

A COMPARISON OF PREVENTIVE EFFECTS OF PUNICA GRANATUM JUICE AND EXTRACT ON WEIGHT AND VOLUME OF STEROID INDUCED MICE KIDNEYS

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ABSTRACT

Objective: To observe the preventive effects of Punica granatum (pomegranate) juice and extract on weight and volume of steroid induced mice kidneys.

Study Design: Lab based randomized control trial.

Place and Duration of Study: Department of Anatomy, Army Medical College (AMC) Rawalpindi, in cooperation with National Institute of Health (NIH) Islamabad, from Apr to May 2015.

Material and Methods: Forty male and female healthy (BALB/c strain) mice with average weight of 25-30gms were divided into four groups, having ten animals each. Group A was a control group and groups B, C and D were experimental groups. Mice in experimental groups were injected Nandrolone decanoate (ND) at the dosage of 1 mg/100 gm of body weight, intramuscularly (I/M) in the hind limb once a week for 8 weeks. Mice in experimental group C were also given pomegranate juice (PJ) at the dosage of 3ml/kg of body weight through oral gavage tube for 8 weeks daily and mice in experimental group D were given pomegranate peel extract (PPE) at the dosage of 200mg/kg of body weight by oral gavage tube for 8 weeks daily. Intergroup comparison of results of experimental groups B, C and D with each other and with control group A was done.

Results: The weight and volume of kidneys were significantly different in ND treated experimental group B and difference in both these parameters was seen in pomegranate administered experimental groups C and D.

Conclusion: Pomegranate in both forms, as pomegranate juice and peels extract, equally prevent the weight and volume of steroid induced kidneys.

Keywords: Pomegranate, Steroids, Volume, Weight.

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INTRODUCTION

Androgenic anabolic steroids (AASs) are synthetic analogues of hormone testosterone, produced by Leydig cell of testis and suggested for the management of many diseases such as renal insufficiency, osteoporosis, male hypogonadism, delayed puberty, endometriosis, growth retardation and hereditary angioedema. Amongst many AASs, nandrolone decanoate (ND), generally known as Deca-Durabolin (DD), is the most common steroid available in market. These substances interact with androgenic receptors (AR), causing androgenic and anabolic effects^{1,2}.

Yet AASs have been violently and

sadistically used both by men and women, athletes and non-athletes in order to improve stamina, agility and deftness. The therapeutic benefits of such steroids come with unwanted side effects also such as elevated cholesterol and triglyceride levels, nephrotoxicity, hepatotoxicity and various hematological disorders³. The obnoxious usage of androgenic steroids leads to excessive facial hair growth, enlarged clitoris, deepening of voice and menstrual irregularities. Ovarian cycle and spermatogenesis is also adversely affected by such substances⁴.

Hepatic and renal systems are mainly related to drug metabolism, so they are predominantly susceptible to oxidative injury. Hepatic and renal destruction because of the oxidative stress involves excessive production of cytokines and growth factors ultimately inducing an inflammatory response⁵.

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Pomegranate was originated in the region of modern day Iran later on introduced in Spain. It is the symbol and icon of the ancient city of Granada in Spain from which the city acquires its name. Pomegranate is used by mankind from the time of emergence of civilization. It is a frugally significant plant and has been important for its nutritive, curative, ornamental and industrial value since ancient times. It has been placed as an imperative medication even in earliest literature by Hippocrates, Soranus, Dioscorides and Pliny^{6,7}.

The fruit is precisely a berry, filled with crispy seeds, each of which is sheathed in a salacious, juicy and slightly acidic pulp that is itself enclosed in an inner white membranous skin and outer tough reddish peel. Edible part of pomegranate fruit is rich in vitamin C and phytochemical compounds, which act as strong

Medical College (AMC), in cooperation with National Institute of Health (NIH) Islamabad and Pathology Department, Army Medical College, Rawalpindi. All experimental protocols were approved by Ethical Committee of Centre for Research in Experimental and Applied Medicine (CREAM), AMC, Rawalpindi. Forty male and female (BALB/c strain) healthy mice with average weight of 25-30 grams were randomly divided into four groups equally, with ten animals each. They were provided with standard laboratory diet pellets and water ad libitum for eight weeks. Group A was a control group and groups B, C and D were experimental groups. Animals in three experimental groups B, C and D were injected ND at the dosage of 1 mg/100 gm of body weight, intramuscularly in the hind limb once weekly for eight weeks¹⁰. Animals in experimental group C were also given pome-

Table: Statistical difference for weight of kidneys, volume of kidneys and RTBWI (relative tissue body weight index) on intergroup comparison of control group A an experimental groups B, C and D.

Parameters	Group A vs. B	Group A vs. C	Group A vs. D	Group B vs. C	Group B vs. D	Group C vs. D
Weight of kidneys (gm)	$p<0.001$	0.201	0.051	$p<0.001$	$p<0.001$	0.907
Volume of kidneys (mm ³)	$p<0.001$	0.102	0.050	$p<0.001$	0.001	0.988
RTBWI	$p<0.001$	0.763	0.328	$p<0.001$	$p<0.001$	0.879

p -value <0.05 is statistically significant.

antioxidants exerting potential therapeutic benefits⁸.

Oxidative stress is responsible for many of the chronic diseases resulting from an imbalance between formation and deactivation of pro-oxidants. High level of antioxidants in pomegranate enhanced the quenching of free radicals inside the cells, so have the ability to protect kidney and liver tissue from oxidative stress damage⁹. The rationale of current study was to observe the effects of steroids on weight and volume of mice kidneys and protection of these effects by two forms of pomegranate.

MATERIAL AND METHODS

This lab based randomized control trial was conducted at the department of Anatomy, Army

granate juice (PJ)¹¹, at the dosage of 3ml/kg of body weight via oral gavage tube for eight weeks daily¹², and animals in experimental group D were given pomegranate peel extract (PPE)¹³, at the dosage of 200mg/kg of body weight via oral gavage tube for eight weeks daily¹⁴.

At the end of experimental period, the animals were sacrificed and dissected. Flanks were opened by giving lateral incision and flaps of abdominal wall were stripped off by fixing them through thumb pins to expose the underneath abdominal organs. Suprarenal glands were identified, separated and kidneys were removed from the paravertebral gutter on dorsal body wall.

Both kidneys of each specimen were weighed and observed for size, shape and color. Removed kidneys were weighed in grams on digital weighing balance and relative tissue body weight index (RTBWI) was calculated using the following formula¹⁵.

$$\text{RTBWI} = \frac{\text{weight of organ in grams} \times 100}{\text{weight of body in grams}}$$

Volume of kidneys were calculated by using water displacement method¹⁶ in mm³. Kidneys were fixed in 10% formalin. Tissues were processed and then cut in 5 microns thick sections. Afterwards sections were stained with Eosin & Hematoxylin and were permanently mounted. IBM SPSS version 21 was used for data

Kidney specimens were observed in all animals. In control group A, kidneys were bean shaped and soft in consistency. In experimental group B, kidneys were grossly enlarged and lighter in color as compared to control group A, while in groups C and D kidneys showed no such change.

In control group A, mean \pm SD of right and left kidneys weight was $0.257 \pm 0.040\text{gm}$, Whereas in experimental groups B, C and D it was $0.767 \pm 0.103\text{gm}$, $0.332 \pm 0.095\text{gm}$ and $0.357 \pm 0.079\text{gm}$ respectively. On intergroup comparison, statistically significant difference was present between control group A and experimental group B ($p < 0.001$). Comparison of experimental group B with experimental groups C and D

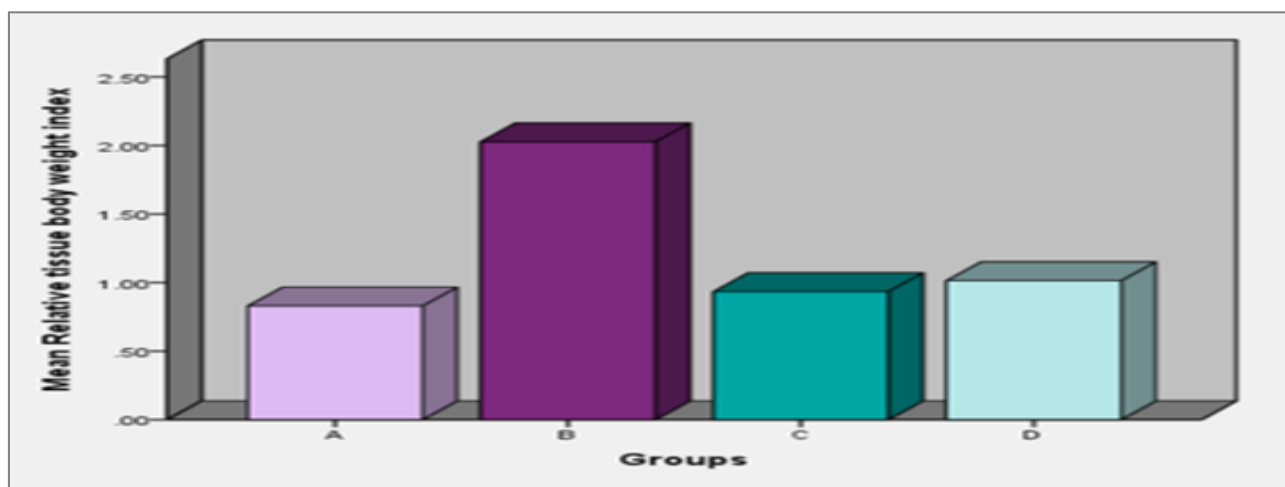


Figure-1: Bar chart showing comparison of mean values of relative tissue body weight index (RTBWI) between the control group A and experimental groups B, C and D.

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 \pm SD). A p -value < 0.05 was considered to be indicative of statistical significance.

RESULTS

This study was conducted to evaluate the protective role of pomegranate juice and peel extract on steroids induced effects on weight and volume of kidneys in mice. For this purpose, forty BALB/c, healthy male and female mice were equally divided into four groups.

showed statistical significance ($p < 0.001$). When experimental group C was compared with experimental group D the change in weight was found to be statistically insignificant (p -value = 0.907) (table).

Mean \pm SD of RTBWI of both kidneys in control group A was 0.828 ± 0.136 , whereas in experimental groups B, C and D it was 2.025 ± 0.287 , 0.933 ± 0.262 and 1.013 ± 0.244 respectively (fig-1). Statistically significant difference was found on intergroup comparison between control group A and experimental group B ($p < 0.001$) and no statistical significance was present when control group A was compared

with experimental groups C and D (p -values=0.763 and 0.328 respectively). Statistical difference ($p<0.001$) was found when RTBWI of experimental group B was compared with experimental groups C and D. No statistical significance was seen when experimental groups C and D were compared with each other (p -value=0.879) (table).

In control group A, mean \pm SD of right and left kidneys volume was $0.380 \pm 0.103\text{mm}^3$, Whereas in experimental groups B, C and D it was $1.418 \pm 0.401\text{mm}^3$, $0.635 \pm 0.200\text{mm}^3$ and $0.670 \pm 0.141\text{mm}^3$ respectively. On intergroup comparison, statistically significant difference

the muscular mass. Many side effects have been associated with AASs misuse, including urogenital tract disorders especially chronic renal diseases¹⁷. The objective of this study was to observe the protective effects of two forms of pomegranate on weight and volume of steroid induced mice kidneys.

Both weight and volume of kidneys in ND administered experimental group B showed significant increase in current study. This was in agreement with the study which reported that AAS abuse leads to development of tumor in kidneys, autonomic dysfunction and mesangial matrix accumulation leading to hypertrophy,

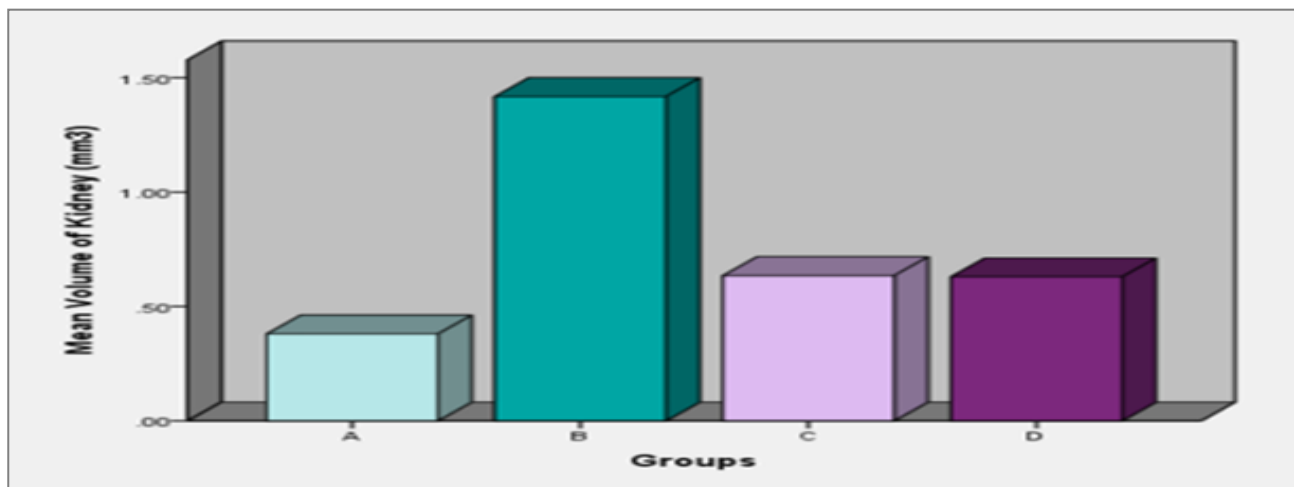


Figure-2: Bar chart showing comparison of mean values of kidney volume between the control group A and experimental groups B, C and D.

was present between control group A and experimental group B ($p<0.001$). The change in kidney volume was found to be highly significant when experimental group B was compared with experimental groups C and D ($p<0.001$). When experimental group C was compared with control group A and experimental group D the change was found to be statistically insignificant (p -values=0.102 and 0.988 respectively) (table, fig-2).

DISCUSSION

AAS's are commonly used by both proficient and amateur athletes to enhance athletic performance, improve their looks and increase

there was an increase in the weight of the kidneys as the dose of the drug was increased¹⁸.

These results also coincide with the results of another study which found that there was increased weight and size of the kidneys in steroid (ND) treated group of mice in five weeks study. Hoseini and colleagues reported that this increase was because of increased collagen deposition in intertubular connective tissue, interstitial edema and tubular dilatation¹⁹. Similar histomorphological changes were observed in kidneys of experimental group B in this study at the end of 8th week.

Pomegranate administered experimental groups C and D showed significant difference in weight and volume of hypertrophied kidneys. Kidneys in both these groups exhibited decrease in length and width when compared with experimental group B and showed no statistical significance when compared with control group A. This was in agreement with the effects of study conducted which demonstrated that pomegranate peel extract consumption (0.5ml/day for 12 weeks) reduced the incidence and severity of collagen induced interstitial nephritis in mice²⁰. Oxidative stress plays a vital role in developing chronic complications in interstitial nephritis and it is associated with increased lipid peroxidation, which can induce DNA fragmentation and stimulate apoptosis in renal tubules leading to end stage renal disease. Therefore, oxidative stress can be the common pathogenic factor of nephrotoxicity also²¹.

The mechanism of liver and kidney destruction because of the oxidative stress involves the secretion of cytokines, mainly tumor necrosis factor TNF- α , interleukin IL-1, and IFN-c which might cause an abnormal production of growth factors. Oxidative stress is initiated by free radicals, which seek stability through electron pairing with biological macromolecules such as proteins, lipids and DNA in healthy human cells and cause protein and DNA damage along with lipid per oxidation. Subsequently, they facilitate the synthesis of extracellular matrix proteins and the depositions in the glomerular level that finally lead to mesangial expansion, glomerular shrinkage, and glomerular basement thickening²².

High levels of phytochemicals (antioxidants) in pomegranate could enhance the reduction of free radicals inside the cells, to protect the kidney tissue from oxidative stress damage. Pomegranate juice and peels extract induces apoptosis in numerous cancer cell lines, thus inhibiting cellular proliferation. Furthermore, PJ considerably reduced the concentration of pro-inflammatory chemokines like IL-6, IL-12p40, IL-1 β and cytokines, thus reducing inflam-

mation and its effects on cancerous growth. Pomegranates contain bioactive chemicals with prospects for the treatment and prevention of abnormal growths and depositions²³.

CONCLUSION

Pomegranate in both forms, as juice and peels extract, has equal preventive effects on steroid induced changes on weight and volume of kidneys.

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CONFLICT OF INTEREST

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

REFERENCES

1. Tugyan K, Ozbal S, Cilaker S, Kiray M, Peketin C, Ergur BU et al. Neuroprotective effect of erythropoietin on nandrolone decanoate-induced brain injury in rats. *Neuroscience letters* 2013; 533: 28-33.
2. Simão VA, Berloff Belardin L, Leite A, Adan G, Almeida Chuffa LG, Camargo IC. Effects of different doses of nandrolone decanoate on estrous cycle and ovarian tissue of rats after treatment and recovery periods. *Int J Experimental Pathol* 2015; 96(5): 338-49.
3. Vargas RA, Oliveira LP, Frankenfeld S, Souza DB, Costa WS, Favorito LA, et al. The prostate after administration of anabolic androgenic steroids: a morphometrical study in rats. *International brazilian journal* 2013; 39(5): 675-82.
4. Mukudai S, Matsuda KI, Nishio T, Sugiyama Y, Bando H, Hirota R, et al. Differential responses to steroid hormones in fibroblasts from the vocal fold, trachea, and esophagus. *Endocrinology* 2014; 156(3): 1000-9.
5. Motamedi F, Nematbakhsh M, Monajemi R, Pezeshki Z, Talebi A, Zolfaghari B, et al. *J Nephropathol* 2014; 3(4): 78-83.
6. Kaneria MJ, Chanda SV. The effect of sequential fractionation technique on the various efficacies of pomegranate (*Punica granatum L.*). *Food Analytical Methods* 2013; 6(1): 164-75.
7. Viuda Martos MA, Perez Álvarez JA, Sendra E, Fernandez Lopez JU. In vitro antioxidant properties of pomegranate (*Punica granatum*) peel powder extract obtained as coproduct in the juice extraction process. *Journal of Food Processing and Preservation* 2013; 37(5): 772-6.
8. Zaouay F, Mena P, Garcia-Viguera C, Mars M. Antioxidant activity and physico chemical properties of Tunisian grown pomegranate (*Punica granatum L.*) cultivars. *Industrial Crops and Products* 2012; 40: 81-9.
9. Dahlawi, H, Jordan Mahy N, Clench, M.R, and Le Maitre, C.L. Bioactive actions of pomegranate fruit extracts on leukemia cell lines in vitro hold promise for new therapeutic agents for leukemia. *Nutrition and cancer* 2012; 64(1): 100-10.

10. Frankenfeld SP, Oliveira LP, Ortenzi VH, Rego-Monteiro IC, Chaves EA, Ferreira AC, et al. The anabolic androgenic steroid nandrolone decanoate disrupts redox homeostasis in liver, heart and kidney of male Wistar rats 2014b; PloS one, 9(9): 102699.
 11. Faria A, Monteiro R, Mateus N, Azevedo I, Calhau, C. Effect of pomegranate (*Punica granatum*) juice intake on hepatic oxidative stress. *Eur J Nutr* 2007; 46(5): 271-8.
 12. Moneim AE, Dkhil MA, Al-Quraishy S. Studies on the effect of pomegranate (*Punica granatum*) juice and peel on liver and kidney in adult male rats. *J Med Plants Res* 2011; 5(20): 5083-8.
 13. El-Habibi, E.M. Renoprotective effects of *Punica granatum* (pomegranate) against adenine-induced chronic renal failure in male rats. *Life Sci J* 2013; 10(4): 2059-69.
 14. Parmar HS, Kar A. Medicinal values of fruit peels from *Citrus sinensis*, *Punica granatum*, and *Musa paradisiaca* with respect to alterations in tissue lipid peroxidation and serum concentration of glucose, insulin, and thyroid hormones. *J Med Food* 2008; 11(2): 376-81.
 15. Waseem N, Butt S.A, Hamid, S. Amelioration of lead induced changes in ovary of mice, by garlic extract. *J Pak Med Assoc* 2014; 64: 798-801.
 16. Okur A, Serin HI, Zengin K, Erkoç MF, Tanık S, Yıldırım U, et al. Relationship between kidney volume and body indexes in the Turkish population determined using ultrasonography. *International Braz J Urol* 2014; 40(6): 816-22.
 17. Belardin LB, Simao VA, Leite GA, Chuffa LG, Camargo IC. Dose Dependent Effects and Reversibility of the Injuries Caused by Nandrolone Decanoate in Uterine Tissue and Fertility of Rats. *Birth Defects Research Part B: Developmental and Reproductive Toxicology* 2014; 101(2): 168-77.
 18. D'Errico S, Di Battista B, Di Paolo M, Fiore C, Pomara, C. Renal heat shock proteins over-expression due to anabolic androgenic steroids abuse. *Mini Reviews in Medicinal Chemistry* 2011; 11(5): 446-50.
 19. Hoseini, L, Roozbeh, J, Sagheb M, Karbalay Doust S, Noorafshan A. Nandrolone decanoate increases the volume but not the length of the proximal and distal convoluted tubules of the mouse kidney. *Micron* 2009; 40(2): 226-30.
 20. Balbir G, Alexandra, Bianca, Yolanda BM, Doron M, Michael A. Consumption of pomegranate decreases serum oxidative stress and reduces disease activity in patients with active rheumatoid arthritis: a pilot study. *Isr Med Assoc J* 2011; 13 (80): 474-79.
 21. Pendergraft WF, Herlitz LC, Thornley Brown D, Rosner M, Niles JL. Nephrotoxic effects of common and emerging drugs of abuse. *Clin J Am Soc Nephrol* 2014; CJN-00360114.
 22. Aboonabi A, Rahmat A, Othman F. Effect of Pomegranate on Histopathology of Liver and Kidney on Generated Oxidative Stress Diabetic Induced Rats. *Journal of Cytology & Histology* 2015; 78(9): 3689-99.
 23. Rahimi HR, Arastoo M, Ostad SN. A comprehensive review of *Punica Granatum* (pomegranate) properties in toxicological, pharmacological, cellular and molecular biology researches. *Iran J Pharmaceut Res* 2012; 11(2): 385-400.
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