CINNAMON EXTRACT PROVIDES MORE NEPHROPROTECTION THAN GREEN TEA IN BISPHENOL - A INDUCED DAMAGE IN PROXIMAL AND DISTAL KIDNEY TUBULES

Huma Beenish, Rehana Rana, Shabana Ali

Islamic International Medical College/Riphah International University Rawalpindi Pakistan

ABSTRACT

Objective: To compare protective potential of cinnamon extract and green tea against Bisphenol A induced changes in tubular histology of rat kidney.
Design of Study: Randomized controlled trial.
Place and Duration of Study: Duration of study was six months and was conducted in Islamic International Medical College in collaboration with NIH.
Material and Methods: Sixty adult male rats of Sprague Dawley strain were placed in 4 cages having 15 rats each. Experimental duration was 30 days. Rats in control group A were subcutaneously injected with distilled water. Rats in experimental group B were given subcutaneous injections of BPA at dose of 30mg/kg/day. Rats in group C were given cinnamon (200mg/kg/day) orally along with s/c injection of BPA while group D rats were given green tea orally along with s/c BPA injections. All rats were dissected after 30 days and right kidneys were taken out to examine histological changes in proximal and distal convoluted tubules.
Results: Histological parameters of proximal and distal convoluted tubules were observed in experimental and control groups. Marked deterioration of tubular structure was observed in group B as compared to control group. Although both groups C and D showed nephroprotection against tubular histological changes but group D offered greater nephroprotection than group C.
Conclusion: Green tea was more effective than cinnamon in combating the oxidative stress induced neprotoxicity caused by BPA. Co-administration of cinnamon and green tea with BPA reduced the histological damage in proximal and distal convoluted tubules of nephrons in rats caused by Bisphenol A.

Keywords: Bisphenol A, Cinnamomumzeylanicum, Kidney, Oxidative stress.

INTRODUCTION

Kidneys, the essential organs of human body can be acutely affected by toxins. This acute kidney damage progresses to chronic renal disease[1]; which is now documented as a global health dilemma[2].

Among the list of environmental toxins, Bisphenol A (BPA) is regarded as the highest volume chemical produced in the world.It is a polymer of polycarbonated plastics and acts as an environmental toxin[3]. Main source of exposure to BPA in humans is through oral route[4] because it is used in inner lining of metal cans, soft drink bottles, plastic food containers, and water supply pipes. Significantly detectable BPA levels have been found in outdoor and indoor air, oil, water and canned food[5]. It is also absorbed through dermal contact as it is used in paper towels[6]. BPA harms kidney by generating oxidative stress leading to damage of kidney tissue[7]. As BPA is eliminated from human body by kidneys, increased levels of BPA have been reported in patients of CKD[8]. BPA affects physiology, morphology, histology and embryology of various organs of humans and animals[9].

Antioxidants have a role in nephroprotectionas they cause apoptosis of myofibroblasts and regenerate epithelial cells[10]. Cinnamon, a commonly used herb, has antioxidative properties which guard human kidneys from injuries caused by diabetes and various toxins[11]. It contains cinnamaldehyde, polyphenols and cinnamic acid.

Correspondence: Dr Huma Beenish, Islamic International Medical College Rawalpindi Pakistan *(Email: huma.beenish@ripha.edu.pk)*
Received: 01 Oct 2018; revised received: 20 Jan 2019; accepted: 21 Jan 2019

Another commonly used nephroprotective herb renowned of antioxidant effects is green tea (Camellia sinensis)[12]. Catechins of green tea include epicatechin-3-gallate (ECG), epigallocatechin-3-gallate (EGCG), epicatechin (EC) and epigallocatechin (EGC). Positive effects of green tea are due to its antioxidative, anticarcinogenic and anti-inflammatory properties. It also protects against kidney injury[13]. This study has been planned to compare the nephroprotective efficacy of cinnamon and green tea againsthistological changes in renal tubules caused by BPA.

MATERIAL AND METHODS

This study was a randomized controlled trial and synopsis of the study was approved by Ethical Review Committee, FHMS, Riphah International University before the conduction of experiment. Duration of research was one year. Sixty adult male rats of Sprague Dawley strain were included in this study by simple random sampling technique. Rats were kept in 4 cages in animal house of NIH with a number of 15 rats/cage. A standard laboratory environment with adjusted dietary supplementation was given to the experimental animals. Rats in group A allocated as controls were injected subcutaneously with 1ml distilled water. Group B rats were injected subcutaneously with 30mg/kg/day BPA[14]. Cinnamon aqueous extract was given to group C rats at dose of 200mg/kg/day[15] while green tea aqueous extract was given orally to the animals of group D at dose of 200mg/kg/day[16]. Both groups received oral dose through gavage tube 2 hr before daily subcutaneous injection of BPA. At the end of experimental tenure of 30 days, rats were euthenized and right kidneys were dissected out through longitudinal incision in the abdominal region[11]. Transverse sections of rat kidney were taken and stained with hematoxylin and eosin. All slides were examined under X4, X10 and X40 power of light microscope. The presence or absence of following qualitative parameters was noted in 4 random fields in each slide of kidney.

- Loss of brush border of PCT
- Cellular debris in lumen of PCT
- Cellular debris in lumen of DCT
- Distorted epithelium of DCT

Loss of eiosinophilic appearance of microvilli directed towards lumen of PCT was labeled as loss of brush border. The presence of sloughed off epithelial cells in lumen of PCT and DCT were labeled as cellular debris[17]. Cells of DCT having when cells showed abnormal shape or discontinuity in basement membrane was labelled distorted epithelium of DCT.

Data was analyzed by using SPSS version 22. Chi square test was applied for the comparison of qualitative variables among groups. A *p*-value less than 0.05 was considered as a significant value.

RESULTS

Loss of brush border was absent in the control group A, present in 100% experimental animals in group B, 80% and 26.7% in experimental animals of group C and D respectively. This parameter showed significant difference between all the groups (p=0.00).

In group A, 13.3% experimental animals showed cellular debris in lumen of PCT while 100%, 66.7% and 20% experimental animals showed this parameter in group B, C and D respectively. Significant difference was found in presence of cellular debris in PCT lumen between all groups (p=0.00).

In group A, 13.3% experimental animals showed cellular debris in lumen of DCT while 100%, 60% and 13.3% experimental animals showed this parameter in group B, C and D respectively. Significant difference was found in presence of cellular debris in DCT lumen between all groups (p=0.00).

In group A, 0% experimental animals showed distorted epithelium in DCT. In group B, distorted epithelium of DCT was present in 93.3% experimental animals. In group C and D 46.7% and 20% experimental animals showed distorted epithelium respectively (p=0.00).

DISCUSSION

In present study, we studied both proximal and distal convoluted tubules to compare the effects of BPA and herbs in histological appearance of both. PCT was looked up for loss of brush border and cellular debris in lumen. Loss of apical brush border in PCT was seen in all rats of experimental group B. In group C , 80% and in group D 26.7% rats showed loss of brush border. Similarly maximum cellular debris was found in lumen of PCT in group B (100%) followed by group C (66.7%) while group D showed this change only in 20% of experimental animals indicating more protection offered by green tea than cinnamon.

In the present study, these manifestations in group B might be explained by vulnerability of tubular membranes' enzymatic system to toxins[18]. In addition, generation of ROS by BPA is the key event involved in necrosis of renal tubules leading to functional disturbances[19]. Ahmed observed same changes in histology of PCT[17].

Improvement of renal parameters of PCT in group C are supported by study of Morgan who showed protective effect of cinnamon against BPA toxicity but did not compare cinnamon with green tea. Cinnamon resulted in decrease in cloudy swelling and obstruction in lumen of PCT. Underlying proposed cause is reduction of oxidative stress by cinnamon[15]. Mishra showed improvement in same histological parameters by treating diabetic rat kidney with cinnamon oil. He deduced that cinnamon decreases oxidative stress in kidneys by virtue of its antioxidant effects[20]. All the above mentioned studies potentiate our results in group C which indicate significant improvement in PCT histology as compared to group B but in present study green tea improved PCT histology more than cinnamon. This might be because of greater reduction in oxidative stress by green tea than cinnamon.

Table: Comparison of protective effect of green tea and cinnamon in BPA induced histological changes in kidney tubules of rat.

	Loss of brush border of PCT	Cellular debris in lumen of PCT	Cellular debris in lumen of DCT	Distorted epithelium of DCT
Group A (control)	0 (0%)	2 (13.3%)	2 (13.3%)	0 (0%)
Group B (BPA)	15 (100%)	15 (100%)	15 (100%)	14 (93.3%)
Group C (BPA + Cinnamon)	12 (80%)	10 (66.7%)	9 (60%)	7 (46.7%)
Group D (BPA + Green tea)	4 (26.7%)	3 (20%)	2 (13.3%)	3 (20%)
p-value	0.000			

Although histological effects of green tea against BPA nephrotoxicity have not been studied; Sardana used green tea against gentamycin induced nephrotoxicity. It was concluded that catechins have role in preventing tubular degenerative changes like epithelial

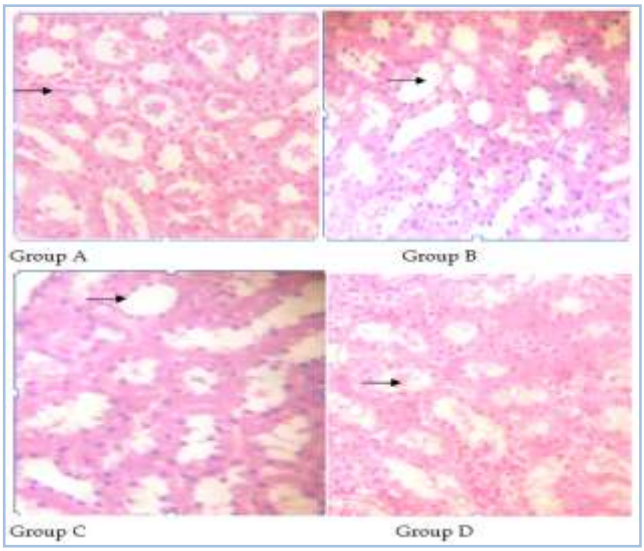

Figure: Group-wise distribution of cellular debris in lumen of PCT.

sloughing and cellular tubular debris due to its antioxidative effects[21].

Advantageous effects of green tea on PCT are potentiated by findings of Zhou who observed similar degenerative changes in PCT while looking for protective effect of EGCG on obstructive nephropathy. Electron microscopy showed that co- administration of EGCG in nephropathy resulted in intact basement membrane of PCT with presence of normal microvilli. The proposed cause was activation of Nrf_2 signaling pathway via antioxidative properties of EGCG[22]. This observation has also been witnessed by Ai Peng who found that EGCG ameliorates the tubular damage caused by immune mediated glomerulonephritis. He proposed that antioxidative effects of green tea reduced oxidative stress in glomerulonephritis[23]. Ryu observed necrosis in cells of proximal tubules of cyclosporine treated rats. Green tea extract ameliorated this change and gave normal appearance to PCT under microscope. He added that green tea inhibits activation of renin angiotensin aldosterone system[24]. Electron microscopic finding of degenerated cells in lumen of PCT and abnormal arrangement of microvilli in aged rats showed improvement after green tea administration. Ability of green tea polyphenols to quench free radicals directly is involved in this mechanism[12].

All above mentioned research work agrees with our findings of significantly greater nephroprotection shown by group D. It can be attributed to the presence of catechins of green tea which are most powerful antioxidants with high safety[22].

The qualitative parameters measured in DCT were cellular debris in lumen and distorted epithelium.

All the rats in group B showed cellular debris in lumen of DCT while group C and D showed presence of cellular debris in 60% and 13.3% experimental animals respectively. Hence both group C and D showed significant improvement in this parameter as compared to B. Regarding this parameter DCT of group Dshowed similar appearance to control group A. Distortion in DCT epithelium was negligible in group A. It was maximum in group B (93.3%) followed by group C (46.7%) while it was minimum in group D (20%).

Our results of cellular debris in lumen of DCT in group B are supported by publication of Ahmed who reported cellular debris in lumen and dilated lumen in DCT of BPA treated rats[17]. The presence of necrosis in epithelium of DCT may be explained by the depletion of ATP caused oxidative stress which finally leads to the death of the cells[25]. Improvement in parameters of DCT in group C are supported by findings of Muhammad who showed ameliorative effect of cinnamon on tubular degenerative changes caused by diabetes[11]. Asmarian proved cinnamon to be protective against altered kidney morphology by use of gelofen[25]. Greater improvement in histology of DCT by use of green tea than cinnamon may be because of greater antioxidative effect of green tea than cinnamon.

CONCLUSION

Co-administration of cinnamon and green tea with BPA reduced the histological damage in proximal and distal convoluted tubules of nephrons in rats. Green tea is more effective than cinnamon to combat the oxidative stress induced neprotoxicity caused by BPA.

ACKNOWLEDGEMENT

All praise is to Allah; We praise Him, seek his help, and ask for His forgiveness. I would like to extend my deep gratitude and appreciation to Gen. Masood Anwar Principal IIMC for providing me all available resources which I need during the course of research.

I gratefully acknowledge kind supervision of Prof. Dr. Rehana Rana HOD Anatomy Department IIMC. The insight she provided was the greatest victory in the effort. Many thanks to my Co-Supervisor Dr. Shabana, Associate Professor Anatomy IIMC, for guiding me during research course.

Author's Contribution

1) Dr Huma Beenish: Substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data.
2) Prof Dr Rehana Rana: Drafting the article or revising it critically for important intellectual Content.
3) Dr Shabana Ali: Final approval of the version to be published.

CONFLICT OF INTEREST

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

REFERENCES

1. Venkatachalam MA, Griffin KA, Lan R, Geng H, Saikumar P, Bidani AK. Acute kidney injury: A springboard for progression in chronic kidney disease. Am J Physiology-Renal 2010; 298(5): f1078-94.
2. El Nahas AM, Bello AK. Chronic kidney disease: the global challenge. Lancet 2005; 365(9456): 331-40.
3. Vandenberg LN, Maffini MV, Sonnenschein C, Rubin BS, Soto AM. Bisphenol-A and the great divide: A review of controversies in the field of endocrine disruption. Endocrine Reviews 2009; 30(1): 75-95.
4. Usman A, Ahmad M. From BPA to its analogues: Is it a safe journey? Chemosphere 2016; 158: 131-42.
5. Schecter A, Malik N, Haffner D, Smith S, Harris TR, Paepke O, et al. Bisphenol a (BPA) in US food. Environmental Science & Technology 2010; 44(24): 9425-30.
6. Shelby M. NTP-CERHR monograph on the potential human reproductive and developmental effects of bisphenol A. Ntp Cerhr Mon 2008; (22): 1-64.
7. Hassan ZK, Elobeid MA, Virk P, Omer SA, El-Amin M, Daghestani MH, et al. Bisphenol A induces hepatotoxicity through oxidative stress in rat model. Oxidative medicine and cellular longevity 2012; 2012.
8. González-Parra E, Herrero JA, Elewa U, Bosch RJ, Arduán AO, Egido J. Bisphenol A in chronic kidney disease. Intl J Nephrology 2013; 2013.
9. Michałowicz J. Bisphenol A–sources, toxicity and biotransformation. Environmental toxicology pharmacology 2014; 37(2): 738-58.
10. Wojcikowski K, Wohlmuth H, Johnson DW, Rolfe M, Gobe G. An in vitro investigation of herbs traditionally used for kidney and urinary system disorders: potential therapeutic and toxic effects. Nephrology 2009; 14(1): 70-9.
11. Mhammad HA, Jubrail AMS, Najeeb MK. Impact of Cinnamon Extract on Liver, Kidneys and Spleen of Diabetic Rats.
12. Gad SB, Zaghloul DM. Beneficial effects of green tea extract on liver and kidney functions, ultrastructure, lipid profile and hematological parameters in aged male rats. Global Vet 2013; 11(2): 191-205.
13. Wang Y, Wang B, Du F, Su X, Sun G, Zhou G, et al. Epigallocatechin-3-gallate attenuates unilateral ureteral obstruction-induced renal interstitial fibrosis in mice. J Histochem Cytochem 2015; 63(4): 270-9.
14. Badawi MM, Soliman MG, Abdel-Kawi NA, Abozaid NM. Physiological and Histopathological studies on Bisphenol-A compound as xenoestrogen in male albino rats. Egyptian J Hospital Medicine 2013; 50: 127-36.
15. Morgan AM, El-Ballal SS, El-Bialy BE, EL-Borai NB. Studies on the potential protective effect of cinnamon against bisphenol A- and octylphenol-induced oxidative stress in male albino rats. Toxicology Reports 2014; 1: 92-101.
16. Isbrucker R, Edwards J, Wolz E, Davidovich A, Bausch J. Safety studies on epigallocatechin gallate (EGCG) preparations. Part 2: dermal, acute and short-term toxicity studies. Food and chemical toxicology 2006; 44(5): 636-50.
17. Ahmed W, Moselhy W, Nabil T. Bisphenol A toxicity in adult male rats: hematological, biochemical and histopathological approach. Glob Veternaria 2015; 14: 228-38.
18. Murmu S, Shrivastava VK. Role of vitamin-c as antidote against bisphenol-a toxicity in kidney of fresh water fish cirrhinusmrigala (HAM).
19. Veljković M, Pavlović DR, Stojiljković N, Ilić S, Petrović A, Jovanović I, et al. Morphological and morphometric study of protective effect of green tea in gentamicin-induced nephrotoxicity in rats. Life sciences 2016; 147: 85-91.
20. Mishra A, Bhatti R, Singh A, Ishar MPS. Ameliorative effect of the cinnamon oil from Cinnamomum zeylanicum upon early stage diabetic nephropathy. Planta medica 2010; 76(05): 412-7.
21. Sardana A, Kalra S, Khanna D, Balakumar P. Nephroprotective effect of catechin on gentamicin-induced experimental nephrotoxicity. Clinical and experimental nephrology 2015; 19(2): 178-84.Zhou P, Yu JF, Zhao CG, Sui FX, Teng X, Wu YB. Therapeutic
22. potential of EGCG on acute renal damage in a rat model of obstructive nephropathy. Molecular medicine reports 2013; 7(4): 1096-102.
23. Peng A, Ye T, Rakheja D, Tu Y, Wang T, Du Y, et al. The green tea polyphenol (−)-epigallocatechin-3-gallate ameliorates experimental immune-mediated glomerulonephritis. Kidney intl 2011; 80(6): 601-11.
24. Ryu HH, Kim HL, Chung JH, Lee BR, Kim TH, Shin BC. Renoprotective effects of green tea extract on renin-angiotensin-aldosterone system in chronic cyclosporine-treated rats. Nephrol Dialysis Transplantation 2011; 26(4): 1188-93.
25. Rahimi O, Farokhi F, Khojasteh SMB, Ozi SA. The effect of Bisphenol A on serum parameters and morphology of kidney's tissue. Biological Forum; 2015: Research Trend.