

Effect of Anabolic Androgenic Steroid on Body Weight and Its Protection by Punica Granatum in Mice Model

Humaira Ali, Uzma Tipu Awan, Sarwar Zia, Khadija Qamar

ABSTRACT

Objectives: To determine the effect of anabolic androgenic steroid (AAS) on body weight and observe the protective role of two forms of Punica granatum (Pomegranate). **Place and duration of study:** Department of Anatomy, Army Medical College (AMC), Rawalpindi in collaboration with National Institute of Health (NIH), Islamabad from May 1st to June 30th, 2015. **Methodology:** In this study, 40 healthy mice (BALB/c-strain) having average weight of 25-30 gms were divided into four groups, each group having five males and five females. Ten animals were kept in one cage. Mice were fed with NIH standard laboratory diet pellets for eight weeks with water ad libitum. Group A which served as a control group received only lab diet. Groups B, C and D served as experimental groups. These three experimental groups were injected ND (Nandrolone decanoate) (1 mg/100 gm of body weight), intramuscularly (I/M), in the back limb (once weekly) for 8 weeks. Experimental group C also received pomegranate juice (PJ) (at the dose of 3ml/kg of body weight) via gastric tube daily for 8 weeks and experimental group D received pomegranate peel extract (PPE) (at the dose of 200mg/kg of body weight) through gastric tube daily for 8 weeks. Net body weight gain of animals was recorded by weighing the animals at the start and end of the study. The results of all the four groups were compiled and compared with each other. **Results:** The weights of animals were increased considerably in experimental groups B, C and D. No significant improvement was seen in pomegranate administered experimental groups C and D. **Conclusion:** Punica granatum has no protective role on steroid's induced total body weight gain.

Keywords: Punica granatum, body weight, Nandrolone Decanoate.

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INTRODUCTION

AAS's are synthetically produced derivatives of male sex hormone, testosterone, secreted by Leydig cells of testis. Currently, more than hundred forms of AAS's drugs have been developed. Even though these drugs are given only on prescription, but in some countries they are sold illegitimately. These medications have been abusively used both by gymnasts and best athletes to augment their performance and for the purpose of bodybuilding. They take the steroids in ten to hundred times more than the recommended physiologic dose which causes a number of adverse effects in hepatic, cardiac, vascular and genital systems¹. Androgenic hormones play a vital role in development of male reproductive system mainly spermatogenesis, prostate development and secondary sex characteristics.

Competitors use drugs in order to enhance their strengths and to improve their physical appearance; this is called "doping", which is prohibited according to International Olympic Committee rules. To reduce

the hazard of developing tolerance to any specific drug, steroids are taken as a mixture of different agents taken at one time. When such drugs are used as cocktail or simultaneously, this is known as "stacking" and these are taken in toxic quantities that causes ten to hundred folds increase in androgen concentrations. Nandrolone Decanoate (ND) is the most commonly abused AAS, as it is available in both oral and injectable form².

Although AAS's are given therapeutically in some wasting conditions such as bone marrow failures, osteoporosis, hypogonadism and aplastic type of anemias. According to researcher's data, beneficial anabolic effects come together with multiple side effects on different body organs. Nephrotoxicity and hepatotoxicity are often related to oxidative stress, as these are main organ systems linked to drug metabolism and clearing. When balance between free radicals and defensive mechanisms gets disturbed, oxidative stress occurs which leads to cellular damage³.

Punica granatum (pomegranate) belongs to the “Punicaceae” family and is among the ancient eatable fruits. Pomegranate is widely cultivated in both near and far east countries, in mediterranean region. This fruit is filled with numerous juicy arils having a colour from white to bright red. Each aril contains husk in the centre with surrounding acidic red juice. Pomegranate is rich source of phytochemicals, flavonoids and tannins such as anthocyanins, ellagic acid, gallic acid and minerals as well.

Antifungal, anthelmintic, antiviral, anti-neoplastic, antimicrobial, antidiarrheal, anti-diabetic, antiulcer and antioxidant properties have been documented for different parts of this plant class⁴. The rationale of this study was to observe the effect of anabolic androgenic steroid on body weight and its protection by pomegranate in mice.

METHODOLOGY

This lab based trial was conducted at Anatomy Department, AMC, Rawalpindi in association with National Institute of Health (NIH) laboratories, Islamabad. Forty healthy mice of BALB/c-species, both male and female with weight range of 25-30 gms were divided into four groups of ten animals each. They were kept in NIH laboratories under controlled environment regarding light and temperature. Group A served as a control group and was administered standard laboratory mice pellets for 2 months. Groups B, C and D served as experimental groups. Mice in these three experimental groups were injected ND at the dose of 1 mg/100 gm body weight, as single I/M injection in the hind limb once a week for 2 months⁵. After preparation, PJ was stored at -20°C after diluting with distilled water to volume of 1:3⁶ and mice in experimental group C was also given PJ at the dose of 3ml/kg body weight⁷ by gastric tube daily for 2

months, and mice in experimental group D was given PPE⁸ at the dose of 200mg/kg body weight⁹ by gastric tube daily for 2 months. This extract was dissolved in plain water and was given to each mouse.

The body weight of all the animals was recorded in grams at the beginning and at the end of study, just before the sacrifice of animals to calculate the total body weight gain. The weight was recorded with the help of triple beam balance. IBM SPSS v21 was used for data analysis. ANOVA test was applied for intergroup comparison of quantitative variables followed by Post Hoc Tukey’s Test that was taken as mean and standard deviations (mean ± SD). A p-value < 0.05 was taken to be indicative of statistical significance.

RESULTS

This study was conducted to evaluate the “effect of anabolic androgenic steroid on body weight and its protection by *Punica granatum* in mice”. For this purpose, forty BALB/c strain healthy male and female mice were equally divided into four groups. General observations showed that animals in control group A and experimental groups B, C and D remained active with normal sleep wake cycle throughout the period of the study.

The initial (at the start of experiment) and final (at the end of experiment) weights of animals were recorded in grams (Fig-1) and weight gain was calculated. In control group A, the mean and standard deviation (SD) of weight gain at the time of sacrifice was 2.260±0.741gm. Whereas in experimental group B, mean and SD of weight gain was 8.710±0.851gm. In experimental groups C and D, mean and SD of weight gain were 6.150±1.229gm and 6.210±0.817gms respectively (Table-1).

Table 1: Mean values of animal weight gain of control group A and experimental groups B, C and D (n=10)

Parameter	Group A Mean±SD	Group B Mean±SD	Group C Mean±SD	Group D Mean±SD	P-value
Animal weight gain (gm)	2.260±0.741	8.710±0.851	6.150±1.229	6.210±0.817	<0.001

Table 2: Statistical difference for animal weight gain on intergroup comparison of control group A and experimental groups B, C and D

	Group B A vs.	Group A vs. C	Group A vs. D	Group B vs. C	Group B vs. D	Group C vs. D
Animal weight gain (gm)	<0.001	0.001	0.000*	0.000*	0.000*	0.990

On intergroup comparison, weight gain in group B was found statistically significant (p<0.001) as compared to control group A. There was also

statistically significant difference of weight gain when experimental groups B and D were compared with control group A (p=0.001). Experimental groups

C and D showed no statistical significance (p -value=0.990) (Table-2, Fig-2).

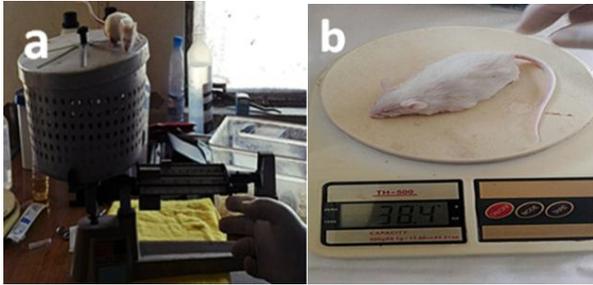


Figure-1: Photograph showing initial (a) and final (b) weight of the animal in animal no. 3 of steroid treated experimental group B.

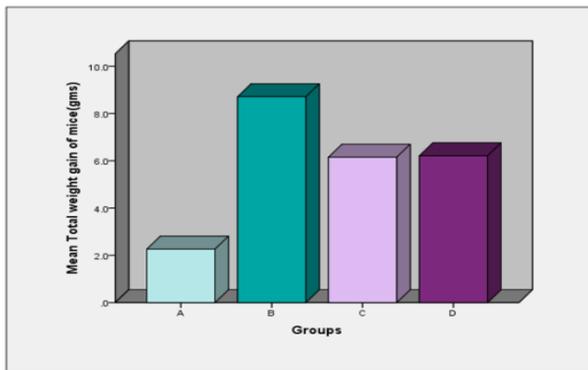


Figure-2: Bar chart showing comparison of mean values of total weight gain of mice between the control group A and experimental groups B, C and D.

DISCUSSION

Androgens and other appearance and performance enhancing substances are widely abused worldwide, either to gain muscle mass or lose body fat. This topic represents an expanding area of research and an issue of growing public-health concern.

In the current study, all animals remained healthy and active throughout the experimental period except in experimental group B. These animals showed significant body weight gain when compared with control group A. The body weight of all the animals in grams was recorded at the beginning and at the end of study, just before the sacrifice of animals. This increase in weight gain in experimental group B was in agreement with the results of Cherici *et al.*, 2009, in which initial body weight was similar among all the groups, but the final body weight was higher in the experimental group receiving 7.5mg ND/kg body weight. This weight gain is related to a significant role of testosterone and other androgens in growth and development of the body by increasing protein synthesis, decreasing protein and amino acid catabolism and maintaining

nitrogen in fat-free body mass¹⁰. Earlier statistics suggested that 4%–12% of high school boys abused AAS, and they are now being used by youngsters for various reasons that include gaining body mass to strengthen their competitive edge at sports as well as to enhance their looks by well-toned body figure and development of bulging muscles¹¹.

Steroids bind to glucocortico-steroid receptors, leading to anti-catabolic effect by blocking the glucocortico-steroid effects of decreased protein synthesis during a tough training. Negative nitrogen balance is transformed to a positive one by increasing the usage of ingested protein leading to more nitrogen retention. Furthermore, they have the capability of inducing protein synthesis in skeletal muscle cell. Athletes report that they recover more quickly from aerobics while using these drugs, experience elevated moods and more aggressive behavior¹². This was also in accordance with the study according to which AAS improves the body's utilization of consumed proteins, which positively alters nitrogen balance leading to muscle mass gain, which described the utility of these drugs in wasting syndromes¹³.

In this study, the change in body weight was also statistically significant when experimental groups C and D were compared with control group A. Experimental groups C and D were daily receiving pomegranate peel extract and pomegranate juice, respectively by oral gavage tube, along with Nandrolone Decanoate which was injected once a week for eight weeks. Although in this study, body weight gain was comparatively less in animals of experimental groups C and D, but this difference was not statistically significant when compared with ND administered experimental group B. These findings were in contrast to the study of which reported that pomegranate peel and leaf extract, as well as pomegranate juice were reported to have beneficial effects on blood lipid regulation and play a positive role in lipid metabolic disorders and obesity¹⁴.

In current study, body weight gain were similar to the findings of Kaneto *et al.*, 1999 who reported that anti-oxidants do not exert an effect on intracellular events such as those involved in apoptosis and gene transcription¹⁵. This explains why pomegranate had no significant beneficial effect on reducing body weight gain. This was also supported by another study which proved that AAS's chiefly stimulate protein synthesis by turning on gene transcription, after binding to androgenic receptors at the cellular level and enhance the production of mRNA, ultimately leading to weight gain¹⁶.

CONCLUSION

It was observed in present study that nandrolone decanoate treated groups showed significant changes in body weights in mice model. The weights of animals were significantly increased in experimental group B, C and D. Punica granatum has no protective role on steroid's induced total body weight gain.

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