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ABNORMALITIES OF TESTICULAR FUNCTION OF MALE WISTAR RATS FED SOME PROTEIN SUPPLEMENTS

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Abstract:-

Athletes and bodybuilder consumed high protein supplements to obtain a lot of energy and enhance the development and strength of their muscles. However, after time, different investigations revealed dysfunctions of their body organs. There are contradictions between scientists concerning the benefits and the alarm of developing body dysfunction. In the present work, we selected three animal groups of male Wistar albino rats (n=10), control, hyper whey (HW) 2.6g/kg body weight and super amino 2500 (SA) 2.6g/kg body weight. The dose was provided through a healthy water tap for 14 weeks. The body weight was regularly measured. At the end of experiment, animals were sacrificed, dissected and blood collected from puncture of heart and the testes were removed and weighed. A biochemical analysis of function testes and histopathological investigations are done. From Morphometrical prospect, the total number of seminiferous tubules were markedly decreased in HW & SA. On the other hand, by using histopathological method on the symbol supplemented by WH, the spermatogenic cells were apparently declined and lacked normal pattern of arrangement. There was a noticeable increased number of necrotic spermatocytes and cell debris occupied the central region of the tubular lumina. Super amino supplementation revealed alterations of the seminiferous tubules. There was a clear reduction of spermatogenic cells. Biochemically. In HW and SA supplemented groups, it has registered a decrease in plasma total testosterone. The levels of T3 and T4 was markedly decreased in HW supplementation and slightly affected in SA fed rats

Key words:-*Protein supplements, blood picture, T3&T4, biochemistry, histopathology*.

INTRODUCTION

Widely distributed fitness gym encourage junior individuals to consume anabolic drugs to maximize body composition. Most athletes and bodybuilders use protein supplements to enhance the development and strength of their muscles and to accelerate the release of growth hormone [1]. Casein, whey, and soy proteins are the most popular protein supplements, having a high caloric value and increases muscle mass. Whey protein contains total cow's milk protein (approximately 20%), lactoglobulin (50%), B_lactalbumin (25%), serum albumin (7%), and immunoglobulins (5%). Due to the high concentrations of amino acid, it plays a significant role in the protein synthesis and carbohydrate metabolism giving energy requirements during exercise [2]. Alarming widespread use of anabolic and amino acid supplements has been associated with pathological abnormalities such as abdominal pain, nausea, vomiting, hypercalcemia, elevated liver enzymes and high levels of amylase, lipase and creatine protein kinase [3]. There is a discrepancies about the benefit and risk of damage about intake of anabolic supplements; whey protein and super amino. Although consumption of whey protein supplementation induce adverse kidney and/or hepatic damage, other human studies have demonstrated that higher protein intakes exerted no adverse effects on renal or liver function markers [4, 5]. Excessive protein intake may lead to dehydration, gout, liver and kidney damage, calcium loss, and gastrointestinal disturbances [6]. Feeding on high casein diet led to marked increase of urinary saturation of calcium phosphate and increased bone resorption in rats [7]. Short and long term creatine supplementation were found to increase creatinine, urea and transaminase [8]. Also, consumption of high protein diets was found to cause a considerable increase in kidney weight, urinary volume and acidity, as well as in the urinary excretion of Ca, with a parallel reduction in the urinary excretion of citrate leading to alterations in renal health status in pig [9] rats [10]. Feeding mice with a 50% casein diet led to a marked increase in serum ALT and AST activities , liver Finkel-BiskisJinkins (FBJ) osteosarcoma oncogene and nuclear receptor subfamily 4, group A, member 1 mRNA levels [11]. High protein/high meat intake in humans were associated with the development of disorders such as bone and calcium homeostasis, renal dysfunction, increased cancer risk, liver dysfunction, and progression of coronary artery disease [12].

The present study aimed to illustrate the role of consuming of two anabolic protein supplements on body weight and structural and function of testes of male albino Wistar rats.

MATERIAL AND METHODS

1. Hyper whey-treatment:

Hyper whey protein matrix produced by Nutrabolics, Richmond (B.C. Canada) was used. It is composed of maltodextrin, glycine, cocoa, natural and artificial flavours, cellulose gum, colour, sucralose as well as amino acids especially glutamine or glutamic acid and taurine (Table, 1). Each rat received daily oral doses of 2.6 g/kg body weight in drinking water for 14 weeks.

2. Super amino2500-treatment:

Super amino 2500 is a highly efficient protein source produced by APN (Launderale, FL 33301, U.S.A). It is composed of a mixture of amino acids (Table, 2). Each rat received daily oral doses of 2.6 g/kg body weight in drinking water for 14 weeks.

3. Experimental Work:

Thirty fertile male Wistar albino rats weighing approximately 60g body weight, obtained from Hellwan Breeding Farm, Ministry of Health, and Egypt were used for experimentation. Free access of standard diet composed of Protein 21.27%, Fat 2.83% and Fiber 2.46% was supplied. Water was allowed *ad-libitum.* They were kept in good ventilation with 12 hour light and dark cycle. The rats were arranged into three groups (n=10) ; Control , fed on standard diet, Hyper whey-treated group (HW) and Super amino 2500-treated group (SA) fed on standard diet plus supplemented the anabolic protein. During intervals of treatment, body weights were recorded every 2 weeks interval till 14 weeks. At the end of treatments, the rats of both control and experimental groups were anesthetized by diethyl ether and sacrificed. Blood was collected by puncture from heart in clean tubes containing EDTA anticoagulants, centrifuged at 3000 rpm and plasma collected. Testes was separated and processed for the following investigations:

3. 1. Histological investigations and morphpmetric assessments:

Testis specimens were incised immediately, fixed in 10% phosphate-buffered formalin (pH 7.4) and processed for histological investigations. Serial 5 µm thick sections were cut and stained with hematoxylin and eosin [13] and examined under bright field microscope. The total number of somniferous tubule were recorded in control and different treatment in five area/each specimen (n=5) at magnification 100. The percentages abnormal swollen seminiferous tubule, active somniferous tubule and non-active seminiferous tubule were recorded. The diameter and germ cell height of the seminiferous tubules were recorded.

3. 2. Biochemical investigation:

Malondialdhyde (MDA) and superoxide dismutase (SOD) were determined in both plasma and testis according to [14]. Plasma Triiodothyronine (T3) and thyroxine (T4) were determined according the instruction of measured by BioVision'Triiodothyronine (T3) (Mouse/Rat) ELISA Kit (Cat #. K7422-100). The level of T3 is decreased in hypothyroid patients and is increased in hyperthyroid patients. BioVision's mouse/rat Triiodothyronine (T3) kit is a solid phase competitive ELISA Kit. The samples, and T3 enzyme conjugate are added to the wells coated with anti-T3 and anti-4 polyclonal antibody. Unbound T3 and T3 enzyme conjugate are removed. The colour develops after addition of

the substrate and its intensity is measured spectrophometrically at wave length 450 nm. A standard curve is prepared and the concentration of the T3 is determined.

Free and total testosterone level was determined in plasma by using Calbiotech' Mouse/Rat Testosterone ELISA Kit (Cat. # TE187S-100) according to [15]. In the incubation, goat anti-rabbit IgG-coated wells are incubated with 25µl of testosterone standards, controls, experimental samples, 100 µl Testosterone-HRP conjugate reagent and 50µl rabbit anti-testosterone reagent at room temperature for 60 minutes. Unbound Testosterone peroxidase conjugate is removed and a solution of TMB reagent is added and incubated for 15 minutes, resulting in the development of blue color which is measured spectrophotometrically at 450nm.

3. 3. Statistical Analysis:
Statistical analysis was carried out between the control and the experimental groups to compare the different values. All values were expressed as means ±SE. The differences were considered statistically significant from the control at $P < 0.05$.

RESULTS

1. Body weight gain:
Figure (1) illustrated the percentages of body weight grain in hyper whey and super amino supplemented groups comparing with the control at intervals 2, 4, 6, 8, 10, 12 and 14 weeks. During 2, 4, 6 and 8 weeks of treatments, there are non-significant changes and variations were still within small ranges. However, at 10, 12 and 14 weeks, significant depletion of body weights were detected in male rats supplemented hyper whey and super amino diet.

2. Absolute and relative testis weight:
From fig. (2), both hyperwhey and super amino 2500 showed non-significant decrease of absolute testis weight comparison with the control. Also, there is no change of relative weight in hyperwhey fed rats, meanwhile SA fed rats showed a non-significant decrease comparison with the control.

3. Histopathological
At light microscopy, the testis section of control animals possessed typical feature of normal arrangement of spermatogenic cells (spermatogonia, spermatocytes and spermatids). Few numbers of sertoli cells were observed resting on the basement membrane of the tubule at fairly regular intervals. Many of the metamorphosed spermatids and spermatozoa were observed near the lumen. The seminiferous tubules were closely packed with each other leaving triangular spaces occupied by a group of interstitial cells of Leydig and rich in blood vessels. Leydig cells appeared as large irregular cells with very dense cytoplasm. The tubules were infiltrated by a thin connective tissue (Fig.3 A and B). In experimental group supplemented HW, the spermatogenic cells were apparently declined and lacked normal pattern of arrangement. There was a detected increased number of necrotic spermatocytes and cell debris occupied the central region of the tubular lumina. The intertubular tissue attained markedly thinning and hyalinized especially in the triangular spaces between SeT. Leydig cells were markedly reduced (Fig.3 A1-C1). Super amino 2500 supplementation revealed marked alterations of the SeT. There was marked reduction of spermatogenic cells. Exfoliation of necrotic germ cells and cell debris were detected within the tubular lumina. Few numbers of spermatogonia and spermatocytes were detected. Hyalinization of the intertubular lumina was also observed (Fig.3 A2-C2).

4. Morphometric observation:
Table (3) illustrated the total number of seminiferous tubules of the frequencies of active & inactive SeT as well as mean diameter and mean germ cell height in male rats supplemented hyperwhey and super amino in comparison with the control. The total number of SeT were markedly decreased in both HW and SA supplemented animal being 21.59±0.85 and 22.86±1.12 respectively comparing with the control of 35.28±1.96. The percentages of abnormal swollen SeT reached to 38.80±6.02 in HW fed rats and 35.20±7.09 in SA fed groups. The percentages of inactive SeT were markedly increased in HW and SA supplemented groups in comparison with the control. The diameters of SeT were markedly increased in HW and SA supplemented groups being 316.40±19.68 and 390±36.74 respectively in comparison with the control 290±23.18. The germ cell height attained a considerable reduction in both HW and SA supplemented animals.

5. Biochemical Observations:
In experiment of HW and SA supplemented group, there was a marked decrease of plasma total testosterone level. The level of T3 and T4 was markedly decreased in HW supplementation and slightly affected in SA .There was a detected decrease of testes super oxide dismutase and marked increase of malondialdehyde in both anabolic protein supplementation (Table. 4).

DISCUSSION
Athletes -and bodybuilders widely consume different supplements containing high protein amounts and HW and SA represent couple of these supplement mixtures designed for development of strength and muscle mass. There are contradictions between scientists concerning the benefits and the harm of these supplements. The present findings revealed a decrease of body weight especially after 10, 12 and 14 weeks of treatments. The observed decreased body weight gain may result from apparent reduction of body fat percentage and bigger muscular weight as mentioned by da

Silva et al. [16]. Helms et al. [17] reported that the depletion of bodyweight reached 0.5 to 1%/wk during maximize muscle retention. Within this caloric intake, most but not all bodybuilders will respond best to consuming 2.3-3.1 g/kg of lean body mass per day of protein, 15-30% of calories from fat, and the reminder of calories from carbohydrate. The observed findings revealed a decrease of testis weight in rats supplemented HW and SA supplemented protein. These was coincides with histopathological and morphometric alterations in testis included apparent reduction of spermatogenic cells, decreased germ cell height, edematous lesions in triangular spaces and swollen of seminiferous tubules.

Although there was no available work in HW and SA, most of the research work come from the usage of anabolic steroids. Androgenic anabolic steroids (AAS) were found to be associated to secondary hypogonadotropic hypogonadism and transient or persistent damage to the testicle with impairment on male reproductive function [18]. The spermatogenesis development in physiological conditions is dependent on hormonal and neurochemical signals transmitted through the axis of the hypothalamus, pituitary, Leydig interstitial cells, Sertoli cells and germinal epithelium [19]. The observed damage of sertoli cells and edematous lesion in triangular spaces with damage of interstitial cells may interfere with disorganization and deformation of spermatogenesis. Also, the observed dramatic effects on testicular structure was confirmed by apparent reduction of plasma testosterone level and increase of malondialdehyde and decrease of superoxide dismutase. Similar findings were reported by Badger et al [20], Gardner-Thorpe et al [21] and Hulmi et al [22] in men supplement soy protein, casein, whey, and SPI protein formula. Similar impairment of spermatogenesis and deformation of sperms were reported in male rabbits treated with 5 mg boldenone/kg [23] and male rat treated with nandrolone decanoate [24]. Degeneration of thyroid follicle cells and deformed lumina of the follicles were confirmed by depletion of plasma T3 level in both hyper whey and super amino 2500, however T4 non-significantly decreased in both treatment. Similar findings were recorded by Deyssig and Weissel [25] in young (20-29 yr old) male body builders recieved androgen-anabolic steroids exhibited apparent reduction of T3 & T4. Rats received Soya protein supplementation exhibited significant depletion of T3 & T4. These was associated with a depletion of hepatic mitochondrial respiration with succinate substrate [26]. The thyroid function (T3 and T4) was markedly reduced by the administration of anabolic-androgenic steroids (AAS) Deca-Durabolin (DECA) [27].

It is well known that T3 and T 4 are synthesized and secreted by thyroid follicle cells. T3 is the most biologically active hormone and most of them formed from T4 after deiodination in liver [28]. Serum levels of thyroid hormones, including T3 and T4 are markers of the thyroid function in humans and experimental animals. Changes in their concentration may reflect dysfunction in their glandular synthesis and secretion [29]. It is well known that triiodothyronine (T3) play a great role in the control of Sertoli cell and Leydig cell proliferation. The spermatogenic cells showed higher receptors to the thyroid hormone T3 [30, 31]. The observed finding revealed apparent depletion of plasma T3 and testosterone hormone which intern reflect on abnormal disorganized testis cytostructure causing infertility of male rats.

Finally, the present wark concluded that WH & SA are interfered with impairment of liver function and depletion of insulin, testosterone and T3 which have great role in promoting testis function causing disruption and infertility of male rats.

Legend of Figures

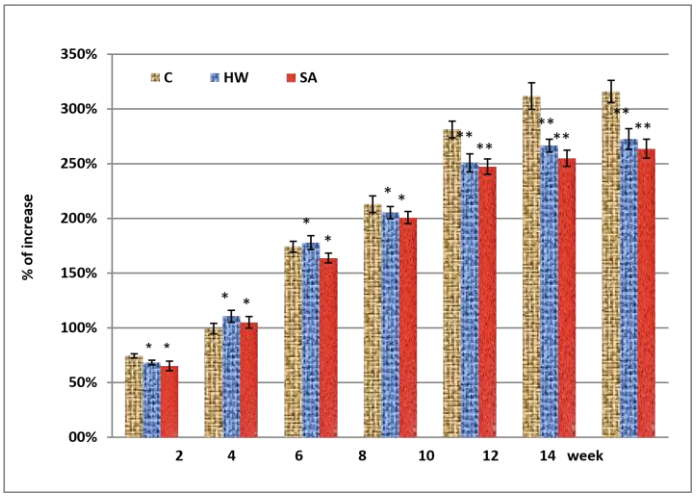

Fig. 1 Percent of increase of body weight of male rats supplemented hyperwhey and super amino 2500 for 14 weeks. Each result represent the mean± SE. (n=10). *means non-significant at P<0.05; ** means significant at P<0.05. Abbreviation; C, Control; HW, Hyperwhey; SA, Super amino 2500.

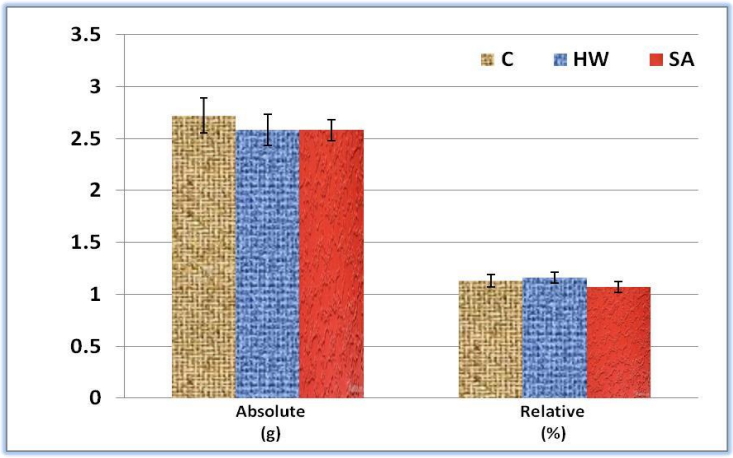

Fig. 2. Absolute and relative testis weight of male rats supplemented hyperwhey and super amino 2500 for 14 weeks. Each result represent the mean ± SE. (n=10).* Significant at P<0.05. Abbreviations; C, Control; HW, Hyperwhey; SA, Super amino 2500.

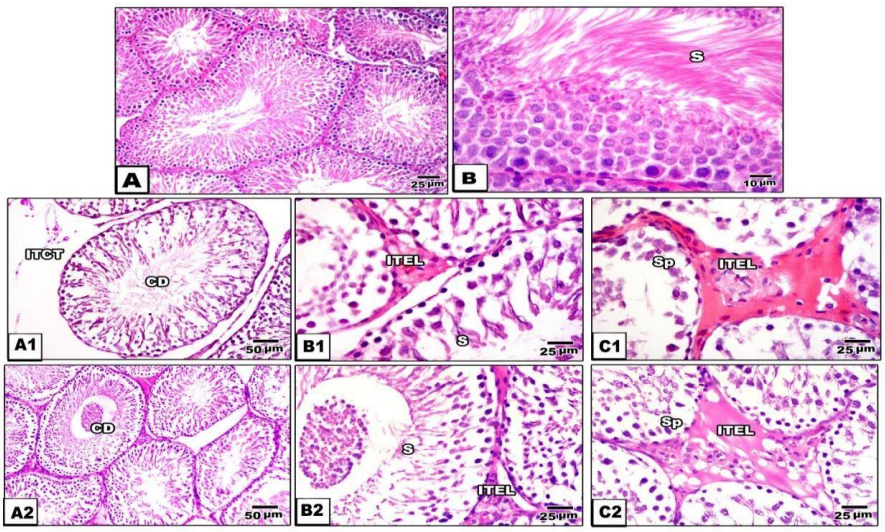

Fig. 3 Photomicrographs of transverse histological section of testis of male rats of control (A&B), hyperwhey (A1-C1) and super amino 2500 (A2-C2) rats. A&B. Control showing normal pattern arrangement of spermatogenic cells and adjoining sperm within lumina of seminiferous tubules. A1-C1. WH showing numerical reduction of spermatogenic cells, comparative decreased germ cell height and intertubular edematous lesions. A2-C2. SA showing numerical reduction of spermatogenic cells, comparative decreased germ cell height, exfoliation of germ cells within tubular lumina and intertubular edematous lesions.

Abbreviation; CD, cell debris; ITCT, inter tubular connective tissue; ITEL, inter tubular edematous lesion; S, sperm; Sp, spermatogonia.

Table.1. Percentage of nutrients constituents of anabolic Hyperwhey (nutrabolics®, Richmond, B. C. Canada V7E 2G1).

Item	Amount & %	
Total Fat	1g	2%
Saturated Fat	0g	0%
Cholesterol	40mg	13%
Total Carbohydrate	5g	2%
Dietary Fiber	1g	4%
Sugar	1g	
Protein	20g	
Sodium	25mg	1%
Potassium	100mg	3%
Calcium		30%
Iron		2%

Table. 2. Amino acid contents of super amino 2500 per 15 g protein (APN, Launderdale, FL 33301, and U.S.A).

Item	Amount
L-Alanine	180mg
L-Arginine	218mg
L-Aspartic Acid	414mg
L-Cystine	154mg
L-Glutamic Acid	1354mg
L-Glycine	108mg
L-Histidine	274mg
L-Isoleucine	276mg
L-Leucine	546mg
L-Lysine	453mg
L-Methinine	174mg
L-Phenylalanine	306mg
L-Proline	624mg
L-serine	348mg
L-Threonine	358mg
L-Tryptophan	172mg
L-Tyrosine	130mg
L-Valine	342mg

Table.3. Mophrometric assessments of somniferous tubules of male rats supplemented hyperwhey and super amino 2500 for 14 weeks. Each result represent the mean ± SE. (n=10).

	C	HW	SA	F Test
Total number of seminiferou tubule (ST)	35.28±1.96	21.59±0.85**	22.86±1.12**	29.184
% of abnormal swollen ST (AsST)	-	38.80±6.02**	35.20±7.09**	20.775
% of active seminiferous tubule (AST)	89.61±4.72	70.11±5.61*	73.17±4.45*	1.730
% of non-active seminiferous tubule	10.39±6.46	29.89±6.60*	26.83±4.45*	0.932
Mean diameter of ST (µm)	290±23.18	316.40±19.68*	390±36.74**	3.542
Mean germ cell height (GH) (µm)	130±9.53	80±9.35*	100±7.90*	1.747

Non-significant at P<0.05; ** Significant at P<0.05. Abbreviation; AST, active somniferous tubule; AsST, Abnormal swollen somniferous tubule; C , Control; GH, epithelium height; HW, Hyper whey; NST, normal somniferous tubule; NAST, non-active somniferous tubule; SA, Super amino 2500; ST, somniferous tubule; STD, somniferous tubule diameter.

Table.4. Plasma testosterone, T3& T4 hormones and super oxide dismutase and malondialdhyde of male rats supplemented hyper whey and super amino 2500 for 14 weeks. Each result represent the mean ± SE. (n=10).

		C	HW	SA	F test
			2.5 g/kg body weight		
Plasma	SOD **(u/100mg)**	290±4.02	186±2.60**	195±2.12**	61.85
	MDA **(u/100mg)**	217.6±8.31	470.2±14.01**	949.4±13.96**	899.37
	Testosterone **(ng/ml)**	3.96±0.25	2.94±0.18**	2.49±0.38*	3.17
	T3 **(ng/dl)**	91.91±1.82	82.75±1.84**	90.26±1.18*	8.8
	T4 **(ng/dl)**	4.79±0.24	4.04±0.17**	4.60±0.27*	2.8

Non-significant at P<0.05; ** Significant at P<0.05. Abbreviation; C, Control; HW, Hyper whey; SA, Super amino 2500.

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