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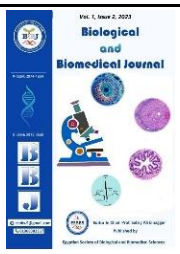

The helpful effect of vitamin D and coconut oil in modulating the histological disorders of the splenic tissue in hyperglycemic mice

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ABSTRACT

The present study aims to study the effect of vitamin D (Vit. D) or/and coconut oil (C.O) on the splenic histological changes of diabetic adult mice induced by streptozotocin (STZ). The mice were divided into 7 groups (n=10), and the experimental duration was 4 weeks. Group 1 (GpI): control group without any treatment; GpII and GpIII: non-diabetic groups, orally received Vit. D in a dose of 500 IU (6.25 ml)/kg b.w/d or C.O in a dose of 7.5 ml /kg b.w/d ,respectively; GpIV: diabetic group injected i.p. with a single dose of STZ (200 mg/kg bw). Gps V, VI and VII: administration of Vit. D or C.O or both together to diabetic group respectively. The results showed no significant changes in the blood glucose (BG), insulin, and splenic weight in GpII and GpIII. GpIV showed a significant increase in BG, significant decrease in insulin levels, and splenic weight values. GpV recorded a moderate decrease in BG, and a moderate increase in insulin levels and splenic weight atrophy. While GpVI or GpVII showed a marked decrease in BG and an increase in insulin levels and splenic weight increment. Histologically, the splenic sections of control or non-diabetic mice received either Vit. D or C.O showed normal structure of the splenocytes. GpIV showed great numbers of giant cells, disarrangement, interference of red and white pulps, and dilated congested blood vessels. Mild improvement was seen in GpV, while in GpVI or GpVII showed a marked improvement in the splenic tissues. In conclusion, diabetic mice received Vit. D/C. O showed strong anti-hyperglycemic effects to restore the glucose and insulin levels close to normal levels. It also restored the splenic weight and histological architecture close to normal status than those administered Vit. D alone.

Keywords:

Hyperglycemia, Spleen, Histology, Glucose, Insulin, Vitamin D, Coconut, Mice

1. Introduction

Hyperglycemia is the hallmark of the most prevalent and dangerous metabolic illness in humans known as diabetes mellitus (DM) disorders (Njolstad et al., 2003). Furthermore, other symptoms such as increase insulin degradation and glucagon secretion enhance catecholamines and insulin resistance, accelerated atherosclerosis development, and cardiovascular risk (Goud, 2015; Galicia-Garcia et al., 2020). Egypt had the eighth-highest rate of diabetes in the world. 15.6% of all adults in Egypt between the ages of 20 and

79 had type-2 diabetes (T2-DM) in 2015 (Hegazi et al., 2015). In 2017, there were 8.222.600 cases of diabetes in Egypt, and this number is expected to double by 2035 (IDF, 2017). The spleen is the largest secondary lymphoid organ in the body. It plays a main role in immune functions besides its roles in haematopoiesis and red blood cell clearance. It recycles iron while discarding old red blood

cells and keeping a blood reserve that might be useful in the case of hemorrhagic shock. It destroys hemoglobin that has been taken out of senescent red blood cells as a component of the mononuclear phagocyte system (Lewis et al., 2019). The heme part of hemoglobin is converted into bilirubin, which is expelled in the liver, while the globin part is reduced to its native amino acids (Swirski et al., 2009). The spleen produces antibodies in its white pulp and expels pathogens and antibody-coated blood cells through lymphatic and blood circulation (Rashad et al., 2020). It has been reported that half of the body's monocytes are found in the spleen's crimson pulp (Jia and Pamer, 2009). When these monocytes move to injured tissue, such as the heart after a myocardial infarction, they develop into dendritic cells and macrophages that help the body repair (Brender et al., 2005) .

Vitamin D (Vit. D) is fat-soluble and boosts calcium, magnesium, phosphate, and zinc absorption in the intestine (Holick, 2006). Different types of Vit. D exist (vitamers). There are two main types of Vit. D: Vit. D2 (ergo-calciferol) and Vit. D3. Without a subscript, Vit. D refers to calciferol, which is the chemical name for either Vit. D2 or D3 or both (Tripkovic, 2012). The biological inactivation of Vit. D from the diet or cutaneous production from sunshine involves an enzymatic conversion throughout the kidney and liver (Berger et al., 2022). Vit. D's effects on insulin secretion and sensitivity may allow it to operate as a functional agent in the regulation of glucose tolerance. Vit. D insufficiency inhibits pancreatic insulin production, and this deficiency is linked to glucose intolerance and T2-DM (Al-Shoumer and Al-Essa, 2015). Vit. D supplementation is considered as a therapeutic agent to T2-DM; it can decrease the oxidation stress in diabetic patients and stimulates the insulin release (Hu et al., 2019; Fathi et al., 2022). Coconut oil (C.O) functions as hormonal support for immune system since they have antifungal, antibacterial, and antiviral effects (Lazo and Dayrit, 1998). C.O oil is great for digestion since it contains a lot of healthy lipids. The best source of lauric acid is C.O. Its antibacterial qualities can aid in the battle against Candida infection and stomach discomfort (Khunnamwong et al., 2015). The antioxidant activities in C.O accomplished through the scavenging reactive oxygen species (ROS) which in turn led to reduce insulin resistance (Siddalingaswamy et al., 2011). C.O improves insulin levels and increases absorption of calcium and magnesium. The superior effect of C.O over calcium is probably because it contains high amount of saturated fats in the form of medium chain triglycerides that are important for calcium absorption from the intestine (Hayatullina et al., 2012). C.O supplementation in a diabetic patient can control the glycemic level (Malaeb and Spoke, 2020). The aim of the work is to study the beneficial role of Vit. D or/and C.O on the blood glucose, insulin levels, in the weight and histopathology of the spleen of STZ-diabetic adult male albino mice.

2. Materials and Methods

Animals

Seventy male albino mice (CD-1) were purchased from Vacsera, Cairo, Egypt, each weighing 25±2 g and aged 6-8 weeks. For one week, the animals were acclimated in plastic cages at the same temperature and with the same natural dark-light cycle. The animals had unrestricted access to food and water during the experiment. The Institutional Animal Ethics Committee of Tanta University approved all care and techniques used for the current experiment, and they also complied with guidelines for the humane handling and use of laboratory animals.

Induction of Diabetes mellitus (DM)

Streptozotocin (STZ) was acquired from Sigma Chemicals Co., (St. Louis, Mo., USA). STZ was dissolved in saline solution at a dose of 200 mg/kg of body weight to induce DM in mice by intraperitoneal (i.p.) injection (Deeds et al., 2011).

Treatment

Vit. D was obtained from "Egyptian Group for Pharmaceutical Industries Co., Egypt." and administered orally. C.O was received from local pharmacy (Al-Badawia Company for Herbal and Oil Extraction, Mansoura, Egypt) and administered orally, by a gastric tube.

Experimental design

After acclimatization, the mice were divided into 7 equal groups (n=10); all were kept under the same conditions and received the same diet. GpI: Normal control mice group was left without any treatments. GpII: Non-diabetic group received vit. D orally in a dose of 500 international units (6.25 ml)/kg/b.w/d for 4 weeks. GpIII: 7.5 ml/kg b.w. of C.O was administered daily to the non-diabetic mice for 4 weeks. GpIV: Diabetic group received a single injection of STZ (200 ml/kg body weight) i.p. GpV: For four weeks, diabetic mice were administered 500 international units (6.25 ml/kg body weight) of Vit. D orally. GpVI: Diabetic mice administered orally C.O in a dose of 7.5 ml /kg/b.w/d for 4 weeks. GpVII: Diabetic mice administered orally with both vit. D and C.O at the same previous doses daily for 4 weeks.

Sample collection and sera separation

The animals were fasted for 14 hours at the end of each session of the experiment, and were anaesthetized with diethyl ether, and then sacrificed. Blood samples were collected from all groups. Before centrifuging the blood for 20 minutes at 1000 rpm, the blood was allowed to clot for 30 minutes at room temperature. Serum samples were then taken and stored at -20 °C until they were used for insulin assay. The spleen specimens were removed and weighted then processed for histological studies (La-Flamme, 1990).

Histological study

Splenic specimens were fixed in 10% neutral buffered formalin for 24 hours, then they were processed in ethyl alcohol in progressive grades, cleared in xylene, embedded in paraffin wax, sectioned at a thickness of 5µm, then stained with H&E (Bancroft and Gamble, 2002) to demonstrate the histological changes. For each pair of duplicate standards, control samples, and the average optical density of the zero standards, the mean absorbance was calculated. On a log-log graph paper, the standard curve was drawn with the common concentration on the X-axis and the absorbance on the Y-axis. The best-fit straight line through the reference points were created considering Bourne, (2007).

Statistical analysis

The IBM SPSS® software package, version 16.0, USA, was used to enter data into the computer and analyse it. S.E. was used to the data analysis together with percentages and numbers. When the data were evenly distributed, the F-test (ANOVA) and Post Hoc test were employed to compare the seven research groups (LSD), according to significance was achieved at p 0.05 (Diggle and Liang, 1994).

3. Results

Effect of vitamin D and coconut oil on blood glucose levels

The induction of diabetes in adult mice by STZ caused significant increase in blood glucose level (GpIV) as compared to the control mice (GpI) (**$p \leq 0.001$). There was significant increase in blood glucose values of diabetic mice administered with Vit. D alone (GpV) (*$p \leq 0.05$) and non-significant increase in mice blood glucose levels of diabetic mice given C.O only (GpVI) or co-administered with Vit.D and C.O together (GpVII) as compared to the normal control mice ($p > 0.05$) (Fig. 1).

Effect of vitamin D and coconut oil on insulin levels

Diabetic mice recorded a significant decrease in blood serum insulin (GpIV) as compared to control mice (GpI) (*$p \leq 0.05$). In addition, a mild decrease in blood serum insulin of the diabetic groups administerd Vit. D or/and C.O (Gps V, VI and VII) was also recoded, as compared to control mice (GpI) ($p > 0.05$) (Fig. 2).

Effect of vitamin D or/and C.O on spleen weight

The induction of diabetes in adult mice by STZ caused highly significant decrease in spleen weight values (GpIV) as compared to the control mice (GpI) (**$p \leq 0.001$). Additionally, a significant decrease in spleen weight values of diabetic mice administered with vit. D alone (GpV) (*$p \leq 0.05$) and a mild decrease in spleen weight values of diabetic mice administered C.O only (GpVI) or administered Vit. D and C.O together (GpVII) were also recorded as compared to the control mice ($p > 0.05$) (Fig. 3).

Histological observations

Control mice group (GpI), the histological structure of spleen sections of a normal control mouse stained with H & E revealed normal structure of splenocytes with normal appearance of periarteriolar lymphoid sheaths of white pulp, red pulp and marginal zone between the red pulp and white pulp. The entire spleen is surrounded by a dense connective tissue capsule from which emerge trabeculae into the splenic parenchyma (Fig. 4a and b). Non-diabetic mice administered either vitamin D or coconut oil (GPII and III).

The histological structures of the spleen sections of non-diabetic mice received vit. D in a dose of 6.25 ml /kg b.w/d or C.O in a dose of 7.5 ml/kg b.w/d, respectively for 4 weeks illustrated similar normal structure as seen in control ones (Fig. 5a, b and 6a, b). The diabetic mice group (GPIV), injected with a single dose of STZ (200 mg/kg/b.w) showed great numbers of giant cells, disarrangement and interference of red and white pulps between them. Dilated and congested blood vessels as well as thin capsule and thick branched trabeculae were also noticed (Fig. 7a and b). The diabetic mice group given Vit. D (GPV). The diabetic mice administered with Vit. D alone in a dose of 6.25 ml/kg b.w daily for 4 weeks demonstrated giant cells and interference between red and periarteriolar lymphoid sheaths of white pulps. Either normal thin capsule or trabeculae were seen (Fig. 8a and b).

The diabetic mice groups administered coconut oil alone or coconut oil with vitamin D (Groups VI and VII). The diabetic mice given C.O alone or C.O administered with Vit. D daily for 4 weeks showed a marked recovery and improvement of red pulps, marginal zone, and white pulps, and restored approximately their normal histological structure. Normal thin capsule and thin trabeculae were illustrated (Fig. 9 and 10).

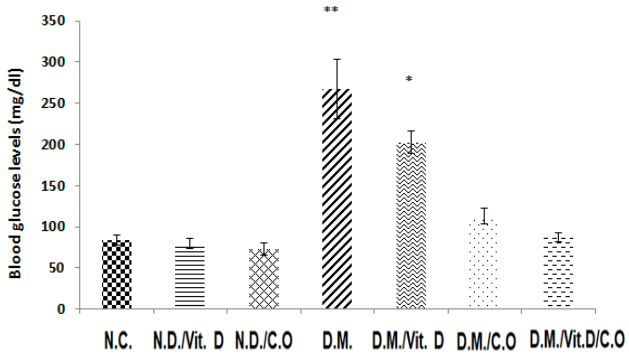


Fig. 1. Effect of Vit. D or/and coconut oil on blood glucose levels. Statistical significance was measured at $p > 0.05$; $*p \leq 0.05$;$**p \leq 0.005$

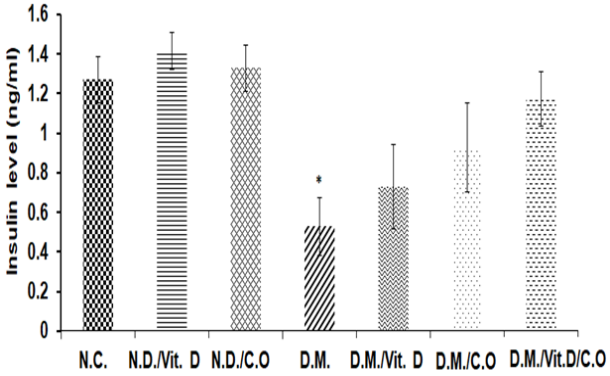


Fig. 2. Effect of Vit. D or/and coconut oil on insulin levels .Statistical significance was measured at $p > 0.05$; $*p \leq 0.05$

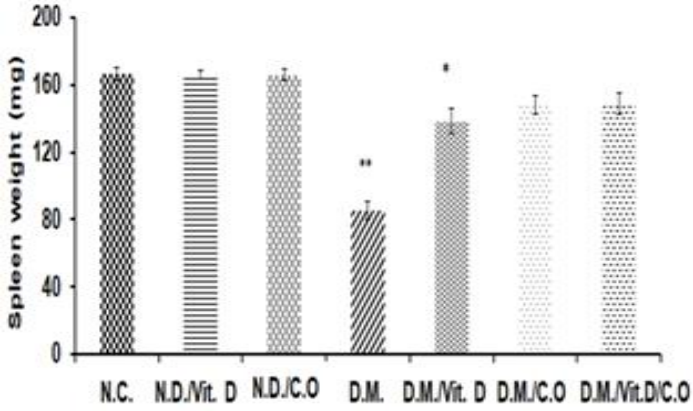
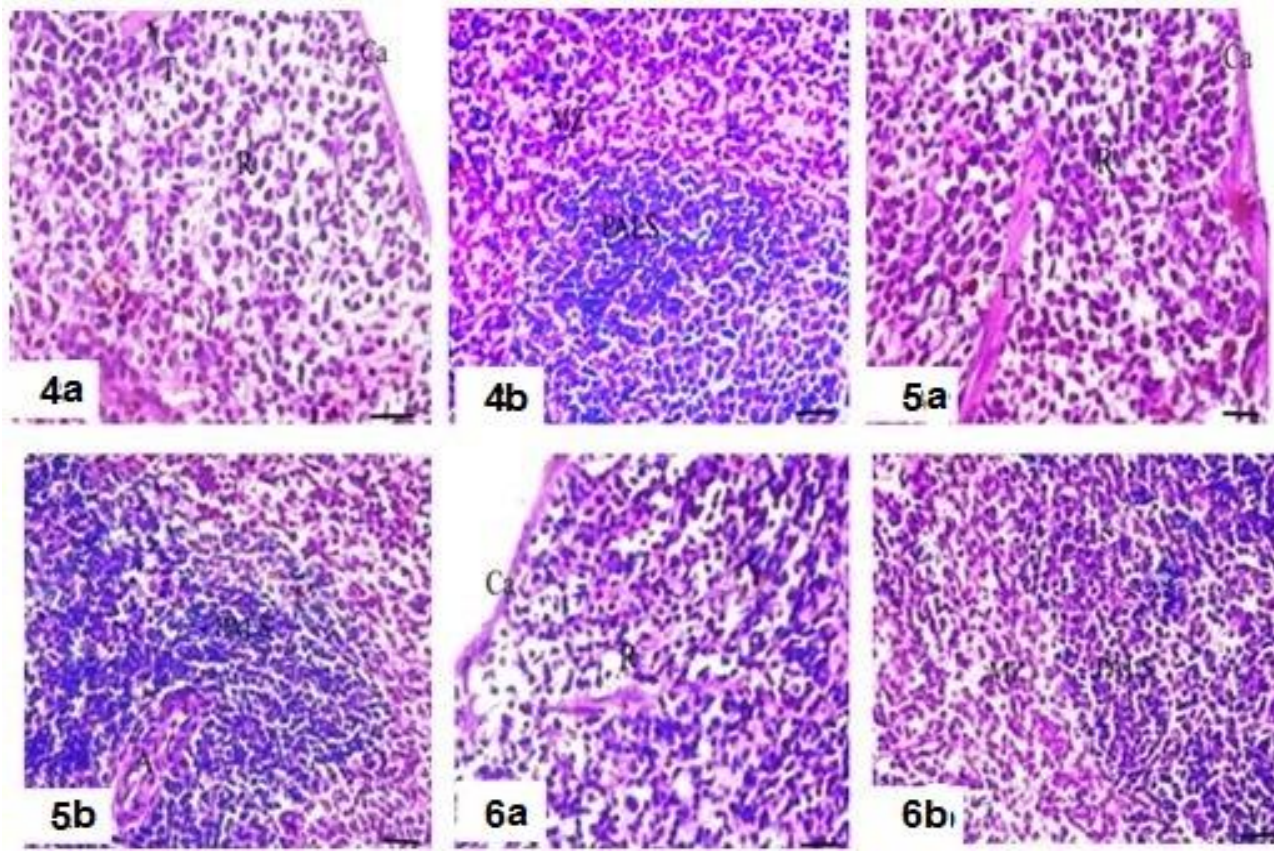
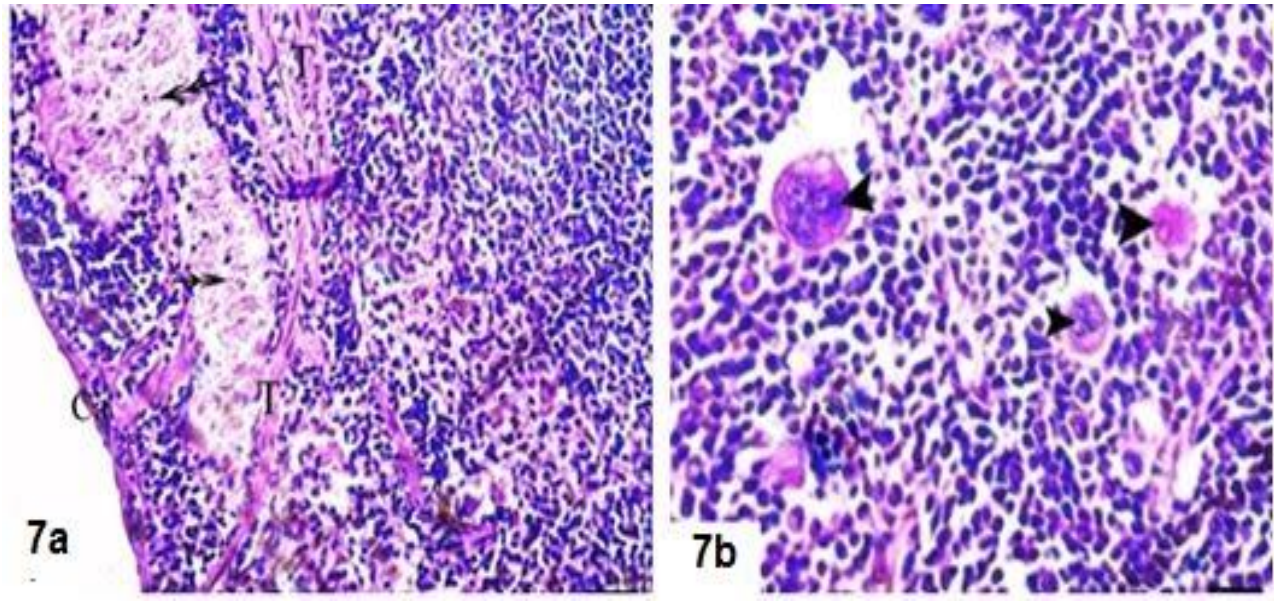


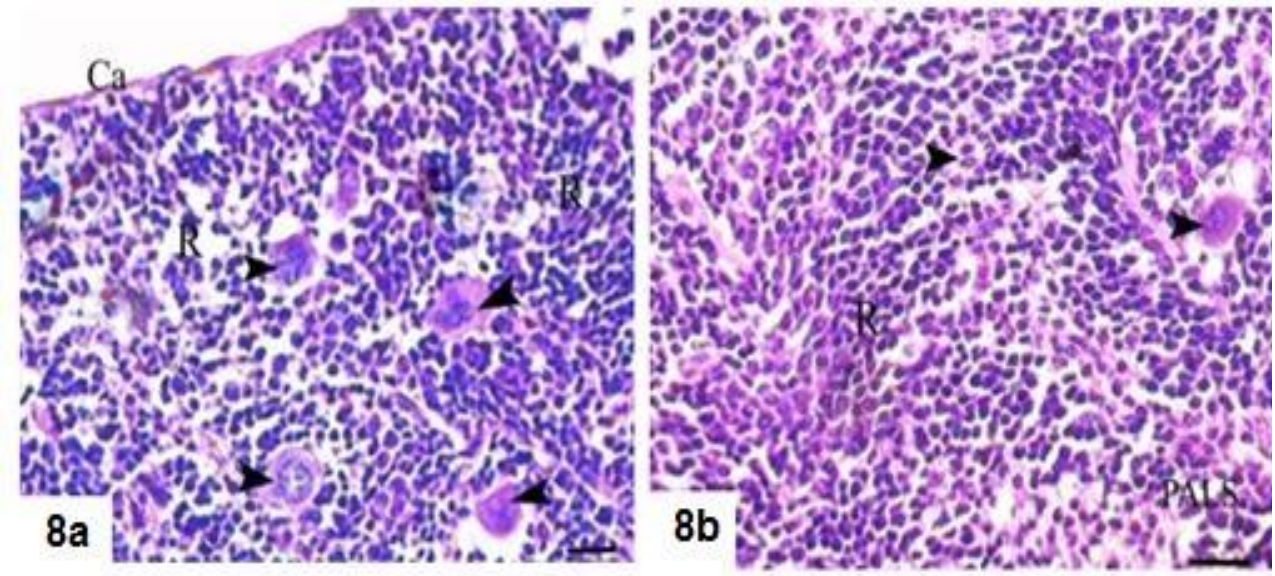
Fig. 3. Effect of Vit. D or/and coconut oil on spleen weight levels. Statistical significance was measured at $p > 0.05$; $*p \leq 0.05$; $**p \leq 0.001$



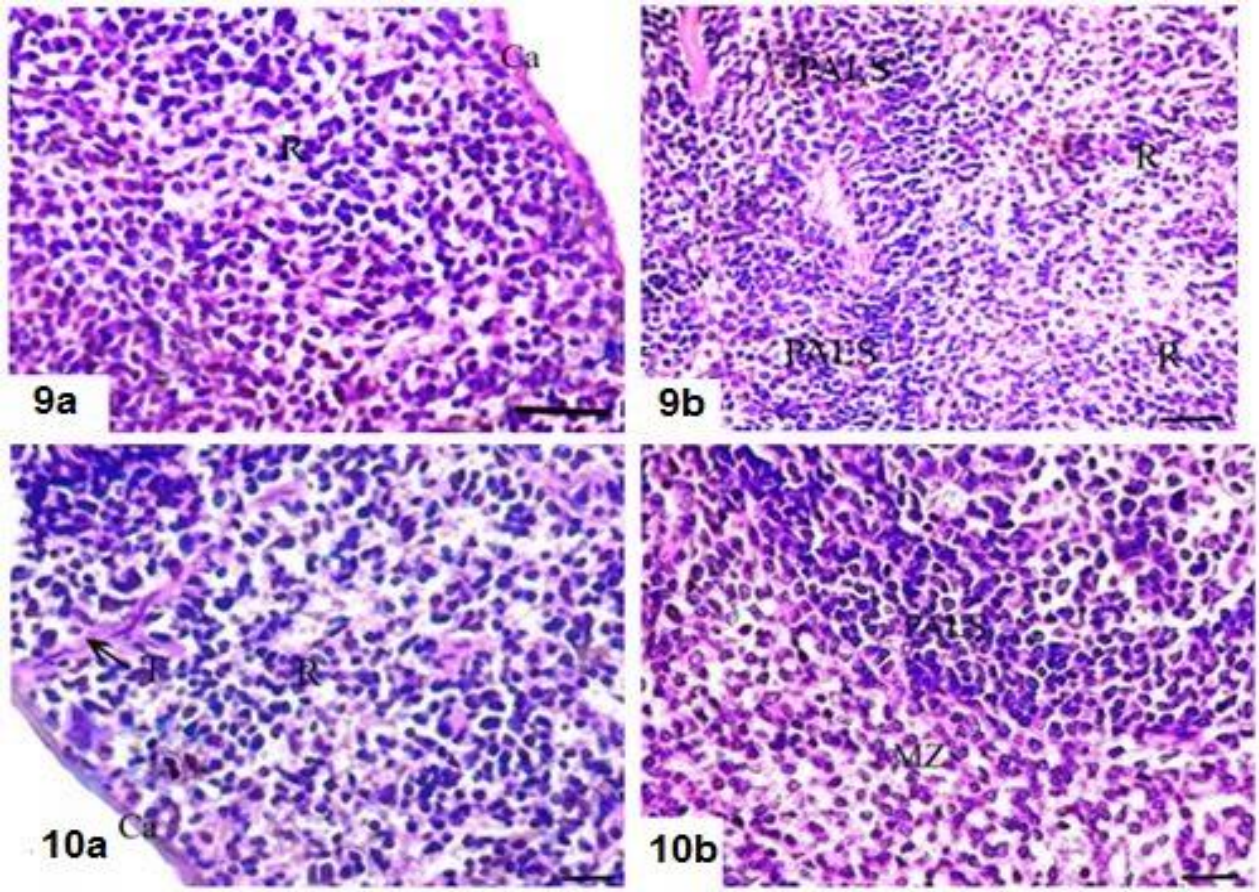
Figs. 4-6. Sections of the spleen showing normal structure of splenocytes with normal appearance of periarteriolar lymphoid sheaths of white pulp (PALS), red pulp (R) and marginal zone (MZ) between the red pulp and white pulp. Entire spleen is surrounded by the capsule (Ca) which sends out trabeculae (T) into the splenic parenchyma in normal control mice (Figs. 4a&b), in non-diabetic mice received vitamin D daily for 4 weeks (Figs. 5a&b), and in non-diabetic mice received coconut oil daily for 4 weeks (Figs. 6 a and b). H&E, all; Bars = 6.25 µm.



Figs. 7a and b. Sections of the spleen of diabetic mice showing dilated and congested blood vessel (double arrows), giant cells (arrow heads), disarrangement of red and white pulps and interference between them. Thin capsule (Ca) and thick branched trabeculae (T) are also noticed. H&E, Bar = 6.25 µm.



Figs. 8a and b. Sections of the spleen of diabetic mice given vitamin D daily for 4 weeks showing disappearance of the blood vessel congestion; while giant cells (arrow heads) and interference between red and periarteriolar lymphoid sheaths of white pulps are still noticed. Normal thin capsule (Ca) and normal thin trabeculae (T) are demonstrated. H&E, Bar = 6.25 µm.



Figs. (9a, b and 10a, b): Sections of the spleen of STZ diabetic mice administered with coconut oil alone or co-administered with vitamin D showing an obvious improvement and arrangement of red pulp (R), marginal zone (MZ) and periarteriolar lymphoid sheaths of white pulp (PALS). Figs (9a&b): diabetic mice received coconut oil daily for 4 weeks, and Figs (10a&b): diabetic mice given Vit. D and coconut oil together daily for 4 weeks. H&E, all; Bars = 6.25 µm.

4. Discussion

Diabetes mellitus (DM) and its complications constitute a severe public health issue facing modern societies. A complete or partial lack of insulin secretion and/or activity causes problems in the metabolism of carbohydrates, proteins, and lipids (Ullah et al., 2016). DM later leads to micro- and macro-vascular complications and becomes a major cause of death (Huang et al., 2017). In the present study, there were no appreciable differences in blood glucose and insulin levels between the non-diabetic mice groups. In the diabetic mice group, blood glucose levels were substantially higher than those of the healthy control mice and insulin levels were significantly lower. The insulin-producing beta cells of the pancreas in mammals are toxic to the glucosamine-nitrosourea compound STZ (Goud, 2015). Alloxan and STZ are toxic glucose analogues

that destroy pancreatic β cells resulting in hyperglycemia via the GLUT2 glucose transporter. Alloxan generates reactive oxygen species in a cyclic redox reaction that selectively inhibits glucose-induced insulin secretion through its ability to inhibit the beta cell glucose sensor glucokinase. STZ targets β cells by its alkylating property corresponding to that of cytotoxic nitrosourea compounds. STZ is split into its glucose and methyl nitrosourea moiety. Owing to its alkylating properties, the latter modifies biological macromolecules, fragments DNA, and destroys the beta cells, causing a state of insulin-dependent diabetes. The targeting of mitochondrial DNA, thereby impairing the signaling function of beta cell mitochondrial metabolism, also explains how STZ is able to inhibit glucose-induced insulin secretion (Ullah et al., 2016).

Compared to the group of diabetics in the current study, diabetic mice administered C.O had a substantial decrease in blood glucose levels and a considerable increase in insulin. Whereas diabetic mice administered Vit. D demonstrated just a mild decrease in blood glucose and a mild increase in insulin levels. The metabolic abnormalities caused by inadequate insulin secretion in T2-DM range from stimulation of glucose uptake and increased hepatic glucose production, to

dyslipidemia, which includes poor fatty acid, triglyceride, and lipoprotein homeostasis (Jain and Patel, 2016). DM is a result of insulin resistance and Vit. D deficiency, the balance of glucose appears to be impacted by Vit. D. By reducing stress in the pancreatic cells and inhibiting the inflammatory responses brought on by cytokines, Vit. D prevents the apoptosis of pancreatic cells. Along with these molecular discoveries, there is a chance that Vit. D may play a role in the prevention of the onset of insulin resistance (Hu et al., 2019). Vit. D ameliorated the harmful impact of diabetes mellitus, probably by increasing insulin secretion and sensitivity, ameliorating β-cell function, decreasing cortisol levels, reduces the number of pro-inflammatory cytokines, and increases the activity of the antioxidant system catalase while reducing lipid peroxidation (Fathi et al., 2022). C.O is a natural antioxidant that contains cotrienols, capric acid, caproic acid, and lauric acid. These compounds act as scavengers of potentially harmful oxygen free radicals, which are essential for the emergence of diabetes, ageing, atherosclerosis, and cancer (Kamsiah et al., 2001; Schaffer et al., 2005; Boateng et al., 2016). C.O's lauric acid is insulin-tropic (Girotti and Thomas, 1984). Antioxidants may be extremely important for diabetes patients in enhancing insulin responsiveness to the loaded glucose and lowering insulin resistance (St-Onge et al., 2003). The gradual decrease in blood glucose in diabetic mice after being treated with C.O daily for 3 weeks has been recorded (Siddalingaswamy et al., 2011). Moreover, Iranloyel et al. (2013) have observed that virgin C.O reduces hyperglycemia and enhances glucose tolerance, likely due to its antioxidant impact, which also enhances insulin production (Malaeb and Spoke, 2020) .

In the present findings, the diabetic mice group recorded a marked atrophy in the weight of their spleen tissues. While after Vit. D or C.O administration daily or combined for 4 weeks; showed an increase in the weight of mice spleen tissues and returned approximately to normal weight. Moreover, the histological findings

showed that control, non-diabetic mice administered Vit. D or C.O had normal-appearing red and white pulps with normal nuclei and a marginal zone separating the red pulp and white pulp when their spleen sections were stained with H&E. Entire spleen is surrounded by the capsule which sends out trabeculae into the splenic parenchyma. The diabetic mice group demonstrated great numbers of giant cells, dilated and congested blood vessels, disarrangement of red and white pulps and interference between them. Thickly branched trabeculae were also noticed. The diabetic mice that administered Vit. D or/and C.O daily for 4 weeks showed a marked improvement and recovery of approximately normal red pulps, marginal zone, and white pulps. Similar results were recorded in diabetic rats by Ebaid et al. (2015); Hashish and Kamal (2015) who illustrated the atrophy of diabetic splenic mice and diffusion of white pulp. Also, with dilated blood arteries, mature lymphocytes in the periphery of the spleen were too significantly decreased. In diabetic guinea pigs, similarly, Kumar et al. (2013) found that the red pulp and white pulp frequently vacuolated with nuclei degeneration. The white pulp of the spleen resembles a lymph node structurally. It has T-cell and B-cell zones and enables the production of antigen-specific immune responses that shield the body from bacterial, viral, and fungal infections that spread through blood. Immune responses that are harmful to the host can be controlled at the spleen (Bronte and Pittet, 2013). Diabetes decreases immune response capabilities, including immune cell function and immune organ atrophy (Ebaid, 2014). Additionally, diabetes and oxidative stress play a vital role in the development of diabetes complications and increase the cell death receptor Fas, which caused splenocytes to die in rats via the Fas/FasL (programmed cell death receptors) pathway, suggesting a potential mechanism for the immunotoxicity of hyperglycemia (Berbudi et al., 2020). The components of inflammation appear to be significantly impacted by Vit. D and its metabolites, insulin synthesis, secretion, action, and all of these may have an influence on the pathogenesis of T2-DM (Zipitis and Akobeng, 2008). Through its immunomodulatory and anti-inflammatory properties, Vit. D appears to play a part in the pathogenesis of diabetes.

By lowering insulin resistance, raising insulin production, and reducing autoimmune inflammation, Vit. D decreases the risk of developing T2-DM and T1-DM. Therefore, it is feasible that early Vit. D supplementation could provide protection against the onset of DM (Hu et al., 2019; Fathi et al., 2022) .

Virgin C.O contains the beneficial component lauric acid. Monolaurin, which has been demonstrated to influence immune cell proliferation and have antibacterial activity (Pereira et al., 2004; Malaeb and Spoke, 2020). It also involved in inflammation for instance, during acute and chronic inflammations, in response to infections or other antigenic chemicals, immune cells become active (Bermas, 2014). According to recent studies, inflammation contributes significantly to several human disorders that are not primarily immune system issues. These include Alzheimer's disease, neurological diseases, ischemic heart disease, cancer, and atherosclerosis (Berbudi et al., 2020). In conclusion, diabetic mice administered either C.O alone or with Vit. D demonstrated strong anti-hyperglycemic effects to recovery the glucose and insulin rates close to normal values and restored the histological architecture of the spleen close to normal structure than those administered Vit. D alone.

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