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Original Article

Ameliorative Effects of Two Forms of Pomegranate on Glomerular Transvertical Diameter in Steroid- Induced Kidney Damage in Mice

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Abstract

Background: To observe the effects of Pomegranate juice (PJ) and Pomegranate peel extract (PPE) on glomerular transvertical diameter in steroid induced mice kidney damage.

Methods: In this experimental study forty healthy adult mice (BALB/c strain), average weighing 25-30 gms were divided into four groups, having ten mice each. Control group A received only standard laboratory diet without alteration.Experimental groups B, C and D were injected ND (Nandrolone decanoate) (1 mg/100 gm body weight), intramuscularly (I/M), in the hind limb once a week for 8 weeks. Experimental group C was also given PJ (3ml/kg body weight) by oral gavage tube daily for 8 weeks and experimental group D was given PPE (200mg/kg body weight) through oral gavage tube,daily for 8 weeks.After the experimental period, the animals were sacrificed and both kidneys of all mice were obtained. Kidneys were processed, embedded and stained for histological study by using Hematoxylin and Eosin (H&E) and Periodic Acid Schiff (PAS) stains. The results were compiled and compared.

Results: After ND administration, glomerular diameter was significantly reduced in experimental group B when compared to control group A.Protective effects were seen when comparison of experimental group B was done with PJ and PPE 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 has ameliorative effects on glomerular transvertical diameter in steroid induced mice kidney disease.

Key words: Pomegranate, Nandrolone Decanoate, Glomerulus, Kidney.

Introduction

About 25 centuries back, the Father of Medicine, Hippocrates stated, "Let food be thy medicine and let medicine be thy food''. Globally,in reference to this statement, , various researches have shown the protective and curative benefits of many fruits, vegetables, pulses, spices and herbs which now plays an important role in health management.[1] Pomegranate is commonly known as "Anar".Current scientific name of pomegranate,"Punicagranatum" is derived from the name Pomum (apple) and granatus (seeded), or granular apple.[2] Pomegranate is a great natural source of phenolic compounds such as gallotannins, anthocyanins, free ellagic acid, ellagic acid glycosides, ellagitannins, punicalagin and punicalin which contribute to its antioxidant, anticancer, anthelminthic and antimicrobial potentials as per research.[3-5]

Anabolic androgenic steroids (AAS's) are synthetically produced drugs correlated to the hormone testosterone, formed in the interstitial Leydig cells of the testes. AAS's are used therapeutically to supplement two different situations; firstly it is administered as androgen replacement therapy because of androgen deficit due to hypothalamic, pituitary or testicular genomic disorders in order to achieve optimum testosterone levels. Secondly, AAS's are given as pharmacological androgen therapy (PAT) in non-androgen-deficient patients having prolonged and devastating diseases to improve the quality of life.[6] Besides having beneficial therapeutic effects, AAS's have been recognized to produce undesirable effects towards patient's health, such as cardiovascular system failure, prostate gland diseases, lipid metabolic disorders, insulin sensitivity,cholestatic jaundice,testicular atrophy, gynecomastia and compromised spermatogenesis.[7]

Stanozolol, oxandrolone, oxymetolon, nandrolonedecanoate and testosteonespionat are commonly abused AAS's.[8] These drugs are capable of growing muscle mass and boosting physical strength, so they are often misused and self-administered by bodybuilders and young athletes to enhance their stamina and performance. [8]Data of renal disorders is intermittently evolving from clinical reports among AAS's users.[9]

Material and Methods

This experimental trial was conducted at Anatomy Department, Army Medical College, Rawalpindi in association with National Institute of Health (NIH), Islamabad.Forty healthy male and female BALB/c mice weighing 25-30 gms were equally divided into four groups, having ten animals each.They were kept in NIH under controlled conditions of temperature and light. Group A served as control and was given standard laboratory micepellets for 8 weeks. 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 8 weeks.[10] After preparation, PJ was stored at-20°C after diluting with distilled water to volume of 1:3 and mice in experimental group C was also given PJ (at the dose of 3ml/kg body weight) by oral gavage tube daily for 8 weeks, and mice in experimental group D was given PPE (at the dose of 200mg/kg body weight) by oral gavage tube daily for 8 weeks. [11-14]This extract was dissolved in plain water and was given to each mouse by oral gavage tube.At the end of experimental period, the animals were sacrificed. Both kidneys of each specimen were washed in saline and observed for size, color or cystic appearance. Texture was appreciated by palpation. The coronal section of each of the right kidney was taken and each left kidney was cut transversely at the level of hilum. Tissues were fixed in 10% formalin solution, dehydrated by passing through graded alcohol, embedded in paraffin waxto form blocks. Blocks were mounted on rotary microtome to obtain sections having thickness of 5µm. H&Eand PAS stains were used for histological study.

Transvertical diameter of glomeruli was measured by using ocular micrometer which was calibrated by a stage micrometer (Figure-1). Three glomeruli were randomly chosen in three different fields in one slide per specimen at 40X magnification. Both transverse and vertical diameters of same glomerulus were measured and then mean of both diameters was calculated, the result was the transvertical diameter of the glomerulus. Three readings were taken and then mean of the three readings was taken as the observed diameter of the glomeruli for the particular specimen. The data was entered and analyzed by using SPSS version 21.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). P-value of <0.05 was taken as significant.

Results

Mean glomerular transvertical diameter ± SD of right and left kidneys of control group A were 50.997±1.591µm which was statistically significant when compared with ND administered experimental group B (p-value=0.000) and nonsignificant when compared with PJ administered experimental group C and PPE administered experimental group D (p-value=0.159 and 0.083) respectively (Table-1) .

Mean glomerular transvertical diameter ± SD in right and left kidneys of experimental groups B was 37.365±1.249 showing renal damage caused by ND. In experimental groups C and D mean glomerular transvertical diameter ± SD were 48.323±23.917 and 47.927±3.414 respectively, (Table-2) showing remarkable improvement in both Pomegranate administered groups. On intergroup comparison, statistical significance was found when experimental group B was compared with experimental groups C and D (p-value=0.000). When experimental group C was compared with control group A and experimental group D (p-value=0.159 and 0.989) respectively,no statistical difference was present (Table-1). No remarkable difference was present when experimental groups C and D were compared with each other (0.989) showing that both forms of pomegranate has nearly equal protective effects on steroid induced renal damage (Figure-2).

Table1: Statistical difference for glomerular transvertical diameter on intergroup comparison of control group A and experimental groups B, C and D

Groups	Group A vs. Group B	Group A Vs Group C	Group A vs Group D	Group B vs Group C	Group B vs Group D	Group C vs Group D
p-value	0.00*	0.159	0.083	0.00*	0.001	0.989

Table-2: Mean values of glomerular transvertical diameter of control group A and experimental groups B, C and D

Glomerular transvertical diameter (µm)	Group A	Group B	Group C	Group D
Mean value±SD	50.99± 1.591	37.36±1.249	48.32±3.917	47.92±3.414

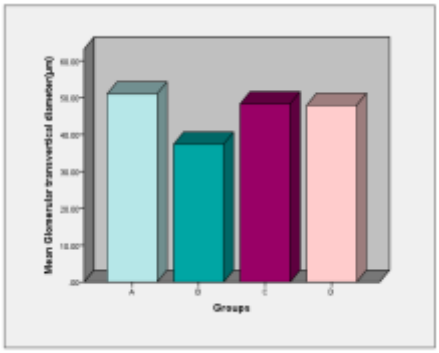

Figure-1: Comparison of mean values of glomerular transvertical diameter between the control group A and experimental groups B, C and D

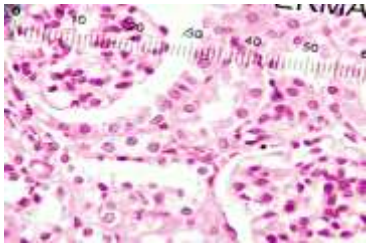

Figure-2: Photomicrograph showing micrometry of glomerulus in animal no. 5 of control group A, H & E 400X

Discussion

Anabolic androgens and other appearance and performance enhancing substances are abused worldwide. Nephrotoxicity and hepatotoxicity are often associated to oxidative stress, as these are major organs involved in metabolism, detoxification and excretion of drugs.[15] Elevated creatinine and decreased GFR (glomerular filtration rate) may occur as a result of rhabdomyolysis in highly muscular androgen users engaged in heavy resistance training.[16]

Numerous studies recommend that anabolic androgens exert a direct toxic effect on podocytes leading to their depletion, glomerular cell damage and accumulation of mesangial matrix.[17] High doses of AASs enhanced androgen receptor expression on glomerular and mesangial cells, increased mRNA levels of the pro-fibrotic cytokine, thus providing a potent pro-apoptotic stimulus to podocytes and promote FSGS (focal segmental glomerulosclerosis), the direct nephrotoxic effect of anabolic steroids.[18,19]

It is reported that bodybuilders abusing high doses of AAS's are diagnosed with end-stage renal disease. Renal biopsy of these patients revealed glomerulosclerosis with discrete obstructive lesions of pre-glomerular vessels and chronic diffuse tubulo-interstitial damage.[20] Fragmentations of glomeruli along with few atrophied elements are also observed.[21,22] These pathological alterations cause an abnormal production of cytokines and growth factors. Consequently, they enhance the synthesis of extracellular matrix proteins and their deposition in the glomerulus that eventually lead to mesangial expansion, glomerular basement thickening and glomerular shrinkage.[23,24] These modifications increase hydrogen peroxide production in the mesangial cells and lipid peroxidation of the glomerulus.[25] A cellular damage and lipid peroxidation products lead to oxidative stress.[26]

Oxidative stress occurs whenever there is an imbalance between pro-oxidant compounds and antioxidant defences leading to modifications of DNA, lipids and proteins .These structural alterations in biomolecules can modify cellular functions and processes, and play a substantial role in causing a variety of common diseases and degenerative conditions.[27]Marked improvement in glomerular diameter was seen in Pomegranate administered experimental groups C and D. Mean diameters were increased and no statistical difference was seen when compared with mean diameters of control group A. Reno-protective effects of pomegranate involve the activation of nitric oxide-dependent and peroxisome proliferator-activated receptor (PPAR-γ) signaling pathway.[28] In another study improvement in renal pathology was observed in Adenine-treated rats co-administered with Pomegranate juice or Pomegranate peel extract and this was also attributed to the activation of PPAR-γ and increased NO (nitric oxide) production.[29] Another research also documented protective role of NO in renal failure, including glycerol-induced renal failure in animal model.[30]

Studies have shown that Pomegranate is very effective scavenger of toxic hydroxyl radicals and is a potent antioxidant. It enhances the antioxidant enzyme activity like of superoxide dismutase, glutathione peroxidase and catalase in conditions of increased oxidative stress and regulate mRNA levels in the cells for these enzymes This antioxidant ability was attributed to large amount of phenolics, flavonoids and proathocyanidins contained both in peel as well as pulp extract.[31-33]Both forms of pomegranate appeared to have markedly high potential to be used as a health supplement rich in natural antioxidants in various chronic and debilitating diseases.

Conclusion

1. Nandrolone decanoate treated group showed significant reduction in glomerular transvertical diameter indicating renal damage. Improvement was seen in pomegranate administered experimental groups C and D.

2. Pomegranate in both forms has nearly equal ameliorative effects on steroid induced renal damage.

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