end of the experiment, the animals were fasted for 14 hour and were anaesthetized by using diethyl ether, and then sacrificed. Blood samples were collected from all studied groups. The blood samples were allowed to clot at room temperature for 30 minutes before centrifugation at 1000 revolutions per minute (rpm) for 20minute; Sera were collected into 1.5 ml epindorph tubes and stored at -20°C till used to measure glucose, insulin TSH, T3 and T4(32). The thyroid gland specimens were removed and processed for light microscopic studies (33).

Immunohistochemical studies (IHC):

The specimens of the thyroid glands were fixed in 10 % neutral buffered formalin for 24h. IHC avidin-biotin technique (34) and monoclonal antibodies against vimentin and cytokeratin were used. Vimentin (V9) was received from Dako Carpinteria, CA 93013 U.S.A. Cytokeratin (CK7) was received from Dako [OV-TL 12/30] U.S.A.

Calculation of the results:

The mean absorbance for each set of duplicate standards, control samples, subtract and the average zero standard optical density were calculated. The standard curve was plotted on log-log graph paper, with standard concentration on the X-axis and absorbance on the Y-axis. The best-fit straight line through the standard points was drawn.

Image analysis:

Digital images were analyzed by a semi-quantitative system (Figi-Image J software, Java based application for analyzing images). The brown colour of intermediate filaments (vimentin and cytokeratin) was immunohistochemically expressed in the thyroid gland sections; the percentage colored stained area(area fraction) per field area was determined by measuring six randomly photographed high-power fields (X400 magnifications) (35).

Statistical analysis:

Data were fed to the computer and analyzed using IBM SPSS® software package version 16.0, USA. Data were analyzed using numbers and percentages ± S.E. For normally distributed data, comparisons between the seven studied groups were analyzed using F-test (ANOVA) and Post Hoc test (LSD). Significance was obtained at $p< 0.05$.

RESULTS

Effect of vitamin D or/and coconut oil on blood glucose levels:

STZ diabetic mice recorded highly significant increase in blood glucose level (Gp.IV) as compared to normal control mice (Gp.I) (**$p \leq 0.001$); a significant increase in blood glucose level of diabetic mice administered with vitamin D alone (Gp. V) (*$p \leq 0.05$); and non-significant increase in mice blood glucose levels

ISSN: 0975-8585

of diabetic mice given coconut oil only (Gp.VI) or co-administered both together for 4 weeks (Gp.VII) as compared to normal control mice (Gp.I) ($p > 0.05$), (Table 1& Graph 1).

Effect of vitamin D or/and coconut oil on insulin levels:

Diabetic mice recorded a significant decrease in blood sera insulin levels (Gp.IV) as compared to normal control mice (Gp.I) (*$p \leq 0.05$); and non-significant decrease in mice blood sera insulin levels of diabetic groups given vitamin D or/and coconut oil (Gps.V, VI and VII)for 4 weeks as compared to normal control mice (Gp.I) ($p > 0.05$), (Table 2& Graph 2).

Effect of vitamin D or/and coconut oil on thyroid stimulating hormone (TSH) levels:

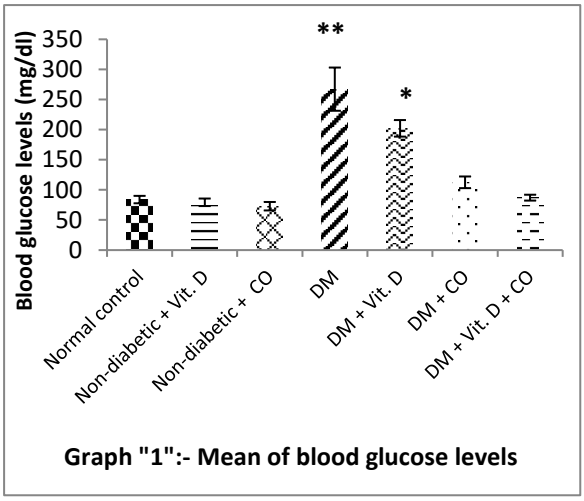
Diabetic mice recorded a significant decrease in blood sera TSH levels as well as diabetic group given vitamin D only (Gps.IV&V)as compared to normal control mice (Gp.I) (*$p \leq 0.05$), non-significant decrease in diabetic mice given coconut oil only (Gp.VI)($p > 0.05$)as compared to normal control mice (Gp.I), and non-significant increase in diabetic mice given vitamin D and coconut oil together (Gp.VII) as compared to normal control mice (Gp.I) ($p > 0.05$), (Table 3 & Graph 3).

Effect of vitamin D or/and coconut oil on T3 and T4 hormone levels:

Diabetic mice recorded highly significant increase in blood sera T3 & T4 levels (Gp.IV) as compared to normal control mice (Gp.I) (**$p \leq 0.001$); a significant increase in blood sera T3 & T4levels of diabetic mice administered with vitamin D alone (Gp.V) (*$p \leq 0.05$), and non-significant increase in mice blood sera T3 & T4 levels of diabetic mice given coconut oil only (Gp.VI) or co-administered of vitamin D and coconut oil together (Gp.VII) ($p > 0.05$), as compared to normal control mice (Gp.I), (Tables 4,5& Graphs 4,5).

Table 1& Graph 1: Effect of vitamin D or/and coconut oil on blood glucose levels:-

Groups	Mean ± SE (mg/dl)
Group I (Normal control)	83.9 ± 6.14
Group II (Non-diabetic + Vit. D)	79.4 ± 6.25
Group III (Non-diabetic + CO)	72.9 ± 7.03
Group IV (DM)	267.27 ± 35.87**
Group V (DM + Vit. D)	202.2 ± 13.74*
Group VI (DM + CO)	112.6 ± 9.68
Group VII (DM + Vit. D + CO)	87.1 ± 4.88



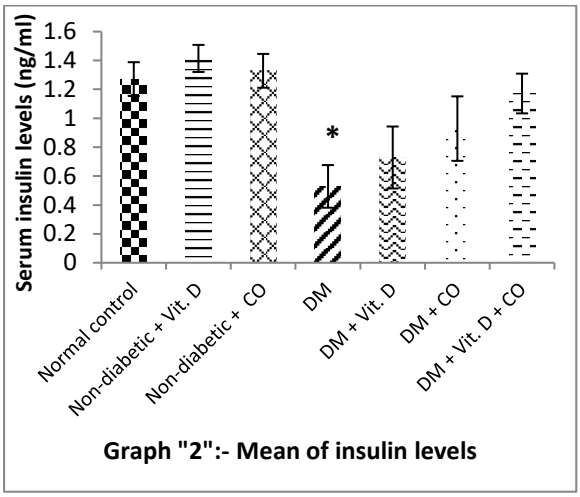
Graph "1":- Mean of blood glucose levels

Statistical significance was measured at $p > 0.05$; *$p \leq 0.05$;**$p \leq 0.001$

ISSN: 0975-8585

Table 2&Graph 2: Effect of vitamin D or/and coconut oil on insulin levels:

Groups	Mean ± SE (ng/ml)
Group I (Normal control)	1.27 ± 0.11
Group II (Non-diabetic + Vit. D)	1.41 ± 0.09
Group III (Non-diabetic + CO)	1.32 ± 0.11
Group IV (DM)	0.52 ± 0.14*
Group V (DM + Vit. D)	0.72 ± 0.21
Group VI (DM + CO)	0.92 ± 0.22
Group VII (DM + Vit. D + CO)	1.17± 0.13

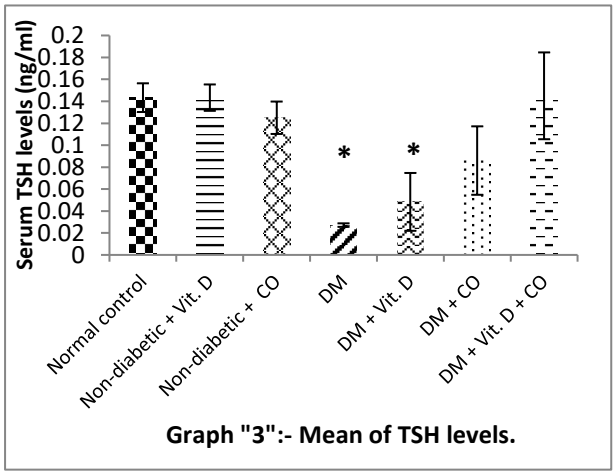


Graph "2":- Mean of insulin levels

Statistical significance was measured at p > 0.05; *p ≤ 0.05

Table 3 & Graph 3: Effect of vitamin D or/and coconut oil on TSH levels:

Groups	Mean ± SE (ng/dl)
Group I (Normal control)	0.143 ± 0.013
Group II (Non-diabetic + Vit. D)	0.143 ± 0.012
Group III (Non-diabetic + CO)	0.125 ± 0.014
Group IV (DM)	0.027 ± 0.001*
Group V (DM + Vit. D)	0.048 ± 0.026*
Group VI (DM + CO)	0.086 ± 0.031
Group VII (DM + Vit. D + CO)	0.145± 0.039

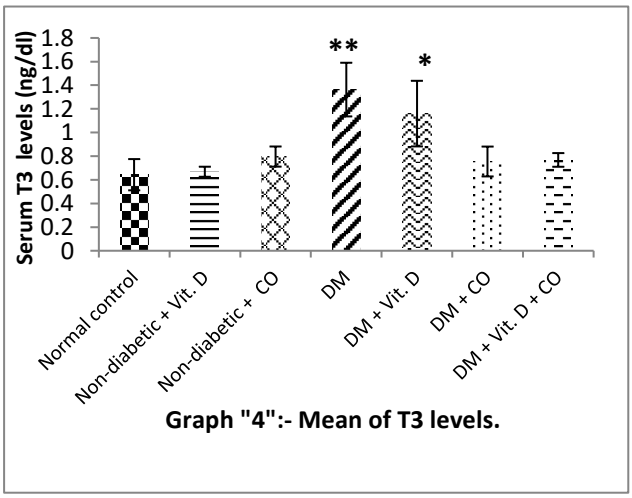


Graph "3":- Mean of TSH levels.

Statistical significance was measured at p > 0.05; *p ≤ 0.05

Table 4 & Graph 4: Effect of vitamin D or/and coconut oil on T3 levels:

Groups	Mean ± SE (ng/dl)
Group I (Normal control)	0.64 ± 0.13
Group II (Non-diabetic + Vit. D)	0.66 ± 0.04
Group III (Non-diabetic + CO)	0.79 ± 0.08
Group IV (DM)	1.36 ± 0.22**
Group V (DM + Vit. D)	1.16 ± 0.27*
Group VI (DM + CO)	0.75 ± 0.12
Group VII (DM + Vit. D + CO)	0.76± 0.05



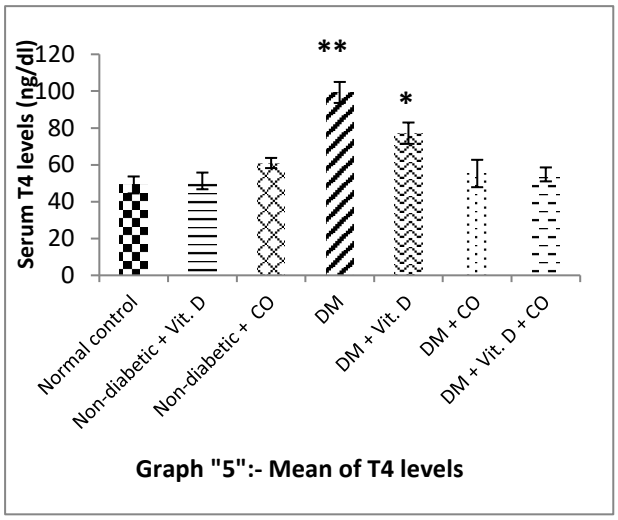
Graph "4":- Mean of T3 levels.

Statistical significance was measured at p > 0.05; *p ≤ 0.05;**p ≤ 0.001

ISSN: 0975-8585

Table 5 & Graph 5: Effect of vitamin D or/and coconut oil on T4 levels:

Groups	Mean ± SE (ng/dl)
Group I (Normal control)	49.08± 4.59
Group II (Non-diabetic + Vit. D)	51.26 ± 4.52
Group III (Non-diabetic + CO)	60.98 ± 2.76
Group IV (DM)	99.30 ± 5.69**
Group V (DM + Vit. D)	77.16 ± 5.80*
Group VI (DM + CO)	55.28 ± 7.43
Group VII (DM + Vit. D + CO)	54.80 ± 3.79



Graph "5":- Mean of T4 levels

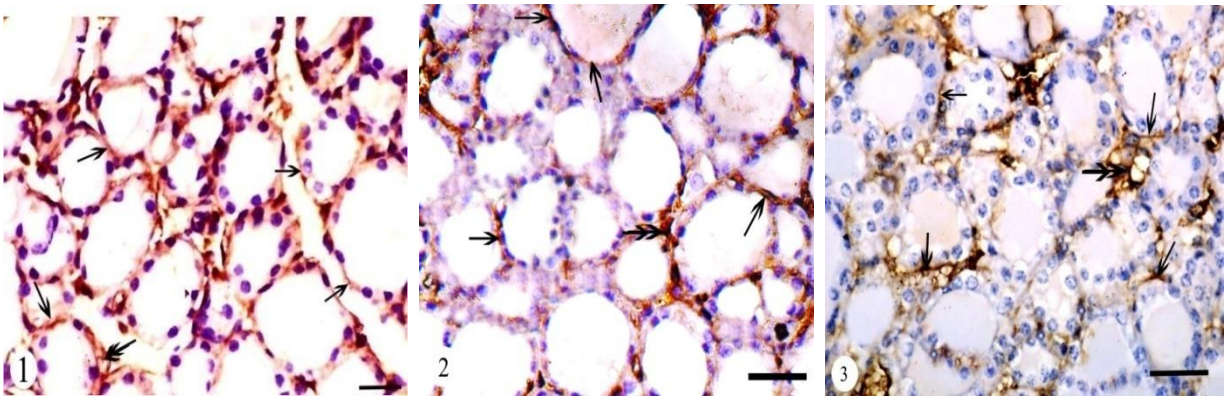
Statistical significance was measured at $p > 0.05$; $*p \leq 0.05$;$**p \leq 0.001$

Immunohistochemical results:

Vimentin:

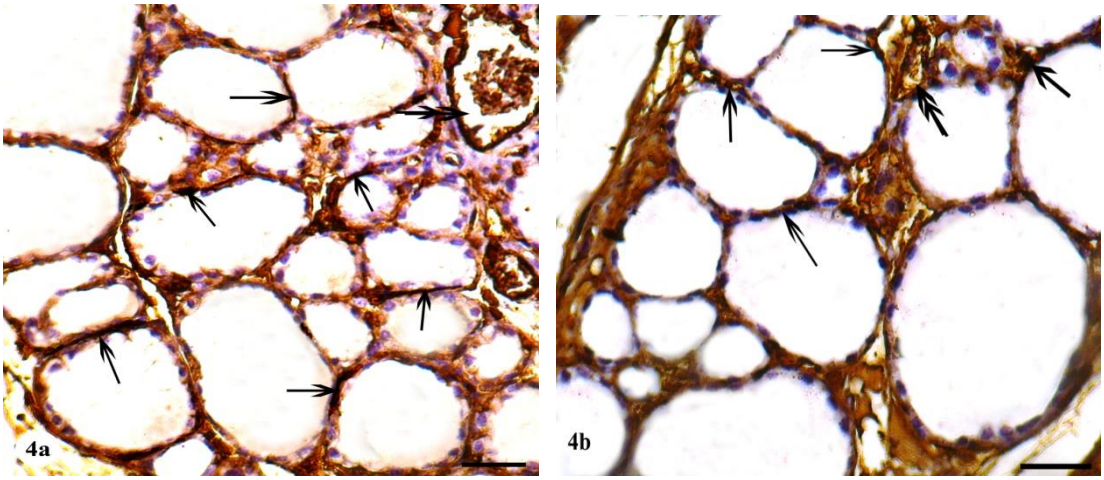
The normal control group and non-diabetic mice groups received either vitamin Din a dose of6.25 ml/kg bw/d or coconut oil in a dose of 7.5 ml/kg bw/d for 4 weeks expressed normal moderate immunoreactivity to vimentin filaments at the basal part of thyrocytes and in blood vessel walls as a brown filamentous colour by using avidin-biotin immune-peroxidase technique (Figs. 1 - 3).

STZ diabetic mice group expressed an obvious intense immunoreactivity to vimentin filaments in the connective tissue at the basal part of all follicles of the thyroid and in the dilated blood vessel walls (Fig. 4a&b).The administration of either vitamin D or coconut oil or both together daily for 4 weeks to diabetic mice expressed a marked improvement of vimentin immunoreactivity *in situ*. However, a marked recovery in vimentin immunostain was expressed in the thyrocytes of diabetic mice received either coconut oil alone or coconut oil with vitamin D together more than those given vitamin D alone(Figs.5 - 7).

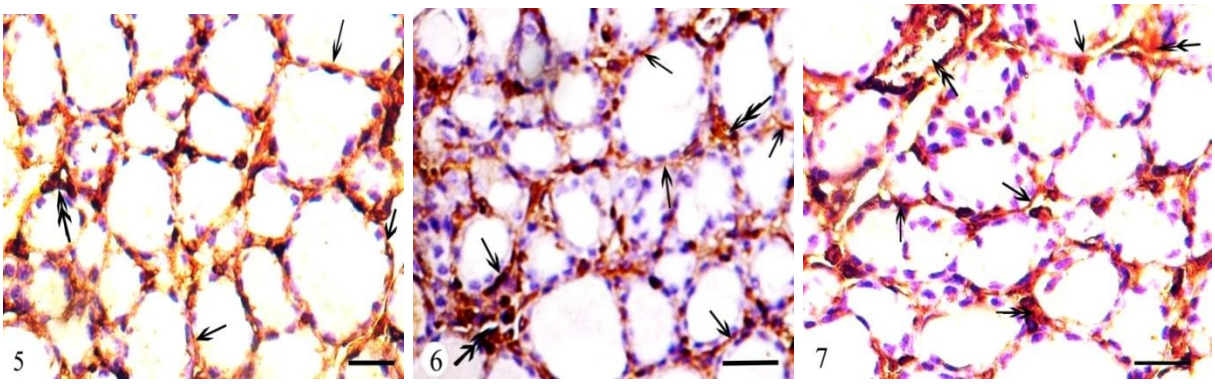


Figs. (1-3): Sections of the thyroid glands stained with vimentin immunostain expressing normal moderate immunoreactivity to vimentin filaments in the connective tissue at the basal part of thyrocytes (arrows) and in blood vessel walls (double arrows) in normal control mice (Fig. 1), in non-diabetic mice received vitamin D daily for 4 weeks (Fig. 2), and in non-diabetic mice received coconut oil daily for 4 weeks (Fig.3). Vimentin immunostain, all; Bars = 6.25 µm.

ISSN: 0975-8585



Figs. (4a&b): Sections of the thyroid glands of STZ diabetic mice expressing intense immunoreactivity to vimentin filaments in the connective tissue at the basal part of thyrocytes (arrows) and blood vessel walls (double arrows). Vimentin immunostain, Bar = 6.25 µm.



Figs. (5-7): Sections of the thyroid glands of diabetic mice given vitamin D or coconut oil or both together daily for 4 weeks and stained with vimentin immunostain expressing improvement of immunoreactivity to vimentin filaments in the connective tissue at the basal part of thyrocytes (arrows) and blood vessel walls (double arrows) received; Fig. 5: vitamin D expressing a reduction and improvement of vimentin immunoreactivity; Fig.(6): coconut oil expressing an obvious improvement of vimentin immunoreactivity, and Fig.(7): vitamin D and coconut oil together expressing a marked recovery of immunoreactivity to vimentin filaments. Vimentin immunostain, all; Bars = 6.25 µm.

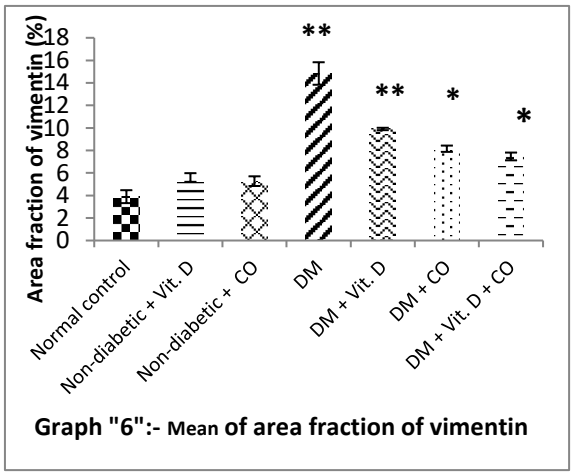
Image analysis of thyroids cytoskeletal vimentin:

The induction of diabetes in adult mice by STZ caused highly significant increase in the area of vimentin (Gp.IV) of the thyroid glands as well as in diabetic mice administered with vitamin D alone (Gp.V) as compared to the thyroids of normal control mice (Gp.I) (**p≤0.001); a significant increase in the area of thyroid vimentin (*p ≤0.05) of diabetic mice given coconut oil only (Gp.VI) or co-administered with vitamin D (Gp.VII) as compared to thyroid vimentin of normal control mice (Gp.I) (p >0.05)**, (**Table 6& Graph 6**).**

ISSN: 0975-8585

Table 6 & Graph 6: % of area (Mean ± SD) of vimentin expression in thyroid glands of all groups

Groups	Mean ± SE (mg/dl)
Group I (Normal control)	3.89 ± 0.57
Group II (Non-diabetic + Vit. D)	5.59 ± 0.35
Group III (Non-diabetic + CO)	5.27 ± 0.43
Group IV (DM)	14.83 ± 1.00**
Group V (DM + Vit. D)	9.90 ± 0.10**
Group VI (DM + CO)	8.15 ± 0.28*
Group VII (DM + Vit. D + CO)	7.46 ± 0.34*

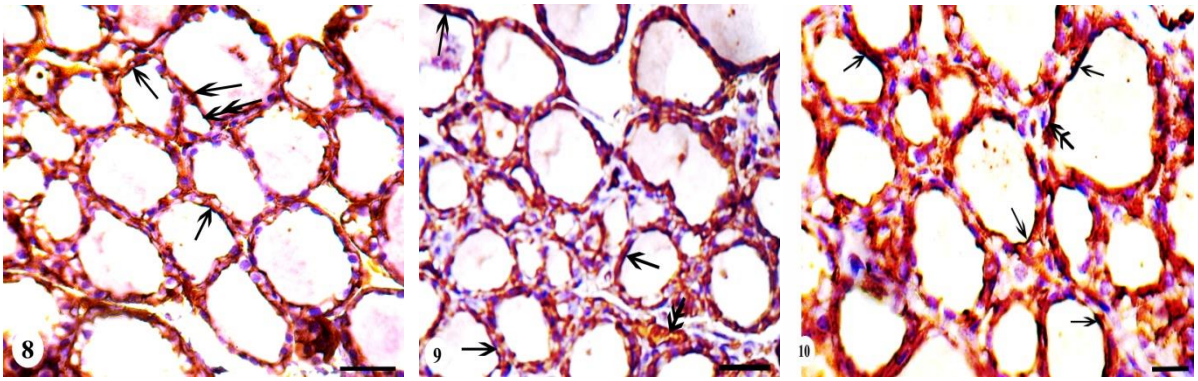


Graph "6":- Mean of area fraction of vimentin

Statistical significance was measured at p > 0.05; *p ≤ 0.05;**p ≤ 0.001

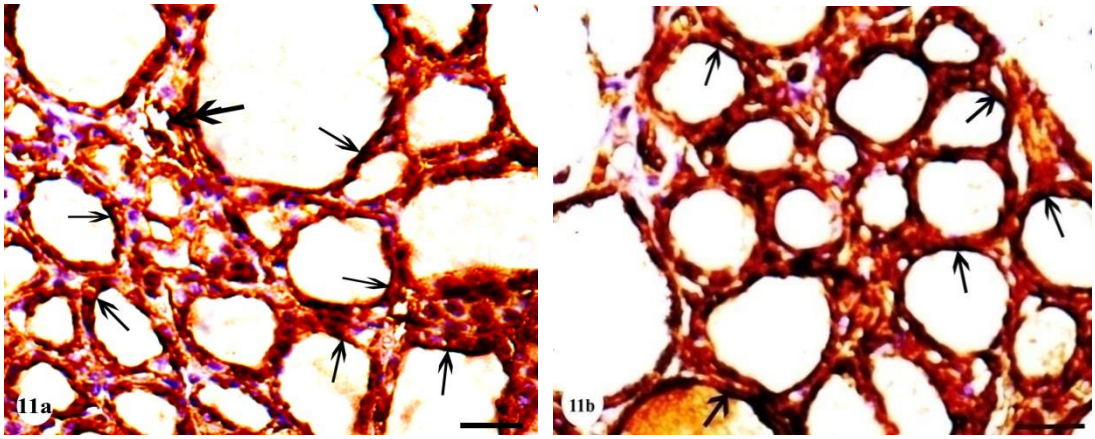
Cytokeratin:

The thyroid glands of normal control and non-diabetic mice received vitamin D or coconut oil expressed normal moderate cytokeratin filaments immunoreactivity as brown colour at the apical surface of epithelial thyrocytes and at the endothelia of blood vessels (Figs. 8 –10). STZ diabetic mice group revealed markedly more intense cytokeratin filaments immunoreactivity at the apical part of follicular cells (Fig. 11a&b). After administration of diabetic mice with vitamin D or coconut oil or both together daily for 4 weeks; a marked improvement and recovery of cytokeratin filaments immunoreactivity to approximately normal expression at the apical surface of epithelial follicular cells of either coconut oil or co-taken with vitamin D more than those given vitamin D alone(Figs. 12 – 14).

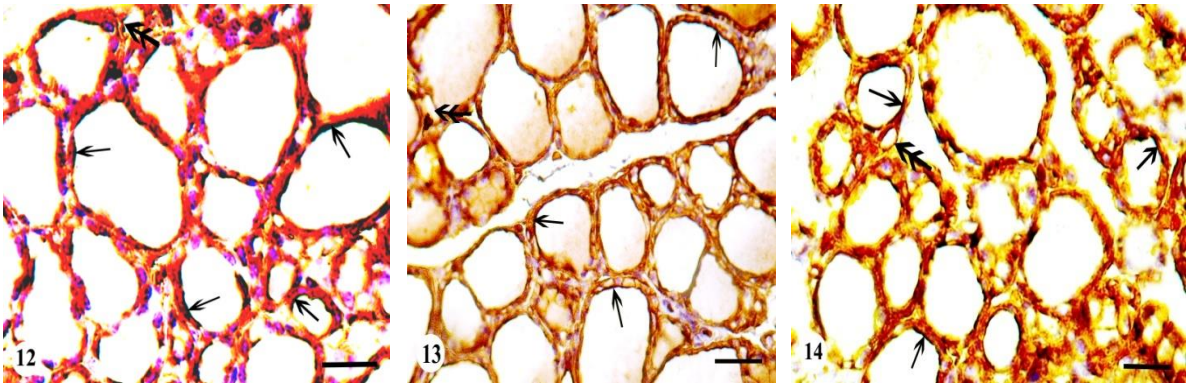


Figs. (8-10):Sections of the thyroid glands with cytokeratin immunostain expressing normal moderate immunoreactivity to cytokeratin at the apical of thyrocytes (arrows) and at the endothelia (double arrows) in normal control mice(Fig. 8), in non-diabetic mice received vitamin D (Fig. 9) or received coconut oil (Fig. 10) daily for 4 weeks. Cytokeratin immunostain, all; Bars = 6.25 µm.

ISSN: 0975-8585



Figs. (11a&b): Sections of the thyroid glands of STZ diabetic mice expressing an obvious intense immunoreactivity to cytokeratin at the apical part of follicular cells (arrows) and at the endothelia (double arrows). Cytokeratin immunostain, Bar = 6.25 µm.



Figs. (12-14): Sections of the thyroid glands of the treatment of STZ diabetic mice stained with cytokeratin immunostain expressing improvement of immunoreactivity to cytokeratin at the apical part of follicular cells (arrows)and at the endothelia (double arrows) of mice received; Fig. 12: vitamin D daily for 4 weeks expressing improvement and a decrease of immunoreactivity to cytokeratin, Figs. (13&14):coconut oil only or both vitamin D and coconut oil together daily for 4 weeks, respectively expressing an obvious improvement and recovery of immunoreactivity to cytokeratin to approximately normal expression. Cytokeratin immunostain, all; Bars = 6.25 µm.

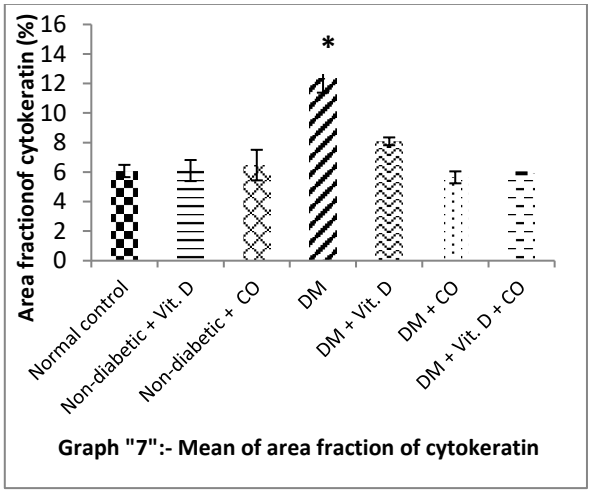
Image analysis of thyroids cytoskeletal cytokeratin:

Diabetic mice recorded a significant increase in the area of thyroid CK (Gp. IV) as compared to normal control mice (Gp.I)(*p ≤0.05); non-significant increase in the area of thyroid CK of diabetic mice administered with vitamin D alone (Gp.V)(p >0.05),and non-significant decrease in area of CK of diabetic mice given coconut oil only (Gp.VI) or co-administered of vitamin D and coconut oil (Gp.VII) as compared to normal control mice (p >0.05), (Tables 7& Graph 7).

ISSN: 0975-8585

Table 7 & Graph 7: % of area (Mean ± SD) of cytokeratin expression in thyroid glands of all groups:

Groups	Mean ± SE (mg/dl)
Group I (Normal control)	6.07 ± 0.41
Group II (Non-diabetic + Vit. D)	6.10 ± 0.71
Group III (Non-diabetic + CO)	6.47 ± 1.03
Group IV (DM)	12.37 ± 0.99*
Group V (DM + Vit. D)	8.08 ± 0.26
Group VI (DM + CO)	5.64 ± 0.40
Group VII (DM + Vit. D + CO)	5.91 ± 0.06



Graph "7":- Mean of area fraction of cytokeratin

Statistical significance was measured at $p > 0.05$; *$p \leq 0.05$;**$p \leq 0.001$

DISCUSSION

Diabetes mellitus (DM) and its complications constitute a severe public health issue facing modern societies**.** It is characterized by disarrangements in carbohydrates, proteins and fat metabolism caused by complete or partial insufficiency of insulin secretion and/or insulin action**.**DM later leads to micro and macro-vascular complications and becomes a major cause of death (36,37).

The present results of non-diabetic adult mice groups received vitamin D or coconut oil recorded non-significant changes in the blood glucose and insulin levels as compared to normal control group. A highly significant increase in blood glucose levels and a significant decrease in insulin were recorded in STZ- diabetic mice group as compared to the normal control ones. Diabetic mice received vitamin D only recorded a slight decrease in blood glucose levels and a slight increase in insulin; while the diabetic mice given coconut oil alone or co-administered with vitamin D recorded a significant decrease in blood glucose levels and a significant increase in insulin as compared to diabetic group.

Streptozotocin (STZ is glucosamine-nitrosourea compound which is toxic to the insulin-producing beta cells of the pancreas in mammals. It is used in medicine for treating certain cancers of the islets of Langerhans and used in medical research to produce an animal model for hyperglycemia by causing damage to the DNA (4,38).

Defective insulin secretion leads to various metabolic aberrations in type 2 DM, spanning from hyperglycemia due to defective insulin-stimulated glucose uptake and up regulated hepatic glucose production, along with dyslipidemia, which includes impaired homeostasis of fatty acids, triglycerides and lipoproteins (5,37).

There are possibilities that vitamin D could have a role on the prevention of the beginning of insulin resistance. Vitamin D may act directly to induce β-cell insulin secretion by increasing the intracellular calcium concentration via non-selective voltage-dependent calcium channels; or it may mediate activation of beta-cell calcium-dependent endo-peptidases to produce the cleavage that facilitates the conversion of pro-insulin to insulin (39). Hypovitaminosis D is associated with insulin resistance leading to DM. Vitamin D seems to affect the glucose homeostasis and diminishes stress in β cells which in turn avoids pancreatic cellular apoptosis (40,41).

Additionally, Siddalingaswamy *et al.* (29) observed gradual decrease in blood glucose in STZ diabetic mice after treated with coconut oil daily for 3 weeks. Iranloye *et al.* (42) also recorded that virgin coconut oil

alleviates hyperglycemia and improves glucose tolerance probably by its antioxidant effect which consequently leads to improvement of insulin secretion.

Coconut oil is rich with natural antioxidants that may play a vital role in improving insulin response to the loaded glucose and may reduce insulin resistance like cotrienols, capric acid, caproic acid, and lauric acid, which damage oxygen free radicals that have been suggested to play an important role in Diabetes mellitus (43). Lauric acid in coconut oil has insulin-tropic properties. The superior effect of coconut oil over calcium was probably because it contains high amount of saturated fats in the form of medium chain triglycerides that are important for calcium absorption from the intestines (30).

In the present results, the diabetic mice expressed significant decrease in thyroid stimulating hormone (TSH) levels and highly significant increase in triiodothyronine (T3) and thyroxine (T4) levels. The diabetic mice given vitamin D only daily for 4 weeks was not approximately effective, while the diabetic mice administered with coconut oil alone or co-administered with vitamin D daily for 4 weeks recorded a significant improvement of TSH, T3 and T4 levels approximately similar to normal control mice.

Similar results were recorded by Ray and Ghosh (44) and Ahmed *et al.* (45) who registered the relationship between thyroid disorders and DM; TSH was significantly lower while T3 and T4 were significantly higher in diabetic patients. They reported that serum T4 entirely originates from the thyroid gland, while more than 80% of T3 is produced by de-iodination of T4 in other tissues. Increment of serum T4 concentration suppresses TSH secretion through the pituitary thyroid feedback mechanism (46).

Insulin and thyroid hormones influence on the metabolism of carbohydrates, proteins, and lipids; hence their interrelation between DM and thyroid disorders (47,48).The hyperthyroidism is usually reserved for thyrotoxicosis caused by excessive production of thyroid hormone. Other forms of thyrotoxicosis include thyrotoxicosis factitia and those associated with different forms of thyroiditis. Overt thyrotoxicosis is defined as the syndrome of hyperthyroidism associated with suppressed TSH and elevated serum levels of T_4 or T_3. Subclinical thyrotoxicosis is devoid of symptoms, but TSH is suppressed although there are normal circulating levels of thyroid hormone (49,50).

Concerning to the cytoskeleton; the present study demonstrated that the thyroid glands of normal control or non-diabetic mice given either vitamin D or coconut oil expressed normal moderate immunoreactivity to vimentin filaments at the basal part of thyrocytes and in blood vessel walls, while CK immunoreactivity expressed at the apical part of follicular cells and at the endothelia of blood vessels. Diabetic mice expressed an obvious intense immunoreactivity to vimentin and CK filaments. However, after the administration of vitamin D or coconut oil or both together daily for 4 weeks to diabetic mice group, a marked improvement and recovery of thyroid vimentin and CK to approximately normal moderate immunostain expression in either coconut oil group or co-administered with vitamin D more than vitamin D group alone.

In accordance, El-Desouki *et al.* (51) reported that the intermediate filament proteins vimentin and CK were evaluated in the mesenchymal and epithelial cells, respectively of normal and pathologically changes in the thyroid tissues. Similar results were seen in the breast tumors using a pre-diabetic, hyperinsulinemic mouse model, that insulin promotes an increased expression of vimentin (52).

There is association between diabetes and stress as well as between stress and autoimmune thyroid disease (AITD) (especially Graves' hyperthyroidism)(53).Chronic stress increased serum corticosterone level (54); this increment leads to releasing corticosterone which induces insulin resistance, thus elevating the serum glucose(55). Also, El-Desouki *et al.* (56&57) reported the acute and chronic stress increased the levels of cortisol in male albino rats and subsequently the increment of the vimentin and CK intensity; intense CK filaments immunoreactivity was expressed at the apical part and lateral borders of the three types of gastric mucosal cells and the vimentin filaments manifested an obvious intense immunoreactivity in the lamina propria of gastric mucosa and in the blood vessels during stress as compared to the control ones(56). Moreover, the immobilized-stressed rats expressed obvious alterations in cytokeratin and vimentin immune reaction in the rat liver tissues. Also, Stress cause neurological disorders whose pathway included intermediate filaments accumulation (58).

Flitney *et al.* (59) also reported that the moderate stress applied to cells for a short period of time caused phosphorylation which was associated with the building of keratins into thick tonofibrils to enhance the ability of cells to resist mechanical stress. Vimentin is crucial for the attachment of lymphocytes to the vascular endothelium and transcellular migration of lymphocytes through endothelial cells (60).Moreover, the increment of prolactin hormone (hyperprolactemia) caused the accumulation of the immunostainto vimentin in mice thyrocytes (51).

Vitamin D3 supplementation to rat model has been found to reduce oxidative stress, improve cell viability and protect cells from death. The mechanisms involved include the prevention of free oxygen radical release and the modulation of the interplay between apoptosis and autophagy (61,62). The neuro-protective action of vitamin D3 is revealed through neuronal calcium regulation, antioxidative pathway, immune-modulation, and detoxification (62).

Virgin coconut oil (VCO) has a lot of benefits including antiulcerogenic, antinociceptive, anti-inflammatory, anti-hypercholesterolemic, antimicrobial and hepato-protective effects (63). Nutrition and antioxidant supplements have been considered to be beneficial for the recovery from oxidative stress (64). This effect may be attributed to the high medium-chain fatty acids that found in VCO which may help prevent the development of stress-induced depression in mice using a similar forced swim test model (65). Coconut oil can help normalize the function of thyroid glands**.** Coconut oil with its unique medium chain fatty acids raises both energy level and metabolism, improves the function of the mitochondria and producing energy inside each cell of the body and prevents both low blood sugar and high blood sugar preventing hypoglycemia and other blood sugar problems (42 ,66,67).

In conclusion, the changes in blood glucose, insulin, TSH, T3 and T4 levels of diabetic mice as well as the immunohistochemical changes in cytoskeletal vimentin and cytokeratin of thyroid glands were restored to normal after administration of coconut oil alone or co- administered with vitamin D, and demonstrated stronger anti-hyperglycemic effects than those taken vitamin D only.

REFERENCES

[1] Kitabchi, A.; Umpierrez, G.; Miles, J. and Fisher, J. (2009): Hyperglycemic crises in adult patients with diabetes. Diab. Care J., 32(7): 1335–1343.

[2] Derosa, G.; Liman, C. and Macias, P. (2014): Dietary and nutraceutical approach to type 2 diabetes. Arch. Med. Sci., 10(2): 336-344.

[3] De-Fronzo, R. (2004): Pathogenesis of type 2 Diabetes mellitus. Med. Clin., 88(4): 787–835.

[4] Goud, B. J. (2015): Streptozotocin - A diabetogenic agent in animal models. Human J., 3 (1): 253-269.

[5] Hegazi, R.; El-Gamal, M.; Abdel-Hady, N. and Hamdy, O. (2015): Epidemiology of and risk factors for type 2 diabetes in Egypt. Ann. Glob. Health, 81(6): 814-820.

[6] International Diabetes Federation, Diabetes Atlas (2017): Diabetes in Middle East and North Africa. 8th edn.. Available at: https:// www.diabetesatlas.org. pp. 1&2.

[7] Brenta, G.; Celi, F.; Pisarev, M.; Schnitman, M.; Sinay, I. and Arias, P. (2009): Acute thyroid hormone withdrawal in thyreotic patients results in a state of insulin resistance. Thyroid, 19(6): 665–669.

[8] Mishra, M.; Sotto, M.; Panta, R.; Miyares, M. and Solanki, R. (2017): Association of Diabetes mellitus and thyroid disorders: A metabolic prospective. Asian Pac. J. health Sci., 4(3): 253-262.

[9] Boron, M.; Walter, F. and Boulpaep, L.(2012): Medical Physiology, 2nd edn, Philadelphia, Saunders, p. 1052.

[10] Hassan, N. M., Harbi, S. O., Shomo, A. I., El-Tahir , Y. I. and Ahmed, M. E. (2014): Cytokeratin 18 and vimentin expression in breast cancer tissue from Sudanese patients. Ann. Biol. Res., 5(1): 9 – 16.

[11] Kumar, N.; Robidoux, J.; Daniel, K. W.; Guzman, G.; Floering, L. M. and Collins, S. (2007): Requirement of vimentin filament assembly for beta3-adrenergic receptor activation of ERK MAP kinase and lipolysis. J. Biol. Chem., 282: 9244-9250.

[12] Baharami, A.; Truong, L. D. and Roj, y. (2008): Undifferentiated tumor: True identity by immunohistochemistry. Arch. Pathol. Lab. Med., 132: 326-348.

[13] Eriksson, J.; Dechat, T.; Grin, B.; Helfand, B.; Mendez, M.; Pallari, H. and Goldman, R. (2009):Introducing intermediate filaments: from discovery to disease. J. Clin. Invest., 119 (7): 266-371.

[14] Katsumoto, T.; Mitsushima, A. and Kurimura, T. (1990): The role of the vimentin intermediate filaments in rat 3Y1 cells elucidated by immunoelectron microscopy and computer graphic reconstruction. Biol. Cell, 68 (2): 139–146.

[15] Ogrodnik, M.; Salmonowicz, H.; Brown, R.; Turkowska, J.; Sredniawa, W.; Pattabiraman, S.; Amen, T.; Abraham, A.; Eichler, N.; Lyakhovetsky, R. and Kaganovich, D. (2014): Dynamic JUNQ inclusion bodies are asymmetrically inherited in mammalian cell lines through the asymmetric partitioning of vimentin. Proc. Nat. Acad. Sci., 111(22): 8049-8054.

[16] Karantza, V. (2011): Keratins in health and cancer: more than epithelial cell markers. Oncogene, 30: 127-138.

[17] Herrmann, H.; Bär, H.; Kreplak, L. Strelkov, S. and Aebi, U. (2007): Intermediate filaments: from cell architecture to nano-mechanics. Nat. Rev. Mol. Cell Biol., 8 (7): 562–573.

[18] Rekhtman, N. and Justin, A. (2011): Quick Reference Handbook for Surgical Pathologists. 1stedn, Heidelberg, pp: 4–8.

[19] Miettinen, M., K. Franssila, V.P. Lehto, R. Paasivuo and I. Virtanen, (1984): Expression of intermediate filament proteins in thyroid gland and thyroid tumors Lab. Invest., 50(3): 262-270.

[20] Holick, M. (2006): High prevalence of vitamin D inadequacy and implications forhealth.Mayo. Clin. Proc., 81 (3): 353–373.

[21] Tripkovic,L.; Lambert, H.; Hart, K.; Smith, C.; Bucca, G.; Penson, S.; Chope, G.; Hyppönen, E.;Berry, J.; Vieth, R. and Lanham, S. (2012): Comparison of vitamin D2 and vitamin D3 supplementation in raising serum 25-hydroxyvitamin D status: a systematic review and meta-analysis. Am. J. Clin. Nutr., 95: 1357–1364.

[22] Hilger, J.; Friedel, A.; Herr, R.; Rausch, T.; Roos, F. and Wahl, D. (2013): A systematic review of vitamin D status in populations worldwide. Br. J. Nutr., 9: 1–23.

[23] Bland, R.; Markovic, D.; Hills, C.; Hughes, S.; Chan, S.; Squires, P. and Hewison, M. (2004): Expression of 25-hydroxyvitamin D3-1alpha-hydroxylase in pancreatic islets. J. Ster. Biochem. Mol. Biol., 1(5): 89-90,121-125.

[24] Al-Shoumer, K. and Al-Essa, T. (2015): Is there a relationship between vitamin D with insulin resistance and Diabetes mellitus?.World J. Diab., 6(8): 1057–1064.

[25] Lazo, S. and Dayrit, C. (1998): Tolerability and bioavailability testing of monoglyceride of lauric acid: A preliminary report, Philippine J. Cocon. Stud. "PJCS", 2: 21-22.

[26] Nevin, K. and Rajamohan, T. (2004): Beneficial effects of virgin coconut oil on lipid parameters and *in vitro* LDL oxidation. Clin. Biochem., 37: 830-835.

[27] Law, K.; Azman, N.; Omar, E.; Musa, M.; Yusoff, N.; Sulaiman, S. and Hussain, N. (2014): The Effects of virgin coconut oil (VCO) as supplementation on quality of life (QOL) among breast cancer patients. Lip. Heal. Dis., 13: 139-144.

[28] Fernando, W.; Martins, I; Goozee, K.; Brennan, C.; Jayasena, V. and Martins, R. (2015): The role of dietary coconut for the prevention and treatment of Alzheimer's disease: potential mechanisms of action. Brit. J. Nut., (1): 1-14.

[29] Siddalingaswamy, M.; Rayaorth, A. and Khanum, F. (2011): Anti-diabetic effects of cold and hot extracted virgin coconut oil. Sci. Res. J. Diab., 1(4): 118- 123.

[30] Hayatullina, Z.; Muhammad, N. and Soelaiman, I. (2012): Virgin coconut oil supplementation prevents bone loss in osteoporosis rat model. Comp. Alter. Med., 3: 1-8.

[31] Deeds, M.; Anderson, J.; Armstrong, D.; Gastineau, H.; Hiddinga, A.; Jahangir, N. and Kudva, Y. (2011): Single dose streptozotocin induced diabetes: Considerations for study design in islet transplantation models; Lab. Anim., 45(3): 131–140.

[32] Hepburn, S.; Farid, S.; Dawson. J, and Goodall, S. (2012): Thyroid function testing. Br. J. Hosp. Med., 73(8): 114–118.

[33] La-Flamme, J. (1990): The Preparation of Materials for Microscopic Study. In L. J. Jamboran D. D. J. Vaughan.3rdedn, Advanced Microscopic Studies of Ore Minerals. Mineralogical Association of Canada Short Course Handbook, 17, pp: 37-68.

[34] Hsu, S.M.; Raine, L. and Fanger, H. (1981): Use of avidin-biotin peroxidase complex (ABC) in immunoperoxidasetechniques.A comparison between ABC and unlabeled antibody (PAP) procedures. J. Histochem. Cytochem., 29: 557-580.

[35] Schindelin, J.; Arganda-Carreras, I.; Frise, E.; Kaynig, V.; Longair, M.; Pietzsch, T.; Preibisch, S.; Rueden, C.; Saalfeld, S.; Schmid, B.; Tinevez, J.; White, D.; Hartenstein, V.; Eliceiri, K.; Tomancak, P. and Cardona, A. (2012): Fiji: an open-source platform for biological-image analysis. Nat. Methods, 9(7): 1-15.

[36] Centers for Disease Control and Prevention "CDC". (2015): Basics about diabetes. www.cdc.gov/diabetes/basics/diabetes.html
[37] Ullah, A.; Khan, A. and Khan, I. (2016): Diabetes mellitus and oxidative stress –A concise review. Saudi Pharm. J., 24: 547–553.
[38] Brentjens, R. and Saltz, L. (2001): Islet cell tumors of the pancreas: the medical oncologist's perspective. Surg. Clin.North. Am., 81(3): 527–542.
[39] Krishna, G.; Bubblu, T. and Amarabalan, R. (2011): Role of vitamin D in diabetes. J. Endocrinol. Metab., 1(2): 47-56.
[40] Henrique, L.; Bandeira, F.; Andrade, M. and Gabbay, L. (2014): Vitamin D and Diabetes mellitus.Arq. Bras. Endocrinol. Metab., 58(1): 1-8.
[41] Souza, C.; Cunha de- Sá, L.; Rocha, D. and Arbex, A. (2016): Vitamin D and Diabetes mellitus. Endocrinol. Metab., 6: 1-7.
[42] Iranloye1, B.; Oludare1, G. and Olubiyi, M. (2013): Anti-diabetic and antioxidant effects of virgin coconut oil in alloxan induced diabetic male Sprague Dawley rats. Diab. Mel. J., 3(4): 221-226.
[43] St-Onge, M.; Ross, R.; Parsons, W. and Jones, P. (2003): Medium chain triglycerides increases energy expenses and decreases adiposity in over weight men. Obes. Res., 11: 395-402.
[44] [44] Ray, S. and Ghosh, S. (2016): Thyroid disorders and Diabetes mellitus: double trouble. J. Dia. Res. Ther., 2(1): 113-121.
[45] Ahmed, A.; Mohamed, S.; Elmadi, S.; Abdorabo, A.; Ismail, M. and Ismail, A. (2017): Assessment of thyroid dysfunctions in type 2 Diabetes Mellitus patients in surman, western-Libya. Int. J. Clin. Exper. Med. Sci., 3(1): 1-4.
[46] Da-Costa, V.; Moreira, D. and Rosenthal, D. (2001): Thyroid function and aging: gender-related differences. J. Endocrinol., 171: 193-198.
[47] Athanasia, P.; Alexios, S.; Anthi, K.; Marina, K.; Petroula, S. and Stavros, P. (2010): Prevalence of Thyroid dysfunction among greek type 2 diabetic patients attending an outpatient clinic. Cli. Med. Res. J., 2 (2): 75-78.
[48] Singh, G.; Gupta, V.; Sharma, A. and Gupta, N. (2011): Evaluation of thyroid dysfunction among type 2 diabetic Punjabi population. Advan. Biores. J., 2 (2): 3-9.
[49] Asmabi, M.; Amit, S.; Shivaleela, B. and Swaliha, Q. (2015): Effect of thyroid dysfunction on metabolic response in type 2 diabetic patients. Uni. J. Med. Dent. Sci., 3(1): 65-69.
[50] Yamada, M.; Shibata, H.; Masugi, Y.; Ishi, M.; Kameyama, K.; Ebinuma, H. and Hasegawa, T. (2017): Histological changes in autoimmune hepatitis with graves'disease: A child case report. Intern.Med., 56: 2139-2143.
[51] El-Desouki, N. I.; Salem, M. L.; Nasef, M. and Abdallah, F. M. (2016): Hyperprolactinemia induced histological and cytoskeletal vimentin alterations in mice thyroid glands. Intern. J. Sci. Eng. Res., 7(4): 170: 180.
[52] Zelenko, Z.; Gallagher, E.; Tobin-Hess, A.; Belardi, V.; Rostoker, R.; Blank, J.; Yemisi, D. and Le-Roith, D. (2017): Silencing vimentin expression decreases pulmonary metastases in a pre-diabetic mouse model of mammary tumor progression. Oncogene, 36 (10): 1394–1403.
[53] Effraimidis, a.; Jan, G.; Tijssen, b.; Jos, F.; Brosschot, C. and Wiersinga, M. (2012): Involvement of stress in the pathogenesis of autoimmune thyroid disease: A prospective study. Psychoneuroendocrinology, 37: 1191-1198.
[54] Gamaro, G.; Manoli, L.; Torres, I.; Silveira, R. and Dalmaz, C. (2003): Effects of chronic variate stress on feeding behavior and on monoamine levels in different rat brain structures. Neurochem. Int., 42: 107–114.
[55] Kenjale, R.; Shah, R. and Sathaye, S. (2007): Anti-stress and anti-oxidant effects of roots of *Chlorophytumborivilianum.* Indian Exp. Biol. J., 45: 974–979.
[56] El– Desouki, N. I.; El-Refaiy, I. A.; Sayed, G. M.; Ibrahim, M. A. and Mohamed, H. N. (2013): Effect of immobilization stress on the cytoskeletal intermediate filaments of rat stomach and the possible curative role of diazepam. Life Sci. J., 10(2): 2211- 2219.
[57] El-Desouki, N. I.; Gabry, M. S. and Nagi, H. M. (2015): Beneficial role of diazepam in the histological alterations of colon post immobilization stress-induced in adult albino rats. IJSER, 6 (5): 432-440.
[58] Israeli, E.; Dryanovski, D.; Schumacker, P.; Chandel, N.; Singer, J.; Julien, J.; Goldman, R. and Opal, P. (2016): Intermediate filament aggregates cause mitochondrial dysmotility and increase energy demands in giant axonal neuropathy. Hum. Mol. Genet., 10: 1-10.

[59] Flitney, E. W.; Kuczmarski, E. R.; Adam, S. A. and Goldman, R. D. (2009): Insights into the mechanical properties of epithelial cells: the effects of shear stress on the assembly and remodeling of keratin intermediate filaments. FASEB J., 23(7): 2110-2119.

[60] Nieminen, M.; Henttinen, T.; Merinen, M.; Marttila-Ichihara, F.; Eriksson, J. E. and Jalkanen, S. (2006): Vimentin function in lymphocyte adhesion and transcellular migration. Nat. Cell Biol., 8: 156-162.

[61] Wang, Y.; Chiang, Y. H.; Su, T. P.; Hayashi, T.; Morales, M. and Hoffer, B. J. (2000): Vitamin D3 attenuates cortical infarction induced by middle cerebral arterial ligation in rats. Neuropharmacology, 39(5): 873–880.

[62] Uberti, F.; Lattuada, D.; Morsanuto, V.; Nava, U.; Bolis, G. and Vacca, G. (2014): Vitamin D protects human endothelial cells from oxidative stress through the autophagic and survival pathways. J. Clin. Endocrinol. Metab., 99 (4): 1367–1374.

[63] Zakaria, Z. A. and Somchit, M. N. (2011): *In vivo* anti-nociceptive and anti-inflammatory activities of dried and fermented processed virgin coconut oil. Med. Princ. Pract., 20: 231–236.

[64] Choi, E. H; Kang, J. I.; Cho, J. Y.; Lee, S. H.; Kim, T. S.; Yeo, I. H. and Chun, H. S. (2012): Supplementation of standardized lipid-soluble extract from maca (*Lepidiummeyenii*) increases swimming endurance capacity in rats. J. Funct. Foods, 4: 568–573.

[65] Shinohara, H.; Fukumitsu, H.; Seto, A. and Furukawa, S. (2013): Medium-chain fatty acid-containing dietary oil alleviates the depression-like behaviour in mice exposed to stress due to chronic forced swimming. J. Funct. Foods, 5: 601–606.

[66] Vanderpas, J. (2006): Nutritional epidemiology and thyroid hormone metabolism. Annu. Rev. Nutr., 26: 293-322.

[67] Boateng, L.; Ansong, R.; Owusu, E. and Steiner-Asiedu, M. (2016): Coconut oil and palm oil's role in nutrition, health and national development.Ghana, Med. J., 50(3): 189–196.