ORIGINAL ARTICLE

Protective Effect of Commercial Green Tea on Ibuprofen-Induced Glomerular Atrophy in Kidney of Adult Rat

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ABSTRACT

Objective: To determine the effect of commercial green tea on ibuprofen-induced glomerular atrophy in kidney of adult rat by using transvertical glomerular diameter as the determining parameter.

Study Design: Laboratory-based experimental study.

Place and Duration of Study: Department of Anatomy, Army Medical College Rawalpindi in collaboration with National Institute of Health (NIH) Islamabad from December 2016 to January 2017.

Methodology: Thirty Sprague-dawley rats, males and females, were selected and divided into three groups. Each group comprised of 10 animals. Group A was used as control. Group B was given ibuprofen 120 mg/kg bodyweight/day. Group C received extract of Lipton green tea 1 ml/100 mg bodyweight/day in addition to ibuprofen 120 mg/kg. The dose was administered once daily for a period of 9 weeks. Animals were sacrificed 24 hours after administration of last dose. Kidney tissue was processed and stained with H&E. Routine histological study was performed. Change in transvertical glomerular diameter was taken as the defining parameter for determining glomerular atrophy. SPSS version 22 was the tool used for statistical analysis. P-value <0.05 was considered significant.

Results: Transvertical glomerular diameter (TVGD) was significantly decreased in both experimental groups B (76.15 $\pm$1.85 µm) and C (89.59 $\pm$1.87 µm) in comparison with control group A (94.27 $\pm$1.82 µm). However, the difference in TVGD between both experimental groups was also significant, asserting the ameliorative effect of green tea against glomerular damage.

Conclusion: Ibuprofen caused glomerular atrophy but administration of green tea along with ibuprofen protected against significant reduction in glomerular diameter, thus preventing atrophy.

Key Words: *Ibuprofen. Glomerular atrophy. Transvertical glomerular diameter. Green tea.*

INTRODUCTION

Histologically, the structural and functional unit of kidney is a uriniferous tubule, which is comprised of parenchyma (glandular or secretory unit) as nephron and its excretory duct as collecting tubule. The Bowman's capsule and glomerulus together form the renal or malphigian corpuscle. The secretory tubule is composed of proximal convoluted tubule, loop of Henle and distal convoluted tubule. The latter joins the collecting tubule.[1]

The kidneys play a vital role in the maintenance of homeostasis by maintaining the body's acid-base balance and electrolytes. They regulate blood pressure by changes in sodium balance and renin secretion. Erythropoietin and 1, 25-dihydroxycholecalciferol are synthesised in the kidney.[2] Kidney is second only to liver in drug metabolism. Most of the nephrotoxic effects of various drugs are manifested through their metabolites.

Non-steroidal anti-inflammatory drugs (NSAIDs), widely used for the treatment of various acute and chronic inflammatory conditions, are not only frequently prescribed all over the world but are also sold over-the-counter. Ibuprofen, classified as a non-narcotic analgesic, is the prototype drug of this group.[3] It is inexpensive and easily available. Ibuprofen is the drug of choice for symptomatic relief, an analgesic and antipyretic at low dose (800-1200 mg/day); as well as for the treatment of inflammation ranging from toothache and dysmenorrhea to rheumatoid arthritis and ankylosing spondylitis at a high dose of 1800-2400 mg/day.[4] In kidney, ibuprofen is well-documented to cause renal papillary necrosis, hyperkalemia, interstitial nephritis, glomerulonephritis, and acute or chronic renal failure.[5] Nephrotoxicity of ibuprofen assumes a major importance considering the fact that ibuprofen has very short plasma half-life (t1/2, 1-3 h), necessitating the need of frequent administration of the drug.[4]

Green tea, made from unfermented, mature leaves of *Camellia sinensis*, is an immensely popular, widely consumed drink worldwide and has long been used for medicinal purposes. It has anti-oxidant, anti-microbial, anti-aging, anti-inflammatory, anti-cancer and anti-mutagenic activities.[6] Green tea has been proven to have highly beneficial effects on a number of diseases including obesity, metabolic syndrome, cardiovascular diseases, cerebrovascular disorders, atherosclerosis, and neurodegenerative disorders such as Parkinson's and Alzheimer's diseases.[7] Green tea has been found to have protective effect against contrast media-induced

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renal damage in male Wistar rats.[8] The chief components of green tea are polyphenols, of which catechins are the most abundant. Green tea polyphenols markedly decrease cyclosporine A-induced kidney damage and improve kidney function. The protective effect of green tea on various organs, especially on kidneys, is attributed to the anti-oxidant activity of catechins (especially epigallocatechin-3-gallate), gallic acid and phenolic acids which are the major component of green tea. The effectiveness of antioxidant activity of green tea catechins surpasses that of Vitamins C and E. These catechins are not only scavengers of reactive oxygen species but also inhibit the generation of the later from the metabolism of various drugs including ibuprofen.[9]

This study was carried out to assess the protective effect of commercial green tea against glomerular atrophy, induced by ibuprofen, using transvertical glomerular diameter (TVGD) as the determining parameter.

METHODOLOGY

This study was approved by Ethical Review Committee of Army Medical Colleges Rawalpindi and was carried out in the Department of Anatomy, Army Medical College, Rawalpindi in collaboration with National Institute of Health (NIH) Islamabad. Thirty male and female Sprague-dawley rats, 9-11 weeks of age, with weights ranging from 200-330 gm. were housed in separate cages in a spacious room with good ventilation. Twelve hours light and 12 hours dark cycles were maintained under a temperature range of 20-26°C through a central temperature regulating system. Rats were fed NIH standardised laboratory diet for two months. Water was provided ad libitum. The doses of both ibuprofen and green tea were administered using oral gavage once daily for a period of 9 weeks.

Ibuprofen syrup (Abbott Laboratories Pvt. Ltd.), containing ibuprofen 100 mg/ 5 mL was used as drug source. Commercial green tea of Lipton brand was used. The green tea extract was prepared by adding 1.25 g of green tea leaves to 25 mL of boiling water. The infusion was cooled to room temperature and then filtered. The same tea leaves were then extracted a second time with another 25 mL of boiling water and filtered; the two filtrates were then combined to obtain an aqueous 2.5% green tea extract (2.5 g of tea leaves/100 mL water).[10]

The catechin content of green tea (Lipton^{TM}) determined using high performance liquid chromatography was found to be 6.19 mg/g, which is second highest among the catechin levels of five green tea brands available in Pakistan.[11]

Rats were divided into three groups (10 animals per group). Rats in group A served as untreated controls. Rats in group B were given ibuprofen 120 mg/kg bodyweight/day, while rats in group C received green tea 1 ml/100 mg bodyweight/day,[10] in addition to ibuprofen 120 mg/kg . The catechin content of green tea dose of 1ml/100 mg bodyweight was calculated to be 2.58 mg. The doses were administered through oral gavage. At the end of 9 weeks, 24 hours after administration of last dose, all animals were sacrificed. Kidneys were removed and placed in 10% formalin in plastic containers. The tissues were then processed and cut into 5 micron thick sections using a rotary microtome, then stained with eosin & haematoxylin for routine histological examination. Photomicrographs were taken at 10X and 40X objectives. Each image was subsequently opened in Image J v.1.48.[12] A scale was set by calibrating Image J with the help of the photomicrograph of a stage micrometer. Five random, approximately circular glomeruli were selected in 5 consecutive high power fields (HPF)[13] at 10X. For each glomerulus, two measurements were taken. The first measurement was taken at the maximum TVGD and the second was taken perpendicular to the first one. Hence, the TVGD was measured by taking the mean of the two diameter values as average diameter = (maximum transverse diameter +maximum perpendicular diameter) ÷ 2.

Statistical Package for Social Sciences (SPSS) version 22 was used for data analysis. TVGD was expressed as mean average diameter + standard deviation. One-way analysis of variance (ANOVA) was followed by post Hoc Tukey test to determine significant difference. P-value <0.05 was considered significant. Confidence intervals were kept at 95%.

RESULTS

Histologically, the control group A showed normal rounded glomeruli and Bowman's space (Figure 1A). Experimental group B, however, showed shrunken and

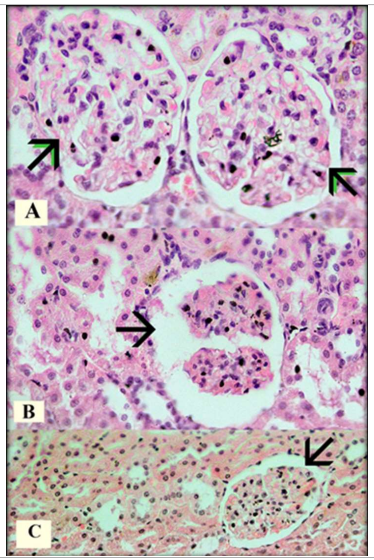

Figure 1: Photomicrograph showing glomerular histology. **(A)** From control group A showing normal glomerular structure. **(B)** From experimental group B showing shrunken distorted and fragmented glomerulus and increased Bowman's space. **(C)** From experimental group C showing slightly decreased glomerular size but no fragmentation or distortion is seen.

Table I: Comparison of mean values of tansverticular glomerular diameter between control group A and experimental groups B and C and intergroup comparison of P-values.

Parameter	Group A Mean ± SD (n=10)	Group B Mean ± SD (n=10)	Group C Mean ± SD (n=10)	Group A vs. B (P-value)	Group A vs. C (P-value)	Group B vs. C (P-value)
Transvertical glomerular diameter (μm)	94.27 ±1.82	76.15±1.85	89.59± 1.69	<0.001	<0.001	<0.001

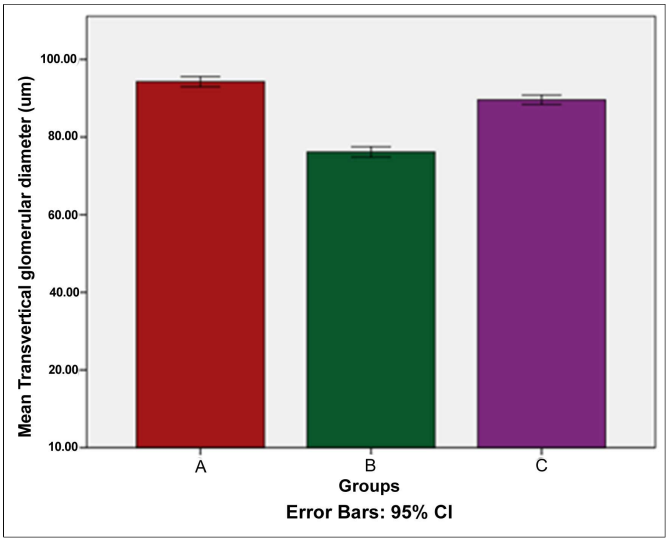

Figure 2: Bar charts showing the comparison of mean values of transvertical glomerular diameter of control group A and experimental groups B and C.

disfigured glomeruli and increased Bowman's space (Figure 1B). Experimental group C showed slight decrease in glomerular size but no shrinkage or disruption was observed (Figure 1C). Mean TVGD in control group A was 94.27 ±1.82 μm which was statistically significant (p-value <0.001) on comparison with both experimental groups B and C (Table I). Mean TVGD + SD in experimental group B was 76.15 ±1.85 μm and in experimental group C was 89.59 ±1.69 μm, which was also found statistically significant (<0.001) when compared between the two experimental groups (Table I).

DISCUSSION

Nephrotoxicity is the most common adverse event reported during the development of a drug. Many of the commonly used drugs, such as gentamicin among others,[14] have damaging effects on kidneys. The proven and well-documented nephrotoxicity of ibuprofen is a rapidly growing concern in modern medicine as the drug is among the most commonly used analgesics and anti-inflammatory agents worldwide. Renal adverse effects of NSAIDs are mainly mediated via cyclooxygenase-1 inhibition and ibuprofen inhibits both cyclooxygenase-1 and 2. Decrease in the synthesis of prostaglandin due to inhibition of the above mentioned enzyme can lead to renal ischemia ultimately resulting in acute renal failure.[15] However, the main trigger for damage to renal architecture is believed to be the oxidative stress caused by the activity of reactive oxygen species produced as a result of ibuprofen metabolism.[16]

The size of glomerulus is one of the imperative parameters to indicate the renal condition.[13] In the present study, oral administration of ibuprofen in experimental group B resulted in significant glomerular atrophy. This finding is supported by a 2012 study,[17] which determined that ibuprofen induced significant changes in glomerular structure. Another study established that ibuprofen significantly decreases glomerular filtration rate.[18] Decreased glomerular filtration rate (GFR) is believed to be the major cause behind NSAIDs-induced acute renal failure. This can be attributed to the generation of reactive oxygen species such as hydrogen peroxide, hydroxyl radical, singlet oxygen and superoxide, from ibuprofen metabolism. Also ibuprofen inhibits cyclooxygenase enzymes necessary for prostaglandin synthesis, which play a vital role in maintenance of glomerular filtration rate. Reactive oxygen species generated by ibuprofen also causes DNA strand scission.[19] This results in loss of genetic information stored in this biopolymer and forms the basis of such pathologies as arteriosclerosis and cataract formation.[20] Irrespective of origin, these reactive oxygen species cause degeneration and fragmentation of glomerulus, degrade the glomerular basement membrane and alter the glomerular cell functions, hence reducing the glomerular filtration rate.[21] Degradation of glomerular basement membrane by ibuprofen results in effacement of podocytes, disrupting the glomerular filtration barrier. This results in proteinuria.[22] The fragmentation of glomeruli results in the release of inflammatory mediators which enhance the synthesis of extracellular matrix proteins and their deposition in the glomerulus. This causes shrinkage of glomeruli and increase in capsular space. Thus, ibuprofen has proved to be more detrimental to glomeruli than NSAIDs, which are cyclooxygenase-2 inhibitors e.g. celecoxib.

In experimental group C, administration of Lipton clear green tea in addition to ibuprofen resulted in prevention of significant degeneration and atrophy, preserving the glomerular architecture. This is in accordance with a study conducted by Peng and colleagues,[23] which determined that a green tea component, epigallocatechin-3-gallate (EGCG), is able to reduce albuminuria and other markers of glomerular damage observed in glomerulonephritis in mice. In the same study, EGCG was found to attenuate the development of anti-glomerular basement membrane antibody-induced glomerulonephritis (anti-GBM-GN), improving glomerular filtration rate and ameliorating the biochemical and histologic abnormalities. Reversal of

gentamicin-induced glomerular atrophy in rats was observed after administration of green tea.[24] In a study conducted by Soon-Jae Rhee,[25] green tea catechins were proved to maintain the glomerular filtration rate on a normal level by significantly decreasing the urinary output of β2-microglobulin.Thus green tea polyphenols not only modulate antioxidant enzymes but are also scavengers of free radicals, thereby exhibiting nephro-protective effects against oxidative renal damage.

Thus, we can establish that in this study, antioxidant activity of commercial green tea by virtue of its components, the most effective being catechins and among them epigallocatechin-3-gallate (EGCG), is responsible for prevention of not only glomerular atrophy but also of general disruption of glomerular architecture and basement membrane disruption caused by ibuprofen in experimental animals.

CONCLUSION

This study confirms that ibuprofen causes glomerular atrophy, showing a reduction in TVGD but concomitant administration of green tea prevents the atrophy and maintains glomerular size.

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