Journal of Education for Pure Science- University of Thi-Qar
Vol.10, No.1 (March., 2020)
Website: jceps.utq.edu.iq Email: jceps@eps.utq.edu.iq

DOI: http://doi.org/10.32792/utq.jceps.10.01.014

Study Effect Hormone Doping (Growth Horemone Releasing Hexo- Peptids(GHRP-(6) and Insuline of Cholesterol, Triglycerides, High and Low Density Lipoprotein to Laboratory Rats

[1]Zaeinab H. Farhood [2]Ali M. Hussein [3] Satar A. Faris

[2] The Sumer of University -college of Agriculture
[1,3]Thi – Qar of University - college of Education pure sciences – Biology Department

Received 2/6/2019 Accepted 8/9/2019 Published 20/1/2020

Abstract:
The study showed the effect of hormonal activity GHRP-6 and insulin used in laboratory animals by 48 males and 48 females divided into six groups. The control group was injected with a 0.1 mg solution and the other groups were injected with a mixture of stimulants at 0.01and 0.01 times syringe ($P < 0.05$) in the first group of males and in the female treatment groups, and a significant increase in the complex triglycerides excluding the group ($P < 0.05$) and both sexes were observed, ($P < 0.05$) was significantly higher in proteins VLDL and HDL in male rats and significantly lower in fameles ($P < 0.05$), significant increase ($P < 0.05$), was significantly higher in Cholesterol in the male rats and significant decrease in fameles ($P < 0.05$) treated with a combination of stimulants (GHRP-6 + Insulin) compared with control group.

Key words –GHRP-6, insuline, cholesterol, VLDL, HDL, T.G.

Introduction: -

It was the first time that hormonal stimulants were prepared in the 1930s, after many years of research by international scientists in the preparation of various forms and uses of peptides, but the first time their uses were recognized for the purpose of increasing athletic efficiency and abuse was in the 1950s, When the weightlifting athletes in the Olympic sports competitions. The main reason for the use of hormonal (peptide) doping by professional athletes, and amateurs is that it enables them to train more energetic and energetic and build their muscles and restrain them from the trouble of training B Faster format. Peptide stimulants have been used over the past two decades by bodybuilders to improve their performance, but the use of hormonal stimulants for these purposes has many adverse effects on the patient's health and state of mind, including physiological distortions and increased aggression (1).

Recently, there has been talk of doping and the use of stimulants by athletes. Doping and its effects have taken on a large area locally and internationally, following their spread in a large and dangerous manner in all countries of the world. Many physical and informal sports institutions (fitness centers and bodybuilding centers) with the aim of upgrading the physical and athletic level or for other purposes through the use of abnormal means and injected into the body or orally before or during competitions. We

have therefore highlighted this issue of concern to athletes, trainers and other users of doping without full knowledge of the resulting damage (2).

Industrial sports steroids are generally hormones and substances produced in the body naturally and stimulate the structure of the tissue and development, but the users of the supply of their bodies from an external source, through injection, for example, and a very high concentration thought they stimulate the building of their muscles. Although some drugs may increase the size and strength of muscles, the price is very high. The spread of these hormones makes it imperative for officials to shed light on the role, types and methods of these hormones in the sport, and to clarify their risks. Athletes are required to check their complications, their medical uses and what their users should do when these complications occur and what they need to be followed and observed during use (3). The most common hormones in the body are growth hormone Releasing Peptide (GHRP-6), also called HexaPeptide, is a non-natural, synthetic protein-stimulating peptide consisting of six amino acids is L-histidyl-D-tryptophyl-L-alanyl-L-tryptophyl-D-phenylalanyl-LI-Lysinamide. This is the hormone peptide of the hormone growth hormone secratogogous (GHS) category. As with all relevant peptides, it is the most common member of the GHS family used for the induction and growth hormone growth hormone by binding to the pituitary receptors and hypothalamis receptors. These receptors that are associated with the activator GHRP-6 differ from the receptors of the hormone stimulating growth hormone The GHRP-6 has a structural structure that is also different, so it does not affect the body inside or it may be a refractory act (4)..

The hormone insulin is the building's structural hormones and is constructed by the transfer of nutrients to the cells. The most important characteristic is its help in building muscle by stimulating the synthesis of protein in the muscle tissue. The cells are ordered to absorb amino acids. The formation of new proteins through the speed of transfer of amino acids, heals muscle faster, and also orders glucose absorption to form muscle Glycogen and transfer the glycose to the rest of the body and convert fatty acids and cholesterol to fat (5).

It has been discovered that when GHRP-6 and insulin are given simultaneously in a mixture, the pituitary response to increased GH secretion is increased to GHRP-6, increasing the consumption of carbohydrates, fatty acids, and calcium (4).

A recent study in rats that have been given GHRP-6 activator mixed with insulin has shown significant differences in body composition from normal mice in muscle growth, glucose metabolism, memory cells and heart function in mice, there are still many questions about this new compound, scientists hope in obtaining a better clinical understanding of peptide by conducting further research over the next few years. (6).

Aims of the study: - The

This study was designed to study the physiological and histological effects of some internal organs that can result from the systematic use of stimulants and the effect of growth hormone stimulating peptides (GHRP- 6) and insulin in the standards to see their effect on some biochemical parameters of male and female laboratory rats which included

1-Calculate the concentration of some lipids (triglycerides, total cholesterol).

2-Calculate the concentration of some lipoproteins (LDL, HDL)

Materials and Method: -

Preparation of experimental animals:

In the present study, female and male *Rattus norvegicus* rats were used in the current study, obtained from the Department of Life Sciences - Faculty of Science - University of Dhi Qar, which ranged in age from

10-12 weeks and weights (200-165 g). Laboratory of the Department of Life Sciences - Faculty of Education for Pure Sciences / University of Dhi Qar and examined by a veterinarian to ensure that they are free from diseases and then isolated as couples for reproduction and use of their sons until the age of (14-12) and tried the hormone activated The animals were left during the study period in the animal house under the conditions of m Atr them in terms of temperature (25-20 m) cycle lighting (12 hours lighting - 12 hour darkness) during the period of the study.

Forty-eight female laboratory rats and 48 other male laboratory rats were divided into six groups. Each group consisted of 16 animals (8 males and 8 females) as follows:

1-Control group: It was injected with normal saline (NaCl 0.9%) for five weeks

2-TheGroup (1): injected with 10 mg of(GHRP-6) mixed with 10 mg of insulin for one week.

3- TheGroup (2) was injected with 10 mg of GHRP-6 mixed with 10 mg of insulin for two consecutive weeks.

4-TheGroup (3): injected with 10 mg of GHRP-6) mixed with 10 mg of insulin for three consecutive weeks.

TheGroup (4): injected with 10 mg of drug (GHRP-6) mixed with 10 mg of insulin for four consecutive weeks.

The Group (5) was injected with 10 mg of GHRP-6 mixed with 10 mg of insulin for five consecutive weeks.

The collection of blood sample: -

After the end of the injection period, the male and female animals were dissected after anesthesia with ether and chloroform. The blood was then pulled directly from the heart through the cardiac pincus using a 5 mL syringe. The blood was divided into two parts. The second section was placed in tubule gel tubes for several minutes in order to obtain sufficient quantity of serum. The serum was then removed from the clotted blood and placed in special tubes until biochemical tests were carried out.

Measurement of Triglycerides concentration in serum:

Adopted method (7), (8) were used to estimate the level THERMAL CELLSERIDES IN THE BLOOD THERMOMETER BY USING THE THREE THERMAL CELISRIDES MEASUREMENT FROM THE Biolabo FRENCH COMPANY.

Measurement of cholesterol concentration in serum:

I used a method (9) was used to calculate cholesterol concentration in the serum using the equipment prepared by the French company Biolabo.

Measurement of High Density Lipoprotein (HDL) concentration in serum:

I used the measurement kit processed by the company Biolabo was used in the estimation of HDL as it was based on the deposition of low density lipoproteins (LDLs), very low density lipoproteins (VLDL) and kilomycrons in the model by phosphotnastic acid and magnesium chloride. The filtration leachate contains HDL, which has been measured using a cholesterol detector.

Measurement of Low Density Lipoprotein(LDL) concentration in serum:

Adopted method (10) method was used to estimate LDL level in serum and according to the following

Equation:

LDL = Total Cholesterol – (HDL + VLDL).

Rrsults: -

Effect of peptide hormone (GHRP-6) mixed with insulin in the concentration of Cholestrol cholesterol, triglyceride and lipoproteins in male laboratory rats:

The results showed a significant decrease in cholesterol concentration in the treated groups GHRP-6 + Insulin, with the exception of the (2) group. No significant differences were found in comparison with the control group at the probability level ($p\leq0.05$). The results showed that when comparing the groups, was significantly reduced in the (1) group when compared with the (2) group and the (3) group when compared with the (4) group and the significant increase in (5) group when compared with the other groups treated below the level of probability mentioned in Table (1).

The results showed a significant increase in the concentration of triglycerides in the treated groups GHRP-6 + Insulin when compared with the control group at the level of probability ($p\leq0.05$). The results also showed a significant decrease in the (2) group when compared with the (1) group and a significant increase (5) Group when compared with (4) Group and (3) Group below the level of probability

With respect to the level of concentration of high density lipoproteins (HDL), the results indicated in the table showed a significant increase in the GHRP-6 + Insulin treated groups, except for the (3) group, a significant decrease was observed when comparing with the control group at the probability level ($p\leq0.05$). Between groups treated with hormone decreased significantly in the (5) group when compared with the (4) group and a significant increase in the (2) group when compared with the (1) group and the (3) group and the (4) under the level of the possibility mentioned.

The results indicated that there was a significant increase in the level of lipoprotein concentration and LDL in the GHRP-6 + Insulin group. Excluding the (2) group, a significant decrease was observed in comparison with the control group at the probability level ($p\leq0.05$). The comparison of the treated groups was significantly higher in the (3) group when compared with the (1) group and significantly higher in the (5) group when compared with the (4) group and the (3) group under the mentioned probability level.

Tabel (1) Effect of GHRP-6 + Insulin in the concentration of cholesterol, triglycerides and lipid proteins of male laboratory rats (N = 8) (average ± standard error

VLDL	HDL (mg/dl)	T.G (mg/dl)	CHOSTROL (mg/dl)	STANDARDS / GROUP
79.8143±11.45531 a	41.4429±2.21143a	76.5714±16.89020 a	136.5714±13.018 04a	CONTROL group
55.3643±10.90969 b	49.8678±4.08346 bd	63.5714±4.96518b	115.6964±11.209 51b	The group (1) GHRP-6+Insulin
81.8714±25.05255 ac	51.5571±5.46952 b	36.4286±10.15325 c	136.4286±25.656 95ac	The group (2) GHRP-6+Insulin
70.5090±13.30809 ad	40.3696±1.20628a c	48.5536±6.63940d	126.0536±14.193 99d	The group (3) GHRP-6+Insulin)
117.7143±3.84035e	48.1429±1.29887 d	113.8571±3.56189 e	188.5714±2.6444 7e	The group (4) GHRP-6+Insulin)
102.4286±6.58229f	43.9429±3.88788e	156.7143±16.2359 3f	164.5714±8.6461 0f	The group (5) GHRP-6+Insulin

The difference of letters indicates moral differences.

Effect of peptide hormone (GHRP-6) mixed with insulin in the concentration of cholesterol, triglyceride and lipoproteins of female laboratory rats:

The results showed a significant decrease in the concentration of cholesterol in the groups treated with GHRP-6 + Insulin. Excluding the fifth group, a significant increase was observed when comparing with the control group at the probability level ($p \leq 0.05$). In the (4) group and the (3) group when compared with the (2) group and the (1) group. The results showed no significant differences between the (1) group and the (2) group and the (3) group and the (4) group under the level of the possibility mentioned.

The results showed a significant decrease in the level of concentration of triglycerides in the treated groups GHRP-6 + Insulin, except for the (١) group, a significant increase was observed, and the (4) group was not significant. In comparison with the control group at the probability level ($p \leq 0.05$). When comparing the groups, there was a significant increase in Group (5) and group (6) when compared with the (3) group and the (4) group under the mentioned probability level.

The results indicated in the table showed a significant decrease in HDL in the GHRP-6 + Insulin groups, except for the (4) group, a significant decrease was observed when compared with control group at($p \leq 0.05$). The results showed that when comparing the treated groups with the hormone, Between the aggregates except the (4) group was significantly lower when compared with the (1) group under the mentioned probability level.

The results showed a significant decrease inLDL the GHRP-6 + Insulin group with the exception of the (5) group when compared with the control group below the level of probability ($p \leq 0.05$). The results showed that when comparing the groups treated with the hormone, there was a significant decrease in the (1) group, the (2) group, the (3) group and the (4) group under the probability level($p \leq 0.05$).

Tabel (1) Effect of GHRP-6 + Insulin in the concentration of cholesterol, triglyceride and lipid proteins of Female laboratory rats (N = 8) (average ± standard error)

VLDL	HDL (mg/dl)	T.G (mg/dl)	CHOSTROL (mg/dl)	STANDARDS / GROUP
120.68±9.983 a	53.65±1.27 a	84.57±12.202 a	191.42±11.528 a	CONTROL group
62.94±12.096 b	49.71±5.022 ab	113.14±14.984 ab	135.28±13.43 b	The group (1) GHRP-6+Insuli
58.65±6.946 b	52.28±1.304 a	127.42±14.875 b	136.42±7.720 b	The group (2) GHRP-6+Insuli
43.22±9.065 b	48.24±2.517 ab	48.85±8. c	98.85±7.938 c	The group (3) GHRP-6+Insulir
43.32±7.87 b	44.35±1.913 b	62.57±14.738 ac	100.0±7.609 c	The group (4) GHRP-6+Insulir
133.85±5.261 a	49.28±2.222 ab	133.14±1.844 b	164.5714±8.646 a	The group (5) GHRP-6+Insuli
70.9	9.3	50.6	35.3	L.S.D

The difference of letters indicates moral difference.

Discussion: -

Effect of GHRP-6 blended with insulin at the level of cholesterol, triglycerides and lipid proteins in laboratory animals .

The results of the statistical analysis showed a significant increase below the level of ($P<0.05$) in the level of cholesterol, T.G, and the high density VLDL and high HDL proteins in the second group only for male laboratory rats, while cholesterol decreased and there were no significant differences in LDL, was significant in the level of triclacride in the female groups treated with GHRP-6 activator compared with the control group.

The cholesterol that is formed depends on several factors, including the location of the temperature is the liver and the material contained in the vaccine and for laboratory animals is considered the source

of cholesterol and any disorder in these two factors affect the level of cholesterol in blood through the study showed the results and sections of the tissue affected liver and affected significantly and delayed affects the yellow, which provides fat analysis and increased blood level. This is evidenced by the statistical analysis. There is an increase in Triclacride and VLDL. This is consistent with (11) when structural steroids are used on male rats and their effect is known after (1, 2, 3) To study a significantly higher level of cholesterol compared with the control group.

In a randomized dose of 25mg per day for a full year, the results showed high cholesterol, LDL, and a slight increase in HDL (12) Compare with control group.

(13) The use of steroids by weightlifters for a long time because of the effect of this hormone on the enzyme (HMGCR) 3hydroxy - 3Methyl - glutaryl - co - A reductase, which causes an increase in cholesterol and the consequent increase in the rate of blood.

The high level of cholesterol confirms the existence of a defect in the function of the liver to transfer cholesterol from the tissues and peripheral vessels to the liver to get rid of it in the form of yellow substance and this confirms (14) that the high level of cholesterol linked to liver tissue damage, which affects the metabolism of fat and following the increase of free fatty acids Free Fatty Acide is the main constituent of cholesterol and triglycerides.

High cholesterol and triglycerides may be due to ROS, which causes fat oxidation and subsequently increases the acidity of unsaturated fatty acids and increases blood levels. (15) suggest that oxidative stress changes the nature of the membranes as a result of the oxidation of lipid proteins, Liver to blood.

It may be explained that the use of building stimulants to increase lean body weight and eliminate excess weight of those suffering from embolization. These stimulants increase TG release of fat cells by increasing LPO activity to analyze LDL in order to reach cells and use them as an energy source (16) In particular, the results of the statistical analysis showed an increase in blood sugar, T.G, cholesterol and LDL concentration. While HDL is low, this indicates a resistance to insulin in the liver, which leads to fatty tissue. Tint Large amounts of cytokines disrupt the normal functioning of insulin in muscle and fat cells may lead to accumulation of fat in the liver and the subsequent release of unsaturated fatty acids and increased glucose in the liver, both of which have the effect of worsening insulin resistance and increase the risk of type 2 diabetes (17). There is (18) a relationship between insulin resistance and the accumulation of TG triglycerides in muscle. This is what is observed in laboratory rats. This resistance was directed to high unsaturated fatty acids, triglycerides and low oxidation of fatty acids in muscle Nak correlation between insulin resistance and metabolism of fats and fatty tissues, especially in the abdominal area and this leads to obesity Visceral.

LDL cholesterol and low HDL cholesterol are associated with high cholesterol and LDL cholesterol. This may be due to changes made by the GHRP-6 inhibitor mixed with insulin on LDL proteins. Most cholesterol is metabolized on LDL and receptor receptors that lead to high blood cholesterol, (19) Low LDL receptor activity leads to decreased protein degradation and inhibition of lipoprotein lipase, which analyzes cholesterol and is followed by elevated blood levels, or may be due to androgens that increase the effectiveness of hepatic triglyceride lipase (HTGL) About Oz The HDL machine of the blood stream reduces the staining and increases the level of LDL (20), the Kindle is due to the increased efficacy of the Hepatic lipase enzyme which reduces the introduction of LDL into the liver (21).

HDL may be due to the effect of GHRP-6 mixed with insulin on the permeability of cellular membranes due to the reduction of calcium ions and the release of cholesterol and an increase in the blood stream. Because HDL is responsible for transporting cholesterol from blood to live.

Reference: -

-١ المنظمة الأردنية لمكافحة تعاطي المنشطات النسخة ألاولى .عمان.المملكة الاردنية .. (٢٠٠٨). **قواعد تعاطي المنشطات(JADO)** الهاشمية .ص١٦٣.

٢- **المنظمة غرب أسيا لمكافحة المنشطات ،(٢٠٠٩).**الدليل المرشد اللاعبين التثقيفي لمكافحة المنشطات الوكالة العالمية لمكافحة المنشطات بتعاون مع المجلس الاولمبي الاسيوي والمنظمة الاردنية لمكافحة المنشطات،النسخة الاولى .طبعة الخامسة .ص٩.

3-Hersch, E.C. & Merriam, G.R. (2008). Growth hormone (GH)-releasing hormone and GH secretagogues in normal aging: Fountain of Youth or Pool of Tantalus? Clin Interv Aging 3, 121-9.

4-Cabrales, A. Gil, J. Fernández, E. Valenzuela, C. Hernández, F. García, I. Hernández, A. Besada, V. Reyes, O. Padrón, G. Berlanga, J. Guillén, G. González, LJ. (2013). *Eur J Pharm Sci. 48 (1–2): 40-*

5-Grapengiesser E, Hellman B, Gylfe E, Dansk H, Salehi A; Gylfe; Dansk; Salehi (2007): Insulin oscillations--clinically important rhythm. Anti-diabetics should increase the pulsative component of the insulin release. Lakartidningen (in Swedish) 104 (32–33): 2236–9.

6-Kafrouni, M. I.; Anders, R. A. and Verma, S. (2007). Hepatotoxicity associated with dietary supplements containing anabolic steroids. Clin. Gastroenterol. Hepatol. 5(7): 809-812.

7-Tietz, N.W. (1999). Text book of clinical chemistry. 3rd Ed. C.A. Burtis, E. R. Ashwood, W. B. Saunders. Pp: 819- 861.

8-Trinder, P. (1969). Ann. Clin. Biochem. (6) Pp : 27-29.

9-Allain, C. C., Poon, L. S., Chan, C. S., Richmond, W. & Fu, P. C. (1974). Enzymatic determination of total serum cholesterol. Clin. Chem. 20(4), 470 - 475.

10-Koren, D. (1955). Clearing Factors: a heparin activated Lipoprotein Lipase. Isolation and characterization of enzyme from normal rats. J. Biol. Chem., 215(1) Pp: 1-14.

11-Saher Mahmood Jwad& Doaa Yousif Mohamme. (2017). Effect of Some Types of Doping Used by Athletes on Liver andKidneys Functional Performance in Albino Male Rats. Journal of Global Pharma Technology. ISSN: 0975 -8542.

12-Ralf Nass, M.D., Suzan, S. Pezzoli, B.A., Mary Clancy Oliveri, M.S. James, T. Patrie, M.S., Frank E. Harrell Jr, Jody L. Clasey, Steven, B. Heymsfield, M.D. Mark, A. Bach, M.D. Mary Lee Vance, M.D. Michael, O. Thorner, M.B. B.S. D.Sc. (2008). Effects of an Oral Ghrelin Mimetic on Body Composition and Clinical Outcomes in Healthy Older Adults: A Randomized, Controlled Trial**.** Published in final edited form as:4; 149(9): 601–611.

13-Garevik, N.; Skogastierna, C.; Rane, A. and Ekstrom, L. (2012). Single dose testosterone increases total cholesterol levels and induces the expression of HMG CoA reductase. Substance Abuse Treatment Prev. Policy. 7(12): 1-6.)

14-Kafrouni, M. I.; Anders, R. A. and Verma, S. (2007). Hepatotoxicity associated with dietary supplements containing anabolic steroids. *Clin. Gastroenterol. Hepatol.* 5(7): 809-812.

15-Elder, K. & Steng, G. (2000). Plasma thyroxin and cholesterol concentration miniature pigs are influenced by thermally oxidized dietary lipids.J. Nutr., 130pp: 116-121.

16-Horowitz, J. F. (2003). Fatty acid mobilization from adipose tissue during exercise. TRENDS in Endocrinol. Metabol. 14(8): 386-392.

and, Sakata, T. (2004). Role of fatty acid composition in the development of metabolic disorders in sucrose-induced obese rats. Exp. Biol. Med. (Maywood) 229, 486–493.

18- Kraegen, EW., Cooney ,GJ. Ye, J.Thompson, AL.(2001). Triglycerides, fatty acids and insulin resistance--hyperinsulinemia.Exp Clin EndocrinDiabetes. ;109(4):S516-26.

19-Yakubu, M.T.; Akanji, M.A. and Oladiji, A.T. (2005). A phrodisiac potentials of the aqueous extract of Fadogia agrestis (Schweinf. Ex Hiern) stem in male albino rats. Asian Journal of Andrology 7 (4) Pp : 399-404.

20-**Barnard, R. (2012).** Body composition and measurements of elite senior South African body builders during a competitive season. Thesis, University of the Free State.

21-Morikawa, A. T.; Maranhao, R. C.; Alves, M. N.; Negrao, C. E.; Silva, J. L. and Vinagre, C.G. (2012). Effects of anabolic androgenic on chylomicron metabolism. Steroids 77(13): 1321-1326.)