Egyptian Journal of Veterinary Sciences

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Neurosteroid, Natural and Anabolic Steroids: Physiological, Immunological and Histopathological Study on Diabetic Albino Rats

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NEUROSTEROIDS are endogenous steroids are produced in the nervous system and act as neurotransmitters or neuromodulators. Steroid hormones, which are primarily produced in the adrenal glands, play an essential role in the regulation of different physiological actions in the body. While anabolic steroids are synthetic substances with effects comparable to those of testosterone.

This study intended to differentiate between the roles of neurosteroid, natural steroid and anabolic synthetic steroids through investigating the effects of some physiological, immunological and histopathological on various target organs such as kidney, liver and spleen in diabetic rats.

Fifty male albino rats were randomly divided into five equal groups. The first group was used as a control fed on standard diet and injected subcutaneously with 0.5 ml normal saline. The second group was injected subcutaneously with Alloxan (100 mg/kg.bw) fed on a standard diet and served as diabetic control group, while the third group diabetic rats were orally administrated with dehydroepiandrosterone (DHEA) (as a neurosteroid, 17.2mg/kg/day) and the fourth group diabetic rats were subcutaneously injected with hydrocortisone (as a natural steroid, 26.1mg/kg/day). Finally, the fifth group diabetic rats were intramuscularly injected with Sustanon (as an anabolic steroid, 66.9 mg/kg/week).

Based on the aforementioned results of the hematological parameters and interleukins (IL-2 & IL-6) improvements after treatment with DHEA. Fasting blood sugar and body weight after Sustanon, and lipid profile after both hydrocortisone and Sustanon. ALP was almost enhanced in all treated groups. Moreover, histopathological examination of kidney, liver and spleen showed numerously distortion.

In conclusion; from the obtained results, it can be reported that DHEA administration can improve immunity, reduce oxidative stress associated with diabetes, and ameliorate tissue damage in the kidney and spleen. Hydrocortisone may improve lipid profile, but has negative effects on immunity and tissue health. Sustanon has positive effects on blood glucose and lipid profile, but its effects on immunity is indistinguishable.

Keywords: Dehydroepiandrosterone, Hydrocortisone, Sustanon and Diabetes.

Introduction

Diabetes Mellitus (DM) is a metabolic disorder characterized by the presence of chronic hyperglycemia with varying degrees of disruption in the metabolism of carbohydrates, lipids and proteins. The cause and etiology of DM can vary greatly; however, it typically involves defects in either insulin secretion or insulin response, or both [1]. Alloxan is a β-cytotoxin and induces

diabetes mellitus by destructing the secretion of the insulin β-cells of the pancreas, which leads to decreased endogenous insulin release and leads to diabetes [2]. Numerous organs including; kidney, liver, spleen, as well as other organs like the heart, brain, and skeletal muscles are it tends to raise the risk of a number of other diseases caused by macrovascular and microvascular damage [3]. Moreover, diabetic patients are more susceptible to infections due to dysfunction in innate and adaptive immune response [4].

Steroid hormones are synthesized mainly in the adrenal cortex, gonads, and placenta which are derived from cholesterol [5]. These hormones are responsible for major endocrine functioning and stress management. They are produced and secreted by the adrenal glands in response to pituitary adrenocorticotropic hormone, and regulated by hypothalamic corticotropic releasing hormone. Steroids have a wide range of biological effects on vertebrates, mostly on homeostasis, which includes metabolism, reproduction, production, cognition, and inflammation [6-8].

Natural hydrocortisone is a glucocorticoid with a rapid short acting that is used to treat inflammation and adrenal insufficiency. Since hydrocortisone and cortisol possess the same chemical structure, they closely imitate the human adrenal hormone, which has both glucocorticoid and mineralocorticoid properties [9]. It also has an essential role in the carbohydrate, protein, and lipid metabolism of the body. Hydrocortisone medical significance arises from its anti-inflammatory, anti-allergic, and immune-suppressive role in the body [10].

Dehydroepiandrosterone (DHEA), which is synthesized by the adrenal cortex from pregnenolone, is later converted to androstenedione, testosterone, and estrogens [11]. Also, it can be synthesized in the central and peripheral nervous system [12]. Due to its actions on brain neurotransmitter receptors, DHEA has been classified as a neurosteroid. DHEA and its sulfated derivative (DHEAS) are multifunctional steroids with a variety of physiological functions, with different mental effects and immune systems activities [13].

Anabolic-androgenic steroids (AASs), commonly known as anabolic steroids, are a large group of molecules including endogenously produced androgens, such as testosterone and synthetically manufactured derivatives. AASs have been widely used for therapeutic purposes

[14]. Sustanon is an oil-based injectable anabolic-androgenic steroid hormone that generally contains four different testosterone esters. It releases testosterone continuously into the blood and maintains a stable testosterone level for a long time, lasting for three to four weeks. It has been supported by several of lines of evidence that testosterone therapy reduces insulin resistance [15, 16].

The purpose of this study is to investigate the physiological, immunological and histopathological effects of neurosteroid, natural steroid and anabolic synthetic steroids on various target organs such as kidneys, liver and spleen in diabetic rats.

Material and Methods

Animal and housing

The experiment of this study took place in the animal house of the Department of Biology, College of Science, University of Duhok, Iraq where the rats were duly bred. According to ethical and accepted laboratory rules, the experimental rats were housed in vented 30×25×17cm polypropylene cages with access to a standard diet and water, which were 12h light: 12h dark light period, at 25ºC. The weight of the adult animals used in the experiment ranged between 150-250gm (8-10 weeks age). The bedding of the cages, especially those of the diabetic group, was frequently changed owing to polyuria.

Induction of diabetes

Forty male albino rats were fasted overnight and injected for successive seven days with a double subcutaneous of 100 mg/kg.bw of Alloxan (THOMAS BAKER. India). Alloxan was dissolved in citrate buffer (PH = 4.5) and weekly injected. The injected animals with alloxan were treated orally with 5% of D-Glucose (Markans Pharma Ltd - India) after half an hour of the injection to prevent the potentially fatal hypoglycemic effects. Symptoms of diabetes appeared within 24 - 72 hours after injections; such as polyuria, polyphagia and polydipsia. The diagnosis of diabetes was further confirmed by testing blood sugar and glucose in urine by urine-strips (Machinery-Nagel/ Germany).

Preparation of Steroid Drugs

Dehydroepiandrosterone drug was provided and used as neurosteroid supplement. The drug was available in the pharmacies (50 mg pills) and known as DHEA PHARMACY Laboratories s.c. Poland. The pills were grinded and dissolved with

normal saline. Daily records of body weight were maintained and accordingly suspension of DHEA was freshly prepared and given orally at dose (17.2 mg/ kg) to the animals daily for a period of six weeks.

Hydrocortisone drug was provided and used as a natural steroid. The drug was available in the pharmacies (100 mg) and known as Hidrokortizone. Hemofarm A.D. Serbia. Daily records of body weight were maintained and accordingly hydrocortisone was duly dissolved with its own specific buffer, and prepared to be injected daily in a subcutaneous route at dose (26.1 mg/ kg) into the animals for a period of six weeks.

Testosterone drug was provided and used as an anabolic steroid contains testosterone esters (testosterone propionate, phenylpropionate, isocaproate and decanoate). The drug was available in pharmacies and ready to be used, known as Sustanon (250 mg). EVER pharmajena GmbH. Germany. The animals' body weight records were maintained and accordingly injected intramuscularly at dose (66.9 mg/kg) on a weekly basis for six weeks interval.

Standard diet

Standard diet was prepared for each one kilogram (kg) as follows: wheat 665.5g, soya 256.2g, sunflower oil 43.5g, limestone 14.9g, Ca_2PO_4 6.4, salt 6.3g, lysine 2.4g, methionine 1.5g, enzymes 0.8g, choline chloride 0.6g, vitamins 0.58g and trace elements 0.5g. [17].

Experimental Design

Fifty male albino rats were randomly divided into five equal groups and treated for 6 weeks as follows:

Group 1 (Control): Normal rats were injected with 0.5 ml normal saline (0.9%) subcutaneously and served as a control group.

Group 2 (Diabetic Control): Alloxan injected rats were fed on standard diet as a diabetic control group.

Group 3 (Diabetic + DHEA): Diabetic rats were gavaged with DHEA (as a neurosteroid) drug. (17.2 mg/ kg rat /day).

Group 4 (Diabetic + Hydrocortisone): Diabetic rats were injected subcutaneously with hydrocortisone (as a natural steroid) at dose of (26.1 mg/ kg rat /day).

Group 5 (Diabetic + Testosterone): Diabetic rats were injected intramuscularly with Sustanon (as an anabolic steroid) (66.9 mg/kg rat / week.

Estimation of hematological and biochemical parameters

At the end of the experiment, the animals were prohibited of food overnight with free access to water. The rats were anaesthetised with diethyl ether (Scharlab S, L, Spain), blood samples were directly obtained via heart puncture. Two ml of blood were collected in EDTA tubes (Arzer Grande- Italy) for hematological parameters analysis using the automated hematological analyzer (Medonic- Sweden). The remaining of blood samples were positioned in gel tubes (Arzer GrandeItaly) for 30 minutes then centrifuged (at 4000 rpm for 15 minutes) to complete the biochemical tests examination such as fasting blood sugar (FBS), serum lipids, electrolytes, serum proteins, renal and liver function tests. The serum parameters were estimated by Cobas 600 (C501) automated chemistry analyzer (Roche/Germany). This apparatus evaluates the tests depending on their reagent's kits (Roche/ Germany) that include: absorbance photometry (enzymes, substrates and specific proteins).

Estimation of IL-2 and IL- 6

Serum level of IL-2 and IL-6 were determined by enzyme - linked immunosorbent assay apparatus (BioTeckUSA) according to the instructions of the kit's manufacturing company (BT LAB – China).

Histopathological study

After dissecting of the animals, kidneys, liver and spleen sections were washed with tab water and fixed in 10% formalin. After the preparation of paraffin blocks, thin segments (4μM) were stained with haematoxylin and eosin ((H&E) by using Auto Staining technique (Leica -Germany). A light microscope (Motic/China) was used to examine the mounted slides, whereas a specialized camera (Anmo -Taiwan) was used to get the pictures of the pointed field [18].

Statistical Analysis

The following statistical means were used to analyze the data of the experiment: Microsoft Excel 2016 and GraphPad Prism 5 (California – USA), analysis of variance (ANOVA) and Tukey's range test to compare between normal control and others treated groups. Results were expressed as mean ± standard errors and P-values <0.05 were considered as a statistically significant.

Results

The results illustrated the effects of neurosteroids, natural and anabolic steroids on

diabetic rats. Hematological parameters, FBS, lipid profile, urea, creatinine, electrolyte, serum calcium, liver enzymes, serum proteins, IL-2, IL-6 and histological examination of kidney, liver and spleen were investigated. The values represent mean ± standard error:

Hematological parameters

The total number of WBCs and monocytes showed highly statistically significant reduction (p<0.001), with statistically significant decrease in lymphocytes (p<0.01), while, granulocytes, MCV and MCH showed highly significant increase (p<0.001) in the diabetic control group compared with the normal control group.

The total number of WBCs were highly significantly decreased (p<0.001) in both diabetic animals treated with hydrocortisone and testosterone compared with diabetic control group, while the diabetic treated group with DHEA was not changed (Figure 1-A). Granulocytes were significantly increased (p<0.001) in the diabetic treated group with hydrocortisone, whereas DHEA and testosterone in the diabetic treated group showed no obvious changes (Figure 1-B). Lymphocytes showed highly significant reduction (p<0.001) in the diabetic treated group with hydrocortisone (Figure 1-C). MCH showed a significant decrease (p<0.01) in the diabetic treated group with testosterone, while treatments with DHEA and Hydrocortisone had no obvious changes, as well as the remnant hematological

parameters had no statistically significant changes as shown in Table 1.

Blood glucose and lipid profile

Diabetic control group showed a highly significant increase (p<0.001) in FBS level, while High - density lipoprotein cholesterol (HDL-cholesterol) showed a highly significant decrease (p<0.001) in comparison with the control group.

FBS level showed a high significant decrease (p<0.001) in the diabetic treated rats with testosterone compared with the diabetic control group. On the other hand, no significant changes were observed in the DHEA and hydrocortisone treated groups (Figure 2-A). Triglycerides (TG) significantly increased (p<0.05) in the diabetic treated group with DHEA; however, the diabetic treated animals with hydrocortisone and testosterone showed highly significant reduction (p<0.001) (Figure 2-B). Very low-density lipoprotein cholesterol (VLDL-C) was highly significantly increased (p<0.001) in the diabetic treated with DHEA. On the other hand, treatment with hydrocortisone and testosterone showed highly significant decrease (p<0.001). While, cholesterol, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein (LDL-C) showed no significant changes in the other treated groups (Table 2).

Urea, creatinine, electrolyte and serum calcium

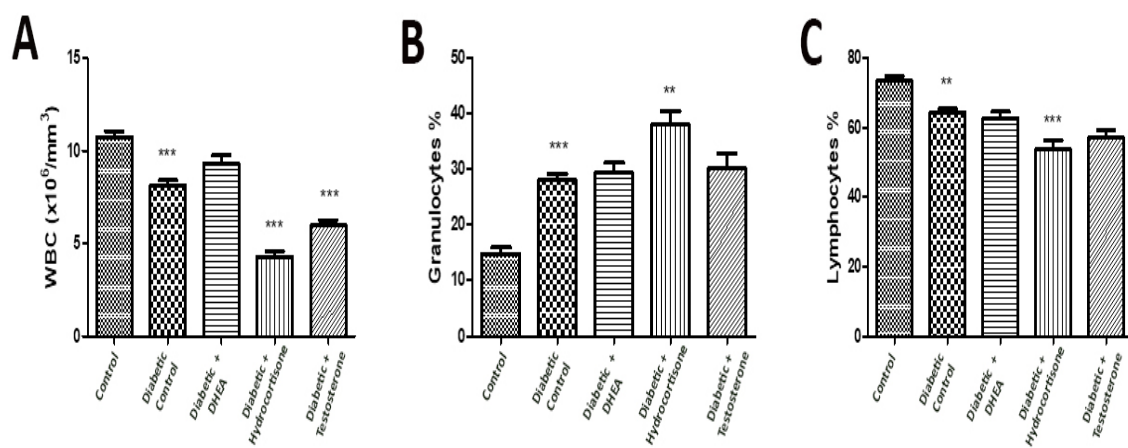Blood urea was significantly increased

Fig. 1. The effect of neurosteroid, natural steroid and anabolic steroid on total A-WBCs count. B-Granulocytes%. C- Lymphocytes%.

TABLE 1. The effects of neurosteroid, natural and anabolic steroids on hematological parameters.

Parameters	Control	Diabetic Control	Diabetic + DHEA	Diabetic + Hydrocortisone	Diabetic + Testosterone
WBCs (x106/mm3)	10.74 ± 0.33	8.115 ± 0.28 ***	9.325 ± 0.42	4.294 ± 0.26 ***	5.953 ± 0.31 ***
Granulocytes %	14.79 ± 1.13	28.12 ± 0.98 ***	29.33 ± 1.82	38.10 ± 2.45 **	30.23 ± 2.75
Lymphocytes %	73.42 ± 1.47	64.35 ± 1.10 **	62.69 ± 1.90	53.85 ± 2.43 ***	57.13 ± 2.08
Monocytes %	9.95 ± 0.44	6.06 ± 0.23 ***	6.23 ± 0.29	6.36 ± 0.32	9.10 ± 0.22
RBCs (x10^6/mm^3)	7.764 ± 0.14	7.282 ± 0.20	7.582 ± 0.07	7.689 ± 0.08	7.849 ± 0.14
Hb (g/dl)	15.06 ± 0.20	14.61 ± 0.26	15.18 ± 0.12	15.33 ± 0.18	15.69 ± 0.21
PCV (%)	44.19 ± 0.99	41.52 ± 1.09	43.80 ± 0.51	44.29 ± 0.66	43.45 ± 0.82
MCV (fl)	53.84 ± 0.71	57.88 ± 0.56 ***	57.69 ± 0.38	57.06 ± 0.44	56.21 ± 0.58
MCH (pg)	19.41 ± 0.10	20.33 ± 0.17 ***	19.98 ± 0.08	19.90 ± 0.08	19.73 ± 0.12 **
MCHC (g/dl)	35.91 ± 0.38	35.30 ± 0.19	34.40 ± 0.20	34.82 ± 0.21	35.13 ± 0.21
Platelets (/mm^3)	578.4 ± 20.37	536.6 ± 12.94	559.5 ± 12.99	574.9 ± 13.19	566.8 ± 9.654

Value expressed mean ± SE. * Significant at $p<0.05$ ** Significant at $p<0.01$ *** significant at $p <0.001$.
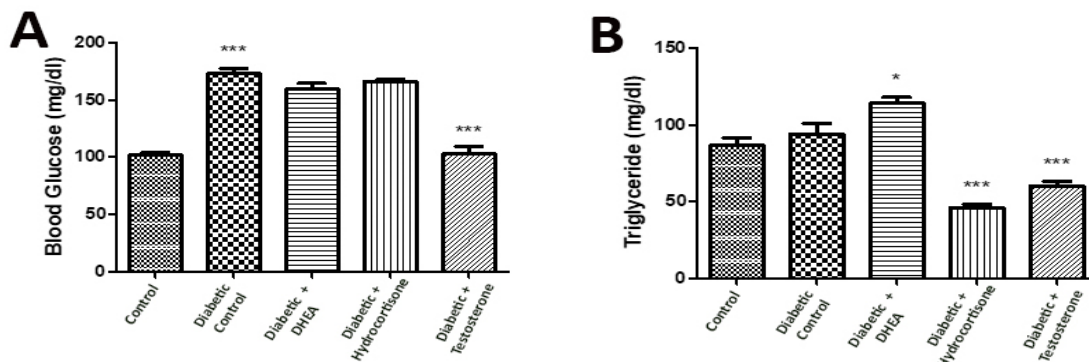

Fig. 2. The effect of neurosteroid, natural steroid and anabolic steroid on A-Blood glucose. B-Triglyceride.

TABLE 2. The effects of neurosteroid, natural and anabolic steroids on FBS and serum lipids level.

Parameters	Control	Diabetic Control	Diabetic + DHEA	Diabetic + Hydrocortisone	Diabetic + Testosterone
Glucose (mg/dl)	101.6 ± 2.38	173.3 ± 3.95 ***	159.2 ± 5.39	166.2 ± 2.14	102.8 ± 6.13 ***
Cholesterol (mg/dl)	69.06 ± 2.32	61.67 ± 2.17	69.81 ± 2.46	65.00 ± 1.79	69.67 ± 1.33
Triglycerides (mg/dl)	86.41 ± 5.04	93.77 ± 7.03	114.2 ± 3.79 *	45.94 ± 1.98 ***	60.07 ± 3.08 ***
High-density lipoprotein cholesterol (mg/dl)	38.00 ± 1.03	32.08 ± 0.92 ***	35.56 ± 0.94	33.38 ± 0.66	33.07 ± 0.85
Low- density lipoprotein cholesterol (mg/dl)	18.24 ± 0.90	22.00 ± 1.01	18.25 ± 1.46	21.81 ± 1.01	22.53 ± 0.81
Very low- density lipoprotein cholesterol (mg/dl)	17.17 ± 0.90	18.75 ± 1.40	22.84 ± 0.75 **	9.188 ± 0.39 ***	12.01 ± 0.61 ***

Value expressed mean ± SE. * Significant at $p<0.05$ ** Significant at $p<0.01$ *** significant at $p <0.001$.

(p<0.05), whereas uric acid was significantly decreased (p<0.05), additionally calcium (Ca) showed highly significant reduction (p<0.001) in diabetic control group compared with normal control group.

Blood urea was significantly increased (p<0.05) in diabetic treated group with hydrocortisone however; no changes were detected in the DHEA and testosterone treated rats. Also, no significant changes showed in serum creatinine level (Figure 3-A). Uric acid was significantly increased (p<0.05) in both DHEA and testosterone treated animals, and no significant changes found in the hydrocortisone group (Figure 3-B). Sodium (Na) was significantly reduced (p<0.01) in diabetic treated rats with DHEA. Also, chloride (Cl) showed highly significant reduction (p<0.001) in the diabetic treated with DHEA, while there were no significant differences in the other treated groups. Moreover, potassium (K) and calcium (Ca) revealed no changes in all mentioned groups in comparison with diabetic control group, as shown in Table 4.

Liver enzymes and serum proteins

TABLE 3. The effects of neurosteroid, natural and anabolic steroids on urea, creatinine, uric acid, serum electrolyte and calcium.

Parameters	Control	Diabetic Control	Diabetic + DHEA	Diabetic + Hydrocortisone	Diabetic + Testosterone
Blood urea (mg/dl)	17.04 ± 0.37	21.10 ± 1.47 *	21.99 ± 0.73	25.18 ± 0.91 *	24.47 ± 1.65
Creatinine (mg/dl)	0.3247 ± 0.013	0.3246 ± 0.028	0.3136 ± 0.014	0.3725 ± 0.051	0.3460 ± 0.025
Uric acid (mg/d)	2.429 ± 0.11	1.936 ± 0.14 *	2.6 ± 0.09 **	2.269 ± 0.088	2.52 ± 0.07 **
Sodium (mmol/L)	128.2 ± 2.49	130.9 ± 0.53	118.2 ± 2.29 **	124.1 ± 3.19	127.5 ± 2.39
Chloride (mmol/L)	89.18 ± 2.49	91.85 ± 0.79	76.69 ± 0.71 ***	95.63 ± 1.05	90.33 ± 1.16
Potassium (mmol/L)	4.824 ± 0.26	4.154 ± 0.15	4.194 ± 0.069	4.344 ± 0.11	4.127 ± 0.081
Calcium (mg/dl)	9.659 ± 0.16	8.931 ± 0.10 ***	9.388 ± 0.097	8.800 ± 0.058	9.033 ± 0.068

Value expressed mean ± SE.   * Significant at p<0.05   ** Significant at p<0.01   *** significant at p <0.001.
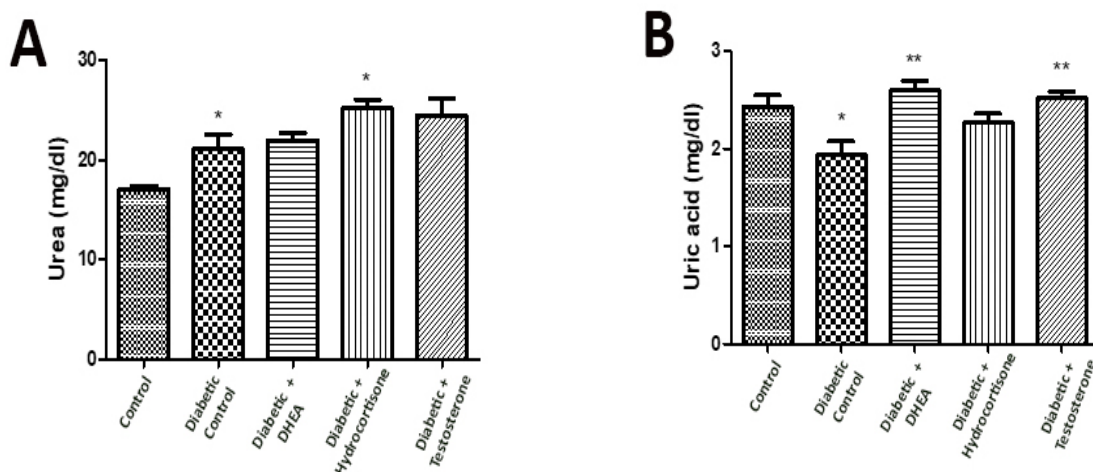

Fig. 3. The effect of neurosteroid, natural steroid and anabolic steroid on A -Blood Urea. B-Serum Uric acid.

TABLE 4. The effects of neurosteroid, natural and anabolic steroids on liver function parameters and serum proteins.

Parameters	Control	Diabetic Control	Diabetic + DHEA	Diabetic + Hydrocortisone	Diabetic + Testosterone
Aspartate amino-transferase (AST) IU/L	113.5 ± 3.14	100.5 ± 2.67	122.9 ± 6.42	99.38 ± 3.19	93.53 ± 3.16
alanine transaminase (ALT) IU/L	44.65 ± 1.03	43.38 ± 2.20	51.75 ± 3.19	38.06 ± 2.04	40.27 ± 2.73
Alkaline phosphatase (ALP) IU/L	350.5 ± 5.95	523.6 ± 8.88 ***	366.1 ± 6.8 ***	253.1 ± 7.7 ***	262.9 ± 5.7 ***
Total Proteins (g/dl)	5.400 ± 0.10	5.646 ± 0.15	8.706 ± 0.21 ***	6.450 ± 0.14 ***	6.700 ± 0.06 ***
Albumin (g/dl)	2.82 ± 0.11	3.235 ± 0.08	3.489 ± 0.12	4.288 ± 0.09 ***	3.231 ± 0.08
Globulin (g/dl)	2.801 ± 0.07	2.157 ± 0.19 *	4.419 ± 0.20 ***	3.213 ± 0.11 ***	3.053 ± 0.11 ***

Value expressed mean ± SE.   * Significant at $p<0.05$ ** Significant at $p<0.01$ *** significant at $p<0.001$.

Alkaline phosphatase (ALP) showed a highly significant increase ($p<0.001$) in diabetic control group, whereas, globulin significantly reduced ($p<0.05$) in the diabetic control group compared with the control group.

Aspartate aminotransferase (AST) and alanine transaminase (ALT) enzymes showed no significant changes in all involved groups. While ALP showed a highly significant reduction ($p<0.001$) in all treated groups (Figure 4-A), the total protein and globulin showed a highly significant increase ($p<0.001$) in all treated groups (Figure 4-B & C). Albumin showed a highly significant elevation ($p<0.001$) in diabetic treated group with DHEA, whereas no significant changes were recorded in the other treated groups (Table 4).

IL-2 and IL-6

IL-2 and IL-6 showed no significant changes in diabetic control group compared with the control group.

IL-2 significantly increased ($p<0.05$) in diabetic treated group with DHEA, whereas there were no significant changes in the diabetic Hydrocortisone and testosterone treated groups (Figure 5-A). On the other hand, IL-6 showed a significant decline ($p<0.01$) in diabetic treated group with DHEA and hydrocortisone (Figure 5-B). However, no significant changes were observed in the diabetic testosterone treatment group compared with diabetic control group (Table 5).

Body Weight

The differences in the body weight were highly declined ($p<0.001$) in the diabetic control group compared with the control group. The diabetic treated groups with hydrocortisone and testosterone also showed a highly significant decrease ($p<0.001$). On the other hand, the diabetic treated group with DHEA were highly significantly elevated ($p<0.001$) compared with diabetic control group (Fig. 6, Table 6).

Histopathological Examination
Kidney

Kidney sections showed almost normal cytoarchitecture appearances of glomeruli (Gs) and renal tubules (PCT & DCT) in the control group (Fig. 7-A). Various damages in kidney section appeared in the diabetic control group such as disintegrations of the Gs, moderate fibrosis (F) in glomerulus and Bowman's capsule, necrosis in PCT and DCT (N-PCT& N-DCT), congestion (C) of blood vessels and hemorrhages (H) (Fig. 7-B). The administration of DHEA in the diabetic rats caused several damages including C, H and N-PCT and N-DCT. The diabetic hydrocortisone treated animals, several structural damages were appeared involving; C in glomerulus, N-PCT, N-DCT, H between renal tubules, F in glomerulus and Bowman's capsule and narrowing in the lumen, which disappeared in brush boarders of some PCT cells (Fig. 7-C & D). Also, in diabetic testosterone treated group, the kidney showed several histopathological changes in renal corpuscle such as glomerulus atrophy and increase in Bowman's space, C, F between renal tubules and N-PCT, N-DCT (Fig. 7-E).
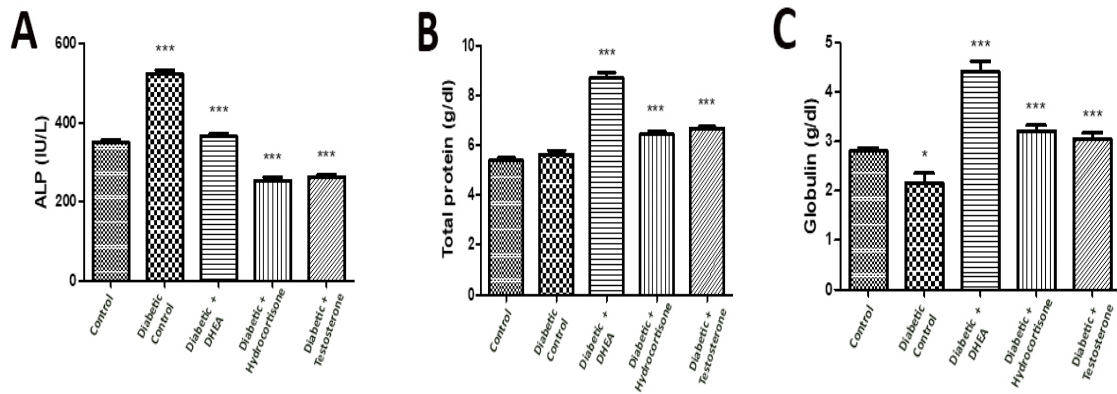
Fig. 4. The effect of neurosteroid, natural steroid and anabolic steroid on A-Alkaline phosphatase. B-Total protein. C- Globulin.
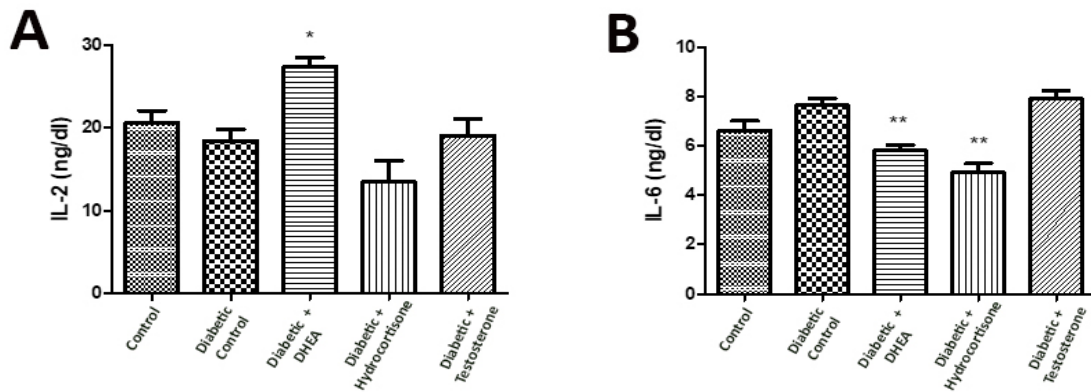

Fig. 5.The effect of neurosteroid, natural steroid and anabolic steroid on A-IL-2. B- IL-6.

TABLE 5. The effects of neurosteroid, natural and anabolic steroids on IL-2 and IL-6.

Parameters	Control	Diabetic Control	Diabetic + DHEA	Diabetic + Hydrocortisone	Diabetic + Testosterone
Interleukin-2 (ng/dl)	20.63 ± 1.46	18.45 ± 1.40	27.42 ± 1.13 *	13.41 ± 2.66	19.10 ± 1.91
Interleukin-6 (ng/dl)	6.64 ± 0.39	7.67 ± 0.27	5.81 ± 0.24 **	4.94 ± 0.34 **	7.91 ± 0.32

Value expressed mean ± SE.   * Significant at $p < 0.05$ ** Significant at $p < 0.01$ *** significant at $p < 0.001$.

TABLE 6ز The effects of neurosteroid, natural and anabolic steroids on Body Weight.

Parameters	Control	Diabetic Control	Diabetic + DHEA	Diabetic + Hydrocortisone	Diabetic + Testosterone
First Day (g)	168.2 ± 3.33	180.6 ± 2.57	189.4 ± 2.96	181.4 ± 3.98	188.2 ± 3.49
Last Day (g)	266.4 ± 5.02	206.2 ± 3.04 ***	224.2 ± 2.80 *	187.0 ± 4.47 *	203.1 ± 3.96
Weight changes (g)	98.20 ± 2.37	25.60 ± 1.57 ***	34.80 ± 0.55 ***	5.6 ± 0.58 ***	14.9 ± 0.77 ***

Value expressed mean ± SE.   * Significant at $p < 0.05$ ** Significant at $p < 0.01$ *** significant at $p < 0.001$.
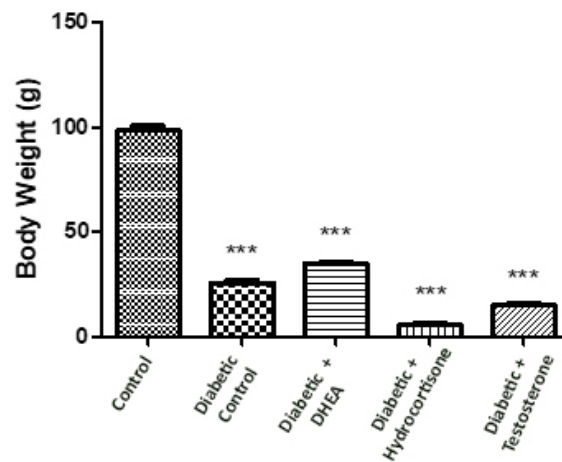
Fig. 6. The effect of neurosteroid, natural steroid and anabolic steroid on body weight change.
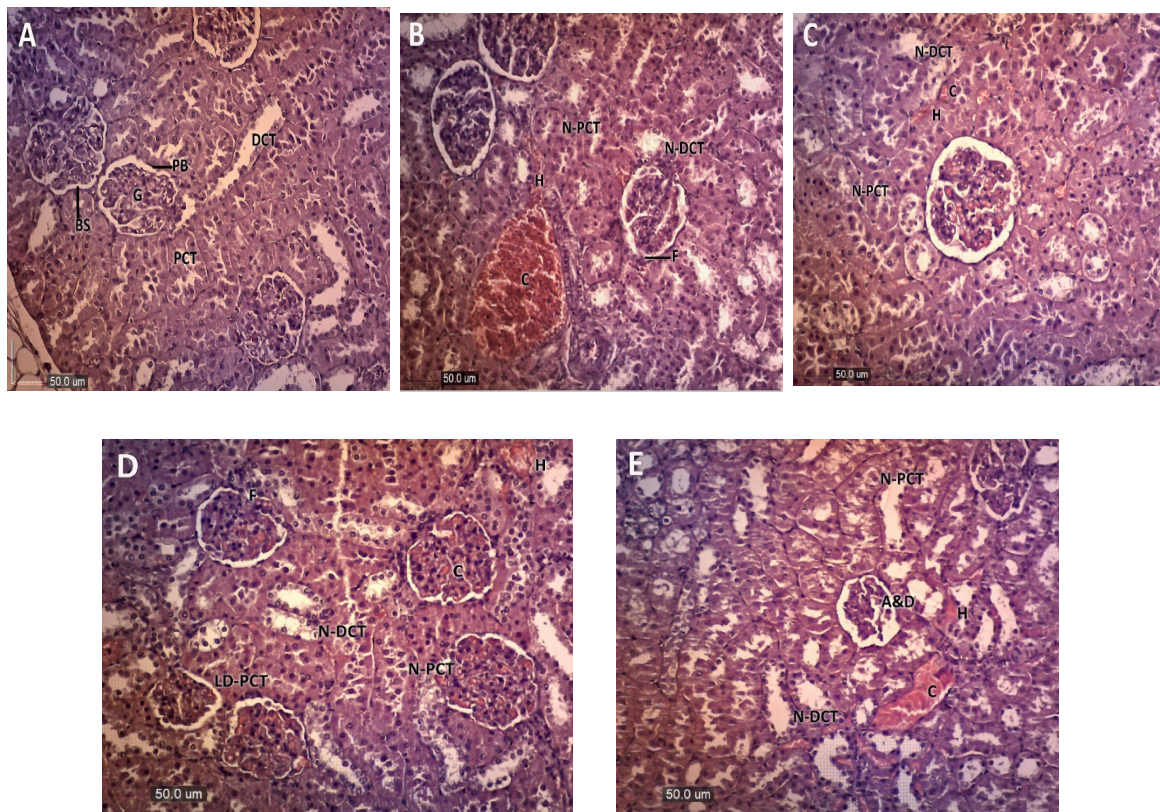

Fig. 7. Cross section of kidney (H&E): A-Control group, showing normal glomerulus (G), with normal proximal & distal convoluted tubules (PCT & DCT). B- Diabetic control group: hemorrhage (H), congestion (C), fibrosis (F) & necrosis in both renal tubules (N-PCT & N-DCT). C- Diabetic + DHEA group: hemorrhage (H), congestion (C) & necrosis in both renal tubules (N-PCT & N-DCT). D- Diabetic + Hydrocortisone group: hemorrhage (H), congestion (C), fibrosis (F), decrease the lumen of proximal convoluted tubule (LD-PCT) & necrosis in both renal tubules (N-PCT & N-DCT) and E- Diabetic + Testosterone group: Atrophy & degeneration (A&D), hemorrhage (H), congestion (C) & necrosis in both renal tubules (N-PCT & N-DCT).

Liver

The histological section of the liver structure in the control group was normal including the size of central vein (CV), clear appearance of hepatocytes (HE) containing large and central nucleus, and the hepatic cord (HC) was obvious (Fig. 8-A). The histological structure of the diabetic control group showed numerous histopathological changes such as moderate C-CV, vacuoles in HE cytoplasm, hepatocytic N and different damages in HE nucleus (Fig. 8-B). Whilst, the diabetic group treated with DHEA displayed different hepatic histopathological alterations including C-CV and C-HS, H in hepatic tissue, hepatocytic N and different damage in nucleus of HE (Fig. 8-C). The diabetic hydrocortisone treated group also exhibited different histopathological damages like C-CV and C-HC, N, increase in hepatic cells, different damages in HE nucleus and dilation in hepatic sinusoidal lumen (Fig. 8-D). On the other hand, the diabetic testosterone treated group revealed a mild to moderate vascular congestion, swelling of HE, and filtration of inflammatory cells (FICs), N and F in the wall of the central vein was observed with C-HC (Fig. 8-E).

Spleen

Fig. (9-A) shows normal spleen architecture with regular appearance of white and red pulps (WP & RP) with clear central arteriole (CA). The histological examination of the diabetic control group showed mild N in both WP and RP (Fig. 9-B). On the other hand, the diabetic treated animals with DHEA exhibited decrease in the size of WP with moderate N-RP (Fig. 9-C). The diabetic treated animals with hydrocortisone revealed a decrease in the size of WP with obvious separation from RP and N-WP and N-RP (Fig. 9-D). Furthermore, the diabetic treated animals with testosterone showed decease in WP size with noticeable departure from RP and N-WP and N-RP (Fig. 9-E).
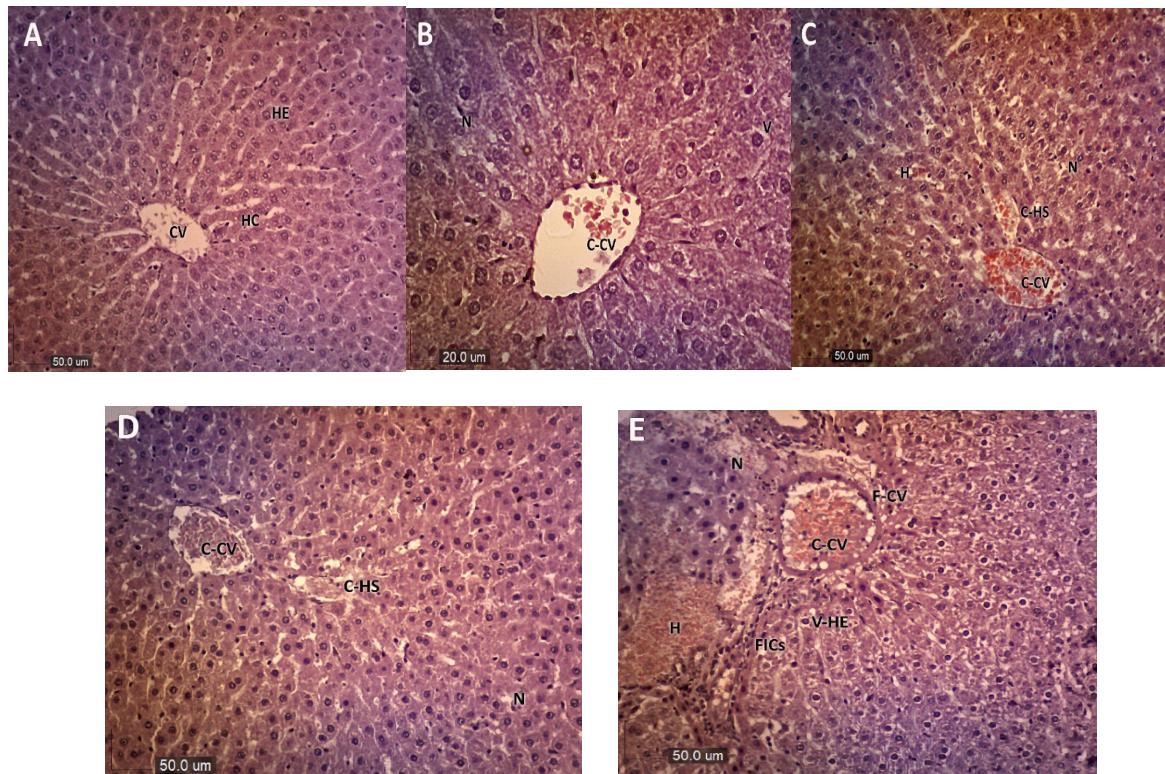

Fig. 8. Cross section of liver (H&E): A-Control group: showing normal central vein (CV), hepatic cord (HC) & hepatocytes (HE). B- Diabetic control group: Central vein congestion (C-CV) & necrosis (N). C- Diabetic + DHEA group: Central vein congestion (C-CV), hepatic sinusoids congestion (C-HS), necrosis (N) & hemorrhage (H). D- Diabetic + Hydrocortisone group: Central vein congestion (C-CV), hepatic sinusoids congestion (C-HS) & necrosis (N) and E- Diabetic + Testosterone group: Central vein congestion (C-CV), filtration of inflammatory cells (FICs), vacuole in hepatocyte cytoplasm (V-HE), fibrosis in central vein walls (F-CV), hemorrhage & necrosis (N).
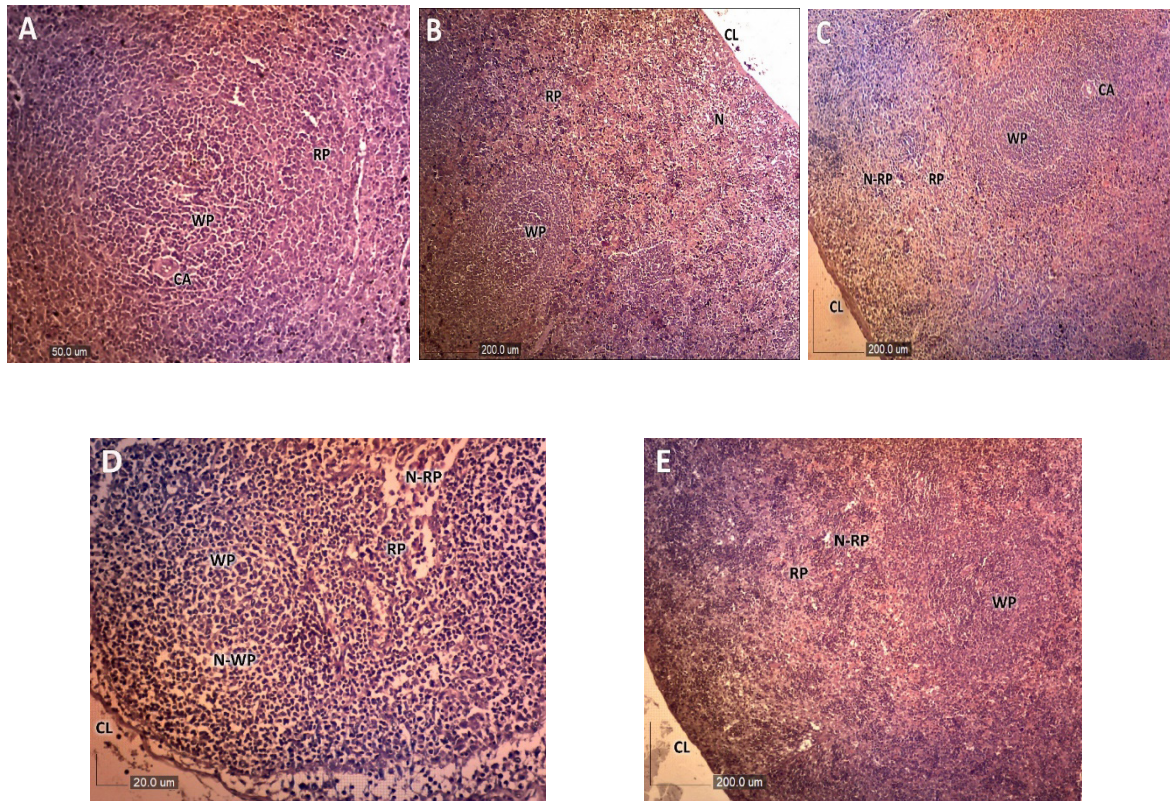
Fig. 9. Cross section of spleen (H&E): A-Control group: showing normal white pulp (WP), red pulp (RP) & central arteriole (CA). B- Diabetic control group: capsule (CL), WP, RP & necrosis (N). C- Diabetic + DHEA group: WP, RP, CA, CL & necrosis in red pulp (N-RP). D- Diabetic + Hydrocortisone group: WP, RP, CL, necrosis in white pulp (N-WP) & necrosis in red pulp (N-RP) and E- Diabetic + Testosterone group: WP, RP, CL & necrosis in red pulp (N-RP).

Discussion

This study aimed to investigate the physiological, immunological and histopathological differences between the effects of neurosteroid, natural steroid and anabolic synthetic steroid on various target organs such as kidneys, liver and spleen in induced diabetic rats.

Circulating steroid hormones, including DHEA and DHEA-S can cross the blood-brain barrier freely and can be converted in the brain to neurosteroids in several regions, including the cortex, the hippocampus and the amygdala. Additionally, DHEA-S together with DHEA have shown to cause anti-glucocorticoid and neuroprotective activities [19]. Also, there is evidence that DHEA-S is synthesized in the glial cells of the rodent brain, which has been termed neurosteroids and can be made and act locally [20]. Glucocorticoids (GCs) are steroid hormones, which are widely used for the treatment of inflammation, autoimmune diseases and cancer.

and exert various broad of physiological and therapeutic effects [21]. Anabolic androgenic steroids are synthetic derivatives of the male hormone testosterone, manufactured to maximize anabolic and minimize androgenic effects [22, 23].

The total WBCs count, lymphocytes and monocytes were significantly decreased, while granulocytes were significantly increased compared with diabetic control group and normal control group. These results are line with Queiroz et al. [24], which suggested the administration of alloxan into the animals caused a decrease in lymphocytes and in general, the total number of circulating leukocytes, while granulocytes increased.

WBCs count showed no significant changes in the diabetic treated with DHEA group, which might be due to the positive effects of DHEA on immunity and its role in the anti-inflammatory response [25]. While, hydrocortisone and

testosterone treated animals showed highly significant reduction in WBCs count compared with the diabetic control group that could be due to the actions of glucocorticoids including the inhibition of leukocytes movement and access of these cells to the site of inflammation as reported by Stahn & Buttgereit, (2008), moreover, hydrocortisone has a negative effect on WBC count [26, 27].

In parallel to our findings, Albano et al. [14], suggested a possible correlation between anabolic steroids abuse and immunodeficiency effects related to a similar action of corticosteroid activity. In another study, testosterone treatment increased circulating leukocytes [28]. The increased granulocytes percentage in diabetic hydrocortisone treated rats in the current study was in comparable with a study conducted by Al-Maliki *et al.*, [27]. Alan and Alan, [29], suggested that the administration of glucocorticoids caused inhibition of neutrophil adhesion to endothelial cells; this effect reduces trapping of neutrophils in the inflamed region and probably is responsible for neutrophilia. The reduction of lymphocytes might be caused by the inhibition of antigen, cytokine production and proliferation by binding to glucocorticoid receptors [8]. Testosterone treatment increased the neutrophil and monocyte, while lymphocyte was not affected. The reduction in the relative proportion of lymphocytes was likely due to the increase in the neutrophil and monocyte [28].

The blood glucose level showed high significant increase, whereas the HDL showed a highly significant decrease in both normal and diabetic control groups. The fact of this highly significant variation might be due to the pathophysiological effects of alloxan treated animals [30].

Despite of the insignificant changes in the DHEA treated group, the level of glucose only declined about 10%, which is still considered to be anti-diabetic agent [31]. The reduction may be due to the role of DHEA enhancing glucose uptake by other cells such as fibroblasts, adipocytes, hepatocytes. Kang *et al*. [32], referred the role of DHEA in regulating serum glucose levels by stimulating glucose conversion to glycogen.

Glucocorticoid therapy can lead to various metabolic complications in glucose level including insulin resistance and hyperglycemia

[33]. Furthermore, glucocorticoids reduce the GLUT2 and glucokinase receptors expression and increase the activity of glucose-6-phosphate dehydrogenase. Additionally, they reduce insulin synthesis and cell mass through the beta cell apoptosis [34].

The glucose level in the diabetic testosterone treated animals was reduced, and this finding is in agreement with previous studies. The reduction was due to an increase in the expression of insulin-regulated glucose transporter mRNA and an androgen-dependent gene. Furthermore, the decrease in fat mass was also accompanied by a decrease in plasma glucose concentration and resistance [35, 36].

Triglycerides (TGs), VLDL, body weight gain were increased in the diabetic rats treated with DHEA. This finding does not correspond with (Labrie, 2010) who found that DHEA decrease serum Lipoproteins, an effect that should be beneficial for cardiovascular diseases [37]. We suggested that male hypogonadism associated with decreased serum testosterone level could occur more frequently in male with diabetes mellitus and metabolic syndrome. Furthermore, decreased serum androgens mainly testosterone is linked with insulin insensitivity, central obesity, increased LDL and hypertension. The study also believes that the effect of DHEA as replacement therapy for male hypogonadism in rats, as the data showed that male hypogonadism increased blood glucose and lipids. Also, DHEA treatment in the control and orchidectomized rats had no effects on serum testosterone level [38]. Moreover, male hypogonadism increased the body weight, BMI, liver weight, and systolic blood pressure [39].

The diabetic hydrocortisone and testosterone treated groups showed highly significant decrease in triglyceride, VLDL and body weight gain. Glucocorticoids had a very important role in energy balance and on lipid metabolism. All possible changes in lipid profile including an improvement in lipid profile have been reported [29]. Furthermore, glucocorticoids play a role in the regulation of triglyceride by regulation of synthesis and hydrolysis of triglycerides [40].

Over physiological doses of Anabolic androgenic steroids are associated with abnormal plasma lipoproteins. Testosterone therapy caused decreased plasma lipid levels [12]. Additionally, testosterone inhibits LPL activity, decreases

triglyceride accumulation, and stimulates lipolysis [41].

Blood urea showed significant increase; this elevation could be associated with the induction of diabetes in the diabetic control group as higher levels of blood urea are linked with increased risk of diabetes [42]. On the other hand, uric acid and serum calcium were significantly decreased, which is in agreement with the findings of Nan *et al.* [43], stated that the serum uric acid was significantly elevated in non-diabetic and reduced after the onset of diabetes. The reduction of calcium, in both types 1 and 2 diabetes mellitus was also associated with profound deterioration of calcium and bone metabolism [44].

DHEA in patients with type 2 diabetic nephropathy decrease gradually with the progress of the disease. This might be explained by the anti-oxidant protective effect of DHEA on diabetic nephropathy [45].

Uric acid was elevated significantly in all diabetic treated groups; a similar result was reported by Xie *et al.* in hydrocortisone treatment [42]. They observed a positive correlation between DHEA and uric acid in both men and postmenopausal women, possibly due to the assumption that DHEA could have activated mineralocorticoid receptors and inhibited glucocorticoid receptors, which might have led to serum uric acid concentration increases by reduced renal uric acid excretion. However, the specific mechanism is still not clear [46]. Furthermore, the high dose of testosterone replacement therapy causes serum uric acid up-regulation [47].

Alkaline phosphatase (ALP) significantly increased in the diabetic control group compared with the control group. This increase was related to the induced diabetic by alloxan, and showed bone fraction isoenzyme in both genders, which consequently elevated serum ALP [48].

All treated groups showed significant reduction in ALP level, while no alterations were displayed in the other liver enzymes. DHEA has been shown to exert a protective effect in hepatocytes against oxidative injury in animal models of oxidative stress [49]. Glucocorticoids therapy does not usually appear clinically though it can cause hepatic steatosis and hepatic enlargement [50]. Also, Werumeus et al. [51], reported a reduction in ALP activity in response to high dose of hydrocortisone. Testosterone treatment dropped the ALP activity

in both genders of the treated rats, which could be explained as the base of alterations of bone turnover due to high dose of testosterone [16, 52].

Plasma proteins revealed high significant increase in all treated groups. DHEA and DHEAS bound to albumin in the plasma [53]. Most glucocorticoids are bound and transported by a specific globulin; glucocorticoid binding globulin (GBG) can also be bound to testosterone and other hormones [54]. Anabolic androgenic steroids are characterized by the activation of protein synthesis, and the over physiological dose may increase the protein mass in treated rats due to amino acid uptake and protein synthesis amelioration, a similar mechanism was described in skeletal muscles where increasing protein synthesis and decreasing protein catabolism were occurred [14].

IL-2 significantly raised in the diabetic animals treated with DHEA, whereas IL-6 significantly declined in the same treated group. DHEA increases the production of cytokines that promote leukocytes activity, while inhibits the production of cytokines responsible for inflammation [55]. It can affect cytokine production, downregulating inflammatory cytokines and upregulating the anti-inflammatory IL-2 synthesis; thus, opposing the glucocorticoids response; it can enhance lymphocyte proliferation [25]. On the other hand, the diabetic hydrocortisone treated group showed significant reduction in IL-6. IL-2 was obviously and clinically reduced, but statistically turned out not to be significant. Glucocorticoids inhibit the secretion and synthesis of inflammatory molecules such as IL-2 and IL-6 [29]. A recent study has indicated that the serum cytokines such as TNF-a, IL-1b, and IL-6 were inhibited by hydrocortisone, which were consistent with previous studies in both animals and humans [56].

The rats with alloxan-induced diabetes had various histopathological abnormalities in their kidney such as increased fibrosis, glomerular hypertrophy, glomerular space narrowing, loss of tubules brush border, and necrotic and thickened basal membranes. As well as, histological changes of liver with alloxan induction including congestion, necrosis, hepatic hyperplasia and acute hepatopathy [57, 58]. Additionally, diabetes leads to several histological alterations in many organs, including the spleen. As collagen accumulation causes pathological disruption of the spleen and necrosis, it is feasible that changes in fiber

distribution in the spleen could clarify the grading of splenic tissue damage caused by diabetes [59].

DHEA's effects on oxidative imbalance may lead to severe kidney nephron destruction and renal injury, including proximal tubular and glomerular damage [60]. Jahn et al. [29], postulated that transforming the growth factor beta1 (TGF- β1) expression was improved by DHEA administration in diabetic rats, which may suggest that the treatment caused an elevation in the levels of the oxidative stress tissues. Moreover, the administration of DHEA reduced liver injury; liver damage was constrained to small areas of congestion, necrosis and a number of apoptotic cells [61]. The dynamics of leukocyte traffic during inflammation may be diminished by DHEA therapy. As a result, the administration of DHEA decreased the number of adherent leukocytes, reflecting an accumulation of splenic cells in contrast to the decreasing frequency of lymphocytes [62]. Moreover, DHEA promotes the production of pro-inflammatory cytokines that have been repressed and could serve as a natural anti-glucocorticoid, reversing the immunosuppressive effects of glucocorticoids [63].

Glucocorticoids may disturb the progress of numerous tissues, causing an advanced effect in organs, such as the liver, lungs, and kidneys. Glucocorticoids can affect the patterns of insulin activity in the plasmatic and tissue lipid profiles as well as the patterns of cellular proliferation and degeneration over the tissue of these organs. It also affects the organization of tissues, and the existence of proteins that binds glucocorticoids has been specifically observed in each of the hepatoma tissue culture cells, fibroblasts, liver and kidney [64]. Renal tubules can be turned into necrotic, and expanded after receiving a high dose of hydrocortisone. One of the factors which influence how the tissue of the liver changes is that glucocorticoids have the strongest effect on adipose tissue. This indicates that glucocorticoids increase the release of fatty acids, while reducing the uptake and utilization of glucose [65]. Furthermore, glucocorticoids suppress the production of prostaglandin and arachidonic acid, which causes hepatic sinusoidal dilatation and congestion [66]. Hydrocortisone treatment causes severe atrophy of splenic white pulp tissue through destroying the tissues and causes diminution of lymphocytes, especially T type lymphocytes and spleen tissue seems quite depleted from T lymphocytes [65, 67].

The administration of anabolic androgenic steroids (AASs) revealed a variety of renal histopathological impacts, including; severe podocyte injury, detachment of the glomerulus basement membrane, which is a site for adhesions to parietal epithelial cells of the Bowman's capsule, renal tubule injury and degeneration, hemorrhage, and necrosis that could be attributed to mitochondrial dysfunction and subsequent cellular ATP reduction [68]. Alterations in the liver's histopathology were brought about by the use of AASs that included an accumulation of fibrosis and necrosis of hepatic cells, mild to severe vascular congestion with inflammatory cell infiltration, and congestion in the sinuses of the liver. AASs caused liver damage through both intrinsic and idiosyncratic hepatotoxicity processes. Depending on the dose, intrinsic hepatotoxins either directly by the drug or indirectly by the drug's metabolite caused hepatocellular damage [69]. Acute changes were observed in the spleen's histological structure in the rats treated with anabolic testosterone such as hyperplasia with modest red pulp destruction, dilation of the white pulp, in addition to testosterone, which is based on the relative energy expended, and the effort required for reproduction may be considered a physiological regulator of immunocompetence as it minimized immunological responses [70].

Conclusion

It is evident from the research provided results that DHEA treatment may improve the amount of leukocytes and IL-2 levels, which could eventually improve immunity. Moreover, DHEA seems to have a preventive impact against the oxidative stress related to diabetes. It also has the capacity to reduce tissue damage to the kidney and spleen. On the contrary, the administration of hydrocortisone can enhance lipid profiles by reducing triglyceride levels, nonetheless it seems to have adverse effects on immunity and the state of tissues. Whereas, Sustanon has a beneficial effect on lipid profiles and blood glucose levels, but its effects on immunity are not clear.

In summary, our results imply that DHEA can represent a treatment alternative with promise for improving immunity and lowering oxidative stress in diabetes. However, there may be trade-offs between hydrocortisone and Sustanon in terms of how they affect lipid profiles, immunity, and the health of tissues.

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الستيرويدات العصبية والطبيعية والمصنعة: دراسة فسيولوجية ومناعية ونسيجية على الجرذان الألبينية المصابة بداء السكري.

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تعتبر الستيرويدات العصبية من الستيرويدات الطبيعية والمنتجة في الجهاز العصبي وتعمل كناقلات عصبية أو محولات عصبية ، وتنتج هرمونات الستيرويد بشكل رئيسي في الغدد الكظرية وتلعب دورًا حاسمًا في تنظيم الإجراءات الفسيولوجية المختلفة في الجسم ، في حين أن الستيرويدات الابتنائية هي المركبات الاصطناعية التي لها تأثيرات مماثلة لهرمون التستوستيرون.

هدفت هذه الدراسة إلى التفريق بين دور الستيرويدات العصبية والستيرويد الطبيعية والستيرويد الابتنائية الاصطناعية من خلال التحقق من تأثيرات على بعض الوظائف الفسيولوجية, المناعية والتشريح المرضي على الأعضاء المستهدفة المختلفة مثل الكلى والكبد والطحال في الفئران المصابة بداء السكري.

تم تقسيم عدد خمسين من ذكور جرذ البيضاء بشكل عشوائي إلى خمس مجموعات متساوية. تم استخدام المجموعة الأولى كعنصر تحكم تم تغذيتها على نظام غذائي معياري وحقنها تحت الجلد مع ٠٫٥ مل من محلول ملحي عادي. تم حقن المجموعة الثانية تحت الجلد مع Alloxan (١٠٠ مجم / كجم من وزن الجسم) التي تم تغذيتها على نظام غذائي قياسي وعملت كمجموعة مراقبة لمرض السكري ، بينما تم تناول جرذان المجموعة الثالثة عن طريق الفم باستخدام (DHEA على هيئة هرمون عصبي ، ١٧٫٢ مجم / كجم / يوما) والمجموعة الرابعة تم حقن الجرذان المصابة بمرض السكر تحت الجلد بالهيدروكورتيزون (على هيئة ستيرويد طبيعي ٢٦٫١ مجم / كجم / يوما) . أخيرًا ، المجموعة الخامسة من الجرذان المصابة بداء السكري تم حقنها عضليًا مع سوستانون (على شكل ستيرويد ابتنائي ، ٦٦٫٩ مجم / كجم / أسبوعيا). بناءً على النتائج المذكورة أعلاه ، وجدت أنه في الجرذان المصابة بداء السكري أظهرت المعلمات الدموية والتحسينات في الإنترلوكينات IL-2 و IL-6 مع DHEA ، سكر الدم الصائم ووزن الجسم مع سوستانون ، ملف الدهون مع كل من الهيدروكورتيزون والسوستانون ، تم تحسين ALP تقريبًا في جميع المجموعات المعالجة. ختاماً ، من النتائج التي تم الحصول عليها ، يمكن الإبلاغ عن أن إدارة DHEA يمكن أن تحسن المناعة ، وتقليل الإجهاد التأكسدي المرتبط بمرض السكري ، وتخفيف تلف الأنسجة في الكلى والطحال. قد يحسن الهيدروكورتيزون صورة الدهون ، ولكن له آثار سلبية على المناعة وصحة الأنسجة. السوستانون له تأثيرات إيجابية على نسبة السكر في الدم وملف الدهون ، ولكن آثاره على المناعة لا يمكن تمييزها.

الكلمات الدالة: ديهيدروإيبياندروستيرون ، الهايدروكورتيز ، السوستانون ، مرض السكري.