AMELIORATIVE EFFECT OF COMMERCIAL GREEN TEA ON IBUPROFEN INDUCED HISTOMORPHOLOGICAL ALTERATION IN LUMINAL DIAMETER OF PROXIMAL RENAL TUBULE

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ABSTRACT

***Objective*:** To study the effects of commercial green tea on ibuprofen induced effects on luminal diameter of proximal renal tubule in adult rat.

***Study Design*:** Laboratory based experimental study.

***Place and Duration of Study*:** Army Medical College Rawalpindi and National Institute of Health (NIH) Islamabad, from Mar 2017 to May 2017.

***Methodology*:** Thirty healthy adult Sprague Dawley rats were divided into three groups, each containing 10 animals. Schedule of intervention was once a day for a period of 8 weeks. Group Awascontrol. Group B received only ibuprofen 120 mg/kg bodyweight once daily. Group C was given green tea 1ml/100 mg bodyweight in addition to ibuprofen 120mg/kg bodyweight, once a day. At the end of 8 weeks, all animals were sacrificed and kidneys were dissected out. Renal tissue was then prepared for histological study. Luminal diameters of proximal renal tubules were measured.

***Results*:** Luminal diameter of proximal renal tubule was found significantly reduced (4.79 ± 0.92µm) in experimental group B (ibuprofen only), as compared to those in control group A (13.073 ± 1.02µm) and in experimental group C (12.67 ± 1.34µm). However, the reduction in proximal luminal diameter in group C was not significant as compared to group A.

***Conclusion*:** Ibuprofen caused reduction in proximal luminal diameter in kidneys of adult rats. However, administration of green tea along with ibuprofen protects against the adverse effects of the drug.

Keywords: Catechins, Ibuprofen, Proximal tubule.

INTRODUCTION

The kidney is actively involved in the metabolism of various drugs and is therefore the organ most at risk of suffering from adverse effects of different drugs[1]. The nephrotoxic effects are caused by metabolic products formed during the biotransformation of the drugs[2]. Damage to renal architecture caused by drugs is a leading cause of patient mortality in the modern medicine[1].

Ibuprofen is a non-narcotic pain relief drug and is the prototype non-selective NSAID (NonSteroidal Anti-Inflammatory Drug)[3]. It is the gold standard against which other analgesics are evaluated[4]. The drug is very commonly prescribed as well as sold over-the-counter[5] for treating a number of minor and major inflammatory conditions as well as providing symptomatic relief from fever and pain[6]. However, ibuprofen has well-documented adverse effects on kidneys, liver and GIT[7]. The nephrotoxic effects of the drug include nephritic syndrome, interstitial nephritis, glomerular nephritis and ultimately, acute or chronic kidney failure[8]. These effects occur when reactive metabolites of the drug cause oxidative damage to renal tissue[9].

Green tea is an immensely popular drink all over the world. It is made from unfermented, mature leaves of the plant Camellia sinensis[10]. The main constituents of green tea are the polyphenols, especially catechins, which have strong antioxidant properties. Thus, green tea has been documented to have protective effects against various diseases caused by reactive oxygen

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species (ROS). Because of its antioxidant properties of green teaprovides ample nephro-protection against peroxidative damage caused by ibuprofen.

In light of the aforementioned information, this study was conducted to evaluate the ameliorative effects of green tea on ibuprofen-induced histomorphological changes in rat kidneys.

METHODOLOGY

This study was approved by Ethical Committee of Army Medical College Rawalpindi (form attached) and was carried out in the department of Anatomy, Army Medical College, Rawalpindi and National Institute of Health (NIH), Islamabad, from 1st March 2017 to 30th May 2017. Brufen suspension, containing ibuprofen 100mg/ 5mL, manufactured by Abbot Laboratories (Pvt.) Limited, was used. Lipton open leaf green tea was purchased from the local market.

By using non probability consecutive sampling technique thirty (30) healthy adult male and female (non-pregnant) Sprague Dawley rats of 9-11 weeks of age and weights ranging from 200-330 gm were used in the experiment and were housed in the animal house of NIH, Islamabad. They were kept in cages in a well-ventilated room, and cycles of 12 hours light and 12 hours dark were maintained under a temperature range of 20-26°C with the help of central temperature regulating system. Male and female rats were kept in separate cages. Rats were fed NIH standardized lab diet for two months. Water was provided ad libitum. Study plan is given in (table-I).

Brufen suspension, containing ibuprofen 100mg/5mL was administered to each rat in experimental groups B and C in pre-calculated dose of 120 mg/kg bodyweight (Dose for 1 rat= 36mg ibuprofen in 1.8ml of suspension)[11,12].

The green tea extract was prepared by adding 1.25 g of green tea leaves to 25mL 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 25mL 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). The green tea extract was administered to each rat in experimental groups B and C by oral gavage at a dose of 1mL/100g body weight (Dose of 1 animal=0.075mg=3ml) at a temperature of 37[13].

At the end of 8 weeks, 24 hours after administration of last dose, the animals were sacrificed and both kidneys were removed. Renal tissue was then processed, stained with H&E stains and slides were prepared. To measure the proximal luminal diameter (PLD), 5 areas from the renal cortex of each animal were randomly selected at 40X objective and images were taken from each field. For each photograph, 10 rounded PT were randomly selected and their luminal diameters were measured from the apical surface of one cell to the apical surface of the opposite cell[14]. Using Image J v1.48[15]. Thus, luminal diameters of 50 PT were measured for each animal in each group (10 rats) and compared among three groups[16].

Data was analysis by using SPSS version 23.00. Mean ± SD were calculated for continuous variables. Anova and post hoc test were used for comparison group A, B and C. *p*-value ≤0.05 considered significant.

RESULTS

This study was conducted to evaluate the effect of green tea on ibuprofen-induced histomorphological changes in proximal luminal diameter in kidneys of adult rat. For this purpose thirty Sprague-Dawley male and female rats were equally divided into three groups (table-I).

In control group A, mean of proximal luminal diameter (PLD) was 13.073 ± 1.02µm. In experimental groups B and C, it was 4.79 ± 0.92µm and 12.67 ± 1.34µm respectively. There was statistically significant difference between group A,B and C (p<0.001), On intergroup comparison, statistically significant difference was found between control group A and experimental group B (p-value <0.001) and also between experimental groups B and C (p-value <0.001). The comparison

between control group A and experimental group C however, did not show any statistical significance (*p*-value=0.698) (table-II).

Green tea, mainly by virtue of its antioxidant properties, has strong nephroprotective effects. It prevents glomerulosclerosis and reduces serum urea and creatinine levels[19]. The drink was also found to have a protective effect against renal tubular damage caused by cyclosporine[20] and against renal ischemia[21].

Table-I: Plan of experiment.

Groups	Exposure
A Control group (n=10)	Rats in this group served as controls. They were given NIH lab diet and water ad libitum for 8 weeks.
B (Ibuprofen only) (n=10)	Rats in this group were given ibuprofen 120mg/kg body weight once daily for 8 weeks.
C (Ibuprofen+green tea) (n=10)	Rats in this group were given green tea 1ml/100g bodyweight in addition to administration of ibuprofen 120mg/kg body weight once dailyfor 8 weeks.

Table-II: Comparison of mean values of proximal luminal diameter of control group A and experimental groups B and C and intergroup (n=10).

Parameters	Group A Mean ± SD	Group B Mean ± SD	Group C Mean ± SD	*p*-value	Group A vs. B	Group A vs. C	Group B vs. C
Proximal luminal diameter (µm)	13.073 ± 1.02	4.79 ± 0.92	12.67 ± 1.34	≤0.001	0.001	0.698	0.001

DISCUSSION

In modern medicine, recognition of adverse effects of pharmaceutical agents on kidney is necessary for the administration of suitable drug dosage and chalking out preventive strategies[17].

Where ibuprofen is widely used for the treatment of a number of diseases, nephrotoxicity of the drug is also well-established and is mainly due to its ability to increase the number of reactive oxygen species which induce peroxidative damage in renal tissue. These includerenal papillary necrosis, tubular necrosis, acute interstitial nephritis, glomerulitis and accelerated chronic renal failure[18].

The reduction of luminal diameters of proximal tubules in experimental group B was found to be statistically significant when compared with control group A and experimental group C. This is attributable to partial occlusion of tubular lumen due to swelling of epithelial cells, shedding off of necrotic cells and accumulation of inflammatory exudates, as supported by 8 who obtained similar results after administration of ibuprofen in albino rats. They also studied the alleviating effects of vitamins B and C on

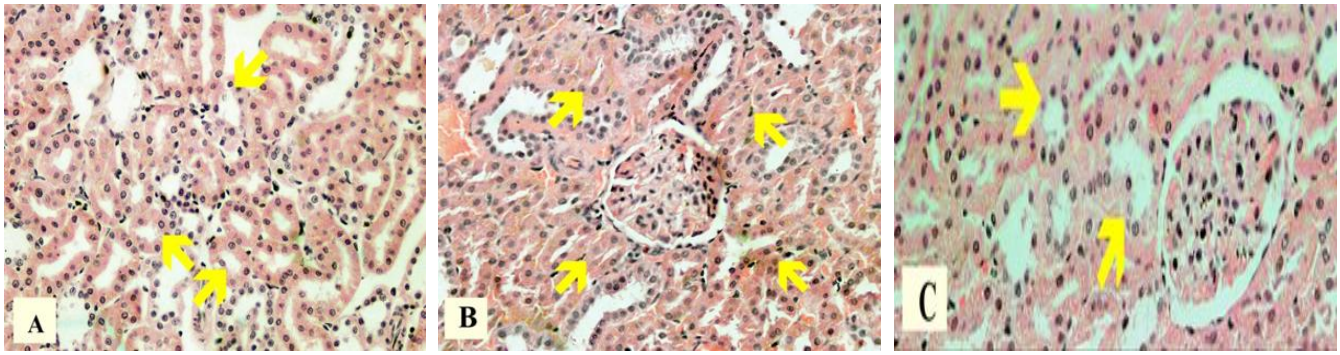

Figure: Photomicrographs A, B and C of rats from control group A and experimental groups B and C respectively, showing normal proximal convoluted tubules in A, necrosed proximal tubules with cytoplasmic swelling, nuclear disintegration and debris in lumen in B, and mildly necrosed proximal tubules with almost normal luminal diameters in C (H&E, 40X).

ibuprofen induced renal damage, determining that the changes could be partially reversed. A study conducted in 2017[22] found similar histological changes in proximal convoluted tubules of albino rats treated with gentamicin. In their study, ibuprofen induced reduction in proximal luminal diameter was reversed by administration of ginseng. In this study, green tea administration along with ibuprofen in experimental group C prevented PCT luminal narrowing by reducing necrosis and inflammation by virtue of strong antioxidant activity of its constituent catechins[23].

Another study conducted in 2016[24] showed that reduction in diameter of tubular lumen increases resistance to the flow of fluid, preventing sufficient reabsorption and reducing renal oxygen consumption by 60%. This leads to renal ischemia, ultimately resulting in acute kidney injury.

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CONCLUSION

Ibuprofen caused swelling of epithelial cells, shedding off of necrotic cells and accumulation of inflammatory exudates in the lumen of proximal tubules, leading to reduction in the proximal luminal diameter. However, concomitant administration of green tea along with ibuprofen protects against the drug-induced damage to renal architecture.

CONFLICT OF INTEREST

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

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