

# Protective Effect of Pumpkin Seed Extract on Bisphenol A Induced Testicular Toxicity in Adult Albino Rats

Pumpkin Seed  
Extract on  
Bisphenol A  
Induced  
Testicular  
Toxicity

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## ABSTRACT

**Objective:** To determine the effects of Cucurbita Pepo (pumpkin) seeds extract on testicular toxicity induced by administration of Bisphenol A in adult male albino rats.

**Study Design:** Experimental study

**Place and Duration of Study:** This study was conducted at the Department of Anatomy, Postgraduate Medical Institute (PGMI), Lahore, Pakistan for a period of 28 days from 15 January 2020 to 11 February 2020.

**Materials and Methods:** This experimental study comprised 30 male adult albino rats divided into three groups, A, B and C with 10 rats in each group. Group A was control and received distilled water for 28 days via oral gavage. Group B received Bisphenol at a dosage of 20mg/kg dissolved in 0.5ml of water for 28 days via oral gavage. Group C received Bisphenol A plus Cucurbita Pepo seeds extract. BPA was administered at a dosage of 20mg/kg body weight dissolved in 0.5ml distilled water and Cucurbita Pepo at a dosage of 300 mg/kg body weight dissolved in 2ml distilled water, once a day for 28 consecutive days through oral gavage method. Gross parameter included weight of both testis and Histological parameters like height of germinal epithelium and number of seminiferous tubules were analyzed.

**Results:** The weight of paired testis of groups A, B and C was in range of 3.16-3.542g, 2.60-3.321g and 3.0-3.43g respectively thus showing most weight reduction in group B. Height of germinal epithelium in groups A, B and C showed significant changes and was in range of 125-158  $\mu$ m, 60-117 $\mu$ m and 92-151  $\mu$ m respectively. The number of seminiferous tubules per field was nearly same as in group A and C but decreased in group B (p-value < 0.001).

**Conclusion:** From the aforementioned results, it is clear about the adverse effects that BPA is inducing in human bodies and that pumpkin seeds have maintained testicular architecture, reduced degeneration of germinal epithelium against BPA induced testicular damage.

**Key Words:** Pumpkin Seeds, Seminiferous Tubules, Germinal Epithelium, Testis

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## INTRODUCTION

Plastic production has been increased over the past 60 years owing to their durability, inexpensiveness and lightweight nature.<sup>1</sup> Bisphenol A (BPA) is added to plastic food packaging as antioxidant to reduce decomposition of plastic polymers<sup>2</sup> and in epoxy resins to form a protective layer in cans to prevent corrosion.<sup>3</sup>

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It is unstable in acidic or basic solutions, higher temperatures and UV light exposure that converts polymeric stable form of BPA into unstable monomeric form which then releases into food and beverages contained in BPA containing containers.<sup>4</sup> BPA is known to have mutagenic, hepatotoxic, nephrotoxic, carcinogenic effects, alters steroidogenesis and adipogenesis.<sup>5</sup> Pumpkin seeds have a unique nutty taste. They are roasted and salted and consumed as a snack in many parts of USA, Mexico, China and other countries. Cucurbita pepo, commonly known as pumpkin, belong to the genus Cucurbita and family Cucurbitaceae.<sup>6</sup> Pumpkin seed extract is useful for immunomodulation and reproductive health.<sup>7</sup> It contains phenolic compounds as tyrosol, vanillic acid, vanillin, ferulic acid, and lulin. The antioxidant property of pumpkin seeds is due to phenols and tocopherols and pumpkin seeds water extract contains 85-92% of total extractable phenolics that is highest percentage as compared to other modes of extraction.<sup>8</sup> Hence pumpkin seeds are able to reduce lipid peroxidation, improve fertility and helps to prevent arteriosclerosis, high blood pressure,

heart diseases and reduce symptoms of benign prostatic hyperplasia.<sup>9</sup>

## MATERIALS AND METHODS

30 male albino Wistar rats, 9-12 weeks and weighing 180-220g were procured from Post Graduate Medical Institute, Lahore. All the animals were examined thoroughly before commencing the experiment. They were individually housed in a climate-controlled

environment and provided with food and water ad libitum.<sup>10</sup> Following acclimatization for a period of one week, experimental procedure was started.

### Therapeutic Agents:

- BPA used in this research was a product of DAEJUNG (Korea).
- Pumpkin seeds (Gen. Name; Cucurbita pepo) extract was prepared at UHS, Lahore.

**Table No.1: Description of the groups, intervention, dose, route & duration of therapeutic agents**

Groups	Intervention & Dosage	Route of Administration	Duration of Administration	Sacrifice Day
A	Distilled water 2ml	By oral gavage	28 days	24hrs after administration of last dose
B	BPA 20mg/kg/day dissolved in 0.5ml distilled water	By oral gavage	28 days	24hrs after administration of last dose
C	BPA 20mg/kg/day dissolved in 0.5ml distilled water plus 300mg/kg/day of pumpkin seed extract dissolved in 2ml distilled water	By oral gavage	28 days	24hrs after the administration of last dose

**Dissection and tissue sampling:** All rats were sacrificed under deep anesthesia 24 hrs after administering the last dose on day 29<sup>th</sup>. Testis were identified lying in the scrotal sac. The testis of each animal was weighed separately and placed in neutral buffered formalin. Tissue was processed for up to 18 hours by using the automatic tissue processor (HISTOTOUCH III-USA). For embedding, liquid paraffin was then poured onto the tissue piece to make tissue block. By using microtome, sections of 3  $\mu$ m thickness were obtained and stained with Hematoxylin and Eosin.<sup>11,12</sup>

### Parameter:

#### Gross:

1. Weight of the paired testis (g).

#### Histological:

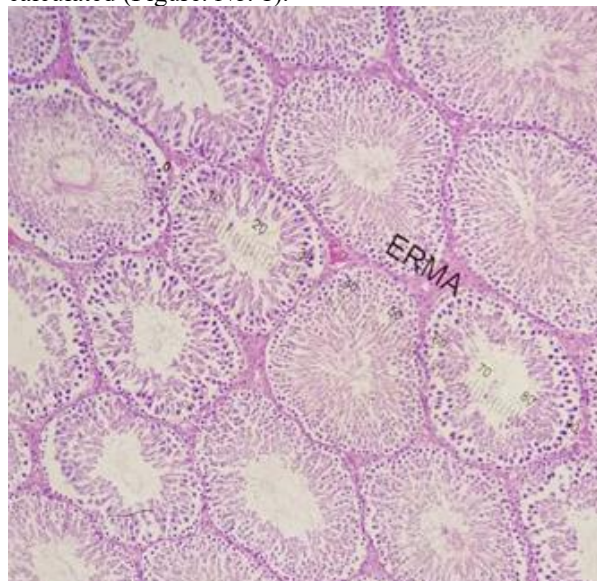
1. Height of germinal epithelium ( $\mu$ m).
2. Number of seminiferous tubules per field.

**Weight of the paired testis:** Weight of paired testicular weight of each animal was recorded immediately after dissection from body on an electronic scale (Sartorius Precision Balance, Germany) and their mean was calculated.

**Height of germinal epithelium:** The height of the germinal epithelium was obtained by subtracting the luminal diameter from the tubular diameter for the same seminiferous tubules under 100X through ocular micrometer and dividing it by 2 (Figure No. 1).<sup>12</sup>

**Number of seminiferous tubules per field:** Number of seminiferous tubules were counted in 3 different fields

under 100X magnification and their mean was calculated (Figure. No. 1).<sup>12</sup>



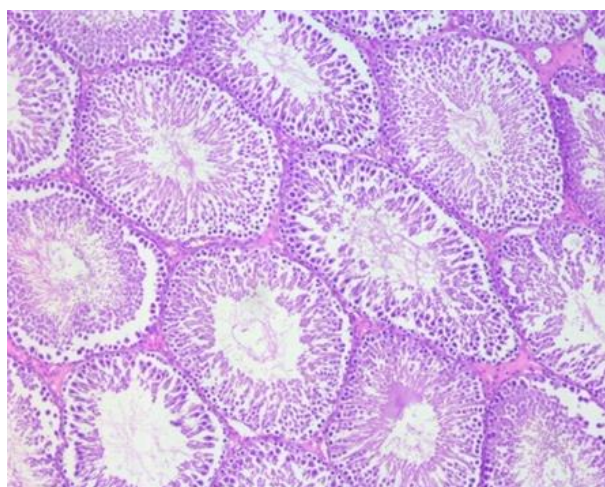
**Figure No. 1: Photomicrograph of testicular section showing micrometry with ocular micrometer for the measurement of height of germinal epithelium & number of seminiferous tubules. 100X.**

**Statistical analysis:** The data was entered and analyzed using SPSS 25. The quantitative data (weight of testis, height of germinal epithelium, number of seminiferous tubules per field) was analyzed using ANOVA followed by post hoc Tukey's test.

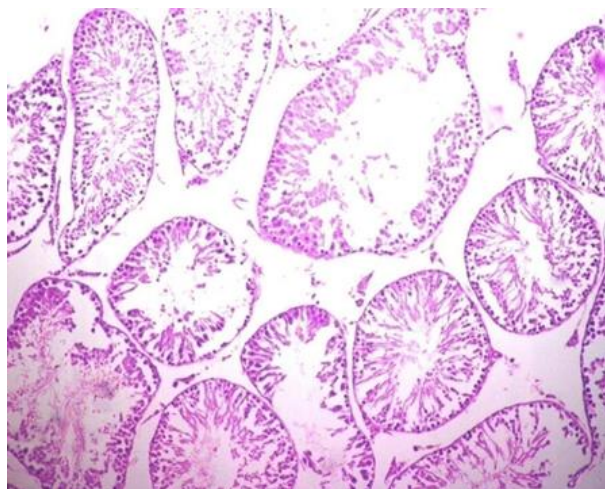
**RESULTS**

**Table No.2: Parameters**

Parameters	Group A	Group B	Group C	P-value#
Weight of paired testis (g)	3.35 ± 0.19	2.96 ± 0.16	3.26 ± 0.15	< 0.001*
Height of germinal epithelium (µm)	139.7 ± 10.1	91.3 ± 19.91	21.4 ± 20.0	< 0.001*
Number of seminiferous tubules per field	20.6 ± 2.6	14.4 ± 3.4	20.4 ± 2.1	< 0.001*



**Figure No. 2: Photomicrograph of testicular section from control group A. H&E stain X.100.**



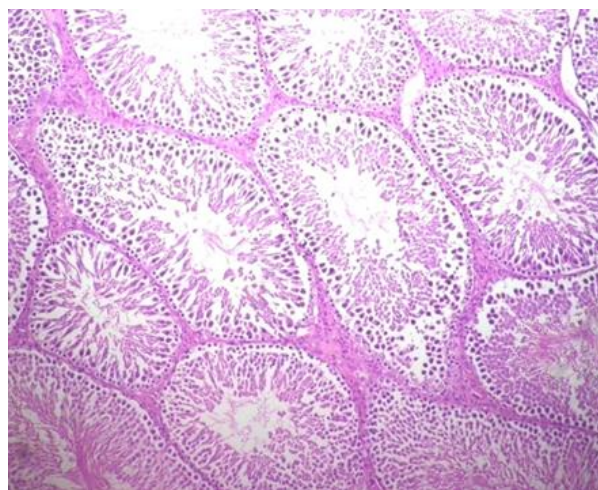
**Figure No. 3: Photomicrograph of group B showing damaged effects of BPA on testicular architecture with reduced germinal epithelium height and decreased number of seminiferous tubules. H& E stain. 100X**

**Weight of Paired Testis:** The weight of both testis in all groups was measured. Paired testicular weight of

groups A, B and C were in range of 3.16-3.542g , 2.60-3.321.g and 3.0-3.43g respectively. It was found that there was significant difference in the mean weight of both testis among groups (p-value < 0.001) (Data Table No. 2).

**Height of germinal epithelium:** Height of germinal epithelium in groups A, B and C was found to be significantly different among groups (p-value <0.001) (Data Table No. 2) (Figure No. 3 & Figure No. 4).

**Number of Seminiferous Tubules per field:** It was found that the average number of seminiferous tubules per field were significantly reduced in Group B (p-value < 0.0001) (Fig. 3). While in that of Group C it was near the control group A (Data Table No. 2) (Figure No. 4)



**Fig 4. Photomicrograph of Group C showing protective effects of extract of pumpkin seeds (Cucurbita pepo) on testicular architecture. Height of germinal epithelium and Number of seminiferous tubules is near control group A. H & E stain. 100X**

**DISCUSSION**

Decrease in paired testicular weight of rats treated with BPA may be attributable to reduced food intake by rats due to BPA induced biochemical changes as documented by Samova et al. (2018).<sup>13</sup> BPA exposure also decreases protein content of testis thus reducing the testicular volume and mass which might be due to reduced availability of sex hormones which are responsible for growth of sex organs.<sup>14</sup> Present work has concluded that administration of BPA to rats in experimental group B has shown a reduction in height of germinal epithelium comparable to the control group A (p value <0.001). Jahan et al. (2016) reported marked atrophy of seminiferous tubules with loss of elongated sperms.<sup>15</sup> It was in line with the studies reporting reduced germinal epithelial height because of oxidative stress induced by BPA.<sup>16</sup> Alboghobeish et al.(2019) also proved that treating testicular tissue with 50mg/kg of BPA reduces height of germinal epithelium by decreasing secretion of sex hormones and by its anti



androgenic effect.<sup>14</sup> The results of group C revealed improvement in height of epithelial lining of seminiferous tubules which is due to anti oxidants present in pumpkin seed extract, that reduces lipid peroxidation of membranes and protects them from oxidative radicals.<sup>17</sup> Current study showed that number of seminiferous tubules per field were reduced in BPA treated group B due to chronic oxidative damage which was significantly improved by pumpkin seeds extract treated group C by anti oxidant role of phenolic compounds present in cucurbita pepo seeds.

## CONCLUSION

From the aforementioned results, it is clear that pumpkin seeds have maintained testicular architecture, reduced degeneration of germinal epithelium by acting as anti oxidant, hence counter-balancing the BPA induced oxidative stress.

### Author's Contribution:

Concept & Design of Study: Uzma Batool  
 Drafting: Qurat ul Ain, Godfrey Paul William  
 Data Analysis: Shumaila Sohail, Hamna Umer, Fatima Jawad.  
 Revisiting Critically: Uzma Batool, Qurat ul Ain  
 Final Approval of version: Uzma Batool

**Conflict of Interest:** The study has no conflict of interest to declare by any author.

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