AMELIORATIVE EFFECT OF PUNICA GRANATUM ON STEROID INDUCED PROXIMAL AND DISTAL TUBULAR DILATATION IN MICE KIDNEY

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ABSTRACT

Objective: To investigate the effects of anabolic androgenic steroid on histomorphology of the proximal and distal renal tubules in mice kidney and amelioration of these effects with Punica granatum (Pomegranate).
Study Design: It was laboratory based randomized control trial.
Place and Duration of Study: Anatomy Department, Army Medical College Rawalpindi, in collaboration with National Institute of Health (NIH), Islamabad, from Apr to May 2015.
Material and Method: Forty healthy male and female BALB/c mice weighing 25-30gms were equally divided into four groups. Group A served as a control group and groups B, C and D served as experimental groups. Mice in these three experimental groups were injected Nandrolone decanoate (ND) at the dose of 1 mg/100 gm body weight, as single intramuscular injection in the hind limb once a week for 8 weeks. Mice in experimental group C was also given pomegranate juice (PJ) at the dose of 3ml/kg body weight by oral gavage tube for 8 weeks daily and mice in experimental group D was given pomegranate peel extract (PPE) at the dose of 200mg/kg body weight by oral gavage tube for 8 weeks daily. The results of experimental groups B, C and D were compared with control group A and with each other.
Results: In experimental group B, proximal and distal tubular and luminal diameters were significantly increased when compared with control group A, and these parameters were improved statistically when compared with pomegranate juice and pomegranate peel extract administered experimental groups C and D, respectively.
Conclusion: Punica granatum in both forms, as Pomegranate juice and Pomegranate peel extract, has nearly equal ameliorative effects on steroid induced renal tubular damage.

Keywords: Anabolic androgenic steroid, Pomegranate juice, Pomegranate peel extract, Renal tubules.

INTRODUCTION

Androgens are a family of hormones that includes testosterone, the naturally occurring androgen, plus numerous synthetic derivatives of testosterone, generated over the last 70 years[1]. These are referred to as "anabolic-androgenic steroids"(AAS). AAS plays a substantial role in treatment of cachexia caused by chronic illnesses such as osteoporosis, breast carcinomas, bone marrow failure syndromes, AIDS, severe burns and renal failure, where dietary and standard care had been shown ineffective[2].

Androlone Decanoate (Anabolic steroid) was proved to be effective in counteracting sarcopaenia in patients on dialysis and was also used as hormone replacement therapy, in male hypogonadism and male hormonal contraception (where progestogens are administered to inhibit gonadotropin secretion)[3].

Androgens are widely abused by athletes to gain muscle mass or lose body fatin order to improve their stamina, performance and appearance, this is known as "doping", which is banned by International Olympic Committee as it causes health hazards to a sportsman[4]. High doses of AAS have adverse effects on the hepatic, endocrine, cardiovascular and renal systems[5]. End-stage renal disease has been documented in body builders abusing high doses of AAS[6].

Toxic hepatic and renal failures are known to induce the excessive production of reactive oxygen species (ROS). As liver and kidneys are important organs of storage, detoxification, metabolism and excretion of many metabolites,

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so they are particularly vulnerable to oxidative damage[7]. However, recent researches indicate that usage of fruits and vegetables rich of phytochemicals can reduce these side effects[8].

Punica granutam, commonly known as pomegranate (Anar) is one of the oldest known eatable and innocuous fruits, first planted during 4000 and 3000 BC, even documented in the Koran and the Bible[9]. Pomegranate 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[10]. Various studies have proven the antihelminthic, antimicrobial, and antioxidant potentials of pomegranate juice and peel extract ingredients, suggesting their protective and curative role[11]. The rationale of this study was to observe the ameliorative effect of Punica granatum on steroid's induced proximal and distal tubular dilatation in mice kidney.

Table: Mean values of proximal and distal tubular diameters of control group A and experimental groups B, C and D.

Parameters	Group A	Group B	Group C	Group D	*p*-value between groups
Proximal tubular diameter (µm)	30.51 ± 1.060	42.34 ± 2.04	32.73 ± 2.64	32.77 ± 1.799	<0.001
Distal tubular diameter (µm)	20.75 ± 1.360	30.54 ± 1.73	22.61 ± 2.20	22.58 ± 1.845	<0.001

MATERIAL AND METHODS

This randomized controlled trial was conducted at the Anatomy department, Army Medical College, in collaboration with National Institute of Health (NIH) Islamabad and Pathology department, Army Medical College, Rawalpindi. All protocols of the experiment 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 weighing 25-30 grams were equally divided into four groups by random number table, each group having ten animals and sampling was done using non-probability convenient sampling technique. They were fed with NIH laboratory diet for eight weeks. Group A served as a control group and 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 intramuscular injection in the hind limb once a week for 8 weeks[12]. Mice in experimental group C were also given pomegranate juice (PJ) at the dose of 3ml/kg body weight by oral gavage tube for 8 weeks daily[13], and mice in experimental group D was given pomegranate peel extract (PPE) at the dose of 200mg/kg body weight by oral gavage tube for 8 weeks daily[14].

Preparation of Pomegranate Juice

Fresh and seasoned pomegranates were obtained from a local market. They were carefully washed and manually peeled. Juice was made through an electrical blender, and filtered by using a filter paper as seeds were not manually separated. It was stored at 20°C after diluting with distilled water to volume of 1:3[15].

Preparation of Pomegranate Peel Extract

Pomegranate peels were separated manually, sun dried and ground to powder. The powder (25g) was extracted by mixing with 100ml methanol at 30°C for 1 hour by using a magnetic stirrer. The extract was then filtered to remove the peel particles, pooled and concentrated under vaccum at 40°C[16].

At the end of eight weeks, the animals were anesthetized and dissected. Both kidneys of each

specimen were weighed and observed for size, color and shape. Kidneys were fixed in 10% formalin. Paraffin wax with melting point of 58°C was used for infiltration and embedding of tissues. The blocks were allowed to solidify on cold plate and cross sections of 5µm thickness were obtained by using rotary microtome. Staining of sections was done with Hematoxylin and Eosin (H&E) for routine histological study and Periodic Acid Schiff Hematoxylin (PASH) for demonstration of basement membrane and microvilli of renal tubules. Tubular diameters were measured by ocular micrometer which was calibrated by a stage micrometer at 40X objective power. One rounded proximal convoluted tubule (PCT) and distal convoluted tubule (DCT) was selected randomly in each of three different fields at 40X objective and the mean was calculated. The external diameter of tubules was measured from the basement membrane of cells on one side of the tubule to the basement membrane of cells on the opposite side of the rounded tubules. Three readings of three rounded tubules in each slide were taken and their mean was calculated as final reading for that specimen.

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 considered to be indicative of statistical significance.

RESULTS

This study was conducted to evaluate the "Ameliorative effect of Punicagranatum on steroid induced proximal and distal tubular dilatation in mice kidney". For this purpose, forty BALB/c, healthy male and female, mice were equally divided into four groups.

In control group A, proximal and distal convoluted tubules could be easily differentiated in H & E stain. Proximal tubules appeared to be more in number and of larger diameter having low columnar cells with brush border while distal tubules were smaller in diameter, less in number, having cuboidal epithelium. PCT were more eosinophilic (dark pink) as compared to DCT.

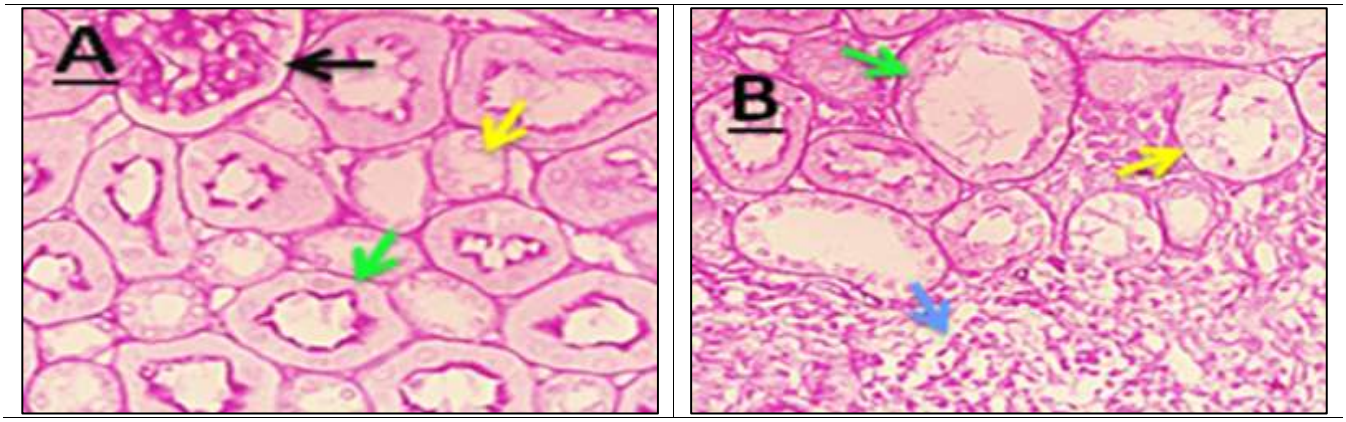

Figure-1: Photomicrograph showing renal architecture in animal no. 5 of control group A, PCT (green arrow), DCT (yellow arrow), mesangium and glomerular basement membrane took magenta colour (black arrow).

Mean tubular diameter ± SD of PCT in both kidneys of control group A were 30.516 ± 1.060 (table) which was statistically significant as compared to experimental group B (p<0.001) and statistically nonsignificant when compared with experimental groups C and D (p-values=0.072 and 0.064) respectively.

Mean tubular diameter ± SD of PCT in both kidneys of experimental groups B, C and D were 42.34 ± 2.054, 32.73 ± 2.604 and 32.77 ± 1.799, respectively (table). When experimental group B was compared with experimental groups C and

D (p<0.001 and p<0.001) statistical difference was observed, but no significance was noted when experimental groups C and D were compared with each other (p-value=1.000).

Mean tubular diameter ± SD of DCT in both kidneys of control group A was 20.757 ± 1.360, and in experimental groups B, C and D it was 30.543 ± 1.723, 22.618 ± 2.20 and 22.584 ± 1.845 respectively.

Intergroup comparison was statistically significant when experimental group B was compared with control group A (p<0.001), but there was no statistical significance when experimental groups C and D was compared with control group A (p-value=0.116 and 0.127) respectively. Statistical significant difference was also present when experimental group B was compared with experimental groups C and D (p<0.001 in both cases). Comparison of experimental groups C and D showed no statistical significance when compared with each other (p-value=1.000).

Dilated & necrosed PCT, dilated and necrosed DCT, inflammatory infiltrate (blue arrow) in animal no. 1 of steroid administered experimental group B, PAS at 400X .

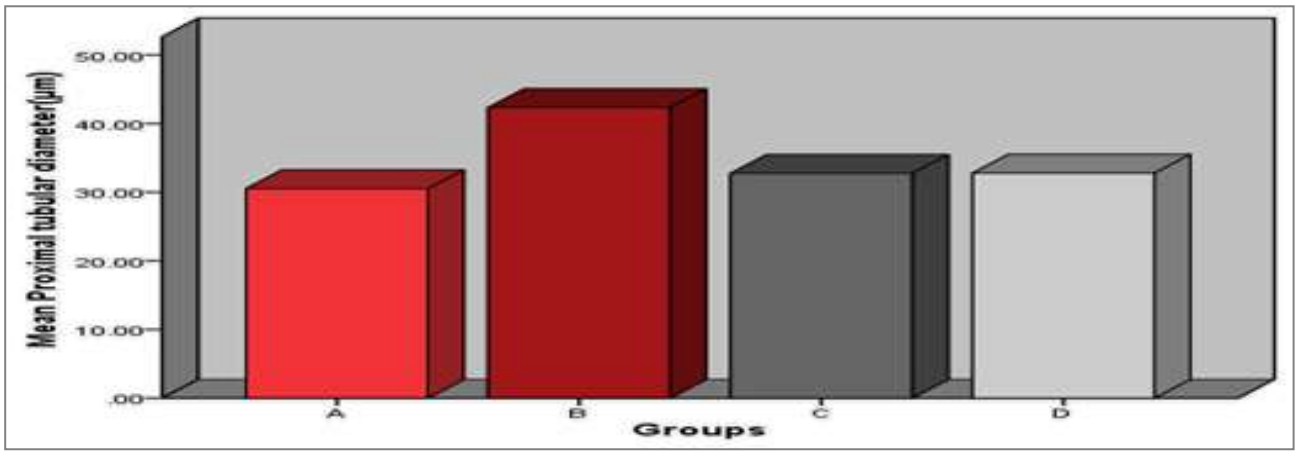

Figure-2: Comparison of proximal tubular diameter between control group A and experimental groups B, C and D.

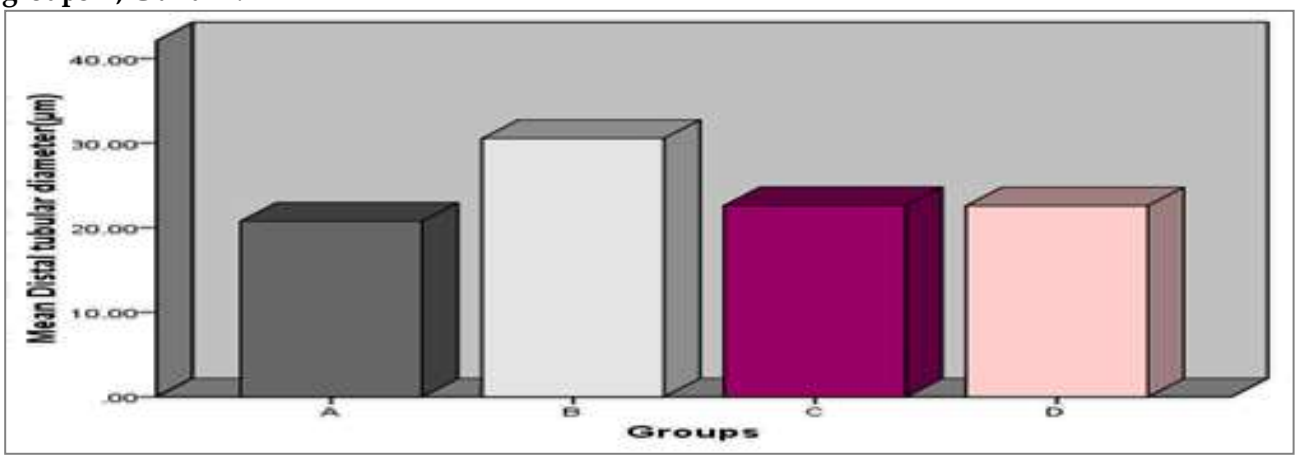

Figure-3: Comparison of distal tubular diameter between control group A and experimental groups B, C and D.

DISCUSSION

The usage of androgenic anabolic steroids (AAS's) and vitamin supplements has reached alarming ratio in the previous decades leading to increased global prevalence of chronic renal disease. Acute kidney injury (AKI) is an

important complication of anabolic steroid and vitamin supplement abuse[17]. The objective of present study was to observe the ameliorative effect of Punica granatum on steroid induced proximal and distal tubular dilatation.

Histomorphological observations in this study showed significant increase in tubular diameters of both proximaland distal convoluted tubules when experimental group B was compared with control group A (fig-1), and it was statistically nonsignificant when compared with experimental groups C and D (fig-2 & 3). The mean proximal convoluted tubules (PCT) diameters were somewhat greater than those for distal tubules.

Gremlin is an embryogenic gene that has been shown to play a key role in kidney pathophysiology, and its expression is generally low in the normal adult kidney. However, raised gremlin expression is found in many human renal diseases, including diabetic nephropathy, steroid induced interstitial nephritis and autoimmune glomerulonephritis. Numerous studies have suggested that gremlin causes renal damage by acting as a downstream mediator of TGF-β (transforming growth factor-β)[18]. In the present study, histological findings suggest tubular dilatation of both proximal and distal convoluted tubules, in experimental group B, and this is in agreement with the study which reported that human gremlin (GREM1)-overexpressing mice have an increased susceptibility to renal damage, supporting the contribution of gremlin in renal damage progression.

To observe the in vivo role of gremlin in nephropathy, Droguett and colleagues made seven viable transgenic mouse lines expressing human gremlin (GREM1) precisely in renal proximal tubular epithelial cells under controlled androgen-regulated environment. These lines revealed 100-200 times increased gremlin expression. Due to the renal damage caused by steroid administration, tubule-specific human gremlin (GREM1) transgenic mice developed histopathological lesions such as tubular dilatation with cellular edema, epithelial flattening, hyaline casts formation and interstitial inflammatory infiltrate mainlymonocytes /macrophages and lymphocytes after two weeks[19].

In another research it was found that the tubular epithelial cells were swollen, reduced in number and vascular congestion was seen with hemorrhagic areas around the tubules, due to the toxic effect of the main metabolite of nandrolone decanoate on these tubules. The cause of renal failure documented was cholestasis and hyperbilirubinemia induced by the use of anabolic steroids, ultimately leading to increased oxidative stress[20]. This view was also supported by the research which demonstrated that the cytotoxic activity of steroids is due to an indirect process interrupting redox cycle, enhancing production of ROS (Reactive oxygen species). ROS interfere with DNA oxidation, especially mitochondrial DNA[21].

Results of present study were comparable with the results of Aboonabi and colleagues who reported that ND exposure can also lead to DNA damage through increase in ROS generation. NOX enzymes are trans-membrane proteins, located on nuclear membrane near DNA, increasing the possibility of its damage. This was also in agreement with another study, who reported the DNA damage, caused by ND in liver, kidneys and heart in rats given a single toxic dose of 5 or 15mg/Kg body weight[22].

In current study, significant reduction in tubular diameters were noticed in both PCT and DCT in experimental groups C and D, as compared to experimental group B. Mean tubular diameters of both these groups were near to control group, and no statistical difference was seen when compared with control group A. This improvement was 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 anti-oxidants, and this was verified by Riezzo et al.

2014 who reported an increase in the 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[23].

CONCLUSION

Punica granatum in both forms, as Pomegranate juice and Pomegranate peel extract, has nearly equal ameliorative effects on steroid induced renal tubular damage.

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CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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