Introduction
In recent years, scientists have increasingly reported evidences that certain pollutants in the environment play important roles in contributing causes of fertility problems. A wide range of compounds, including components from pesticides to plastics, from detergents to cosmetics registered as pollutants. Bisphenol A (2, 2-bis (4-hydroxyphenyl) propane) (BPA), is the chemical found in polycarbonate plastics, leaches from plastic containers during normal usage, and detectable amounts can be found in many commercial food products and dental sealant (Goodson et al., 2004; Mounfort et al., 1997). It is reported that 10-20 μg of BPA in canned food is leached from the lacquer lining (Brontons et al., 1995). In the salivas of patients who had been treated with a dental sealant, 20-30 μg of BPA/mL was detected (Olea et al., 1996). The current reference dose [50μg/kg/day (U.S. EPA 1993)] determined by the U.S. Environmental Protection Agency (EPA) is 1/1000 of the level that exerts the lowest adverse effect (LOAEL; 50 mg/kg/day).

It is chronically ingested by humans, 95% of adults and children testes have detectable total urinary BPA (Matsumot et al., 2003; Calafat et al., 2008). BPA has also been measured in maternal serum and ovarian follicular fluid, as well as in fetal plasma and amniotic fluid, indicating passage across the placenta (Schonfelder et al., 2002; Ikezuki et al., 2002). BPA has both estrogenic and anti-androgenic effect (Akingbemi et al., 2004; Lee et al., 2001; Felder et al., 2002; Ikezuki et al., 2002). BPA has also been measured in maternal serum, amniotic fluid, indicating passage across the placenta (Schonfelder et al., 2002; Ikezuki et al., 2002). BPA has both estrogenic and anti-androgenic effect (Akingbemi et al., 2004; Lee et al., 2001; Felder et al., 2002; Ikezuki et al., 2002). BPA has also been measured in maternal serum, amniotic fluid, indicating passage across the placenta (Schonfelder et al., 2002; Ikezuki et al., 2002). BPA has both estrogenic and anti-androgenic effect (Akingbemi et al., 2004; Lee et al., 2001; Felder et al., 2002; Ikezuki et al., 2002). BPA has also been measured in maternal serum, amniotic fluid, indicating passage across the placenta (Schonfelder et al., 2002; Ikezuki et al., 2002). BPA has both estrogenic and anti-androgenic effect (Akingbemi et al., 2004; Lee et al., 2001; Felder et al., 2002; Ikezuki et al., 2002). BPA has also been measured in maternal serum, amniotic fluid, indicating passage across the placenta (Schonfelder et al., 2002; Ikezuki et al., 2002). BPA has both estrogenic and anti-androgenic effect (Akingbemi et al., 2004; Lee et al., 2001; Felder et al., 2002; Ikezuki et al., 2002). BPA has also been measured in maternal serum, amniotic fluid, indicating passage across the placenta (Schonfelder et al., 2002; Ikezuki et al., 2002). BPA has both estrogenic and anti-androgenic effect (Akingbemi et al., 2004; Lee et al., 2001; Felder et al., 2002; Ikezuki et al., 2002). Bisphenol A: Cause of Male Infertility

Ms.Priya Gupta
Department of Zoology, University of Rajasthan, Jaipur

Dr.Seema Srivastava
 Associate Professor Department of Zoology University of Rajasthan Jaipur * Corresponding Author

ABSTRACT
The aim of this study was to evaluate the effect of bisphenol A (BPA), a contaminant of resin-based dental composites and sealants, on the fertility of male adult wistar albino rats. Adult male albino rats of strain weighing approx. 150-200gm. were divided into four groups of 10 each. BPA (5, 50 and 100 μg /100gm/bw) was administered orally daily to the rats in the test groups and olive oil to the control group for 90 days. Male fertility was assessed periodically by mating each rat with two untreated females. There were significant reductions in the absolute weights of the testes and disrupted spermatogenesis was observed at 50 and 100μg dose level of BPA whereas at 5 μg dose level did not show any such significant effect. Epididymal epithelium degeneration was observed at 5, 50 and100μg dose level. Cauda sperm were found to be reduced in the 100μg dose treated animals. Reduction in the serum testosterone was also observed in treated animals. Study concludes that bpa affects reproduction with impaired fertility in male rats.

Material and Method
Adult male Wistar albino rats (Rattus norvegicus), 3 months old, weighing 150-200 grams, were used in present investigation. The animals were maintained in the Departmental Experimental Facility with light and dark (12h: 12h) schedule in individual polypropylene cage (size 43-27×15cm). Animals were fed with rat pellet diet and water ad libitam. The animals were maintained under perfect veterinary supervision and accordance to the guidelines of CPCSEA (CPCSEA, 2010).

Test Chemical
Bisphenol A (2, 2-bis (4-hydroxyphenyl) propane) (<99% pure) was purchased from sigma Aldrich. This compound was diluted in olive oil to obtain final concentration of the 5, 50 and 100μg/100gm body weight of the animals respectively.

Experimental Design
Animals were divided into four groups with ten in each and olive oil was used as vehicle.

Group I: Control (vehicle treated)
Group II: Oral administration of 5μg BPA/100 g/bw
Group III: Oral administration of 50μg BPA/100 g/bw
Group IV: Oral administration of 100μg BPA/100 g/bw

Doses were given for consecutive 90 days. On 91th day of experiment, animals were sacrificed by overdose of anesthetic ether.

Body and reproductive organ weight:
The body weights of all animals were measured fortnightly. The weights of all reproductive organs of all animals will be obtained at the time of scarification schedule of each group.

Fertility Test:
The treated adult males were mated with the normal adult females. Each cage contains one male and two female. Mating was confirmed by visual appearances and presence of spermatozoa in the vaginal smear. Following mating pregnant animals will be allowed to complete the term and pregnancy.

Histopathology:
A portion of reproductive organs testis, epididymis, were fixed in 4% paraformaldehyde, dehydrated in ethanol, cleared in Xylene and embedded in paraffin wax. Five micron thick sections were stained with haematoxylin and eosin for light microscopic observation.
Hormone analyses:
Circulatory levels of testosterone in all treated group of rats exposed to 5μg BPA/100 g/bw, 50μg BPA/100 g/bw and 100μg BPA/100 g/bw and control group of rats which were vehicle treated assayed by ELISA kit.

Statistical analysis:
The mean values will be compared using respective standard error of mean followed by statistical comparison between control and test groups. The significance of differences among means was evaluated using one-way ANOVA. Differences at $P < 0.05$ were considered statistically significant.

Results

Body and Reproductive organ weight
Weights of all animals were within the normal range. No significant alteration was observed in the body weight of treated animals at different doses of BPA as compared to control. Reductions in reproductive organ weight were observed. No significant alteration was observed in group I i.e. control animals. Non significant decline in the weight of testis and epididymis was observed in Group II and III whereas Group IV animals treated with 100μg BPA/100 g/bw showed a significant alteration. (Table 1) (Figure 1 a and 1b)

<table>
<thead>
<tr>
<th>Treatment (For 90 days)</th>
<th>Initial Body weight (gms.)</th>
<th>Final Body weight (gms.)</th>
<th>Reproductive organs weight (mg/100 gm.)</th>
<th>Testosterone level(ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Testis</td>
<td>Cauda epididymus</td>
</tr>
<tr>
<td>Group I</td>
<td></td>
<td></td>
<td>control (Vehicle Treated)</td>
<td>120± 1.66</td>
</tr>
<tr>
<td>Group II</td>
<td>5μg/100g/bw/day</td>
<td>113.33±5.77</td>
<td>10±1.66</td>
<td>1498± 33.02</td>
</tr>
<tr>
<td>Group III</td>
<td>50μg/100g/bw/day</td>
<td>115±5.77</td>
<td>10±1.66</td>
<td>1139 ±50.69</td>
</tr>
<tr>
<td>Group IV</td>
<td>100μg/100g/bw/day</td>
<td>142.5±5.77</td>
<td>10±1.66</td>
<td>1050±71.62*</td>
</tr>
</tbody>
</table>

($P<0.05$) Group II, III & IV Compared with Group I

Fertility test
Fertility test was performed every 15 days and we found that the treated rats mated normally to the females and they began to mount within 30 min and ejaculate after insertions. The females delivered normal pups. The litter numbers were 5 to 6.

Influence of BPA on spermatogenesis
Histologically group II, III and IV animals showed altered spermatogenesis as observed by reduction in the number of round spermatids, elongated spermatids and no spermatids as compared to control, in the three treated groups respectively. (fig:2)

Effect on epididymis
Histologically group II, III and IV animals showed degenerative changes as observed by altered epithelium and almost nil sperms in the treated animals of group IV. (fig:3)

Effect on testosterone level
The levels of serum testosterone were lower in the BPA treated rats than those in the control rats in all the three groups of treated animals compared to control. (fig: 4) These results indicate that the reduction in testosterone levels by BPA arrests the process of spermatination.

Figure 4 Level of testosterone in BPA treated groups
mal epithelium, (L) denotes lumen of the epididymis
day and 100 μg/100g/day, respectively. (E) denotes epididy-
treatment. A: Control

Figure 2: Photomicrograph showing histological changes in
testis of rats after 90 days of bisphenol A treatment. A: Control; B1-epididymis (R) denotes round spermatid, (E) de-

Figure 3a: Photomicrograph showing histological chang-
es in caput epididymis of rats after 90 days of bisphenol A
treatment. A: Control; B (-) D: 5μg/100g/day, 50 μg/100g/
day and 100 μg/100g/day, respectively. (E) denotes epididy-
mal epithelium, (L) denotes lumen of the epididymis

Figure 3b: Photomicrograph showing histological changes
in corpus epididymis of rats after 90 days of bisphenol A
treatment. A: Control; B (-) D: 5μg/100g/day, 50 μg/100g/
day and 100 μg/100g/day, respectively. (E) denotes epididy-

Figure 3c: Photomicrograph showing histological changes
in cauda epididymis of rats after 90 days of bisphenol A treat-
ment. A: Control; B (-) D: 5μg/100g/day, 50μg/100g/day and
100μg/100g/day, respectively. (E) denotes epididymal epi-
thelium, (L) denotes lumen of the epididymis

Discussion
The testes are most vulnerable organ to measure the fertility ef-
flect. Changes in homeostasis of hormonal level and disrupted
spematogenesis process show the toxicity of the compound on
fertility. In our study decrease in serum testosterone level was
observed in rats exposed to BPA which indicates that sperma-
togenesis also get disrupted. Histological examination of different
regions of testes shows the slowing down of spermatogenesis process and epididymal epithelium show degenerative changes. Lesser
amount of sperms in cauda epididymis indicates that sperm count also gets reduced Consistent with our results, Tohei et al., 2001
and Mendiola et al., 2010 reported that exposure of adult rats to BPA decreased the plasma concentration of testoster-
one. Wisniewski et al., 2015 also reported that exposure of BPA
at 5mg and 50mg/kg/day dose level for 60 days decreased the
plasma level of testosterone in adult male rats which effect the
sperm concentration.

The doses in our study are selected with human applicability in
mind. Studies suggest that an adult is exposed to low concen-
trations of BPA, no more than 0.4–1.5 μg BPA/kg bw/day (Lak-
ind JS, et al., 2008).The environmentally human-relevant dose of BPA in rodent experiments is considered as 20 μg/kg bw/
day(Susiarjo M, et al., 2007). Although the dose of BPA (5, 50
and 100μg/100 bw/day) that induced significant reproductive impairment in our study cannot be considered truly environ-
mentally relevant, it can be considered low. Based on the avail-
able data, a conference sponsored by the National Institute of
Environmental Health Sciences in 2007 predicted that internal
BPA exposure (plasma or serum concentrations) in humans is
435 μg/day (500μg/kg/day) (Vandenberg LN, et al., 2007). More-
over, the actual exposure level may be much higher than the
current accepted level in some countries or areas (Huang YQ
et al., 2012). The effects of bpa have been documented in vari-
ous previous studies using short term exposure and by inject-
ing directly. The documented action of bpa includes orally in-
gested 25 and 100 μg kg⁻¹ BPA for 28 days showed a significant
reduction in testicular sperm counts and in the efficiency of
sperm production(Al-Hiyasat,2002). Reduced sperm count and
epididymal epithelium degeneration in Wistar rat at 20μg/kg /
day dose level in 60 days exposure study was observed. (Chitra,
Latchoumycandane, & Mathur, 2003). Similarly, our result also
shows these effects on 5 μg, 50 μg and 100μg/100gm bw/day
dose level in 90days exposure. In another study oral administra-
tion of low dose of BPA (2μg/kg) for consecutive 14 days in adult
rats also impairs spermatogenesis (Pengpeng Jin,et al.,2013) but
in our study at 5μg dose this effect is less. At 50μg and100μg
dose level BPA affect spermatogenesis process more. In rodent
testes, the multiple types of germ cells are arranged in charac-
teristic cellular associations that succeed each other in a given
area of the seminiferous tubule, which are known as the stages
of spermatogenesis. Their succession in time has been defined as
the cycle of the seminiferous epithelium. These cycle of spermat-
ogenesis are essential for continuous sperm production, which
is dependent upon numerous factors, both intrinsic (sertoli cell
and germ cell) and extrinsic (androgen and retinoic acid) as well
as species specific (Rex A, Hess et al., 2008).

Interestingly, in our study, the BPA treated groups at 5 μg
dose level lesser no. of round spermatid were observed where as at
50 μg dose level the whole lumen of seminiferous tubule filled
with cell debris and round spermatid showed a higher frequency
of stage VII and a lower frequency of stage VIII compared with
control animals, suggesting a possible delay in the progression
of spermatogenesis, which takes place at stage VIII. These results are consistent with previous studies showing that environmental toxin-induced lower daily sperm production and this sperm count reduction is highly associated with the disruption of spermatogenesis. The sex hormones important for controlling the process of spermatogenesis were also altered after BPA treatment but some studies also reported that at 2, 20 and 200μg/kg/d orally administered dose given for 60 days, any significant decrement in testes weight of Wistar rats not observed (C Liu, W Duan et al., 2013). During the treatment of different doses of BPA the body weight of rat did not affected but the weight of testes and cauda epididymis got decreased which was also reported in earlier studies by short term exposure treat-ment.

In conclusion, results from present study demonstrate that BPA has an adverse effect on testicular spermatogenesis at different dose level when exposed orally for long duration of 90 days. BPA also suppressed the level of testosterone hormone which is secreted by Leydig cells and also degenerate the epididymal epithelium.

REFERENCE