

## ORIGINAL ARTICLE

**Comparative Effects of Cinnamon Extract and Green Tea Against Bisphenol- A Induced Damage in Rat Kidney**

Huma Beenish, Rehana Rana, Shabana Ali Muhammad, Asma Ali Khan

**ABSTRACT**

**Objective:** To compare protective efficacy of cinnamon extract and green tea against Bisphenol A induced histological changes in rat kidney.

**Study Design:** Randomized control trial.

**Place and Duration of Study:** The study was conducted at animal house of national institute of health sciences, Islamabad from 16<sup>th</sup> September 2016 to 16<sup>th</sup> March 2017.

**Materials and Methods:** Sixty adult male Sprague Dawley rats were placed in 4 confines having 15 rats each. Rats in control group A were given distilled water subcutaneously for 30 days. Rats in experimental group B were given Bisphenol a (BPA) s/c at dose of 30mg/kg/day for 30 days. Rats in group C were given cinnamon (200mg/kg/day) orally along with s/c injection of BPA while group D rats received green tea orally along with s/c BPA injections. Dissection was done after 30 days and right kidney of all rats were dissected out and examined grossly for weight and appearance and histologically for changes in glomerulus and Bowman's capsule. Data was entered and analyzed in SPSS version 22. Mean and standard error was calculated for the quantitative variables. Categorical variables were presented by frequency and percentage.

One way analysis of variance (ANOVA) was applied for the mean comparison of quantitative variables between control and experimental groups. Post hoc tuckey's test was applied for the multiple comparisons among groups.

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:** Gross and histological parameters were observed in experimental and control groups. Deterioration of kidney structure was greatest in group B as compared to control group. Although both group C and D protected kidney against these changes but group D offered more nephron protection than group C.

**Conclusion:** BPA adversely affects morphology and microstructure of kidneys by causing oxidative stress and green tea is more beneficial than cinnamon in ameliorating these nephrotoxic effects of BPA.

**Key Words:** *Bisphenol A, Cinnamomum Zeylanicum, Kidney, Oxidative Stress.*

**Introduction**

Kidneys, vital organs of human body, remove metabolic wastes of human body and conserve fluids and electrolytes.<sup>1</sup> The acute renal injury is induced by toxins ultimately progresses to chronic kidney disease which is now recognized as a global public health problem.<sup>2</sup> Bisphenol A (BPA), a polymer of

polycarbonated plastics, is one of highest volume environmental toxin produced worldwide.<sup>3</sup> Human exposure to BPA occurs mainly through food<sup>4</sup> as it is used in plastic food containers, soft drink bottles, toys, inner lining of metal cans and water supply pipes. Significant BPA levels have been detected in indoor and outdoor air, water, oil and canned food.<sup>5</sup> It is used as color developer in electric invoices and paper towels. Hence absorbed through dermal contact.<sup>6</sup> BPA causes oxidative stress induced tissue damage in the kidneys<sup>7</sup> as it is eliminated by kidneys, therefore, increased level of BPA are observed in CKD.<sup>8</sup>

BPA affect embryology, physiology, histomorphology of various organs of animals and humans.<sup>9</sup> Antioxidant therapies have nephron protective effect because they cause epithelial cell sparing and

*Department of Anatomy*

*Islamic International Medical College*

*Riphah International University, Islamabad*

*Correspondence:*

*Dr. Huma Beenish*

*Assistant Professor, Anatomy*

*Islamic International Medical College*

*Riphah International University, Islamabad*

*E-mail: huma1186@gmail.com*

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regeneration and cause apoptosis of my fibroblasts.<sup>10</sup> Cinnamon possess ant oxidative effects which protect kidney from damage caused by diabetes and various toxins.<sup>11</sup> Active components of cinnamon include polyphenols, cinnamic acid and cinnamaldehyde. On the other hand, green tea (*Camellia sinensis*) also a nephron protective antioxidant derived from plants.<sup>12</sup> Catechins present in green tea include epigallocatechin-3-gallate (EGCG), epicatechin-3-gallate (ECG), epigallocatechin (EGC) and epicatechin (EC). Beneficial effects of green tea include its ant oxidative, anti-inflammatory and ant carcinogenic properties. It is also protective against renal injury.<sup>13</sup> There were no histological studies seen for protection offered by green tea against BPA induced nephrotoxicity. This study has been designed to compare the modulatory effect of cinnamon n green tea consumption on daily basis to counteract the nephrotoxic changes caused by BPA.

### Materials and Methods

This study was a randomized control trial. It was conducted in animal house of national institute of health sciences, Islamabad from 16<sup>th</sup> September 2016 to 16<sup>th</sup> March 2017. Research took time span of six months before results were achieved. Ethical Review Committee gave approval of synopsis of the study prior to conduction experiment. Research was carried out on sixty adult male rats of Sprague Dawley breed, weighing approximately 250 to 300 grams. Female rats and rats with any conspicuous pathology were rejected.

Simple random sampling technique was used. Under supervision of animal house NIH, rats were kept in 4 confines with a number of 15 rats/confine. A standard laboratory condition indistinguishable to their class with adjusted dietary supplement was given under temperature of 27±3°C.

Rats were randomly divided by lottery method into 4 groups. (15 animals in each group). Rats in Group A serving as controls were given 1ml refined water subcutaneously. Group B rats were given 30mg/kg/day BPA, subcutaneously.<sup>14</sup> Group Crats were given 200mg/kg/day cinnamon aqueous extract<sup>15</sup> via gavage tube 2 hr before daily subcutaneous injection of BPA. Group Drats were given 200mg/kg/day green tea aqueous extract.<sup>16</sup> via gavage tube 2 hr before daily subcutaneous injection

of BPA. At the end of 30 days experimental duration, rats were anesthetized, kidneys were exposed through longitudinally cutting in the abdominal region.<sup>11</sup> Right kidneys of all animals were dissected out and observed for gross appearance by comparing them with normal kidneys of control group. Weight and gross appearance of each kidney was noted. Transverse sections of kidney were taken and stained with hematoxylin and eosin. Slides were examined under X4, X10 and X40 power of light microscope. The presence or absence of following qualitative parameters was confirmed by observing four random fields in each slide of kidney.

1. Contracted glomerulus
2. Swollen glomerulus
3. Hemorrhage in glomerulus
4. Ruptured Bowman's capsule

The glomeruli were labeled contracted when they appeared shrunken with increased urinary space as compared to glomeruli of control group.<sup>17</sup> The glomeruli with negligible urinary space were regarded as swollen. SPSS version 22 was used for data analysis. One way analysis of variance (ANOVA) was applied for the mean comparison weight between control and experimental groups. Post hoc turkey's test was applied for the multiple comparisons among groups. 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

Mean weight of rat kidney was significantly decreased in group B (rats were given 30mg/kg/day BPA, subcutaneously) as compared to control group A. Green tea administration in group D (rats given 200mg/kg/day green tea aqueous extract) significantly increased weight of kidney as compared to group C (rats given 200mg/kg/day cinnamon aqueous extract). Appearance of kidneys was swollen in 80% experimental animals in group B while in group D only 27% kidneys were swollen. The difference of appearance of kidneys was significant between all groups (p=0.00). (Table I) (Fig. 1).

All experimental animals in group B possessed contracted glomeruli while Group C and group D showed contracted glomeruli in 53.3 % and 13.3 % of experimental animals respectively. The difference of contracted appearance of glomeruli was significant

between all the groups (p=0.00). (Table II). In control group a none of experimental animals showed contracted glomeruli, while in group B contracted glomeruli were present in 100% of experimental animals. Group C and group D showed contracted glomeruli in 53.3 % and 13.3 % of experimental animals respectively. Swollen appearance of glomeruli was neither observed in control group A nor in experimental groups B, C and D. (Table II). Hemorrhagic glomeruli were present in 86.67% experimental animals. In group B while in group C and D hemorrhage was present in 73.3 % and 26.7% of experimental animals respectively. The difference of hemorrhagic appearance of glomeruli was significant between all groups (p=0.00) (Table II). Ruptured Bowman's capsule was present in 86.7% of experimental animals in group B. In groups C 46.7% while in group D 20% experimental animals showed ruptured Bowman's capsule. The difference in presence of ruptured capsule was significant between all groups. (Table II).

**Table I: Distribution of Kidney Weight (g) and Appearance between Experimental and Control Groups N=60**

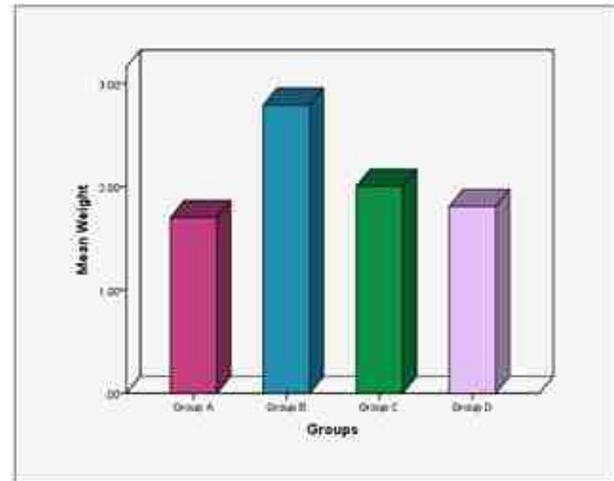
Groups	Kidney Weight Mean ± SEM	Gross appearance of kidney		
		Normal	Shrunken	Swollen
A	1.701±.0338	15(100%)	0(0%)	0(0%)
B	2.789±.061	3(20%)	0(0%)	12(80%)
C	2.003±.045	5(33%)	0(0%)	10(67%)
D	1.808±.041	11(73%)	0(0%)	4(27%)

**Table II: Distribution of Contracted Glomerulus, Swollen Glomerulus, Hemorrhage in Glomerulus and Ruptured Bowman's Capsule between Experimental and Control Groups N=60**

Groups	Contracted glomerulus	Swollen glomerulus	Hemorrhage in glomerulus	Ruptured Bowman's capsule
Group A	0(0%)	0	0 (0%)	0 (0%)
Group B	15(100%)	0	13 (86.7%)	13 (86.7%)
Group C	8(53.3%)	0	11 (73.3%)	7 (46.7%)
Group D	2(13.3%)	0	4 (26.7%)	3 (20%)
P value	0.000			

**Discussion**

Present study was designed to compare the nephron protective efficacy of cinnamon extract and green



**Fig 1: Distribution of Mean Weight between Control and Experimental Groups**

tea against toxicity caused by BPA by measuring gross as well as microscopic parameters. It was found that nephrotoxicity caused by BPA is ameliorated by both cinnamon and green tea but green tea showed best results in protection of kidney against BPA induced damage.

BPA leads to increase in kidney weight. Results showed that both cinnamon and green tea reduced kidney weight but comparison of both showed that green tea caused significantly greater reduction in kidney weight than cinnamon.(p=.021).

Increase in kidney weight in group B is indicating underlying change in morphology of kidneys. The reduction in kidney weight observed in present study in group C is accordance with results of Qusti who reported a similar decrease in kidney weight caused by treatment of diabetic rat kidney with cinnamon. Author related this change with the ant oxidative properties.<sup>18</sup> Noori brought to light that cinnamon is unable to decrease weight of kidney in absence of toxic insult.

Green tea polyphenols also act as antioxidants and combat the oxidative stress induced injuries.<sup>19</sup> Decrease in weight of kidney in group D is potentiated by findings of Hama Douche. Author demonstrated that increase in kidney weight caused by lead induced oxidative stress shows a relative decrease after treatment with green tea.<sup>20</sup> Green tea polyphenols reduced the weight of diabetic rat kidney significantly along with reduction in serum creatinine and MDA levels.<sup>21</sup>

Improvement in renal parameters in both groups C

and D along with maximum improvement in green tea group D is also supported by a study conducted by Hasanein. Author compared the affects of green tea cinnamon and black tea on renal function tests in obese diabetic rats.

The kidney dysfunction associated with diabetic rats was found to be linked with oxidative damage caused by diabetes. Both cinnamon and green tea exerted ant oxidative effects because of presence of polyphenols but RFTs improved more in green tea group.<sup>22</sup> Author did not focus the histological features of kidney in green tea and cinnamon treated groups.

Review of literature shows most studies on BPA and antioxidants have not focused on appearance of kidneys. Study conducted by Tan support our results of swollen kidneys in group B.<sup>23</sup> We were unable to find effects of cinnamon and green tea on gross appearance of kidneys in literature.

Hence all above literature supports our findings of maximum improvement in renal parameters with green tea as compared to cinnamon.

Structure of glomeruli was observed in four different fields of vision and all the glomeruli in group B were found to be contracted. Group D showed significant improvement in this parameter than group C.

Improvement in morphological appearance of glomeruli in group C in present study is supported by work of Morgan who showed glomerular congestion and hyper cellularity caused by BPA showed improvement with cinnamon administration.<sup>15</sup> This can be explained by presence of polyphenols in cinnamon.<sup>24</sup> Qusti reported that diabetes causes damage to renal corpuscle shown by shrunken glomeruli which become close to normal appearing glomeruli histologically after treatment with cinnamon.<sup>18</sup> But present study proved that green tea causes more improvement in above mentioned histology of glomerulus than cinnamon.

Sardana noticed that degeneration in glomerular wall caused by gentamycin improved with catechins. He explained that catechins reduce NADPH oxidase activity. Hence they reduce oxidative stress.<sup>25</sup>

Various studies support improved glomerular histology with green tea in present study.<sup>26</sup> Yokozawa showed that disturbed glomerular morphology in diabetic kidney restored to normal with green tea.<sup>21</sup>

Hama douche proposed similar positive impact of green tea against nephrotoxicity by lead. Lead causes

induction of oxidative stress while epicatechin serve as scavengers of free radicals.<sup>20</sup> Hence all above findings strengthen our results of positive effects of green tea on glomerular histology.

In present study BPA caused rupture of Bowman's capsule. Although both cinnamon and green tea improved this parameter but green tea caused significant improvement in histology of Bowman's capsule than cinnamon.(p=0.00) Sakr noticed that same degenerative changes in renal corpuscle after cypermethrin treatment along with their improvement with cinnamon administration.<sup>27</sup>

These results support our finding of improved capsular histology in group C but improvement was greater in green tea group D. In present study we found that BPA causes nephrotoxic effects in rat kidney. Effects of BPA on human kidney should be explored with reference to its accumulation and excretion by human body.

### Conclusion

Bisphenol A causes significant changes in histomorphology of rat kidney by induction of oxidative stress. Present study shows that co administration of cinnamon and green tea along with BPA causes improvement in gross and microstructure of kidneys. Comparison of both shows that green tea provides better nephron protection than cinnamon against BPA toxicity.

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