The Pharmaceutical and Chemical Journal, 2018, 5(2):34-39

Available online www.tpcj.org

Research Article

ISSN: 2349-7092
CODEN(USA): PCJHBA

Protective Effect of Gallic Acid against Bisphenol A-Induced Morphological Alterations of the Prostate Gland of Wistar Rats

Olufunke E. Ola-Davies[1], Samuel G. Olukole[2]*, Damilare O. Lanipekun[2]

[1]Department of Veterinary Physiology and Biochemistry, University of Ibadan, Nigeria
[2]Department of Veterinary Anatomy, University of Ibadan, Nigeria

Abstract The main objective of this study was to investigate the ameliorative effect of Gallic acid (GA) in BPA-induced toxicities of the prostate gland of adult male Wistar rats.Forty adult male Wistar rats were randomly assigned into four groups of ten animals each as follows: Group 1 (Control rats): 0.2 ml of corn oil; Group 2 (GA-treated rats): 20 mg/kg/day GA (dissolved in distilled water); Group 3 (BPA-treated rats): 10 mg/kg/day BPA suspended in 0.2 ml corn oil; Group 4 (BPA+GA-treated rats); BPA (10 mg/kg/day) with a concomitant GA (20 mg/kg/day). All treatments were orally administered for 14 days.BPA significantly increased ($P<0.05$) prostatic index and epithelial height of prostatic acini while inducing vascular congestion, hyperplasia of prostatic epithelium and atrophic tubules of the prostate gland. Concomitant treatment with GA ameliorated these alterations. BPA caused morphological alterations of the prostate gland of rats while concomitant treatment with GA ameliorated the alterations. Hence, low dose of GA serves a protective function against BPA-induced toxicity of the prostate gland of rats.

Keywords Bisphenol A, Gallic acid, hyperplasia, prostatic index, vascular congestion.

Introduction

Bisphenol A (BPA), 2,2-bis(4-hydroxyphenyl) propane, a xenoestrogens has been reported to be one of the most utilized industrial chemicals in the world [1]. BPA is a widely used endocrine disrupting chemical (EDC) whose annual rate of manufacture is greater than 3.1 million tons [2]. BPA is ubiquitous due to its high resistance to shattering, high temperature and electricity, thus making BPA highly valued in the creation of polycarbonate plastic and epoxy resins [3].

There have been conflicting reports on the effect of low doses of BPA on male reproduction in rodents. Having evaluated the discrepancies in the scientific evidence on low-dose effects of BPA on male reproduction, the US National Toxicology Program concluded that there are no low-dose effects of BPA [4]. However, the possibility of alow-dose effect of BPA due to a number of variables that may be difficult to control has been identified [5].

Gallic acid (GA, 3, 4, 5-trihydroxybenzoicacid), found in several natural products including lemon, red wine, strawberries, pineapples, bananas, gallnuts and tea leaves is widely used in the traditional medicine due to its anti-allergic, anti-mutagenic, anti-inflammatory, anti-oxidant as well as anti-cancer activities, and has been used in biological research as antioxidants against toxicities due to EDCs [6].

A number of chemo-protective agents including geinstein, indole-3-carbinol and selenium have been used to ameliorate EDCs-induced toxicity of the prostate gland [7-9]. Also, investigations on the effect of BPA with respect to the prostate gland of rodents have focused on gestational and perinatal BPA exposure, thereby leaving many gaps

on the effect of BPA in the prostate gland of adult rats [7, 8]. The present study was therefore designed to investigate the effects of GA on BPA-induced morphological alterations of the prostate gland of adult rats.

Materials and Methods

Chemicals

BPA and GA used in the study were purchased from Sigma-Aldrich Co. (St Louis, Missouri, USA). Every other reagents used in this study was of standard grades.

Experimental Animals

Forty adult male Wistar rats were used for the study. They were kept in the Animal House, Faculty of Veterinary Medicine, University of Ibadan. Commercial rat feed pellets and water was given *ad libitum*. The rats were stabilized for two weeks before the commencement of the treatment protocol. All procedures were carried out according to the guidelines for the care and use of experimental animals (National Institute of Health (NIH), USAand was approved by the University of Ibadan Animal Care and Use Research Ethics Committee. The rats used in the study were randomly assigned into four groups often animals each as follows:

- Group 1 (Control rats): 0.2 ml of corn oil orally administered.
- Group 2 (GA-treated rats): 20 mg/kg per day body weight GA (dissolved in distilled water) administered orally for 14 days [10].
- Group 3 (BPA-treated rats): 10 mg/kg per day body weight BPA suspended in 0.2 ml corn oil, orally administered for 14 days [11].
- Group 4 (BPA+GA-treated rats): Orally administered BPA (10 mg/kg per day body weight) with a concomitant GA (20 mg/kg body weight) orally administered.

Necropsy

Twenty-four hours after the last treatment, animals were weighed before being euthanized and blood samples were collected into plain sample bottles and centrifuged at 3000 rpm for 20 minutes, at 4° C to isolate the serum. The entire prostate gland was retrieved and weighed. Prostate samples were collected for histopathological and microstereological analyses.

Histopathology and Microstereology

Samples of the prostate gland of rats were fixed in buffered neutral formalin and embedded in paraffin blocks. Briefly, sections 2-4 µm thick were stained with Haematoxylin and Eosin (H&E) for histopathological and stereological analyses. The slides were analyzed and microscopic fields digitized using a light microscope (Olympus BX63 with a DP72 camera) and sections were observed for lesions of the prostate gland. Stereological analyses of sections were performed with the aid of GIMP 2 Software using 10 histological fields per section of the prostate gland stained with H&E from 5 different rats randomly chosen per group, totaling 50 serial sections per parameter (epithelial height and luminal diameter).

Statistical Analysis

Quantitative data were recorded as means and standard deviation. Comparison of means was performed using one-way ANOVA and followed by post hoc Tukey's test. Statistical significance among parameters was considered at $p < 0.05$. Graphical presentation of data was performed using GraphPad Prism 5 software (GraphPad Software, Inc. La Jolla, California, USA).

Results

There were no significant differences ($p.>0.05$) in the body weights of the rats across the groups (Fig. 1A). BPA significantly increased the prostatic index of the rats compared to the control, while GA ameliorated the condition (Fig. 1B). There was no significant difference in prostatic index between the control and GA-treated rats. However,

the BPA and the BPA + GA as well as the GA and the BPA + GA groupsall showed significant differences with respect to prostatic index.

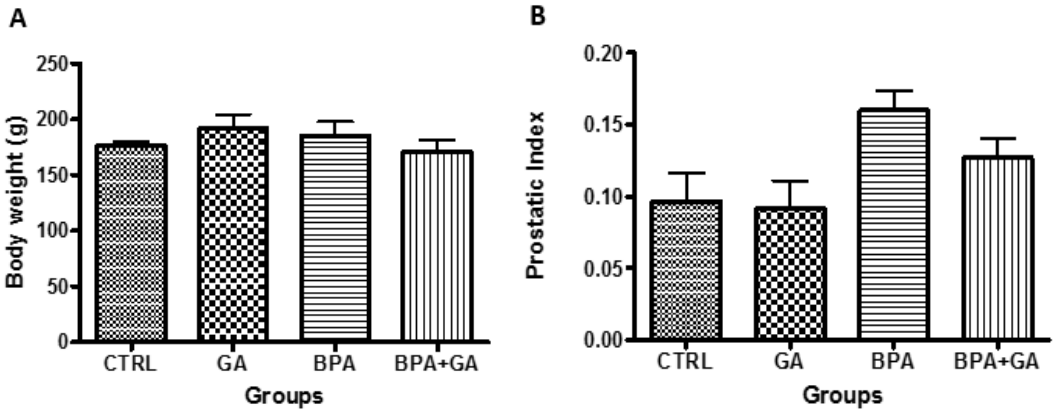

Figure 1: Effect of GA on BPA-induced changes in body weight and prostatic index in rats. A: Body weight; B: Prostatic index.

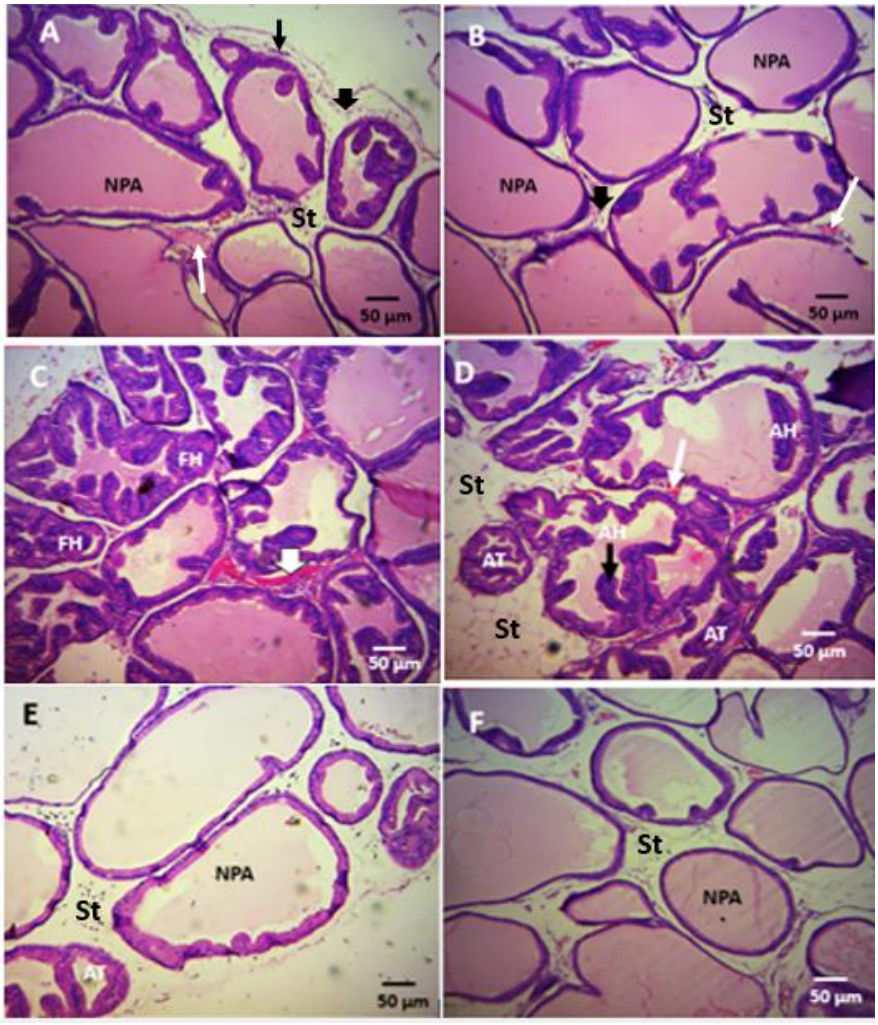

Figure 2: Representative histological sections of the prostate gland of rats (H&E).

A: Control group showing normal architecture of prostate gland. NPA: Normal prostatic acini; St: Stroma; White arrow: blood vessels; Black arrow: smooth muscle; Black Arrow head: fibro-cartilage.
B: GA-treated group showing normal glandular architecture. NPA: Normal prostatic acini; St: Stroma.
C: BPA-treated group showing functional hyperplasia (FH), vascular congestion (White arrow head)
D. BPA-treated group showing atypical hyperplasia (AH); vascular congestion (White arrow); atrophic tubule (AT) and eroded stromal elements (St). E and F: BPA + GA-treated group showing Normal prostatic acini (NPA) and empty stroma (St).

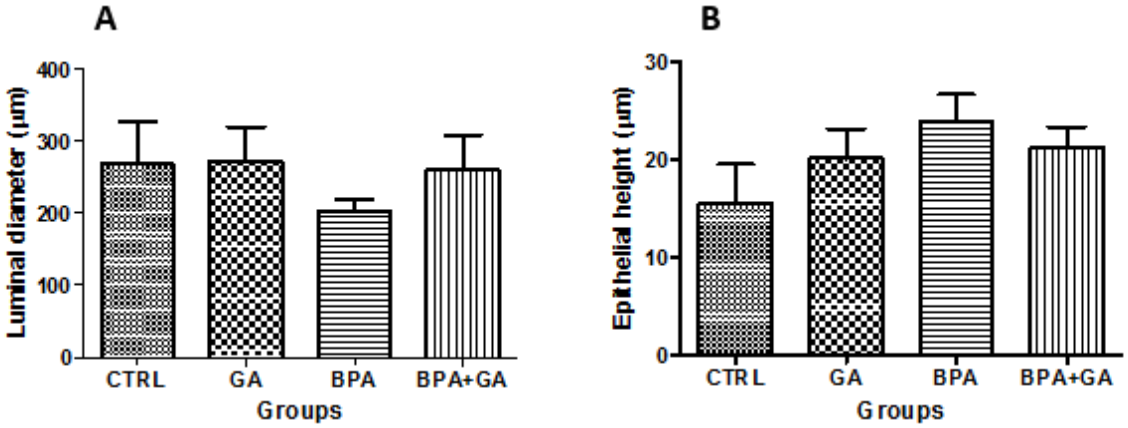

Figure 3: Ameliorative effect of GA on BPA-induced alteration stereological parameters of the prostate gland. A: Luminal diameter. B: Epithelial height.

Histopathological findings in the control and GA-treated rats revealed structural regularity in the glandular acini with their mucosal surfaces lined by simple columnar epithelium and surrounded by a stroma composed of smooth muscles, fibro-collagen and blood vessels (Fig. 2A & B). The BPA-treated rats presented a number of histopathological features of the prostate gland including inflammation of stromal elements, atypical hyperplasia showing a cribriform pattern and epithelial stratification; functional hyperplasia characterized by increased in-folding of the glandular epithelium resulting in the decrease in glandular diameter, atrophic tubules as well as congestion of blood vessels (Fig. 2 C& D). However, the BPA + GA treated rats showed improved glandular architecture with epithelial cells predominantly simple columnar with a reduced pathology (Fig. 2 C&D). In terms of incidence of prostatic lesions in the BPA-treated rats, vascular congestion was the most encountered while atypical hyperplasia was the least (Table 1). However, the BPA + GA-treated rats showed significant decrease in prostatic lesions compared to the BPA-treated rats. BPA caused a significant increase in epithelial height of prostatic acini with a concomitant significant decrease in acini luminal diameter (Fig. 3 A & B). However, these alterations were ameliorated in the BPA+ GA-treated group.

Table 1: Incidence of morphological alterations in the prostate gland of rats

Parameter	Control	GA	BPA	BPA + GA
Number of animals	n=10	n=10	n=10	n=10
Functional hyperplasia (%)	0	0	6 (60)	3 (30)
Atypical hyperplasia (%)	0	1 (10)	2 (20)	0
Inflammation (%)	0	0	6 (60)	3 (30)
Atrophic tubules (%)	0	0	4 (40)	2 (20)
Vascular congestion (%)	1(10)	2 (20)	7 (700)	3 (30)

Discussion

The present study shows that sub-acute exposure of adult rats to low dose of BPA is capable of inducing estrogenic actions including morphological alterations of the prostate gland and that concomitant treatment with GA ameliorates these alterations. These observations are in agreement with those of other authors on the effect of antioxidants in EDC-induced toxicity of the prostate gland [12, 13]. Studies have shown that environmental

estrogens such as BPA are not only nonsteroidal but also man-made chemicals that possess the ability to invade human or animal's body by ingestion or adsorption, subsequently antagonize hormones and disrupt endocrine function have great potential for compromising reproductive health in humans and animals [14].

The BPA-induced significant increase in prostatic index observed in the present study shows that low dose BPA is capable of inducing toxicity in the prostate gland of adult rats. Previous authors have also reported this [7, 8]. Similar observations have been reported following oral BPA administration in rats. Oral exposure of seven weeks old Sprague-Dawley rats, for 28 days, to BPA (40-600 mg/kg per day) induced marked endocrine-related effects, including decrease in prostate, seminal vesicle and pituitary weights, increased testicular weight, atrophic changes of the prostate, seminal vesicle and mammary gland, as well as degenerative changes in the testes of male rats [15].

The morphological alterations of the prostate gland observed in the present study positively correlates with those earlier reported in the prostate gland of rats exposed to BPA [7, 8]. Interestingly, our findings have demonstrated the ability of GA to ameliorate hyperplasia, vascular congestion, as well as atrophic tubules of the prostate gland induced in adult rats by BPA. Hence, the consumption of natural products including lemon, red wine, strawberries, pineapples, bananas, gallnuts and tea leaves (from which GA can be obtained) can be suggested as a means of protecting actions against BPA-induced morphological alterations of the prostate gland. Also the observed BPA-induced significant increase in epithelial height and the concomitant reduction in acini luminal diameter of the prostate gland in the present study is suggestive of a reduced ability of the prostate gland to secrete its fluid into the semen of the rats. This is capable of inducing reduction in fertility in the rats by directly reducing the volume of semen as well as reducing the motility of spermatozoa in the rats. However, the ability of GA in ameliorating these morphological alterations as well as the incidence of BPA-induced lesions of the prostate gland shows that it attenuates the noxious effects of BPA on male fertility by improving on the condition of the prostate gland.

Conclusion

In conclusion, the present study has shown that sub-acute oral administration of BPA at 10 mg/kg BW/day induces significant increases in prostatic index and epithelial height, increases morphological lesions of the prostate gland as well as reducing the luminal diameter of acini in the prostate gland of the adult rats. Concomitant treatment with oral GA (20 mg/kg BW/day) has been shown to ameliorate these conditions. Hence, low dose of GA serves a protective function against BPA-induced toxicity of the prostate gland of rats. It is however recommended that further studies involving protein and gene regulation be carried out to elucidate the mechanisms of the protective effect of GA on BPA-induced toxicity of the prostate gland of rats.

Acknowledgements

The authors acknowledge the technical support of the Department of Pathology, College of Medicine, University of Ibadan, Ibadan. Nigeria.

References

1. Ribeiro, E., Ladeira, C., & Viegas, S. (2017). Occupational exposure to Bisphenol A (BPA): a reality that still needs to be unveiled. *Toxics*, *5*(3), 22.
2. Cabaton, N. J., Canlet, C., Wadia, P. R., Tremblay-Franco, M., Gautier, R., Molina, J., Sonnenschein, C., Cravedi, J.P., Rubin, B.S., Soto, A.M., & Zalko, D. (2013). Effects of low doses of bisphenol A on the metabolome of perinatally exposed CD-1 mice. *Environmental health perspectives*, *121*(5), 586-593.
3. Tian, J., Ding, Y., She, R., Ma, L., Du, F., Xia, K., & Chen, L. (2017). Histologic study of testis injury after bisphenol A exposure in mice: Direct evidence for impairment of the genital system by endocrine disruptors. *Toxicology and industrial health*, *33*(1), 36-45.
4. Melnick, R., Lucier, G., Wolfe, M., Hall, R., Stancel, G., Prins, G., Gallo, M., Reuhl, K., Ho, S.M., Brown, T., & Moore, J. (2002). Summary of the National Toxicology Program's report of the endocrine disruptors low-dose peer review. *Environmental health perspectives*, *110*(4), 427-431

5. Kato, H., Furuhashi, T., Tanaka, M., Katsu, Y., Watanabe, H., Ohta, Y., & Iguchi, T. (2006). Effects of bisphenol A given neonatally on reproductive functions of male rats. *Reproductive Toxicology*, *22*(1), 20-29.
6. Jadon, A., Bhadauria, M., & Shukla, S. (2007). Protective effect of Terminalia belerica Roxb. and gallic acid against carbon tetrachloride induced damage in albino rats. *Journal of ethnopharmacology*, *109*(2), 214-218.
7. Bernardo, B. D., Brandt, J. Z., Grassi, T. F., Silveira, L. T. R., Scarano, W. R., & Barbisan, L. F. (2015). Genistein reduces the noxious effects of in utero bisphenol A exposure on the rat prostate gland at weaning and in adulthood. *Food and Chemical Toxicology*, *84*, 64-73.
8. Brandt, J. Z., Silveira, L. T. R., Grassi, T. F., Anselmo-Franci, J. A., Fávaro, W. J., Felisbino, S. L., Barbisan, L.F. & Scarano, W. R. (2014). Indole-3-carbinol attenuates the deleterious gestational effects of bisphenol A exposure on the prostate gland of male F1 rats. *Reproductive Toxicology*, *43*, 56-66.
9. Sakr, S. A., Mahran, H. A., & Nofal, A. E. (2012). Effect of selenium on carbimazole-induced histopathological and histochemical alterations in prostate of albino rats. *American Journal of Medicine and Medical Sciences*, *2*(1), 5-11.
10. Rather, S. A., Sarumathi, A., Anbu, S., & Saravanan, N. (2013). Gallic acid protects against immobilization stress-induced changes in wistar rats. *Journal of Stress Physiology & Biochemistry*, *9*(1), 136-147.
11. El-Beshbishy, H. A., Aly, H. A., & El-Shafey, M. (2013). Lipoic acid mitigates bisphenol A-induced testicular mitochondrial toxicity in rats. *Toxicology and industrial health*, *29*(10), 875-887.
12. Anjum, S., Rahman, S., Kaur, M., Ahmad, F., Rashid, H., Ansari, R. A., & Raisuddin, S. (2011). Melatonin ameliorates bisphenol A-induced biochemical toxicity in testicular mitochondria of mouse. *Food and chemical toxicology*, *49*(11), 2849-2854.
13. Othman, A. I., Edrees, G. M., El-Missiry, M. A., Ali, D. A., Aboel-Nour, M., & Dabdoub, B. R. (2016). Melatonin controlled apoptosis and protected the testes and sperm quality against bisphenol A-induced oxidative toxicity. *Toxicology and industrial health*, *32*(9), 1537-1549.
14. Haubruge, E., Petit, F., & Gage, M. J. (2000). Reduced sperm counts in guppies (Poecilia reticulata) following exposure to low levels of tributyltin and bisphenol A. *Proceedings of the Royal Society of London B: Biological Sciences*, *267*(1459), 2333-2337.
15. Yamasaki, K., Sawaki, M., Noda, S., Imatanaka, N., & Takatsuki, M. (2002). Subacute oral toxicity study of ethynylestradiol and bisphenol A, based on the draft protocol for the'Enhanced OECD Test Guideline no. 407'. *Archives of toxicology*, *76*(2), 65-74.