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Bisphenol-A induced damage in testicular structure and its amelioration by Vitamin E and *Tinospora cordifolia*

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ABSTRACT

Present study was aimed to investigate the effect of Bisphenol-A (BPA) on testicular tissue of goat and its reversal by antioxidant Vitamin E and Tinospora cordifolia. Testicular tissue culture was carried for 4 and 8 hours. In Group 1, testicular tissue was exposed to two doses of BPA (10^{-2} and 10^{2} nM/ml) while in Group 2 and 3, different doses of BPA (10^{-2},10^{2} nM/ml) were supplied along with Vitamin E (0.1µM/ml) and Tinospora cordifolia (250µg/ml), respectively. It was observed that BPA induced significant damage in the testicular cells. Various degree of histomorphological alterations observed were: reduction in population of spermatids, spermatozoa, vacuolization in lumen, damage in seminiferous epithelium, pyknosis in germ cells and reduced number of luminal spermatozoa. Supplementation with Vitamin E and Tinospora cordifolia resulted in reduction in vacuolization, desquamation of germ cells in lumen and decline in pyknotic cells. Population of sperm cells were elevated and significant restoration in shape of seminiferous tubules was observed as compared to treatment groups. It is concluded that Vitamin E (antioxidant) and Tinospora cordifolia restores fertility in BPA exposed goat testis and exhibit protective effect against BPA induced damage in testis in vitro.

Key words: BPA, *Tinopsora cordifolia*, Vitamin E, spermatids and spermatozoa.

INTRODUCTION

Bisphenol-A [2, 2-bis (4-hydroxyphenyl) propane] is a well-known endocrine disrupting chemical (EDC) that has received particular attention because of its widespread distribution in environment. It is used in the manufacture of polycarbonate plastics and epoxy resins. BPA is found in many end products, including dental sealants, coatings for food cans, linings for metal cans,

polyvinyl chloride and medical equipment [1, 2]. Human exposure to BPA may occur in the workplace through inhalation during production, but the most common route of exposure is by oral intake. BPA exposure to humans was associated with damage to sperm DNA and decline in semen quality [3]. Vom Saal *et al*. (1998) confirmed the role of BPA on rats and recorded a decrease in the weight of epididymis, seminal vesicle and a decrease in sperm density [4]. Various studies confirmed the toxicological role of BPA in the testis of rats and mice [5, 6]. Li *et al*. (2011) have reported that BPA is associated with low semen quality, which directly affect human spermatogenesis [7].

A number of plants have been used as a traditional medicine throughout the world from time immemorial and continued to be used as remedies for humankind and different animals. ***Phytoandrogens*** are substances produced in plants which have effects similar to testosterone in animals.

Tinospora cordifolia, commonly known as Giloy in hindi, is one of the most widely used shrub having medicinal history and is reportedly used as a source of medicine and plays a direct role in antidiabetic, immunomodulatory, antihepatotoxic and antipyretic action [8].

Tinospora cordifolia is reportedly known, to possess antioxidant activity in ram semen therefore protects the normal structure and function of spermatozoa [9].

Vitamin E is lipid soluble Vitamin, with a major antioxidant property. It acts as a major membrane protectant against ROS and lipid peroxidation and therefore plays a direct role in protecting spermatozoa from peroxidative damage and mobility loss [10].

Wang and co-workers have also studied that supplementation of Vitamin E reduced various alteration in testis and increased testicular weight in rats. It was also found that its administration helps in declining testicular cell apoptosis and recovering normal structure [11].

Information related to the BPA induced abnormalities in goat testis along with the protective effect of *Tinospora cordifolia* and Vitamin E was fragmentary. Keeping in view these lacunae, the present study was aimed to determine the nanomolar effect of BPA on structure of goat testis along with the ameliorative effect of *Tinospora cordifolia* and Vitamin E.

MATERIALS AND METHODS

The testis from the mature goat (*Capra hircus*) was obtained from slaughter houses around Kurukshetra (29° 6'N, 76 °50'E) and was brought to lab at 4°C in normal saline.

Collection of plant material

The stem of *Tinospora cordifolia* (commonly known as Giloy) was collected from the Botanical garden of Kurukshetra University, Kurukshetra and was authenticated from the Department of Botany.

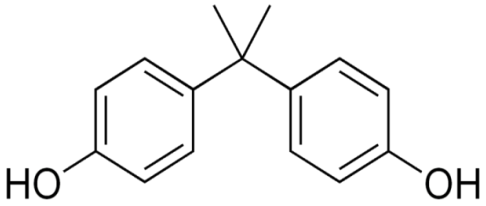
Extraction of *Tinospora cordifolia* stem (maceration method): The stem was shade dried and grinded coarsely. The coarsed powder was mixed with 70% alcohol in 1: 9 ratios. The mixture was shaked on a shaker for 2 days and kept on a water bath for 9-10 hours. The extract was collected in a small container. For mitigating the effects of BPA, the concentration used in the present work of *Tinospora cordifolia* hydroalcoholic extract was 250µg/ml.

Figure 1: Photographs of *Tinospora cordifolia* plant and coarsed powder used for making hydroalcohlic extract.

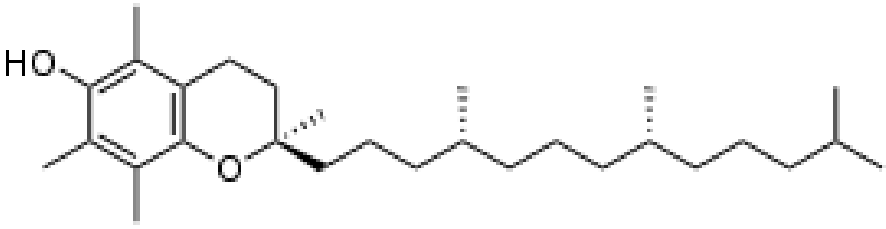
After decapsulation, testis was cut into smaller pieces approximately $1mm^3$ in size. Testicular tissues were washed thrice with TCM-199 and placed on nucleopore filter and floated on medium. The medium constituted TCM-199 and antibiotics (200 unit penicillin 100 IU/ml and streptomycin 100 g/ml). The testicular tissue culture was divided into three experimental groups along with their respective control maintained in similar conditions for 4 and 8 hours. In group1, the tissue was exposed to the different doses of BPA viz. (10^{-2}, 10^{2} nM/ml). In group 2 and 3, Vitamin E (0.1µM/ml) and *Tinospora cordifolia* (250µg/ml) were supplied, respectively along with the different doses of BPA. The culture plates were kept at 39°C, 95% humidity in CO_2 incubator.

Testicular tissues were fixed in Bouin's fixative for 24 hours; washing was done for 3 hours followed by dehydration through series of alcoholic grades. The tissues were embedded in paraffin wax and were sectioned serially at 5 micrometer thickness followed by stretching and dewaxing by xylene for 15 minutes and transferred to absolute alcohol. The slides were passed

through different grades of alcohol and stained with Haematoxylin and Eosin [12]. The slides were observed under light microscope and photographs were taken.



Structure of Bisphenol-A. Chemical formula: $(CH_3)_2 C (C_6H_4OH)_2$. Molar mass: 228.29 g/mol.



Structure of Vitamin E. Chemical formula: $C_{29} H_{50} O_2$. Molar Mass: 430.71 g/mol.

RESULTS

During the present study, the histopathological analysis revealed the normal structure of testicular section under the control group. The germ cells viz. spermatogonia, spermatocytes, spermatids and spermatozoa were well organized in seminiferous tubules. The germ cells were fostered by Sertoli cells and Leydig cells were located within connective tissue surrounding the seminiferous tubules (Figure 2). In experimental group, treated with a dose of 10^{-2} nM/ml (BPA) revealed histoarhitecture alterations which include vacuolization, distortion of seminiferous tubules along with desquamation of cells in the lumen as compared to control (Figure 3). With the increase in dose of BPA (10^2 nM/ml), the testicular sections were found to be more damaged. There was an increase in number of vacuolization, dislodging of germ cells. Spermatogonia and spermatocytes cells got decreased in number as compared to dose of 10^{-2} nM/ml (Figure 4). Upon amelioration with Vitamin E and *Tinopsora cordifolia*, there was a recovery in lumen size and restoration of seminiferous tubule structure with increase in germ cell population viz. spermatogonia, spermatocytes, spermatids and spermtozoa as compared to treated group (Figures 5 and 6).

With an increase in exposure duration from 4 to 8 hours and a dose level of 10^{-2} nM/ml (BPA), testicular section showed damage in seminiferous epithelium, pyknosis and vacuolization (Figure 7). Upon administration with a dose level of 10^2 nM/ml of BPA, there was an increase in alterations in the testicular section as seminiferous tubules got detached from one another with empty

spaces between seminiferous tubules. Shrinkage of seminiferous tubule and empty space was clearly observed in tubules (Figure 8). Vitamin E supplemented tissue showed a decline in damage induced by BPA viz. reduction in vacuolization, pyknosis and intact structure of germinal epithelium. There was a significant restoration in the germ cells towards the peripheral region of seminiferous tubules (Figure 9). The supplementation of *Tinopsora cordifolia* along with the exposure of BPA caused a significant reduction in the abnormalities as compared to the BPA induced tissue. There was a prominent recovery in the size of seminiferous tubules and lumen was filled with spermatozoa (Figure 10).

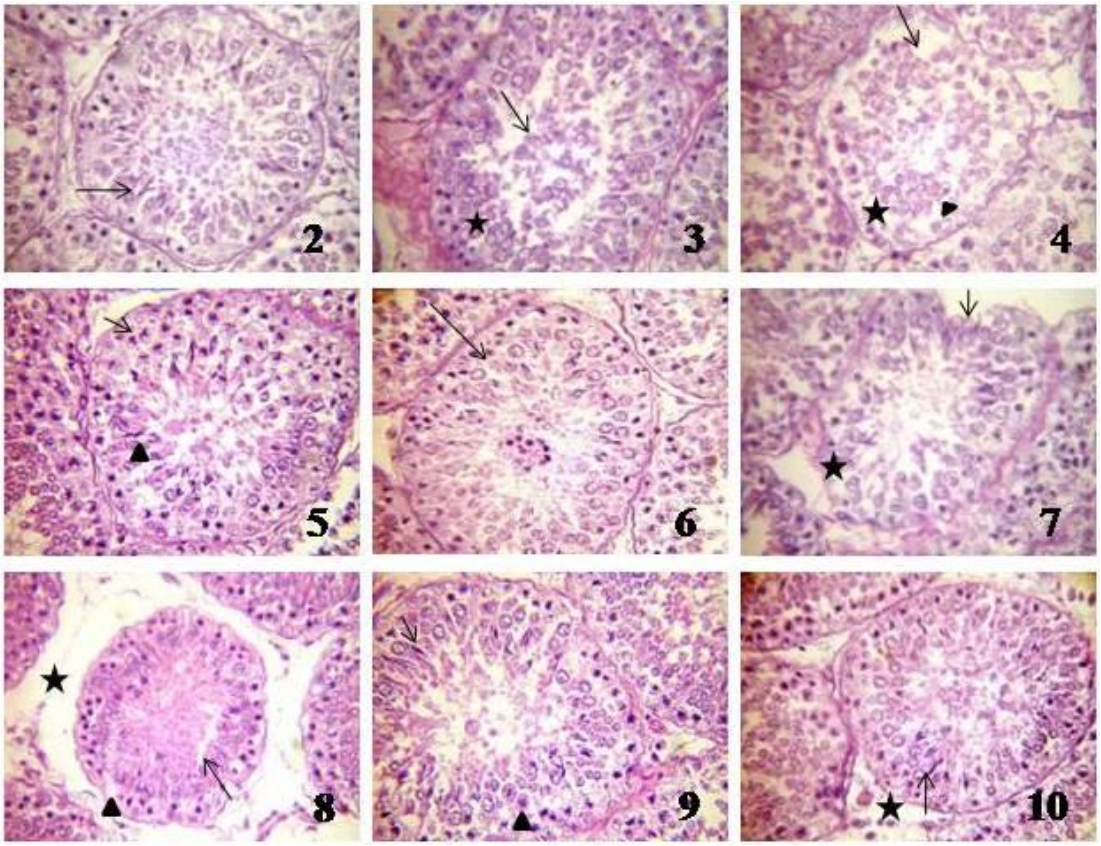


Figure 2: At 4 hour exposure duration, transverse section of testis revealing intact seminiferous epithelium with proper array of germ cells viz. spermatogonia, spermatocytes, spermatids and spermatozoa (arrow) in the seminiferous tubules. **Figure 3:** Dose of 10^{-2} nM/ml of BPA showing seminiferous tubules filled with desquamated germ cells (arrow) and slight vacuolization (star). **Figure 4:** 10^{2} nM/ml of BPA revealing dislodging of germ cells from the basement membrane to the lumen (star), vacoulization (arrow), decreased number of spermatozoa (arrow head). **Figures 5 and 6:** Upon amelioration with Vitamin E and *Tinopsora cordifolia*, seminiferous tubules showing restoration of seminiferous tubule structure with increase in germ cell population. **Figure 7:** Exposure duration of 8 hr and dose of 10^{-2} nM/ml of BPA showing damage in seminiferous epithelium (arrow) and pyknosis (star). **Figure 8:** Testicular section showing increase in damage viz. detachment (star), widening

gaps (arrow) and shrinkage of seminiferous tubules (arrow head) at 10^2 nM/ml of BPA. **Figures 9 and 10:** Amelioration by Vitamin E and *Tinopspora cordifolia* showing a prominent recovery in seminiferous tubules and germ cells as compared to treated groups.

DISCUSSION

The results of the present study have revealed that Bisphenol-A induced histopathological changes in testicular tissue of goat. The various alterations observed were vacuolization, desquamation of germ cells in the lumen, decrease in spermatogenic cell population, lumen diameter, pyknosis, damage to seminiferous epithelium and shrinkage of seminiferous tubules. Qiu *et al*. (2013) have also observed various types of abnormalities in BPA administered on rat testicular tissue such as vacuolization, degeneration of cells and reduction in number of spermatids, spermatozoa [13]. During the present study, Vitamin E showed a significant recovery in testicular structure. The seminiferous tubules showed enhanced germ cell population as there was an increase in number of spermatogonia, spermatocytes, spermatids towards the peripheral region of seminiferous tubules. Lumen showed a normal structure with a number of maturing spermatozoa. Sharma *et al*. (2012) have also reported the similar morphological changes in treated goat testis upon supplementation of Vitamin E [14]. The morphological changes observed were reduction in vacuolization, pyknosis, dislodging and fragmentation of nuclei. The present findings are also in agreement with the findings of Amjadi *et al*. (2015) who have reported atrophy and vacuolation in the germinal epithelium by BPA which got decreased when supplemented with Vitamin E [15].

The results of present study also revealed that the goat testicular tissue upon administration with *Tinospora cordifolia* (250 µg/ml) along with BPA, revealed a marked increase in the germ cell population, recovery of damaged germinal epithelium, restoration of shape of seminiferous tubules, increase in number of spermatozoa and proper alignment of spermatogonial cells. These results are in accordance with the observations of Leung and Wong (2013) who had reported enhanced germ cell population in amifostine exposed mice [16]. Jung *et al*. (2015) have revealed that Korean red ginseng protects against histological damage induced by busulfan treatment, the testis from normal mice had regular spermatogonia, primary/secondary spermatocytes, spermatids, spermatozoa, Sertoli cells in the seminiferous tubules and Leydig cells between the tubules [17]. Sharma *et al*. (2011) have reported that *Tinopsora cordifolia* showed a higher degree of recovery in germ cells and most of the testicular structure was reestablished, but certain alterations were left which includes vacuolization [18]. The results of the present study refutes the earlier findings of Gupta and Sharma (2003) who have studied that oral administration of *Tinopsora cordifolia* resulted in decrease in number of sperm count and germ cells in mature rats [19]. It became evident from the present study that BPA induced alterations in the normal structure of testis. While the administration of Vitamin E and *Tinospora cordifolia* ameliorates the toxic effect induced by BPA.

CONCLUSION

From the present study, it is concluded that even in nanomolar concentration BPA has a drastic effect on the reproductive system of mammals, resulting in impairment of spermatogenesis. Administration of Vitamin E and *Tinospora cordifolia* showed a significant recovery in normal structure of testis. It is recommended that Vitamin E and *Tinospora cordifolia* may be included in the diet to mitigate the toxic effects of plasticizers.

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