

# EFFECTS OF POMEGRANATE JUICE AND PEEL EXTRACT ON STEROID INDUCED INFLAMMATION IN MICE KIDNEYS

Humaira Ali,<sup>1</sup> Kishwar Naheed,<sup>2</sup> Aamna Khalil,<sup>3</sup> Khadija Qamar,<sup>4</sup> Zarmina Saga<sup>3</sup>

## ABSTRACT

**Background:** Athletes use anabolic androgenic steroids in excess of normal therapeutic levels to improve their stamina and performance, and this apparently describes the increase in their toxic side effects. **Objective:** To determine the ameliorative effects of Pomegranate Juice and Pomegranate Peel Extract on steroid induced inflammation in mice kidneys. **Methodology:** Study Design: Laboratory based Randomized control study. Duration and Place of Study: Department of Anatomy, Army Medical College, Rawalpindi, in cooperation with National Institute of Health (NIH), Islamabad, from 1<sup>st</sup> May to 30<sup>th</sup> June 2015. Forty healthy male and female BALB/c mice with average weight of 25-30 gms were randomly distributed into four groups, each group with five male and five female mice. Group A worked as a control group and groups B, C and D worked as experimental groups. Mice in experimental groups B, C and D were given a single injection of (Nandrolone decanoate) ND intramuscularly, at the dose of 1 mg/100 gm body weight, in the hind limb once weekly for 8 weeks. Mice in experimental group C was also given pomegranate juice (PJ) at the dosage of 3ml/kg body weight via oral gavage tube daily for 8 weeks and mice in experimental group D was given pomegranate peel extract (PPE) at the dosage of 200mg/kg body weight via oral gavage tube daily for 8 weeks. The animals were sacrificed after the experimental period was over and kidney tissues were processed for sectioning and staining. The results of all the groups were compiled and compared by using SPSS 21. Comparison of qualitative variables between the groups was done by applying Chi square test. **Results:** Inflammatory infiltrate were significantly higher in steroid administered experimental group B and statistical improvement was seen when comparison of experimental group B was done with PJ and PPE administered experimental groups C and D, respectively ( $p=0.001$  and  $0.000$ ). Comparison of experimental groups C and D showed no statistical significance ( $p=1.0$ ). **Conclusion:** It was concluded that both forms of Pomegranate has ameliorative effects on steroid induced inflammatory changes in mice kidneys.

**Key words:** Nandrolone Decanoate, Renal interstitium, Pomegranate juice, Pomegranate peel extract.

## INTRODUCTION

Androgenic anabolic steroids (AAS's) are synthetic derivatives of testosterone hormone which is the main androgenic and anabolic steroid, naturally, formed in the interstitial cells of Leydig, in testes.<sup>1</sup> The androgenic properties of these hormones are those instigating masculinization and the anabolic properties are those leading to nitrogen fixation and protein synthesis. Therapeutically AAS's are used to counter two different situations; firstly it is utilized as androgen replacement therapy, generally applied in androgen deficient persons either in hypothalamic, pituitary or genetic testicular disorders. Secondly, AAS's are usually given in pharmacological androgen therapy (PAT) in non-androgen-deficient conditions with debilitating illnesses in order to improve the quality of life by scoring adequate testosterone effects.<sup>2,3</sup>

Androgens are misused worldwide for enhancement of performance and presentation. In a survey among people attending gyms equipped for bodybuilding, the proportion of AAS's users was around 25–50%.<sup>3</sup> AAS's causes moral complications when they are abused to increase performance by sportsmen in rivalries and are

often misused and self-administered by bodybuilders to rapidly increase muscle mass, with subsequently increased long term hazards. Severe side effects such as cardiac failure, prostatic and metabolic disorders as well as insulin sensitivity are induced by uncontrolled usage of AAS's.<sup>3,4</sup>

Hepatic and excretory systems are mainly involved in breakdown, absorption and elimination of metabolites, so they are chiefly in danger of oxidative damage. Special effects of androgenic steroids on the liver, heart and kidneys are well established showing that oxidative stress disturb the redox balance in these organ systems leading to increased generation of Reactive oxygen species (ROS).<sup>5</sup> However, numerous studies indicate that usage of phytochemicals rich fruits and vegetables can reduce these side effects by keeping ROS within normal range.<sup>6</sup> The objective of this study was to explore inflammatory effects of androgenic steroids and protective effect of pomegranate juice and peel extract on kidney's histomorphology.

## METHODOLOGY

This laboratory based controlled trial was conducted in Department of Anatomy, Army Medical College Rawalpindi, in cooperation with National Institute of

1. Heavy Industries Taxilla educational Complex-Institute of Medical Sciences, National University of Medical Sciences (NMS) Rawalpindi, Pakistan

2. Liaquat National Medical College, Karachi, Pakistan.

3. Rawal Institute of Health Sciences, Islamabad, Pakistan.

4. Army Medical College Rawalpindi, National University of Medical Sciences (NMS) Rawalpindi, Pakistan

Correspondence: Prof. Dr. Khadija Qamar, Anatomy Department, Army Medical College, Rawalpindi, Pakistan.

E-mail: colkhadijaqamar@gmail.com

Mobile: +92 3335171653

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Health (NIH), Islamabad, from May 1<sup>st</sup> to 30<sup>th</sup> June 2015. Experimental protocols were approved from "CREAMLAB" which is Ethical Committee of Centre for Research in Experimental and Applied Medicine, Army Medical College, Rawalpindi. Forty healthy mice (BALB/c strain) including both male and female, having an average weight of 25-30 gms were divided equally into four groups, with five male five female in each. All groups were kept in NIH under controlled conditions of temperature and light, and were given standard laboratory mice pellets for eight weeks. Group A was a control group and groups B, C and D were experimental groups. Animals in experimental groups B, C and D were intramuscularly injected with Nandrolone decanoate (ND) at the dosage of 1 mg/100 gm of body weight<sup>7</sup>, in the hind limb once weekly for eight weeks. Animals in experimental group C was also given pomegranate juice (PJ) at the dosage of 3ml/kg of body weight<sup>8</sup> through oral gavage tube every day for eight weeks, and animals in experimental group D was given pomegranate peel extract (PPE) at the dosage of 200mg/kg of body weight<sup>9</sup> through oral gavage tube every day for eight weeks.

**Preparation of Pomegranate Juice (PJ):** The juicy and seasoned pomegranates, without any blotches or apparent blemishes were obtained from a local market. They were thoroughly washed and manually peeled. Juice was made by using an electrical blender. As seeds were not manually separated, juice was filtered with a filter paper. It was stored at -20°C after diluting with distilled water to volume of 1:3.<sup>10</sup>

**Preparation of Pomegranate Peel Extract (PPE):** Pomegranate peels were manually separated, sun dried and grounded to powder. The powder (25g) was extracted by mixing using a magnetic stirrer with 100ml methanol at 30°C for 1hr. Filtration of an extract was done to eliminate the peel particles. The extract was pooled and concentrated 40°C under vacuum.<sup>11</sup>

When experimental period was over, mice were sacrificed. Both of the kidneys of each mouse were washed in saline and observed for shape, size and color, weighed and then fixation was done by using 10% formalin solution. Tissues were dehydrated by passing through graded alcohol, processed in paraffin wax and then cut in 5 microns thick sections with help of microtome. Afterwards sections were stained with Eosin &

Hematoxylin and were permanently mounted. These slides were examined for inflammatory infiltrate by using following criteria. The data was entered and analyzed by using SPSS version 21. Chi square test was applied for intergroup comparison and P-value of <0.05 was considered significant.

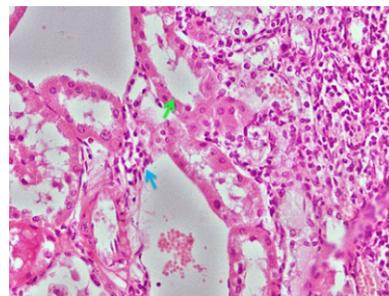
#### Interstitial scoring for inflammatory cells:

Slides were observed for inflammatory cells infiltrate in the interstitium of renal cortex and medulla. It was taken as percentage of the total area involved at 10X objective on a semi-quantitative score adopted as grade 0: No Change, grade 1: lesion involving less than 25% of interstitium, grade 2: lesion involving 25-50% of interstitium, grade 3: lesion involving >75% interstitium and grade 4: lesion involving entire area.<sup>12</sup>

## RESULTS

In all 10 (100%) specimens of Control group A, no inflammation was seen in any slide. In experimental group B, 10% of specimens showed grade 1 infiltrate, 30% has grade 2 inflammation, 30% has grade 3 inflammation and rest of the 30% showed grade 4 inflammation (Figure-1, Table-1). When findings of group B were compared with control group A (p=0.000), experimental groups C (0.000) and D (p=0.000, it was statistically significant.

**Figure I: Photomicrograph of histological section of kidney in animal no. 7 of steroid treated experimental group B showing marked dilatation of renal tubule (green arrow), inflammatory infiltrate mainly showing lymphocytes (blue arrow), H&E at 400X.**



In group C, inflammatory infiltrate was recorded at grade 0 in 60% of cases whereas 40% showed grade 1 inflammation. On comparison of group C with group A (p-value=0.087) no statistical significance was seen but the difference was statistically significant when compared with group B (P=0.001). Where as in experimental group D, infiltrate showed grade 1 in 40% of the cases and rest of the 60% had no infiltrate.

On intergroup comparison, it was found not to be statistically significant with control group A (p-value=0.087), but significantly was seen when compared with experimental group B (p-value=0.001). No statistical significance was seen between groups C and D (p-value=1.000)

**Table I: Comparison of Interstitial scoring for inflammatory cells between the control group A and experimental groups B, C and D**

Scoring of inflammatory infiltrates	Group A	Group B	Group C	Group D
No change	10 (100%)	0 (0.0%)	6 (60.0%)	4 (40.0%)
>25% area involved	0 (0.0%)	1 (10.0%)	4 (40.0%)	6 (60.0%)
26-50% area involved	0 (0.0%)	3 (30.0%)	0 (0.0%)	0 (0.0%)
<75% area involved	0 (0.0%)	3 (30.0%)	0 (0.0%)	0 (0.0%)
Entire area involved	0 (0.0%)	3 (30.0%)	0 (0.0%)	0 (0.0%)

Statistical difference for inflammatory infiltrate Group A and B = 0.000\*, Group A and C = 0.087, Group A and D = 0.087, Group B and C = 0.001 Group B and D = 0.000\* Group C and D = 1.000

## DISCUSSION

Drug-induced renal damage is gaining a lot of attention these days as it is quite common, and accounts for most cases of CRD (chronic renal disease) in teenagers. The objective of this study was to the ameliorative influence of Pomegranate on steroid induced inflammation in mice kidneys. In this study, slides were observed for inflammatory cells infiltrate in the renal interstitium and graded according to percentage of the total area involved at 10X objective on a semi quantitative score adopted by Schwarz et al.

All animals of control group A showed no inflammatory cells while varying degree of inflammation was observed around the degenerating tubules in experimental group B, C and D. Three out of ten animals in experimental group B exhibited grade 4 infiltrate, three of them lie in grade 3, three lie in grade 2 while only one of them showed grade 1 infiltrate. The results of current study are comparable with the results of earlier *in vitro* study which indicated that the administration of toxic doses of nandrolone decanoate augments interleukin-1beta (IL-1 $\beta$ ), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and inflammatory cytokines level in human peripheral blood lymphocyte cultures.<sup>13,14</sup>

Chronic inflammation is a main cause contributing to prolonged and debilitating diseases such as nephropathies and cancers.<sup>15</sup> In

addition, oxidative stress decreases levels of reduced glutathione and this is an important contributory factor leading to TNF- $\alpha$ -induced cell death.<sup>15</sup>

TNF- $\alpha$ , a cytotoxic variety of cytokine causes apoptosis such as in kidney tubular cells by interacting with its membrane-bound receptor, TNFR1. Cell death can be initiated either through a signaling pathway (i.e. binding of TNF- $\alpha$  to TNFR1) or via intrinsic pathway through mitochondrial membrane and depletion of reduced glutathione (GSH) enzyme level.<sup>16</sup> These mechanisms lead to endothelial membrane injury with compromised vasodilatation, causing tubular and glomerular impairment with gradual loss of kidney function.<sup>17</sup>

In this study significantly reduced inflammatory infiltrate was seen in pomegranate administered experimental groups C and D both of which showed no statistical significance when compared with control group A. Statistical difference for inflammatory infiltrate between group A and C was 0.087, between group A and D was 0.087 and group C and D was 1.000. These findings were in accordance with the study which demonstrated the useful effects of pomegranate peel extract by decreased activity of cytokines, in patients suffering from periodontitis. Patients suffering from this type of oral infection were given intra-gingival PPE impregnated chips ultimately leading to decreased cytokines (IL-1beta, TNF- $\alpha$  & IL-6) cytotoxicity few months after treatment.<sup>18</sup>

The renal protective effects of pomegranate in clinical trials were chiefly associated to its antioxidant and anti-inflammatory properties, which was also in support of this study.<sup>19</sup> This opinion was also reinforced by the findings of Ismail *et al.*, 2012 who suggested that PPE activates the immune system within the cells to initiate the anti-inflammatory reactions, due to high levels of phenolic compounds, which are strong antioxidants in pomegranate.<sup>20</sup>

## CONCLUSION

Pomegranate peel extract and juice, both forms exhibited almost equal ameliorative effects on steroid induced renal inflammation.

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## Conflict of interest

The authors have declared no conflict of interest.

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