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POTENTIAL PRESERVATION OF HORMONAL STABILITY AND HISTOGENESIS OF EMBRYONIC RENAL IN DIABETIC RATS BY RED ALGAE SUPPLEMENTATION

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Abstract

Diabetes mellitus (DM), a complex metabolic disorder, is the most common etiological type (90-95% of cases) and is due to the gradual loss of insulin secretion against the background of insulin resistance. Where There is no study using Focusvesiculosus with the purpose of demonstrating the potential protective effects of gestational diabetes against histopathological changes in fetuses during 18 days of gestation (before birth) respectively for pregnant female rats treated with alga vesicular algae at a concentration of 50 mg/kg. In the current study, the results of this study showed a significant decrease ($P < 0.05$) in the glucose level of expectant mothers of the two groups treated with F.vesiculosus and the fourth after treatment with F.vesicular algae compared with the S T Z group. The study also showed a significant increase ($P < 0.05$) in the level of progesterone hormone for expectant mothers for the two groups treated with F.vesiculosus and the fourth after treatment with F. vesicular algae compared with group S T Z. The results of this study showed a significant increase. ($P < 0.05$) in the estrogen level of expectant mothers of the two groups treated with F.Vesiculosus and the fourth after treatment with Algae vesicular compared to the control group and the ST Z group. The results of this study also showed a significant ($P < 0.05$) increase in the level of HCG hormone of expectant mothers in the two groups treated with Algae Fuchs vesicular and the fourth compared to the STZ group after treatment with F. vesicular. As for the histological sections, the results showed in the kidney tissues the presence of histological changes represented in small glomeruli, incomplete and irregularly shaped glomeruli, blood congestion, bleeding, damage and complete loss of renal glomeruli, as well as damage to the tubules. nephropathy in the STZ group compared with the control group. As for the moss F.vesicularisgroup, the tissue sections showed the complete formation of the glomerulus, the absence of a wide space between Bowman's capsule, the absence of blood congestion and the absence of damage to the renal tubules. Therefore, we concluded from our study that F. vesicularis stabilizes pregnancy hormones during the third group and also controls the process of histogenesis of renal tissue.

1. Introduction

Maternal diabetes greatly increases the risk of developing congenital teratogen, a syndrome known as diabetic anomaly or diabetic embryopathy (Lovredo*et al.,* 2001). These defects can affect many developing organ systems, but the neural tube, heart, and skeletal system are the most affected In diabetes, failure to get rid of insulin-stimulated glucose, especially in the fat, muscle and liver of diabetics, results in an abnormal rise in circulating glucose (hyperglycemia). Several studies using rodent modelshavedescribed hyperglycemia-induced oxidative stress with
diabetic teratogen(Sivan *et al.,* 1996).A decrease in GSH in fetuses of diabetic mice, causing an increase in free radicals was used as a rat model for diabetic pregnancy and showed that transporting excess glucose to the fetus significantly increases neural tube defects (NTDs) (Vine *et al.,* 1999), and it is one of the most common defects caused by diabetic pregnancy. A pregnancy resulting from diabetes carries two to three times a greater risk of a mother giving birth to a child with a birth defect than a normal pregnancy (Mills, 1982). The reasons for the increased rate of slag formation are not clear. Genetic and environmental factors are involved in the deformation (Eriksson, 1986). Studies have shown a structural anomaly, where some fetuses showed a defect in the sacral formation, as well as the absence of a tail and the absence of

staining of the bone and cartilage tissue in the caudal sacral region, and here indicates the complete absence of the sacral formation. Offspring of diabetic puppies tend to drink more water, are smaller in size and tend to have a slightly smaller placenta than that found in non-diabetic mice. It was also reflected in body weight, kidneys, blood levels, carbohydrates and lipid metabolites were slightly different among them (Anderson et *al.,*2016). Diabetes is associated with impairment Reproduction in both men and women is due to widespread changes in the male reproductive system in many individuals with diabetes. Studies have documented abnormalities in testicular function as well as abnormal sperm formation in male diabetic rats . (Scarano*et al.,* 2006). Diabetes causes the sperm to have severe structural defects (Baccetti*et al.,* 2002), lack of movement, and has difficulty penetrating theegg Therefore, giving high doses of streptozotocin (STZ) to male rodents results in a decreased level of testosterone production (Oyewopo and Oremosu .,2011).

2.Materials and methods

2.1.Experimental animals.

The study was conducted on female white rats, the type of Norwegian rats, whose ages ranged from 11 to 12 weeks, with an average of 205 gm obtained from Animal House College of Science / Department of Biology / University of Kufa and then transferred to the Animal House Department-College of Education for Pure Science / ThiQar-University The rats were placed in special plastic cages, 60 cm long and 25 cm wide, to be multiplied with metal covers and the cages were cleaned of sawdust, and the appropriate environmental conditions were controlled in a constant light period (12 hours a day/ 12 hours at night) cycle, ventilation, temperature between 22 and 25 degrees Celsius, the Rats were taken to the veterinarian to ensure their health and free of diseases. The rats were kept in clean cage conditions by changing sawdust twice every week, and the animals were given an adequate amount of water and food from a local source (wheat 33%, barley 20%, corn 25%., Animal protein 10%, milk powder 11%, salt 1%) These ingredients were ground and mixed with some oil and water to become a cohesive paste (Tayfour, 2013) and placed in the food area in the cages. In animal husbandry, a mature female was placed with an entire adult male for a whole day and the next morning the females were examined for a vaginal tampon (Saadallah, 2009). The date of the mating is written on the cages. Mating day is day zero (D0) of conception and the following day is the first day of conception (Bogumil, Wlodarczyk, Minta, 2000).

2.2.Induction of Diabetes Mellitus.

The Female rats intraperitoneally injected by a single dose Streptozotocin (Sigma, Chemical Co., St. Louis, MO), 60 mg/kg body wt, induces diabetes within 3 days a mean blood glucose > 180 mg/dL.

2.3.Design of the study

In the present study, 24 pregnant rats were divided into two groups according to the following arrangement :

1-The control group: consisting of six (6) rats that were treated with normal saline Nacl (0, 9%) by orally.

2.The diabetes group: consisting of six (6) rats Group 2 serves as a as Streptozotocin is given as (60 mg / kg) of body weight(Omolaoye,2018).

3. The treated group: consisting of six (6) rats serves as a as Streptozotocin is given as (60 mg / kg) of body weightthat were treated withF.vesiculosus (50 mg / kg)(Rodrigues,2013).It was given orally via a gavage tube for a period (1-18) during pregnancy.

4. The control group: consisting of six (6) rats that were treated with F.vesiculosus (50 mg / kg)It was given orally via a gavage tube for a period (1-18) during pregnancy.

2.5.Serum collection.

On day 14 of gestation, pregnant rats were anesthetized with cotton pads moistened with ether in a glass container, and blood was collected using a thin needle (5 cc) by cardiac puncture. The collected blood samples were placed in a blood collection gel tube and allowed to coagulate at room temperature and separated by centrifugation for 15 min at 3000 rpm. The serum was then taken and kept in a normal tube at −20 °C (Kaneko et al., 2008).

.2.6.Isolate rats embryos

On day 14 of gestation, pregnant rats were anesthetized with cotton pads moistened with ether in a glass container,Where the animals were cut and the embryos extracted with the placenta, then the kidney and liver of the embryos were extracted, and the samples were kept in formalin for the purpose of tissue cutting operations(Shea and Geijsen 2007).

2.7.TheHistological procedures:

The histological procedures were according to (Bancroft and Gamble, 2008).

2.8.Statistics:

All data are expressed as means ± standard error of mean One-way analysis of variance was used to compare the differences between groups while multiple comparisons were performed by Tukey's post hoc test (SPSS 26) A probability value (P) of ,0.05 was considered as the minimum level of statistical significance.

3. Results

The results indicated a significant increase (P 0.05) in the glucose level of the STZ group compared to the control group. There was a significant increase in the STZ group compared with the F**.** vesicular group. But there was a significant decrease in the glucose level in the group F**.**vesicularis compared with the fourth group. There was a significant decrease in the fourth group compared with the control group. There was a significant decrease in the fourth group compared with the control group. There was a significant decrease in the fourth group compared with the STZ group.

Table (1) Level of Glucose in all groups

Parameters / Groups	Glucose(mg/dl) Mean ± SE
Group 1	85.67 ±1.961
Group 2	338±20.34**
Group 3	150.8±14.60
Group 4	76.50±1.839
P value	<0.0001

** It indicates a significant difference P ≤ 0.01

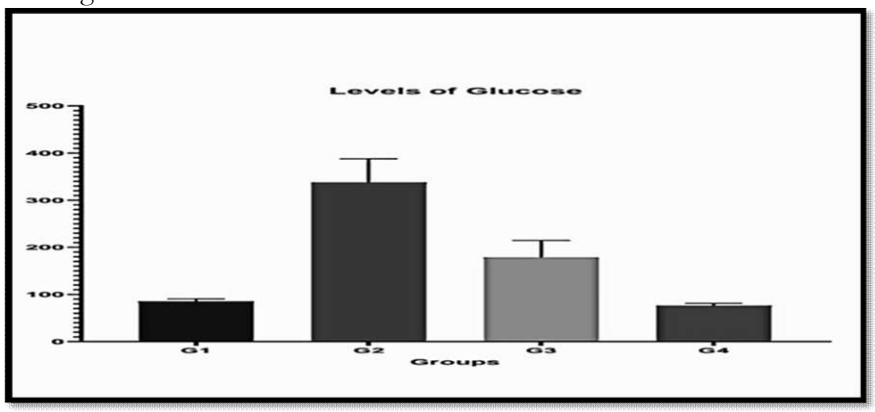

Figure (1) Level of glucose in all groups

3.2.Effect Diabeteson Levels of on Human ChorionicGonadotrophin(HCG) and progesteroneand Estrogenduring the 14 day of pregnancy:

The results indicated a significant decrease (P 0.05) in the HCG hormone for the group STZcompared with the control group. Also, there was a significant decrease in the group STZcompared with the group Fuchsia vesicular. But there was a significant increase in HCG in the Fuchus vesicular group compared with the STZgroup. There was a significant increase in the fourth group compared with the group STZ. But there was no significant decrease in the hormone hCG in the control group compared with the fourth group.

Table (2) the Levels of Human ChorionicGonadotrophin(HCG) and progesterone and Estrogen in all groups

HORMONES			
Parameters	**PROGNg/ ml**	**E2*Pg/ ml***	**HCG*mUI/ml***
Groups	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**
Group 1	**71.8±8.826**	**48.87±4.262**	**41447±3434**
Group 2	**38.9± 2.252****	**26.92±3.577****	**18588±911.2****
Group 3	**69.2±3.931**	**42.94±4.325**	**33940±3542**
Group 4	**80.7±6.209**	**51.96 ±1.555**	**44211±3532**
P value	**0.0026**	**0.0067**	**0.0020**

** It indicates a significant difference P ≤ 0.01

In the same table (2) The results indicated a significant decrease (P 0.05) in the progesterone hormone for the group STZ compared with the control group. Also, there was a significant decrease in the group STZcompared with the group F. vesicular. But there was a significant increase in HCG in the F. vesicular group compared with the STZgroup. There was a significant increase in the fourth group compared with the group STZ. But there was no significant decrease in the hormone progesterone in the control group compared with fourth group.

All so In the same table (2) The results indicated a significant decrease (P 0.05) in the Estrogen hormone for the group STZcompared with the control group. Also, there was a significant decrease in the group STZcompared with the group F. vesicular. But there was a significant increase in HCG in the F. vesicular group compared with the STZ group. There was a significant increase in the fourth group compared with the group STZ. But there was no significant decrease in the hormone Estrogen in the control group compared with the fourth group.

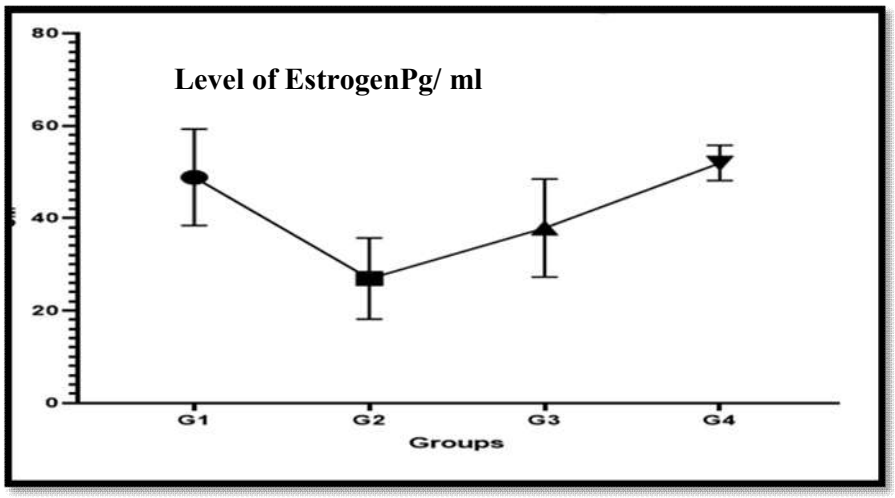

Figure (2) Level of Estrogen in all groups

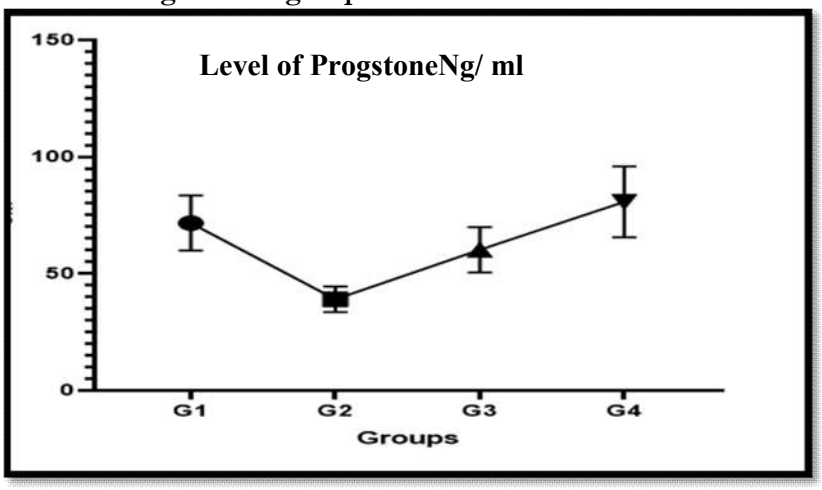

Figure (3) Level of Progstonein all groups

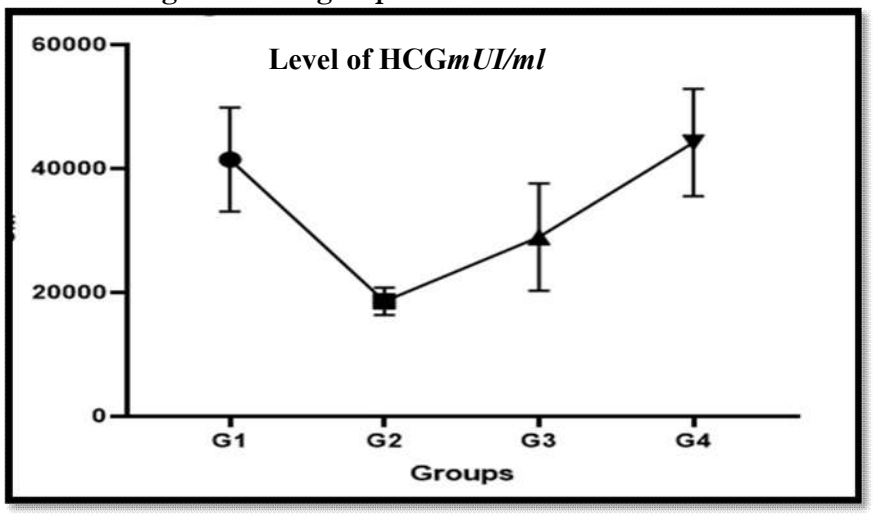

Figure (4) Level of HCG in all groups

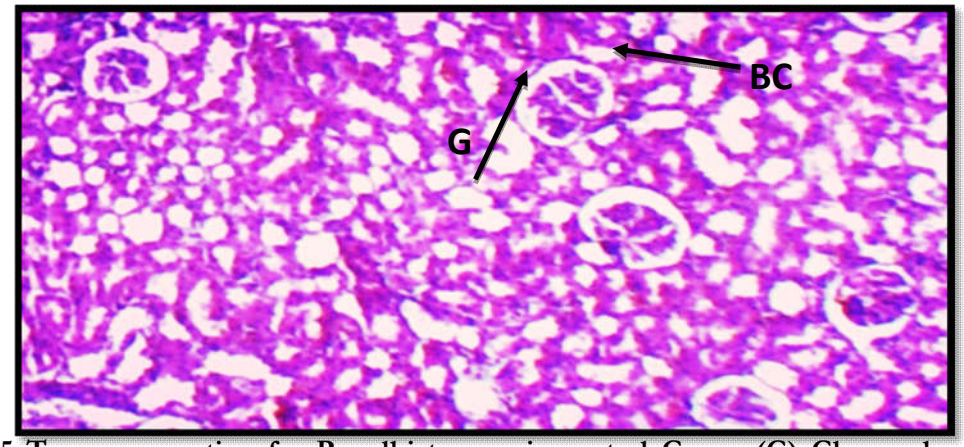

Figure 5 Transvers section for Renalhistogenesis control Group (G) Glomerulus (BC) Bowman's capsule (H&E) (40x).

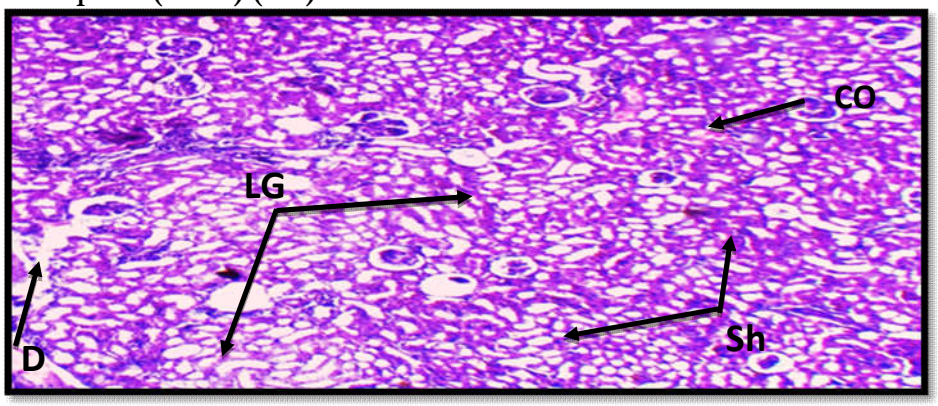

Figure 6 Transvers section for Renal histogenesis diabetes control received streptozotocin (60mg / kg) (Sh) Shrinkage (LG) Loss OF glomerulus (CO) Congestion(D) Damage(H&E) (100x).

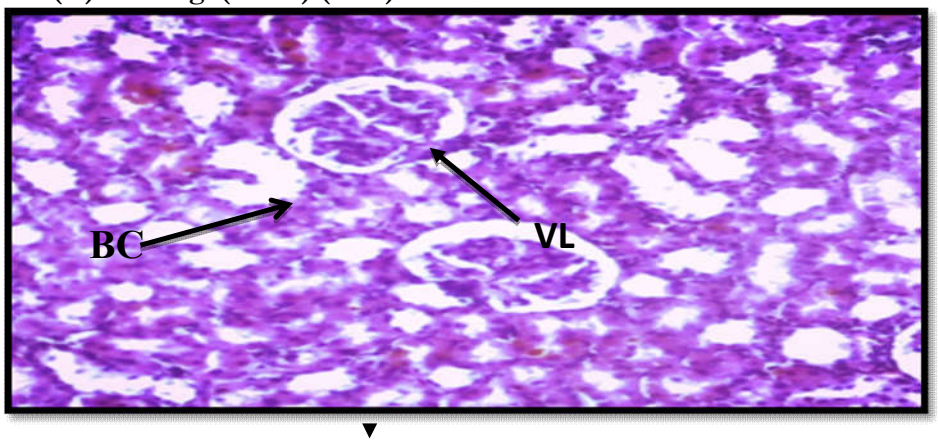

Figure 7 Transvers section for Renal histogenesisGroup received streptozotocin (60mg / kg) treatedwith F. vesiculosus (50 mg / kg) (BC) Bowman's capsule (VL) Visceral layer (H&E)(200x).

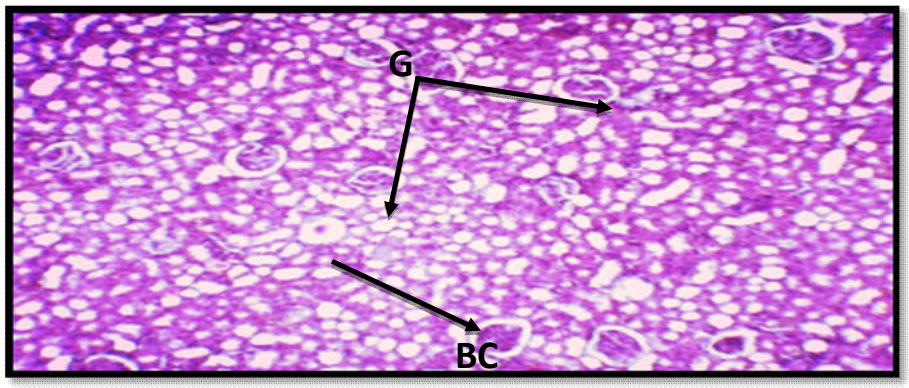

Figure 8 Transvers section for Renal histogenesisGroup 4, given with Standard food and water, then treated with F.vesiculosus (50 mg / kg) (G) Glomerulus) (BC) Bowman's capsule (H&E)(100x).

Table 3 showing the number of damaged and healthy renal Glomerulus in all groups

Control Group		STZ Group		Focus Group		Fourth Group	
Damage	Healthy	Damage	Healthy	Damage	Healthy	Damage	Healthy
4%	96%	32%	68%	10%	90%	5%	95%
2%	98%	19%	81%	5%	95%	9%	91%
5%	95%	35%	65%	9%	91%	7%	93%
6%	94%	38%	62%	12%	88%	11%	89%
3%	97%	20%	80%	15%	85%	4%	94%
5%	95%	26%	74%	11%	89%	6%	96%

4.Discussion

The results showed a significant increase in glucose levels in the diabetic groups compared to the control group. When using F. vesiculosis lowers glucose in the third and fourth groups (F. vesiculosis group) compared to the groups of patients with diabetes. Diabetes symptoms were identified by elevated blood glucose, lipid and carbohydrate change, increased chances of diabetes and oxidative stress (Davis *et al.,* 2006; Al-Assaf *et al.,* 2012). Low-dose streptozotocin is known to cause rapid destruction of beta-cells in the pancreas resulting in impaired glucose-stimulated insulin secretion and insulin resistance, both hallmarks of type 2 diabetes (Sundaram *et al.,* 2013). Elevated blood glucose is a consequence of decreased glucose uptake in muscle and adipose tissue and increased gluconeogenesis, hepatic glucose production and glycogen breakdown (Guignot and Mithieux 1999; Sundaram *et al.,* 2013). Mice with STZ-induced diabetes revealed signs of binge eating. The results of the current study showed a significant decrease in the hormones progesterone, estrogen and hcg in the diabetic group compared to the control group. The explanation for this is that due to high sugar, it closes the hormone receptor pathways in the placenta, which leads to a decrease in its concentration in the blood serum. (Gangestad *et al.*,2012). and the decrease in pregnancy hormones can be explained by the effect of free radicals caused by oxidative stress caused by diabetes, which affects cell membranes, as these radicals oxidize the unsaturated fatty acids that form cell membranes, and then lead to loss of membrane permeability. Membrane damage increases the effect of foreign substances on the cell and its components such as proteins and unsaturated amino acids, which leads to the breakage of double and single bonds, which leads to the formation of lipid peroxide. (Mahjoobi - Summit 2005) The occurrence of hormonal and morphological changes such as necrosis and placental degeneration is evidence of decreased activity, which reflects the damage caused by diabetes as it disrupts the work of the placenta and prevents the exchange of food and transport of important elements and then a

decrease in the production of hormones, in addition to the effects of oxidative stress. A previous study found that iodine deficiency for mothers Pregnant women with diabetes It should be noted that iodine deficiency may stimulate Pathological processes in the thyroid gland by insulin-like growth factor-1 (IGF-1) which promotes the growth of endocrine cells ex vivo (Hofbauer, 1995) Perhaps the reason can be traced back to insulin, as it has recently become a cause of iodine deficiency and insulin resistance. Related (Al Mansourian, 2011). Perhaps due to iodine deficiency, the T3 hormone decreases due to the inhibition of T3 receptors and the breakdown of the proteins that transport these hormones as they interfere with them and impair their work. Or the decrease can be attributed to oxidative stress and decreased body defenses, as shown (Bhimte *et al.,* 2012) where it increases reactive oxygen groups (ROS) and damages mitochondria and their energy production and decreases accompanied by affecting cellular components that negatively affect the Hormone levels, the results of the current study showed a significant increase in the third group (Fuchus group) compared to the group of diabetic patients. The improvement in hormones is due to the effect of vesicular Fuchs on hormone receptors located on the surface of the basement membrane in epithelial cells that activate the enzyme Adenylcyclase located in the cell membrane and leads to an increase in the synthesis of cAMP, a secondary transporter that stimulates the primary enzymatic system of the placenta, as a result of which an increase in the secretion of placental hormones and growth its tissues, which was confirmed by a study (Garg, 2017). The reason may be due to the fact that fucus contains powerful antioxidants such as vitamin C, which works on the oxidation of long-chain fatty acids in the mitochondria and the production and maintenance of energy for cells, and works to balance the amount of fat and muscle mass, because it is an indicator of the balance of the amount of proteins in the body.Through our study we reached, an increase in the level of pregnancy hormones in the fucoid groups compared to the group of diabetics, so we think because of the presence of the fucoxanthin compound in the fucos is considered an antioxidant, as the study agrees with what was mentioned, as it was reported (Bai *et al.,* 2020) It reported having strong biological activities such as antioxidant and antidiabetic activities, mainly due to the unusual fibrous bond and functional oxygen group in its structure (Bay *et al.,* 2015). The results showed that the effect of gestational sugar caused nephrotoxicity in varying degrees, and the damage was increased by increasing the level of Sugar, as it was found that sugar causes cell death, as well as the destruction of blood vessels, which leads to bleeding in the kidney tissues of the fetus and infiltration of cells in the group of diabetics. And that these disorders are caused by the fact that the kidneys are a sensitive organ and are the main site for excreting toxins from the body. Another study showed that diabetes causes alkylating DNA or protein and DNA-to-DNA or protein binding (Evenson et al., 1993; Steckl., 2000; Gomez Martin et al., 2015), we think ACE is involved with the renin system. -Angiotensin-aldosterone that converts angiotensin I to angiotensin II. Therefore, angiotensin II is a powerful vasoconstrictor, stimulates the secretion of aldosterone by the adrenal cortex and increases the absorption of sodium and water. Activation of renin-angiotensin-aldosterone increases blood pressure, causing microvascular and macrovascular complications of diabetic patients (Vo *et al.,* 2018; le *et al.,* 2016).Therefore, aldose reductase inhibitors can inhibit sorbitol formation through a pathway Therefore, the results in the third group showed a clear shape in the glomerulus, completeness of its structure, no disintegration of tissues, and no other abnormalities, due to the presence of fucofuroeckol A compound in the vesicular hatchlings, because it acts as an angiotensin-converting enzyme inhibitor with the renin system. Angiotensin-aldosterone, which has the ability to lower blood pressure, and thus help reduce

complications of diabetes, as well as because algae contain many active compounds of omega-3 essential fatty acids and this is consistent with what has been reached (Zanwar *et al.,* 2011).

Conclusions

we concluded from our study that F. vesicularis stabilizes pregnancy hormones during the third group and also controls the process of histogenesis of renal tissue.

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