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STUDY OF BIOCHEMICAL CHANGES IN KIDNEY AND LIVER FUNCTIONS IN THE BLOOD SERUM OF RATS TREATED WITH DIANABOL

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Abstract: The current study aimed to reveal the cytogenetic effects of Methandienone in white mice Mus Musculus, which is widely used in gyms, by studying some blood images through tests by choosing a third of the dose (0.8) mg/kg of the body weight of the laboratory mouse. The results showed that the increase in the drug has the ability to produce _____ white blood cells and ___ red blood cells at an equal rate. In conclusion, the results of this study show that the drug has mutagenic effects.

Keywords: -

Introduction

Anabolic steroids, also known as anabolic-androgenic steroids[1], were developed in the 1930s and are now used therapeutically in medicine to stimulate muscle growth, increase appetite, induce puberty in males, and treat conditions associated with chronic wasting, such as cancer and AIDS. They are also used for energy and as performance-enhancing drugs in sports and racing.

Bodybuilding is controversial because of its negative effects and the potential to gain an unfair advantage in competitive physical competition. Their use is therefore banned by most major sporting bodies. The American College of Sports Medicine recognizes that anabolic steroids in the presence of an adequate diet can contribute to increased body weight and that increases in muscle strength achieved through intense training and proper diet can also be enhanced by the use of steroids in some individuals[2]. Health risks can result from long-term or excessive use of steroids.[3][4] These effects include harmful changes in cholesterol levels, increased low-density lipoprotein, decreased high-density lipoprotein, acne, high blood pressure, liver damage, and serious changes in the structure of the left ventricle of the heart.[5] Clinical trials in humans involving oral doses of methyltestosterone or injections of testosterone propionate began as early as 1937. Rumors that German soldiers were taking anabolic androgenic steroids during World War II to increase their aggression and endurance were often reported, but this has not been proven.[6] Steroid programs have been used to enhance Olympic performance, especially in weightlifters. In response to the success of Russian weightlifters, Olympic team physician John Ziegler worked with chemists to develop synthetic steroids with reduced androgenic effects.[7][8] The legal status of steroids varies from country to country, with some using them with a prescription despite them being illegal in many countries. In the United States, they are currently listed as Schedule III controlled substances under the Controlled Substances Act, making possession of these substances without a prescription a federal crime punishable by up to one year in prison for a first offense, and unlawful distribution or possession with intent to distribute as a first offense punishable by up to ten years in prison.[9] By the early 1990s, several pharmaceutical companies had stopped manufacturing or marketing steroids in the United States, including Ciba, Searle, Syntex, and others. Under the Controlled Substances Act, anabolic androgenic steroids are defined as any drug or hormonal substance chemically and pharmacologically related to testosterone that promotes muscle growth. The law was amended by the Anabolic Steroid Control Act of 2004, which added prohormones to the list of controlled substances effective January 20, 2005[10].

Aim of study

Study or determine the hematologic toxicity of the drug by studying the complete blood count and comparing it with standard samples of untreated mice.Literature revise

What are steroids:

Any substance or drug taken to enhance activity, raise athletic performance and increase body mass.[11]

2-2 How are steroids taken: Oral steroids are used almost exclusively in the form of esters given by intramuscular injection that act as a long-acting drug precursor. Examples include (testosterone, testosterone oxypone, testosterone enanthate, testosterone propionate, nandrolone, nandrolone phenylpropionate and nandrolone decanoate) In addition to oral activity, these steroids are highly toxic to the liver, although this is uncommon and occurs only after prolonged use [12.[13][

3-2 Adverse effects of steroids: These include harmful changes in cholesterol levels, increased low-density lipoprotein, decreased high-density lipoprotein), acne, high blood pressure, and liver damage ([14] . Side effects specific to women include increased body hair, permanent deepening of the voice, enlargement of the clitoris, and a temporary decrease in menstrual cycles. (It can also occur in females, altering fertility and polycystic ovary syndrome[15] when taken during pregnancy and can affect fetal development by causing male characteristics to develop in a female fetus and female characteristics in a male fetus[16].

Neuropsychiatric effects: A 2005 review in the field of neuropharmacology found that

significant psychiatric symptoms including aggression, violence, mania, and less commonly psychosis and suicide have been associated with steroid abuse. Long-term steroid users may develop dependence and withdrawal symptoms when they stop using anabolic androgenic steroids[17] , and no Currently, large-scale long-term studies of the psychological effects on users of anabolic androgenic steroids are lacking. A 13-month study published in 2006 involving 320 bodybuilders and athletes suggests overuse. [18] Physiological effects: Depending on the length of time the drug is used, there is a possibility of immune system damage. Most of these side effects are dose-dependent, with the most common being increased blood pressure, especially in those with pre-existing hypertension.[19] Steroids have been shown to alter blood sugar tests.[20][21][22] Testosterone also increases the risk of cardiovascular disease or coronary artery disease, and acne is also common due to the stimulation of sebaceous glands by increased testosterone levels.[23] Conversion of testosterone to dihydrotestosterone can increase the rate of premature baldness in genetically predisposed males, but testosterone itself can produce baldness in females.[24] A number of severe side effects can occur in adolescents. For example, it can prematurely stop bone lengthening, leading to stunted growth. Other effects include accelerated bone growth, increased frequency and duration of erections, and early sexual development. Use of anabolic androgenic steroids in adolescence is also associated with more serious health conditions[25].

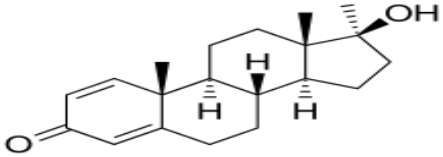
Cardiovascular: Side effects can include changes in the structure of the heart, such as dilation and thickening of the left ventricle, which impairs contraction and relaxation.[26][27][28] Possible effects of these changes on the heart are (high blood pressure, irregular heartbeat, congestive heart failure, heart attacks, and sudden cardiac death). These changes are also seen in athletes who do not use the drug, but steroid use may speed up this process. However, the relationship between changes in the structure of the left ventricle and decreased heart function, and steroid use, is still controversial.[29. [30]

Effects on the kidneys: Kidney tests revealed that nine out of ten steroid users developed a condition called focal glomerulosclerosis, a type of scarring within the kidneys. The kidney damage in bodybuilders is similar to that seen in obese patients but appears more severe.[31]. Steroids can be detected in the following ways: (urine analysis - blood analysis) [32][33][34.[35].

4-2 Dianabol Drug

Scientific name:[36] Methandienone

Molecular formula:[36] $C_{20}H_{28}O_2$



Chemical composition of Dianabol [36].

Dianabol is an anabolic androgenic steroid, sold under the brand name Dianabol among others.[36][37][38][39] It is also used non-medically for physique and performance enhancing purposes.[37] Metandienone was originally developed in 1955 by Ciba and marketed in Germany and the United States.[37][38][39][5][6] Metandienone has become widely used among professional and amateur athletes, and remains the most commonly used non-medical substance.[42][43][40][44] It is currently a controlled substance in the United States.[45][46] Several successful bodybuilders have admitted to using metandienone long-term before the drug was banned, including Arnold Schwarzenegger.[47][48] Methandrostenolone was first described in 1955, and was released to the

U.S. prescription drug market in 1958 under the brand name Dianabol by Ciba Pharmaceuticals.[2] Ciba developed methandrostenolone into a drug with the help of Dr. John Ziegler, who was the physician for a number of U.S. Olympic teams including weightlifters. According to Ziegler, the hormone had notable side effects, and one athlete developed a profound prostate enlargement that forced him to urinate with the aid of a catheter. While working with Ciba, the company tested a steroid that had reduced androgenicity compared to testosterone, but by 1960 Dianabol had sparked a major wave of steroid abuse in competitive sports. Dr. Ziegler's early recommendations, which depending on sources called for as little as 5 mg per day or as much as 15 mg per day.[49] Side effects: Acne, oily skin, increased facial/body hair growth, and scalp hair loss (baldness). Gynecomastia and fluid retention can also occur. Metandienone is dangerous to the liver and its use over long periods of time can lead to liver damage without proper precautions.[37]

5-2 Cyclophosphamide drug: Cyclophosphamide is used to treat many cancers such as lymphomas and leukemia, as it slows or stops cell growth and reduces the immune response to many diseases (Brock, 1996). It is an alkalizing agent and its toxicity comes from its chemical metabolism and transformation into its active metabolite forms (Phosphoramide and Acrolein). Many studies have proven the genetic toxic effects of this drug, as its mutagenic effect increases when it is metabolically activated by liver enzymes, mainly Cytochromes-p450, where the process of adding a hydroxyl group (Hydroxylation) takes place to it, forming the compound 4-hydroxy-cyclophosphamide, which in turn transforms into two metabolites (Phosphoramide mustard and Acrolein) (Ren et al., 1997;Yule et al., 1995). These metabolites break and form cross-links between DNA-DNA strands, thus destroying and stopping the mechanisms of cell division, especially germ cells. These metabolites also have the ability to induce chromosomal aberration and sister chromatid exchange (Codrington et al., 2004; Bishop et al., 1997). At the immune system level, the drug, through its metabolites, works to cause a reduction in immune activities through an acute effect on bone marrow cells and lymphoid organs such as the spleen, as it causes a reduction in the number of blood cells (whites, reds, and platelets) and regulatory T cells (T-regulatory cells) and the degree of their expression of some receptors (CD25+, CD4+) (Lutsia et al., 2005; Turner and Richens, 1978). The effectiveness of the drug is affected by many factors, as its effectiveness increases with the presence of Acrolein, which is one of its metabolites, and decreases with the presence of vitamin C and the compound Indole-3-carbinol, as well as with the presence of carotenoids (Shukla et al.,23; Durnev et al.,1998).

Methods

1. Laboratory animals used: Animal preparation:mA male white laboratory mouse was used, obtained from the Biotechnology Laboratories of Al-Nahrain University, aged 11-14 days, which were arranged in the laboratory in the animal house / Biotechnology Branch in plastic cages with an iron clip cover and were given water and complete feed.
2. The drug used in the research: The drug used in the research is Daynable and was used in specific concentrations. The drug is given at a rate of one dose (1 ml) every day throughout the experimental period for each concentration by dosing methods for 35 days.
3. Measurement of blood urea
   The (Jaffe) method was used to measure blood urea concentration [51]
4. Measurement of blood creatinine
   The creatinine concentration was measured using the (Jaffe) method [51]
5. Measurement of Alkaline phosphatase Activity

The (King & Kind) method was used to measure the enzyme activity [52]

6. Estimation of the amount of pyruvate and oxalate transaminase enzymes (GOT & GPT)
   The (King & Kind) method was used to measure the enzyme activity [52]
7. Statistical Analysis: Statistical analyses were conducted for the information obtained by finding the mean and finding the variance according to the following equation (n∑▒〖xi〗^(2) -(∑▒xi)^2)/(n(n-1)) S^2=, and the standard deviation Standard deviation S.D=√(s^2), and then the standard error Standara Erro SE=SD/√n, in order to extract the value of choosing t (t-test) through the following equation t=(means between the differences)/(for the two samples the standard error sum) (t-test=(m_1-m_2)/(〖SE〗_1+〖SE〗_2)), so if the value of t equals two or more, this indicates the presence of a significant difference, depending on a special table for the t-test, noting that the number of degrees of freedom df equals (n_1+n_2-2) where n_2 and 〖n〗_1 are the number of individuals in the samples and comparison, in order to estimate the amount of significant difference or not [50].

Results and Discussion

Urea

The current study confirmed the values of kidney functions (urinary system) which indicate a decrease in those values Table (1) in the urea rate in the blood serum of the group of positive control mice treated with the mutagenic and carcinogenic substance CFA at a concentration of 40 mg/kg. The t-test values showed a significant difference ($P \leq 0.01$) compared to the untreated negative control mice. It is also noted that there is a significant decrease ($P \leq 0.05$) in the serum urea rate of mice treated with the drug after one week of treatment and also after the third week ($P \leq 0.01$), while an increase in urea values is noted after the fifth week of treatment due to the cumulative effect of the drug or its metabolic compounds, and that this last increase may be caused by damage to the collecting tubules due to what the kidney suffers from the deposition of chemicals released by these drugs, which leads as a result to a decrease in the kidney's efficiency in filtering blood urea [53], which was indicated by [54] in his study on the effect of the drug cortisone on the permeability or osmosis of kidney tissues, which leads to a decrease in the kidney's efficiency in filtering uric acid, which causes an increase in its presence in the blood.

Table (1) Urea values for laboratory mice treated with the drug and untreated mice

Creatinine

Table No. (1) shows the creatinine values in the blood of the negative and positive

		Mg/dl	The status
35 Day	21 Day	7 Day	
		SE ± mean	
		2.34±40.14	negative standard sample
		0.79±13.88	CFA positive standard sample
0.4±51.23	0.58±28.12	0.3±30.75	Treatment

control mice and the mice treated with the drug, indicating a decrease in the values for the treated mice compared to the negative control mice (P≤ 0.05). Any defect in the level of Creatinine in the blood serum is due to a defect in its formation or excretion by the kidney. Creatinine is formed in the liver and kidney and is transported to other parts of the body by binding to phosphatase compounds with mechanical energy, then it returns to be converted into Creatinine outside the organs in which it is located. The study showed a significant increase (p≤ 0.01) in the level of Creatinine in the blood serum of the group of mice treated with the drug (0.69 mg/dl) compared to (0.52 mg/dl) Table (2). This increase may be explained on the basis that high blood urea causes damage to the body's tissues as primary toxins, which causes an increase in the excretion of Creatinine in the kidney, which affects the kidney's function and efficiency in excreting Creatinine, which raises its percentage in the blood serum. [55] indicated that high blood urea levels cause a noticeable increase in Creatinine in the blood serum due to the mechanical and chemical damage caused by urea in the tissues. The body, all the brain and the liver.

Table (2) Creatinine values for laboratory mice treated with the drug and the positive negative control

		K.A.U/dl	The status
35 Day	21 Day	7 Day	
		SE ± mean	
		0.13±38.6	negative standard sample
		0.79±29.4	CFA positive standard sample
0.7±75.19	0.22±35.5	0.35±25.41	Treatment

Alkaline phosphatase enzyme

The association of increased activity of enzymes (ALP, GOT, GPT) with each other is a result of the destruction or damage to the cells of some organs, which in turn leads to an increase in their activity in the blood serum, as enzymes in general, including the alkaline phosphatase enzyme, are auxiliary materials of great importance in the life processes in cells through their role in accelerating chemical reactions. Therefore, any defect that affects these cells affects these enzymes and thus the reactions responsible for them. [53] The results of the studies showed a significant increase (P≤0.05), especially after the fifth week, in the activity of the alkaline phosphatase enzyme in treated mice. This increase may be due to the effect of the activity of the enzyme on the oxides released by the chemicals included in the composition of these pills. [56] indicated that there was an increase in the ALP enzyme in a group of patients who take sedatives excessively.

Table (3) ALP values for both negative and positive control mice and mice treated with the drug

		Mg/dl	The status
35 Day	21 Day	7 Day	
		SE ± mean	
		0.06±0.52	negative standard sample
		0.04±0.73	CFA positive standard sample
0.04±0.69	0.03±0.35	0.03±0.29	Treatment

Amino transferase enzymes (GOT& GPT)

These two enzymes diagnose many pathological conditions by increasing their effectiveness in blood serum when liver cells are destroyed. The enzymes that transport the amino group are very important in the process of synthesizing non-essential amino acids and releasing energy from proteins in cells. If there is an irregularity in the activities of these enzymes, this leads to a defect in the formation of proteins. The increase in the activities of these enzymes occurs when any damage occurs in the liver cells as a result of liver cancer, cirrhosis, or viral infection of the liver. The results of the study showed a significant increase ($P \leq 0.05$) in the activity of the enzymes (GOT & GPT) in the group of mice treated with the drug. A significant increase ($P \leq 0.05$) was found in the activity of the enzyme (GOT) in mice treated with the drug when compared to the control group, Table (3,4), which confirmed the existence of an increase, which confirms the relationship between taking such a drug among young people and It resulted from the accumulation of chemical compounds in the liver.

The study also confirmed that the activity of the enzyme (GPT) in the treated mice was significantly higher ($P \leq 0.05$) when compared with the control group (V/L) and that this increase may be due to the high level of chemical compounds and their other oxides in the liver, which leads to damage to liver cells and thus increases the activity of the enzyme from the blood serum.

This result is consistent with what was stated in the study [56], where an increase in the level of the enzyme (GOT) was observed in many patients who take painkillers.

Table (4) GOT values for both negative and positive control mice and mice treated with the drug

		V/L	The status
35 Day	21 Day	7 Day	
		SE ± mean	
		0.41±19.3	negative standard sample
		0.32±22.6	CFA positive standard sample
0.85±31.25	0.39±28.5	0.98±292	Treatment

Table (5) GPT values for both negative and positive control mice and mice treated with the drug

V/L			The status
35 Day	21 Day	7 Day	
		SE ± mean	
		0.10±23.6	negative standard sample
		0.28±27.2	CFA positive standard sample
0.2±65	0.75±25.18	0.49±100.75	Treatment

Conclusion

The study suggests that the use of anabolic steroids, specifically Dianabol, can significantly affect physiological and biochemical functions of the body. It found that Dianabol can cause changes in various parameters, such as urea and creatinine levels and enzyme activity like alkaline phosphatase, GOT, and GPT. Specifically, the increased urea and creatinine levels in Dianabol may cause kidney damage, which can affect the health of the kidneys. The study also found that the toxic effects of Dianabol were cumulative, with more harmful effects after longer use periods. Therefore, it is crucial to avoid using this steroid and maintain its health before using it.

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