



ISSN:2456-9739

Available Online at <http://www.bjbmr.org>

BRITISH JOURNAL OF BIO-MEDICAL RESEARCH

Cross Ref DOI: <https://doi.org/10.24942/bjbmr.2021.831> Volume 05, Issue 04, July - August 2021

Research Article

Toxic Effect Of Bisphenol A On Human Spermatozoa And Its Amelioration By Quercetin: In Vitro Study

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ARTICLE INFO

Article History:

Received on 11th July 2021
Peer Reviewed on 19th July 2021
Revised on 16th August 2021
Published on 27th August 2021

Keywords:

Bisphenol, In Vitro, xenoestrogen, spermatotoxic, Male reproductive health

ABSTRACT

Bisphenol A is a well-known xenoestrogen, present in large number of household products. Experimental studies suggests that BPA causes negative health effect in humans and animals. Quercetin, a flavonol occurring in fruit and vegetables is a food component with proven beneficial impact on health. Semen sample were treated with bisphenol A and its effects were observed on sperm functional parameters. Aim of present study was to analyze the dose dependent and time dependent effect of bisphenol A on human spermatozoa. Sperm suspensions were prepared from Semen collected from 10 healthy adult donors. BPA treated tubes containing 0.5 ml of sperm suspension and different concentrations of BPA (25-200 µg/ml). In another experiment different concentrations of quercetin (0-30 µg/ml) along with BPA (200 µg/ml) added in sperm suspensions. BPA treatment shows dose dependent and time dependent reduction in motility and viability of spermatozoa. Quercetin administration shows amelioration in the sperm parameters. The outcomes conclude that BPA have spermatotoxic effect in *in vitro* condition which can be mitigated by quercetin.

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INTRODUCTION

Male reproductive health has deteriorated significantly in the recent years. Environmental contaminants are regarded as the underlying cause of reduced male fertility. Bisphenol A (BPA) is a well-known xenoestrogen. It is produced in large quantities for use primarily in the production of polycarbonate plastics and epoxy resins (Ballesteros-Gómez *et al.*, 2011; Hoekstra *et al.*, 2013). Due to the broad spectrum of use of BPA to manufacture products used in many applications, it has been speculated that human exposures to BPA may be widespread and it has been postulated that these exposures may reach high levels (Tsai, 2006; Ranjit *et al.*, 2010; Vandenberg *et al.*, 2013; Rochester *et al.*, 2013). It is mainly used in plastics and food can liners manufacture. Hence, it becomes an integral part of the food chain. Food is acknowledged to be a foremost source of exposure to BPA as it migration from food containers. (Biles *et al.*, 1997; Ballesteros-Gómez *et al.*, 2009; Schechter *et al.*, 2010).

Quercetin, a flavonol occurring in fruit and vegetables is a food component with proven beneficial impact on health. Various studies suggests that quercetin could be a substantially promising organoprotective agent against toxic effects and perhaps against other toxic metal chemicals or drugs (Hamza *et al.*, 2015; Kumar *et al.*, 2016; Kocahan *et al.*, 2017).

The present study was undertaken to investigate the dose dependent and time dependent effect of bisphenol A on human spermatozoa and its amelioration using quercetin.

MATERIALS AND METHODS

Sample Preparation

Semen samples were collected in vials from 10 healthy adult donors (age 23-25 years). For this *in vitro* study, semen samples with sperm counts above 50 million/ml with normal morphology, rapid, linear, progressive motility and viability above 90% were considered.

Effect of BPA on spermatozoa:

After liquefaction semen samples were used for preparation of sperm suspension in PBS. To

study the effect of BPA on spermatozoa following sets of tubes were prepared.

1. Control tubes containing 0.5ml sperm suspension
2. BPA treated tubes containing 0.5 ml of sperm suspension and different concentrations of BPA (25-200 µg/ml).

Final volume was made up to 1.0 ml with addition of normal saline and incubated at 37°C for 60 min. Sperm motility was determined by counting both motile and non-motile spermatozoa in at least 10 separate and randomly selected fields as mentioned earlier. Motility was measured at different time intervals (0, 15, 30, 45, 60 min). Percent motility was calculated and expressed.

Sperm viability (using trypan blue) was determined by counting live and dead spermatozoa in at least 10 separate and randomly selected fields as mentioned earlier. Viability was measured at different time interval (0, 15, 30, 45, 60 min). Percent viability was calculated and expressed.

Ameliorative effect of quercetin on BPA induced toxicity on sperm suspension

Sperm suspensions were prepared as explained earlier. Dose of BPA is selected on the bases of results of set A.

Following sets of tubes were prepared for the ameliorative study;

1. Control tubes containing 0.5ml sperm suspension
2. Antidote control tubes containing 0.5 ml sperm suspensions and quercetin (30 µg/ml)
3. Different concentrations of quercetin (0-30 µg/ml) + BPA (200 µg/ml) + 0.5 ml of sperm suspensions.

The volume of each tube was made up to 1.0 ml with additional saline in order to have the required concentrations and proceed as mentioned earlier.

Statistical analysis:

The results were expressed as the mean \pm S.E.M. The data were statistically analyzed using one way analysis of variance (ANOVA) followed by Tukey's test in Graph pad prism 6 (Graph pad, software, USA). Statistical significance was accepted with $p < 0.05$. Correlation coefficient was measured to estimate the strength of linear association between two variables. Pearson's correlation analysis was used to find the correlation between dose administration and alteration in biochemical parameters in liver and serum of mice.

RESULTS**Toxic effect of BPA on spermatozoa.**

Semen sample were treated with bisphenol A and its effects were observed on sperm

functional parameters. Toxic effect of BPA was seen in spermatozoa. Addition of BPA (0-300 $\mu\text{g}/\text{mL}$) to sperm suspension caused significant ($p < 0.05$) decrease in sperm motility *in vitro* as compared with control that is shown in table 1. This decrease was concentration-dependent ($r = 0.952, 0.992, 0.991, 0.997, 0.998, 0.996$ respectively) as well as time-dependent ($r = 0.995, 0.994, 0.998, 0.998, 0.997, 0.992$, respectively). Similarly, addition of BPA to sperm suspension caused significant decrease in sperm viability as compared with control which was concentration -dependent ($r = -0.966, -0.985, -0.899, -0.860, 0.969, 0.957$ respectively) as well as time-dependent ($r = 0.995, 0.992, 0.950, 0.976, 0.988, 0.947$, respectively) (Table 2).

Table 1: Bisphenol A-induced changes in motility (%) of human spermatozoa

Groups	BPA Conc. ($\mu\text{g}/\text{ml}$)	Duration of treatment (min)					
		10	20	30	40	50	60
Control							
Control	0	88.25 \pm 0.48	86.75 \pm 0.47	86.25 \pm 0.63	85.50 \pm 0.28	83.50 \pm 0.64	82.25 \pm 0.85
Vehicle control	0	87.00 \pm 0.40	87.00 \pm 0.47	84.75 \pm 0.25	85.00 \pm 0.58	83.00 \pm 0.50	82.75 \pm 0.25
Bisphenol A- treated							
1	10	75.50 \pm 1.4 ^a	75.50 \pm 0.28 ^a	69.00 \pm 0.4 ^a	93.00 \pm 0.40 ^a	61.0 \pm 0.40 ^a	54.25 \pm 0.85 ^a
2	50	59.75 \pm 0.2 ^a	59.75 \pm 0.58 ^a	44.50 \pm 1.47 ^a	39.25 \pm 0.65 ^a	37.25 \pm 1.11 ^a	35.25 \pm 0.95 ^a
3	100	49.50 \pm 0.4 ^a	43.50 \pm 2.52 ^a	29.50 \pm 0.64 ^a	24.75 \pm 0.62 ^a	21.00 \pm 1.29 ^a	16.50 \pm 0.64 ^a
4	150	39.50 \pm 0.6 ^a	33.50 \pm 0.82 ^a	24.75 \pm 0.47 ^a	14.00 \pm 1.29 ^a	8.75 \pm 0.94 ^a	4.750 \pm 0.47 ^a
5	200	30.25 \pm 0.8 ^a	20.75 \pm 1.03 ^a	14.50 \pm 0.64 ^a	7.25 \pm 0.47 ^a	3.25 \pm 0.63 ^a	1.25 \pm 0.75 ^a
6	250	24.25 \pm 1.6 ^a	16.50 \pm 1.32 ^a	8.75 \pm 1.75 ^a	4.75 \pm 0.48 ^a	2.50 \pm 1.01 ^a	0.00
7	300	19.00 \pm 0.9 ^a	7.50 \pm 0.64 ^a	3.00 \pm 0.41 ^a	0.00	0.00	0.00

Sperm viability was 93.50% at 0 min.

Values are mean \pm S.E.M., n=10

Level of significance ^a $p < 0.05$, as compared to vehicle control

Ameliorative effect of Quercetin on BPA induced spermatotoxicity

Treatment of 200 $\mu\text{g}/\text{ml}$ BPA in sperm suspension shows maximum reduction in sperm motility and viability. Addition of different concentrations of quercetin along with

BPA shows retardation in toxic effect of BPA. The effect was dose-dependent.

Table 3 shows, addition of quercetin (0-30 $\mu\text{g}/\text{ml}$) along with BPA (250 $\mu\text{g}/\text{ml}$) significantly ameliorates BPA-induced reduction in sperm motility as compared to treated. This ameliorative effect was dose-

dependent ($r=0.992, 0.911, 0.765, 0.997, 0.998, 0.996$ respectively) as well as time-dependent ($r= 0.916, 0.857, 0.827, 0.998, 0.983, 0.992$ respectively).

Similarly, quercetin (0-30 $\mu\text{g/ml}$) also ameliorates BPA (250 $\mu\text{g/ml}$)-induced

reduction in sperm viability as compared to treated. This ameliorative effect was dose-dependent ($r= 0.965, 0.988, 0.992, 0.995, 0.992, 0.987, 0.993$ respectively) and time-dependent ($r=-0.900, 0.981, 0.998, 0.997, 0.990, 0.998$ respectively) (Table 4).

Table 2: Bisphenol A-induced changes in viability (%) of human spermatozoa

Groups	BPA conc ($\mu\text{g/ml}$)	Duration of treatment (min)					
		10	20	30	40	50	60
Control	0	90.75 \pm 0.48	89.00 \pm 1.29	87.75 \pm 1.62	87.00 \pm 1.29	85.75 \pm 1.65	84.00 \pm 0.91
Vehicle control	0	91.25 \pm 0.47	89.25 \pm 1.79	87.25 \pm 0.85	86.25 \pm 0.85	85.25 \pm 1.62	84.25 \pm 1.32
1	10	77.25 \pm 0.85 ^a	73.75 \pm 0.47 ^a	69.50 \pm 0.64 ^a	64.50 \pm 0.65 ^a	64.75 \pm 0.48 ^a	60.25 \pm 0.27 ^a
2	50	69.75 \pm 0.85 ^a	64.50 \pm 0.29 ^a	61.75 \pm 0.75 ^a	59.25 \pm 0.48 ^a	51.25 \pm 0.47 ^a	39.50 \pm 0.65 ^a
3	100	59.75 \pm 0.85 ^a	57.75 \pm 0.49 ^a	52.25 \pm 0.85 ^a	41.00 \pm 0.41 ^a	31.75 \pm 0.85 ^a	21.50 \pm 0.64 ^a
4	150	52.00 \pm 0.41 ^a	47.25 \pm 0.47 ^a	37.00 \pm 0.40 ^a	30.75 \pm 0.48 ^a	21.00 \pm 0.71 ^a	10.25 \pm 0.85 ^a
5	200	38.25 \pm 1.18 ^a	35.25 \pm 0.68 ^a	29.75 \pm 0.85 ^a	16.25 \pm 0.48 ^a	7.00 \pm 0.70 ^a	0.00 \pm 0.00 ^a
6	250	27.75 \pm 0.85 ^a	20.50 \pm 1.04 ^a	9.00 \pm 0.4 ^a	5.25 \pm 0.47 ^a	0.60 \pm 0.07 ^a	0.00 \pm 0.00 ^a
7	300	27.25 \pm 0.85 ^a	11.25 \pm 0.47 ^a	6.25 \pm 0.48 ^a	2.00 \pm 0.20 ^a	0.20 \pm 0.04 ^a	0.00 \pm 0.00 ^a

Sperm viability was 94.00% at 0 min.

Values are mean \pm S.E.M., n=10

Level of significance ^ap<0.05, as compared to vehicle control

Table 3: Ameliorative effect quercetin on bisphenol A induced changes in motility of human spermatozoa.

Groups	Concentration (ml)		Duration of treatment (min)					
	Bisphenol A	Quercetin	10	20	30	40	50	60
Control								
Control	0	0	88.00 \pm 0.58	86.50 \pm 0.50	85.75 \pm 0.25	85.25 \pm 0.25	83.25 \pm 0.48	82.00 \pm 0.91
Vehicle control	0	0	86.50 \pm 0.64	85.50 \pm 2.38	84.25 \pm 0.75	84.00 \pm 1.41	82.75 \pm 0.85	82.25 \pm 0.47
Antidote Control	0	30	88.00 \pm 0.57	86.5 \pm 0.64	85.75 \pm 0.25	84.75 \pm 0.94	83.25 \pm 0.75	82.00 \pm 0.82
Bisphenol A- treated								
BPA	250	0	24.00 \pm 1.87 ^a	16.25 \pm 1.43 ^a	8.50 \pm 1.70 ^a	5.00 \pm 0.70 ^a	1.75 \pm 0.62 ^a	00
Bisphenol A +quercetin –treated								
1	250	10	49.75 \pm 0.85 ^b	44.25 \pm 1.10 ^b	39.00 \pm 1.68 ^b	31.75 \pm 1.31 ^b	19.75 \pm 1.93 ^b	10.25 \pm 0.85 ^b
2	250	20	69.00 \pm 1.68 ^b	59.25 \pm 2.49 ^b	54.50 \pm 1.55 ^b	51.25 \pm 1.25 ^b	47.25 \pm 0.85 ^b	39.50 \pm 1.71 ^b
3	250	30	87.25 \pm 1.10 ^b	86.25 \pm 0.85 ^b	84.25 \pm 1.31 ^b	82.50 \pm 1.32 ^b	82.00 \pm 1.08 ^b	81.75 \pm 1.54 ^b

Sperm viability was 93.00% at 0 min.

Values are mean \pm S.E.M., n=10

Level of significance ^ap < 0.05 as compared to vehicle control

^bp < 0.05 as compared to BPA-treated (250 $\mu\text{g/ml}$)

Table 4: Ameliorative effect of quercetin on bisphenol A-induced changes in viability (%) of human spermatozoa.

Groups	Concentration (µg/ml)		Duration of treatment (min)					
	Bisphenol A	Quercetin	10	20	30	40	50	60
Control								
Control	0	0	91.50±0.64	91.00±0.41	88.25±1.43	86.25±0.85	85.00±1.78	84.75±1.37
Vehicle control	0	0	91.25±0.75	89.50±0.86	87.50±1.70	86.75±1.37	85.25±0.48	84.25±0.48
Antidote Control	0	30	91.50±0.29	89.00±1.47	84.75±1.88	86.00±1.08	85.25±1.31	84.25±0.91
Bisphenol A- treated								
BPA	250	0	27.00±0.91 ^a	19.75±1.18 ^a	9.25±0.85 ^a	5.12±0.96 ^a	0.52±0.11 ^a	0.00
Bisphenol A +quercetin –treated								
1	250	10	54.50±2.33 ^b	44.25±0.85 ^b	36.75±1.49 ^b	29.25±1.49 ^b	24.50±1.26 ^b	16.50±1.04 ^b
2	250	20	74.00±3.91 ^b	65.00±1.08 ^b	60.25±2.05 ^b	53.75±1.49 ^b	48.25±2.68 ^b	43.75±1.31 ^b
3	250	30	90.00±1.41 ^b	88.25±0.85 ^b	85.50±1.19 ^b	84.25±1.31 ^b	83.25±0.94 ^b	82.50±0.65 ^b

Sperm viability was 93.75% at 0 min.

Values are mean ± S.E.M., n=10

Level of significance ^ap < 0.05 as compared to vehicle control

^bp < 0.05 as compared to BPA-treated (250 µg/ml)

DISCUSSION

In recent decades men are being exposed to various toxicants which can reduce fertility. Experimental studies suggest xeno-estrogens are one of them. Bisphenol is known for having its toxic effect on vital organs in animal study. Various experimental study shows that BPA generates ROS in *in vivo* condition.

Present study reveal that BPA exposure caused dose dependent and time dependent reduction in sperm motility as well as viability. Overproduction of ROS can be detrimental to sperm. The sperm plasma membrane contains a high amount of unsaturated fatty acids. Therefore, it is particularly susceptible to peroxidative damage (Tirado et al., 2016; Guvvala et al., 2016). If spermatozoa are exposed to excessive levels of ROS then their fertilizing capacity and genetic integrity could be compromised (Aitken et al., 2014; 2016).

According to our study oral administration of BPA for 45 days causes reduction in sperm motility, viability as well as sperm count. Various biochemical studies have demonstrated that, apart from furnishing an osmotically

balanced ambiance for the spermatozoa, the seminal plasma also contains substances such as sugars, enzymes, hormones, inorganic ions and polyamines (Chitra et al., 2003; Lahnsteiner et al., 2005). The function of these substances in relation to sperm transport and motility is still speculative (Vom Saal et al., 1998; Prins et al., 2008).

In present *in vitro* study, BPA shows significant dose dependent reduction in sperm functional parameters. Various experimental studies suggests that BPA affects mitochondrial enzymes and mitochondrial dysfunction may affect vital testicular functions including sperm production. Moreover, it caused significant (p<0.05), dose-dependent decrease in sperm motility and sperm viability as compared to control groups. Similar observations have also reported by various investigators (Hass et al., 2016; Shi et al., 2017). Unfavorable condition for sperm leads to deterioration of the fertility rate (Mínguez-Alarcón et al., 2016).

Our previous *in vivo* study (Samova et al., 2016) concluded that BPA caused concentration-dependent morphological

abnormalities in sperm such as swollen head, round head, bent neck, swollen mid piece, decapitation, coiled tail, tail deformities, head-head agglutination, tail-tail agglutination and head-tail agglutination in mice spermatozoa (Hsu et al., 2016; Othman et al., 2016).

In Silico Study revealed that quercetin reduces BPA induced alteration in steroidogenesis. (Samova et al., 2018) Quercetin owing to its specific chemical structure, counteracts oxidative stress generated as a result of reactive oxygen species (ROS), which contributes to the genesis of various negative health effects. Quercetin is natural antioxidant present in various fruits and vegetables. Various experimental showed that quercetin is beneficial against ROS toxicity.

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How to cite this article:

Sanman Samova, Hetal Doctor and Ramtej Verma Toxic Effect Of Bisphenol A On Human Spermatozoa And Its Amelioration By Quercetin: In Vitro Study Br J Bio Med Res , Vol.05, Issue 04, Pg.1742 - 1748, July - August 2021. ISSN:2456-9739 Cross Ref DOI : <https://doi.org/10.24942/bjbmr.2021.831>

Source of Support: Nil

Conflict of Interest: None

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