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Histological assessment for the effect of Growth hormone one the albino mice kidney, followed by administration of Sage extract as a trial for treatment

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ABSTRACT

This study used (40) laboratory mice whose average weight was (30 ± 3) grams, divided equally into four groups. The first group was injected subcutaneously with growth hormone at a concentration of 0.2 kilograms/mg between one day and another at a dose of 0.25 ml. for a period of three months. While the animals of second group were injected with 0.2mg/kg BW growth hormone and dosed orally with sage extract at a concentration of 50 mg/kg of body weight for three months. The third group was dosed with sage extract only, while the fourth group (control group) was not dosed or injected. The animals were dissected and samples were taken from the animals' kidneys and prepared for histological study to study the tissue changes resulting from injection with growth hormone and dosing with sage extract. The results showed the absence of normal tissue pattern in the group of male mice injected with growth hormone, in contrast to the group of mice injected with growth hormone and dosed with sage extract, while no tissue changes appeared in the kidneys of mice dosed with sage extract.

Introduction: Steroids are the various industrial substances (solid, liquid, gaseous) that an athlete takes with the intention of increasing the athlete's physical, psychological, and mental efficiency. Unnatural substances and means are used by injection, mouth, or nose during training and preparation for competitions, or during competitions for the purpose of illegal gain. Which may result in health damage [1]. One of the most famous and important of these steroids is the growth hormone HG, also known as Somatotropin, which is one of the peptide hormones. It consists of 191 amino acids, its molecular weight is 22,124 Daltons, and its half-life is 30 minutes. in the blood , . Growth hormone is secreted from the anterior part of the pituitary gland, specifically from somatotrophs, which regulates the body's growth rate and stimulates cell proliferation and renewal [2]. Growth hormone plays an important role in cell division and differentiation, bone formation, and muscle structure, as it works to This includes nitrogen retention, the transport of amino acids to the muscles, the generation of insulin-like growth factor I (IGF-I) and insulin-like growth factor-binding protein 3 (IGFBP), it also works on the decomposition of stored fat, and the retention of sodium or phosphorus, which leads to cell swell to add In addition, growth hormone can convert T4 to T3 and active cortisone to its inactive form [3]. These reasons are what make growth hormone one of the most important steroids used by athletes , The kidney is one of the most important sites of GH metabolism after the liver [5,4]. Research has shown that the thujones present in the volatile oil of the sage plant, Salvia officinalis, are a strong antiseptic and carminative, in addition to being a stimulant for the production of estrogens, [6] which is partly responsible for the hormonal effect of sage, especially in reducing the production of the prolactin hormone. In addition, rosmaric acid, which is one of the Phenols are a powerful anti-inflammatory, and are also used for mouth sores and ulcers in the gums. Because sage is an astringent, it is useful for mild diarrhea. In addition, sage is considered a tonic and digestive stimulant, in addition to helping to calm and stimulate the nervous system. [7] In addition to the above, sage is a valuable treatment for irregular menstrual cycles, as it stimulates menstrual blood flow, and its action in generating estrogen makes it a suitable treatment for menopause, as sage helps the body adapt to the hormonal changes occurring in it [8]. The present study was designed to evaluate the effect of Growth hormone on renal tissue followed by administration of Sage extract (Salva officalis)

2. Material and Methods:

Material and Methods

• Preparation of animals: This study used 40 male albino mice, their ages ranged between 10-12 weeks, and their weight ranged from 27-32 grams . They were obtained from the animal house in the pharmacology department of the Samarra Pharmaceuticals and Medical Supplies Industry Company (SDI). The animals were distributed into Four groups for which appropriate laboratory conditions were created. The ventilation and temperature ranged between 26-24 degrees Celsius, with a lighting system of 12:12 hours of light to darkness. The mice were given water and the concentrated diet and left to acclimate for at least a week before the start of the experiment. Care was continued in cleaning the cages of mice and sterilizing them with 70% ethanol alcohol disinfectant once a week.

Experimental groups: The animals were divided into four groups, each group having ten animals.

•The first group (G1) was injected subcutaneously with growth hormone at a concentration of 0.2 mg/kg BW, repeated four days a week for three months [9].

• The second group (G2) was injected with growth hormone at a concentration of 0.2 mg/kg BW and dosed with aqueous sage extract at a concentration of 50 mg/kg body weight [10]

• The third group (G3) was dosed with sage extract only at a concentration of 50 mg/kg of body weight for three months.

• The fourth group is the control group, which was not dosed with the extract or injected with the hormone.

Preparing samples for study with an optical microscope

At the end of experiment period,, the kidneys were dissected and taken from the animals. These samples were fixed in 10% Neutral buffered formalin solution for forty eight hours, Samples were processed by routine histological processing method to get histological sections of 5 micrometer thickness.

Then, it was filled with paraffin wax with a melting point of (58-56) °C in an oven at 60 °C for three hours, then the models were cast in special L-shape molds using clean paraffin wax, then they were cut using a rotary microtome to a thickness of (5) micrometers. The sections were surfaced at a temperature of (45-48)°C in a special water bath to spread the sections, then they were placed on glass slides, stained with Haematoxyllin and Eosin, and prepared for examination and imaging with an optical microscope using a digital camera [11].

3.Results and Discussion

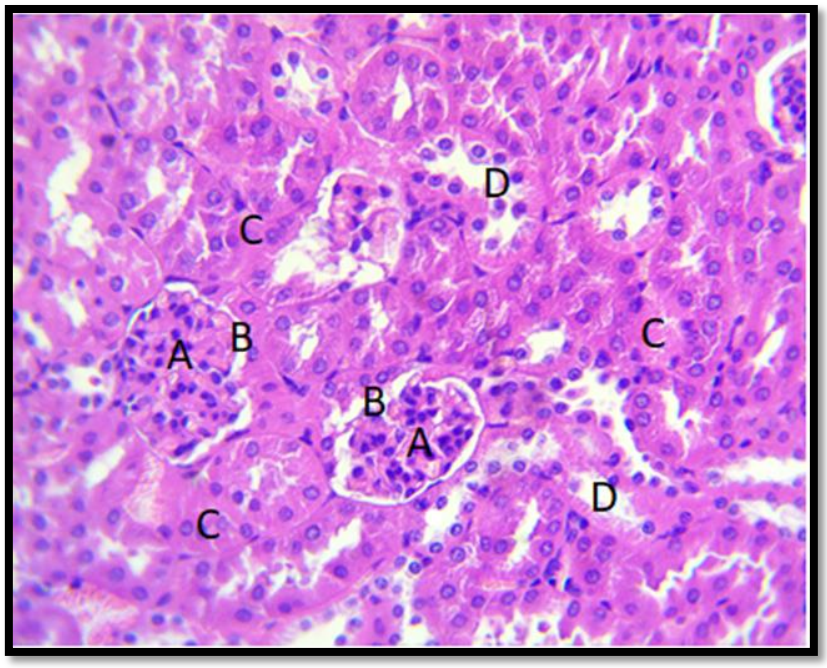

Figure (1) A cross-section of the kidney of a mouse from the control group showing the normal histological pattern. It Showed the kidney cortex in addition to (A) renal glomeruli with white blood cells on their surface (B) the Bowman's space (C) the proximal renal tubules (D) the distal convoluted tubules (H&E X40)

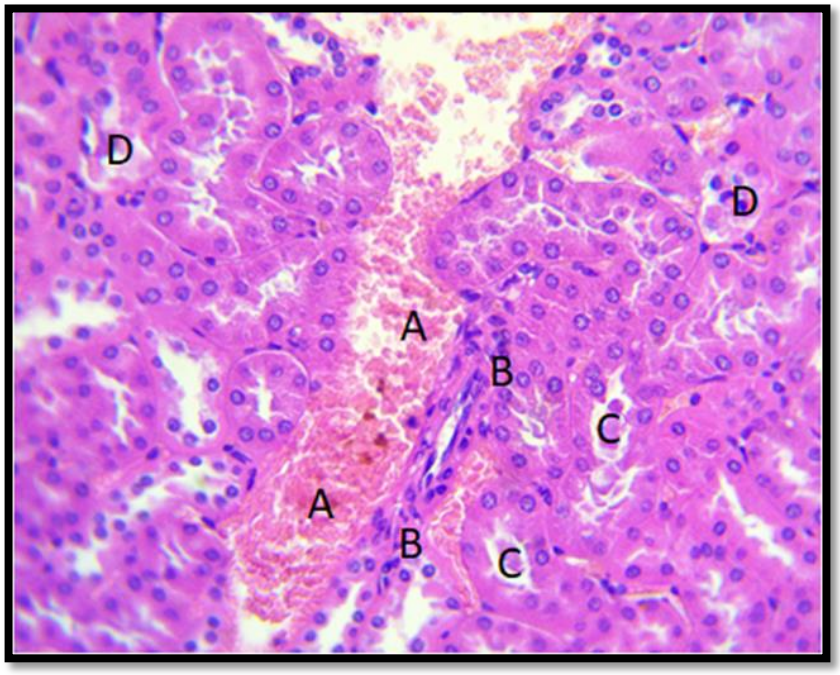

Figure (2) A cross-section of the kidney of a mouse from the first group (G1) showing (A) intertubular hemorrhage (B) infiltration of inflammatory cells (C) proteinaceous casts (D) lumen of convoluted tubules with sloughing of epithelial cells in the lumen of some tubules (H&EX40)

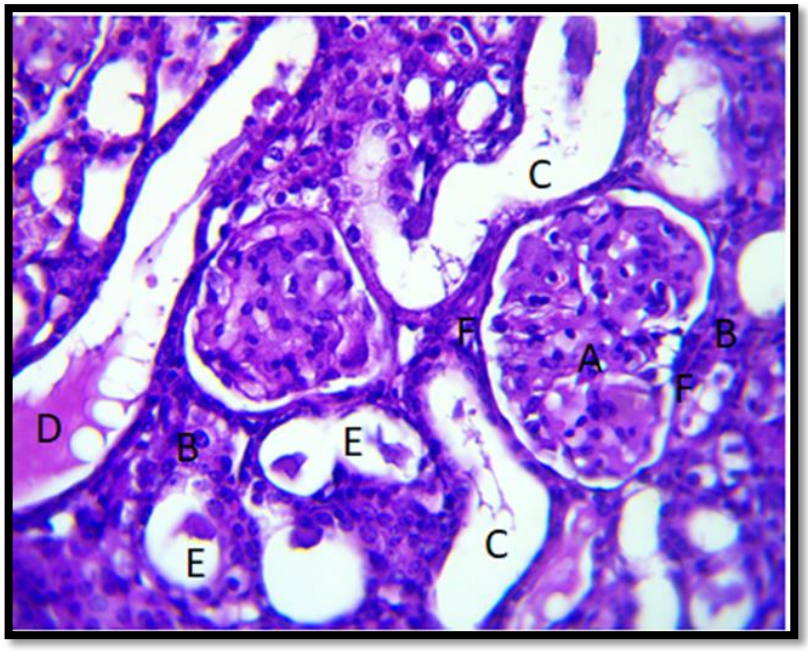

Figure (3) A cross-section of the renal cortex of a mouse from the first group (G1) showing (A) glomerular hypertrophy (B) infiltration of inflammatory cells in the interstitium (C) expansion of the lumen of the distal convoluted tubules (D) inflammatory edematous infiltrates (E) sloughing Epithelial cells in the tubule lumen (F) thicken Bowman's capsule (H&EX40).

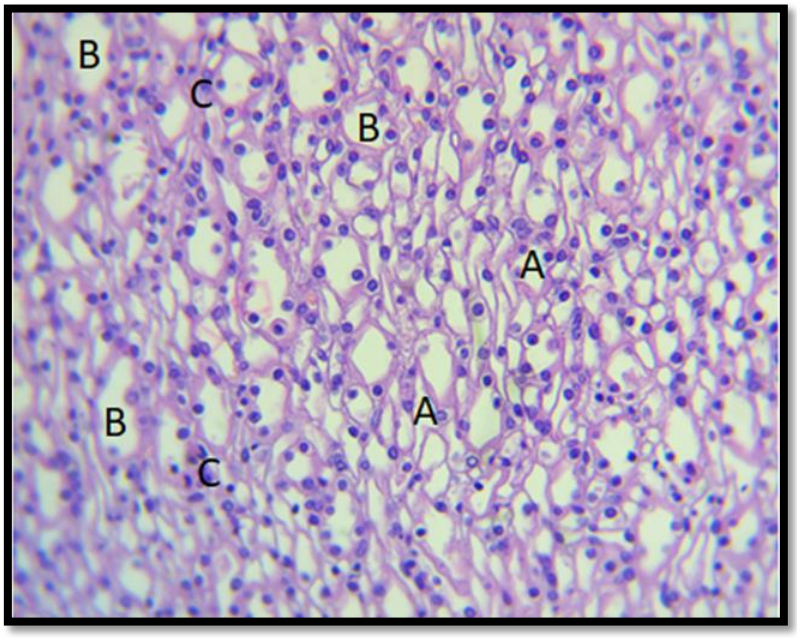

Figure (4) A cross-section of the kidney medulla of a mouse from Group 1 (G1) showing (A) thin fragments of loops of Henle lined with simple squamous cells (B) renal tubules lined with simple cuboidal cells (C) inflammatory edema in the interstitium (D) inflammatory cells (H&EX40)

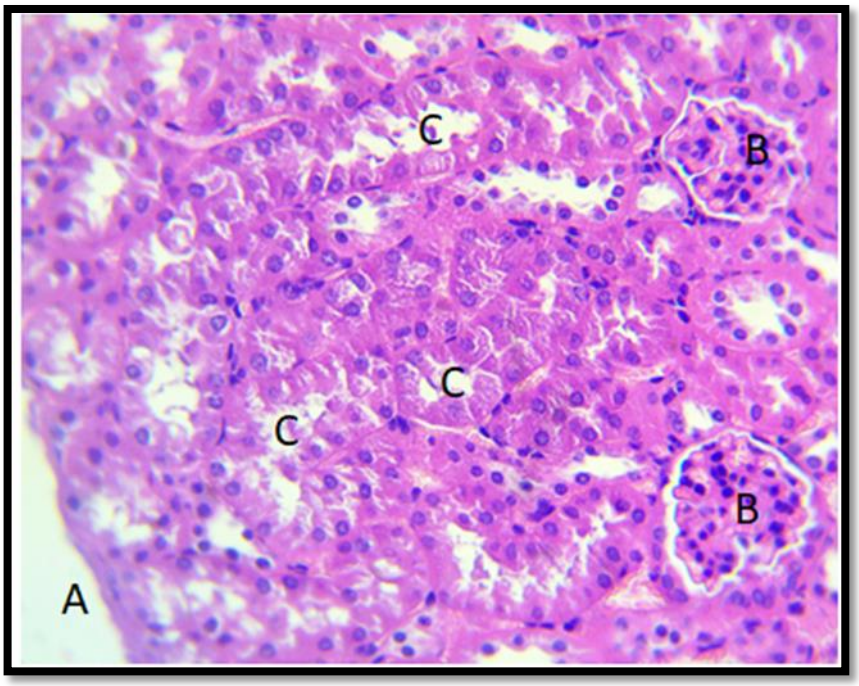

Figure (5) A cross-section of the kidney cortex of a mouse from the second group (G2) showing (A) the kidney capsule (B) normal-shaped renal glomeruli (C) sloughing of epithelial cells in the lumen of some convoluted tubules (H&EX40)

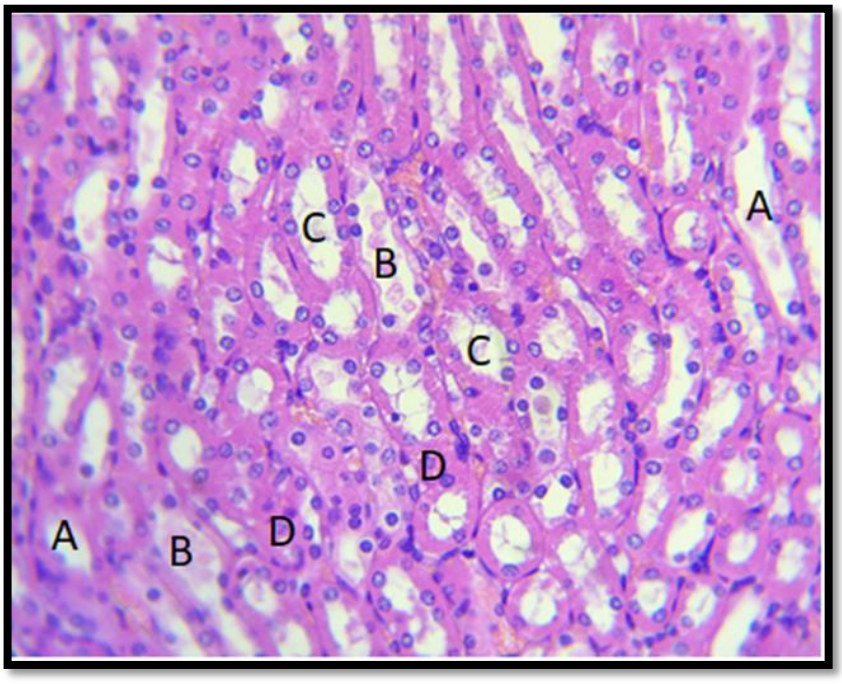

Figure (6) A cross-section of the kidney medulla of a mouse from the second group (G2) showing (A) renal tubules lining the cuboidal cells (B) renal infiltrate in the lumen of some tubules (C) fibrinous edema (D) infiltration of some white blood cells (H&EX40).

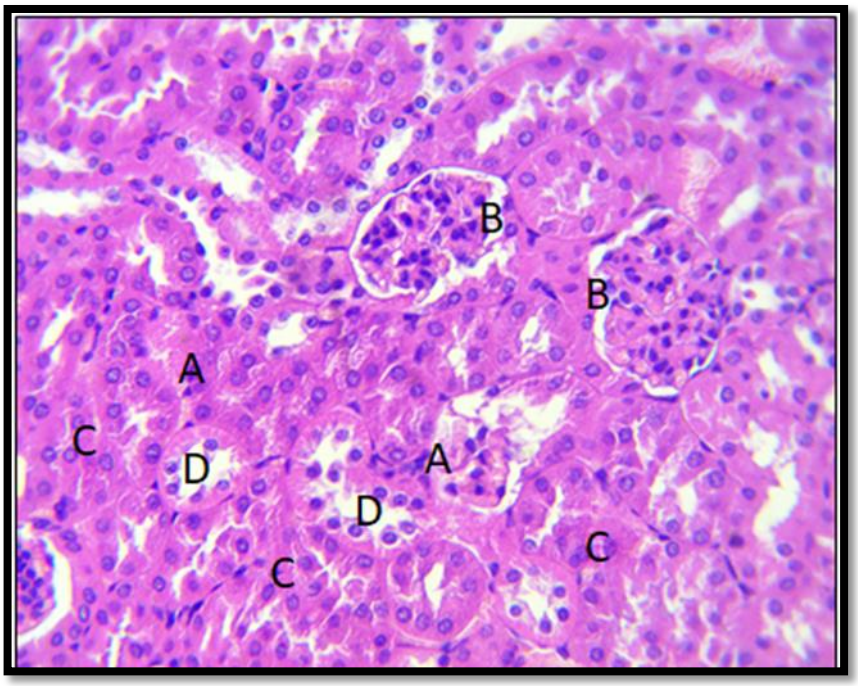

Figure (7) A cross-section of a kidney from a third group (G3) mouse showing the normal histological pattern as it Showed (A) renal glomeruli with white blood cells on their surface (B) the capsular space (C) the proximal renal tubules (D) the distal convoluted tubules (H&E X40)

Discussion The present study was conducted to focusing a high light on the negative impact resulting from the wrong use of growth hormone and to try to find a treatment from a natural plant source represented by sage plant extract. The kidney is one of the most important organs in the human body, as it performs many functions, the most important of which is its work to purify and filter the blood from toxic substances and wastes of the metabolic process in the body. [12] The kidney is also responsible for maintaining the volume of fluids in the body and balancing the amount of elements and their ions in addition to salts. [13], The kidney has an important role in the fulfillment of growth hormone functions, as the kidney is the second organ after the liver in the process of stimulating hormone functions directly or indirectly through the synthesis of Insulin-like growth factor-I (IGF-I). [14] Both GH and IGF-I affect different aspects of the kidney, as GH receptors, IGF-I receptors, IGF-II receptors, and IGF-binding proteins are expressed in kidney tissue, which leads to a change in the size and shape of the kidney [14] and this What appeared in the cross-sections of the kidneys of the mice from the first group, Figure No. (2), which were injected with growth hormone, was that blood bleeding appeared between the tubules and infiltration of white blood cells with the shedding of epithelial cells in the lumen of some of the tubules. In addition to what a cross-section of the kidney

cortex of two mice from the same group Showed: hyperinflation of the glomerulus, accumulation of white blood cells in the interstitial tissue, expansion of the lumen of the distal convoluted tubules, the appearance of an inflammatory edematous infiltrate, and sloughing of epithelial cells in the tubule lumen, in addition to the thickening of Bowman's capsule (Figure 3). This has been confirmed by studies, as administration of growth hormone in female rats leads to kidney enlargement and the absence of the normal tissue pattern [15,16] also confirmed the results represented by the occurrence of inflammatory edema in the interstitial tissue and the appearance of thin fragments of the loops of Henle (Figure 4). While the results of the histological study in the cortex area of the kidneys of mice injected with growth hormone and dosed with aqueous extract of the sage plant showed a normal histological pattern of the renal glomeruli with the appearance of sloughing of epithelial cells in the lumen of some convoluted tubules (Figure 5). The study also showed in the medulla area of the kidney for the same group, some infiltration. White blood cells, the occurrence of fibroedema in some areas, and the appearance of most renal tubules in a normal manner, Image (6) These results can be explained by the effective effect of many compounds contained in the S. officinalis extract, the most important of which are 1,8-cineole and 13-epimanol, which work to regulate the special cellular receptors IGF-I [17], which explains the results. Histological study in the group that was injected with growth hormone and dosed with sage extract. Sage extract is also widely used in the pharmaceutical and cosmetic industries because it contains a variety of anti-inflammatory properties [18], This explains the therapeutic effect of sage plant extract, as the extract works to reduce the inflammatory effect and the infiltration of white blood cells. While no histological change appeared in the tissue sections of the kidneys of mice dosed with sage extract in the third group, figure (7), if previous studies confirmed the safe side of dosing with sage extract if the extract did not cause any toxicity or deaths among mice that were dosed with repeated doses. Up to 1000 mg/kg for 50 days [19].

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تقييم التغيرات الفسلجية جراء الحقن بهرمون النمو والتجريع بمستخلص نبات الميرمية كمحاولة علاجية في الفئران البيضاء

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الملخص

استخدمت في هذه الدراسة (40) فأراً مختبرياً متوسط وزنها (30 ±3) جراماً، قسمت بالتساوي إلى أربع مجموعات. تم حقن المجموعة الأولى تحت الجلد بهرمون النمو بتركيز 0.2 كيلوجرام/ملجم بين يوم وآخر بجرعة 0.25 مل. لمدة ثلاثة أشهر بينما حقنت حيوانات المجموعة الثانية بهرمون النمو بتركيز 0.2 ملجم/كجم من وزن الجسم وبنفس حجم الجرعة وجرعت فمويا بمستخلص الميرمية بتركيز 50 ملجم/كجم من وزن الجسم لمدة ثلاثة أشهر يضا.اما المجموعة الثالثة تم تجريعها بمستخلص الميرمية فقط ولنفس المدة ، بينما المجموعة الرابعة (المجموعة الضابطة) لم يتم جرعتها أو حقنها. تم تشريح الحيوانات وأخذ عينات من كلى الحيوانات وإعدادها للدراسة النسيجية لدراسة التغيرات النسيجية الناتجة عن الحقن بهرمون النمو والجرعات بمستخلص الميرمية. أظهرت النتائج عدم وجود نمط نسيجي طبيعي في مجموعة ذكور الفئران المحقونة بهرمون النموفقط ، على عكس مجموعة الفئران المحقونة بهرمون النمو والمجرعة بمستخلص نبات الميرمية، بينما لم تظهر أي تغيرات نسيجية في كلى الفئران المجرعة بمستخلص نبات الميرامية فقط.

الكلمات الدلالية: دراسة نسيجية ، الكلية ، هرمون النمو ، نبات الميرامية .