ORIGINAL ARTICLE

Protective Role of Pomegranate Juice and its Peel Extract on Steroid Induced Raised Serum Creatinine in Mice

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ABSTRACT

Objective: To determine the protective role of Pomegranate Juice (PJ) and Pomegranate Peel Extract (PPE) on steroid induced serum creatinine level in mice.

Study Design: Randomized control trial.

Place and Duration of Study: Department of Anatomy, Army Medical College Rawalpindi, in collaboration with the National Institute of Health (NIH), Islamabad, from May 1st to June 30th, 2015.

Materials and Methods: Forty healthy mice (BALB/c strain), weighing 25-30 gms on average were randomly divided into four groups, each group having five male and female mice. Group A was control group and groups B, C and D were experimental groups. Mice in experimental groups were injected Nandrolone decanoate, 1 mg/100 gm body weight, intramuscularly in the hind limb once a week for 8 weeks. Mice in experimental group C were also given pomegranate Juice daily, 3ml/kg body weight by oral gavage tube for 8 weeks. Mice in experimental group D were given Pomegranate Peel Extract daily, 200mg/kg bodyweight through oral gavage tube for 8 weeks. After eight weeks 5ml blood was drawn from mice through cardiac puncture just before the sacrifice of animals. Data were entered and analyzed by using SPSS version 21. ANOVA test was applied for intergroup comparison of quantitative variables. P-value of <0.05 was taken as significant.

Results: Serum creatinine level was significantly higher in experimental group B. It was improved statistically when compared with pomegranate juice and pomegranate peel extract administered experimental groups C and D, respectively. When results of experimental groups C and D were compared with each other no statistical significance was present.

Conclusion: Both forms of pomegranate equally ameliorate the steroid induced effects on serum creatinine level in mice.

Key Words: *Androgenic Anabolic Steroids, Pomegranate Juice, Pomegranate Peel Extract, Serum Creatinine Level.*

Introduction

Androgenic anabolic steroids (AAS) are synthetic drugs closely related to the hormone testosterone, capable of increasing muscle mass and physical strength. The anabolic effects of AAS are associated with protein synthesis in bone and skeletal muscle while androgenic effects of these hormones can be generally considered as those associated with masculinization. Recently, more than hundred AAS drugs have been developed. Androgens and other appearance and performance enhancing substances are abused worldwide.

Survey among people attending gyms equipped for bodybuilding, the proportion of AAS's users was around 25–50%. AAS drugs cause ethical problems when they are used to increase performance by sportsmen in competitions and are often misused and self-administered by bodybuilders to rapidly increase muscle mass. Androgen abuse first spread from elite athletics and into the general population in the 1980s, and thus the oldest users – those who began androgens as youths in the 1980s – are only now reaching middle age, with consequently increasing risk of long-term adverse effects. Restrained usage of AAS may also induce serious side effects such as cardiovascular system failure, prostate gland diseases, lipid metabolic disorders or insulin sensitivity.[1]

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Nandrolone decanoate (ND) is the conjugation of Nandrolone and Decanoic acid. Chemical name of Nandrolone decanoate is 19-nortestosteron decanoate. Its trade names are Decadurabolin (DD), Deca-Durabol and Retabolil. ND is available as both intramuscular (I/M) and subcutaneous (S/C) injections, dissolved in a vegetable oil vehicle and produced under 25-200mg /ml dose range.[2] Such injections provide continuous androgen release into the blood stream and have remained the main route of pharmacological androgen therapy (PAT) for the last few decades.

Therapeutic uses of these substances include the treatment of hypogonadism, as in men, androgens are necessary for reproductive function as well as they play a vital role in cognitive function and a feeling of well-being. They are also beneficial in bone mineralization, some muscle wasting disorders, bone marrow failure syndromes, osteoporosis in postmenopausal women and aplastic anemias.[3]

Besides having therapeutic effects, AAS have been recognized to produce some undesirable effects, most documented of which include elevated blood pressure, cardiac arrhythmias, myocardial infarction, altered RBC morphology, cholestatic jaundice, hepatocellular hyperplasia and adenoma. Furthermore, male reproductive side effects are testicular atrophy, gynecomastia, compromised spermatogenesis, masculinization and menstrual disorders in female.[4]

Although AAS are taken only on prescription, but in some countries they are sold illegally. Athletes use these drugs to improve their stamina and performance; this is known as “doping”, which is banned by International Olympic Committee. A variety of AAS are often taken simultaneously, this is called “stacking” and drugs are taken in doses that cause ten to hundred times increase in androgen concentrations, and this apparently describes the increase in toxic effects.[5]

Pomegranate is the figure and icon of the historical city of “Granada” in Spain – from which the city acquires its name. In earliest times it was mentioned as possessing powers of fertility, abundance and good luck. Apart from being eaten fresh, pomegranates are used to make juice, which is a worldwide popular beverage as well. About 100 g arils provide 72kcal of energy, 7mg vitamin C, 16.6g carbohydrate, 1.0g protein, 1mg sodium, 379mg potassium, 12mg magnesium, 13mg calcium, 0.7mg iron, 0.17mg copper and 0.3mg niacin. Pomegranate peel is a great natural source of phenolic compounds such as gallotannins, anthocyanins, free ellagic acid, ellagic acid glycosides, ellagitannins, punicalagin and punicalin. Numerous studies have demonstrated the antihelminthic, antimicrobial, and antioxidant potentials of pomegranate juice and peel extract ingredients, suggesting their protective and curative role.[6]

Pathophysiology in kidney, liver and heart are often associated to oxidative stress, as these are major organs involved in drugs metabolism, detoxification and excretion. This oxidative stress is described by interruption of control regarding redox signaling. A cellular damage and lipid peroxidation products lead to an inflammatory response.

This study was conducted to observe the nephrotoxic effects of AAS, to cause awareness especially, in young abusers. When prescribed in chronic illnesses, AAS have both therapeutic as well as undesirable effects side by side. The present study was planned to determine the protective role of Pomegranate Juice (PJ) and Pomegranate Peel Extract (PPE) on steroid induced serum creatinine level in mice.

Materials and Methods

This randomized control trial was conducted in the Department of Anatomy, Army Medical College, Rawalpindi, in collaboration with National Institute of Health (NIH), Islamabad from 1st May- 30th June 2015. Experimental protocols were carried out with the approval of Ethical Committee of Centre for Research in Experimental and Applied Medicine (CREAM), Army Medical College, Rawalpindi.

Forty healthy male and female BALB/c mice average weighing 25-30 gms, were randomly divided into four groups, each having five male and five female animals. They were housed in NIH under controlled environment of light and temperature, and were fed with NIH standard laboratory diet for eight weeks. Group A served as a control group and groups B, C and D served as experimental groups. Mice in experimental groups B, C and D were injected ND at the dose of 1 mg/100 gm body weight, as a single intramuscular injection in the hind limb once a week for 8 weeks.[7] In addition, mice in experimental group

C was also given PJ at the dose of 3ml/kg body weight by oral gavage tube for 8 weeks daily,[8] and mice in experimental group D was given PPE at the dose of 200mg/kg body weight by oral gavage tube for 8 weeks daily.[9]

Preparation of Pomegranate Juice: The fresh and seasoned Pomegranate fruits were purchased from a local market. They were washed thoroughly and peeled manually. Juice was prepared by using an electrical blender. As seeds cannot be manually separated, juice was filtered through a filter paper. It was stored at-20°C after diluting with distilled water to volume of 1:3, at-20°C.[10] In this study, 3ml of juice is dissolved in 27ml of plain water to make 30ml solution and 1ml was given to each mouse by gastric tube.

Preparation of Pomegranate Peel Extract:

Pomegranate peels were manually separated, sun dried and grounded to powder. Extract was prepared by mixing the powder (25g), by using a magnetic stirrer, with 100ml of methanol at 30°C for 1hr. First it was was filtered to remove the peel particles, and then the extracts was pooled and concentrated under vaccum at 40°C.[11] In this study, 5.6mg of extract was dissolved in 2ml of plain water and was given to each mouse by a gastric tube.

Five ml sample of blood was taken through cardiac puncture[12] in a test tube (Figure 1), for the quantitative measurement of serum creatinine level just before the sacrifice of the animals. After collection, the blood was centrifuged and serum was collected. The blood samples were labeled according to the groups as control group A and experimental groups B, C and D. Serum creatinine level were measured on spectrophotometer using enzymatic method. Data was entered and analyzed by using SPSS version 21. ANOVA test was applied for intergroup comparison of quantitative variables. P-value of <0.05 was taken as significant.

Results

Results of experimental groups B, C and D were compared with control group A and with each other. Mean serum creatinine level ±SD of control group A was 0.130±0.048mg/dl, and in experimental groups B, C and D it was 1.09±0.280mg/dl, 0.360±0.142mg/dl and 0.350±0.217mg/dl respectively (Table I). Intergroup comparison was statistically significant when control group A was compared with experimental group B (p-value=0.000*) and experimental group C (p-value=0.053) but there was no significant difference when compared with experimental group D (p-value=0.069).

Statistical significance was seen when experimental group B was compared with experimental groups C and D (p=0.000* in both cases). When experimental group C was compared with control group A (p-value=0.053) statistical difference was noted but no statistically significant difference was seen when experimental groups C and D were compared with each other (p-value=0.999).

Table I: Mean values of serum creatinine level in control group A and experimental groups B, C and D.

Parameter	Group A	Group B	Group C	Group D	Normal value
Serum creatinine level	0.130± 0.048 mg/dl	1.090± 0.280m g/dl	0.360± 0.142m g/dl	0.350± 0.217m g/dl	0.08–0.11 mg /dl

Fig 1: Blood sample collection for serum creatinine estimation

Discussion

The Father of Medicine, Hippocrates, about 25 centuries ago, stated, “Let food be the medicine and let medicine be the food”. In regard to this statement, globally, the interest in nutraceuticals has increased widely, and this plays a vital role in health management. Numerous studies have shown the beneficial effects of many vegetables, fruits, pulses, spices and herbs.[13]

Pomegranate has achieved the title of “superfood'', due to its presumed safety and potential nutritional as well as therapeutic benefits. Pomegranate juice contains phenolic compounds such as anthocyanins,

ellagic acid, gallic acid, glucose, ascorbic acid, rutin, iron, and amino acids which have been shown to scavenge free radicals, decrease lipid peroxidation and macrophage oxidative stress, as well as increase the plasma antioxidant capability in humans.[14]

The objective of this study was to observe the ameliorative effect of two forms of Pomegranate on the steroid induced changes in serum creatinine level in mice. It was found that the difference of serum creatinine level in all animals of experimental group B in comparison with control group A was statistically significant. Statistical significance was also seen when experimental groups C and D were compared with control animals, although marked improvement was seen in both these experimental groups.

Drug related renal damage is acquiring a lot of attention these days as it is the commonest cause of CRD (chronic renal disease) in teenagers. It has been reported that seven out of ten bodybuilders abusing high doses of AAS's were diagnosed with end-stage renal disease.[15] Pathophysiology in kidney, liver and heart are often associated with oxidative stress, as these are major organs responsible for drugs detoxification, metabolism and excretion. This oxidative stress causes interruption of control regarding redox signaling. A cellular damage and lipid peroxidation products lead to an activation of inflammatory signaling cascades and increases pro inflammatory cytokine production, causing noticeable decrease in capability of some organs to tolerate injury. Reactive oxygen species (ROS), e.g. superoxide and hydrogen peroxide (H_2O_2), can be formed by xanthine-oxidase, mitochondrial electron transport chain or cytochrome P-450, as a product of metabolism, or directly by the nicotinamide adenine dinucleotide phosphate-oxidase (NADPH-oxidase) (NOX) group of enzymes.

The biological functions of NOX are known in various cellular processes, such as cellular differentiation and multiplication, synthesis and release of hormones, enzyme formation, but their overexpression is related to pathophysiology of various diseases. NOX enzymes are transmembrane proteins and are situated at nuclear membrane, near to DNA, increasing the possibility of its damage. Antioxidant systems react with these molecules forming less reactive compounds to maintain ROS levels in limits.[16]

Improvement in experimental groups C and D is attributed to pomegranate's ability to lower the oxidative stress. Edible part of pomegranate fruit is rich in vitamin C and phenolic compounds, which are strong antioxidants[17], and this was verified by Riezzo et al., 2014 who reported an increase in the malondialdehyde (MDA) level (marker of lipid peroxidation) as well as enhanced activity of antioxidant enzymes (GR and GPx) in the Pomegranate administered groups, resulting in the increased ability of the kidneys to scavenge toxic free radicals such as hydrogen peroxide and lipid peroxides.[18] Furthermore, PJ considerably reduced the concentration of pro-inflammatory chemokines like IL-6, IL-12p40, IL-1βand cytokines, thus reducing inflammation and markedly improved kidney and liver functions.[19]

This was also in agreement with the results of study conducted by Balbir et al., 2011, who described that oxidative stress plays a vital role in developing chronic complications in case of interstitial nephritis and it is related to increased lipid peroxidation, which can induce fragmentation of DNA leading to apoptosis in renal tubules causing an end stage renal disease. Pomegranate peel extract consumption at the dose of 0.5ml/day for 12 weeks considerably reduced both the occurrence and severity of collagen-induced interstitial nephritis in mice. High level of phytochemicals (antioxidants) in pomegranate could enhance the reduction of free radicals inside the cells, to protect the kidney tissue from oxidative stress damage.[20]

These findings were in accordance with another study conducted by Sastravaha and colleagues who demonstrated the beneficial effect of PPE by reducing cytokine activity, in patients having periodontitis. Patients suffering from this type of oral inflammation received intra-gingival PPE impregnated chips which resulted in reduced inflammatory cytotoxic cytokines (IL-1beta, IL-6 and TNF-α) levels few months after treatment.[21] The reno-protective roles of pomegranate in clinical trials were chiefly associated to its antioxidant and anti-inflammatory properties. This opinion was also reinforced by the findings of Viuda-Martos et al., 2013 who suggested that PPE activates the anti-inflammatory responses of the immune system within the cells.[22]

Future researchers may focus their attention to explain AAS's (Anabolic androgenic steroids) and Punica granatum interactions in conditions of long term co-exposure and their consequences for health in human population. Additional studies are recommended to see if the effects noticed in the current study are transitory or long-lasting in order to evaluate the use of Punica granatum as a potential agent to be used as antidote along with anabolic steroids in clinical settings.

Conclusion

There was significant increase in serum creatinine level of animals in experimental group B, indicating renal damage, and improvement was seen in Pomegranate administered experimental groups C and D. Therefore, it is concluded that Pomegranate in both forms has equal antitoxic effects on steroid induced renal damage. Advance studies are required to be conducted to explain the exact mechanism of action, in protection provided by Punica granatum, on histomorphology of kidneys during steroid induced renal damage.

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