Possible therapeutic effects of Quercetin against Bisphenol A induced testicular damage in Male Sprague Dawly Rats: A histological study

By

Qurat-ul-Ain

DEPARTMENT OF ANIMAL SCIENCES

FACULTY OF BIOLOGICAL SCIENCES

QUAID-I-AZAM UNIVERSITY

ISLAMABAD

2015

Possible therapeutic effects of Quercetin against Bisphenol- A induced testicular damage in Male Sprague Dawly Rats: A histological study

A dissertation submitted in the partial fulfillment of the requirements for the Degree of Master of Philosophy

IN

ANIMAL SCIENCES

(REPRODUCTIVE PHYSIOLOGY)

By

Qurat-ul-Ain

DEPARTMENT OF ANIMAL SCIENCES

FACULTY OF BIOLOGICAL SCIENCES

QUAID-I-AZAM UNIVERSITY

ISLAMABAD

2015

“In the Name of ALLAH, the most Beneficent, the most Merciful”

Dedicated to My Parents and Family members

Who have given me the opportunity to

Study from the best institutions and

Support throughout my life.

DECLARATION

I hereby declare that the work presented in the following thesis is my own effort and the material contained in this thesis is original. I have not previously presented any part of this work elsewhere for any other degree.

Qurat-ul-Ain

CERTIFICATE

This dissertation submitted "Possible therapeutic effects of Quercetin against Bisphenol A induced testicular damage in Male Sprague Dawly Rats: A histological study" by **Qurat-ul-Ain** is accepted in its present form by the Department of Animal Sciences, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad as satisfying the thesis requirements for the degree of Master of Philosophy in Reproductive Physiology.

Supervisor: _____

Dr. Sarwat Jahan

External Examiner: _____

Chairman: _____

Dr. Irfan Zia Qureshi

Dated:

List of Contents

List of Tables

List of Figures

List of Abbreviations

BPA	Bisphenol A
µg	Microgram
µm	Micrometer
ng	Nano gram
µl	microliter
dL	Deciliter
µmol	Micro molar
mmol	Mili molar
KU	Kilo unit
TCDD	2,3,7,8-Tetrachlorodibenzo-p-Dioxin
ELISA	Enzyme linked immunosorbent assay
Fe^{2+}	Ferrous ion
Cu^{2+}	Cuprous ion
H_2O_2	Hydrogen peroxide
ROS	Reactive oxygen specie
HRP	Horse radish peroxide
TMB	Tetra methyl benzidin
LH	Luteinizing hormone
FSH	Follicle stimulating hormone

ACKNOWLEDGEMENTS

*All praises to **Almighty Allah**, Who puts the sun's seal on the tablets of the flowing waters and throws clouds to the skies, Who distills the waters of the clouds over the seas to conceive the pearl in the womb of the oyster, Who creates fire in every stone, colour in the fire, radiance in the colour, Who gives voices to the dust, word to the voices, and life to the world, Who created us as a Muslim and blessed us with knowledge to differentiate between right and wrong. All prays to Him as He blessed us with the **Holy Prophet, Hazrat Muhammad** (SAW) for whom the whole universe is created and who enabled us to worship only one God. He (SAW) brought us out of darkness and enlightened the way of heaven.*

*It is a matter of great pleasure to express my sincere regards to my honorable Supervisor Assistant Professor **Dr. Sarwat Jahan**, Department of Animal Sciences for her affectionate supervision, inspiring attitude, masterly advice and encouragement. Without her useful intellectual suggestions, it would have been impossible for me to complete this tedious work. I would like to extend my thanks to **Dr. Irfan Zia Qureshi,** Chairman, Department of Animal Sciences, for providing me the opportunity and making the department facilities available*

*I am really thankful to my senior lab fellows Ayesha and special thanks to **Hizbullah Wazir** for special help during my research work. I wish to extend my greatest appreciation gratitude and thanks to my seniors **Gul Rukh and Sidra Alvi**. I will never forget their honest efforts, valuable Suggestions about my work. I have special thanks for my batch fellows **Sara Anum, Benish Farooq and Asma Ahsan** for their affectionate efforts, support, encouragement and cooperation during my research work.*

Qurat-ul-Ain

Abstract

Bisphenol A (BPA) is well known environmental toxicant and endocrine disruptor that can mimic endogenous estrogenic activity. BPA is monomer and used in manufacturing of food packing material and plastic products. It may be leached from walls of food cans into circulating blood and can cause cytotoxic effect. The present study was designed to investigate possible protective effect of quercetin against BPA induced reproductive toxicity. Twenty adult male Sprague Dawley rats (75-90 days old, 190-250g) were divided into four groups. First group served as control and was provided with normal saline. Second group of rats was treated with 50 mg/kg of BPA dissolved in normal saline. Third group was provided with oral gavage of 50 mg/kg quercetin while fourth group was treated with quercetin (50 mg/kg) along with BPA (50 mg/kg). All the treatments were carried out for 52 days. Rats were dissected and their testicular tissues and epididymis were used for histology while blood plasma was used for hormonal and biochemical analysis. Results of present investigation revealed adverse morphological and histopathological changes in rat testis and epididymis after BPA administration. These degenerative changes include significant decrease in seminiferous tubule diameter and epithelial height and increased luminal diameter. In addition, number of spermatogonia, primary spermatocytes, secondary spermatocytes and spermatids were significantly reduced ($p<0.001$) due to impaired spermatogenesis. Furthermore, BPA treatment significantly reduced ($p<0.05$) plasma testosterone level (ng/ml) while elevated estrogen level (ng/ml). Similarly, this also caused significant increase ($p<0.001$) in blood urea nitrogen (BUN) levels, serum creatinine levels as well as in lipid profile. Interestingly, these harmful effects of BPA were ameliorated by quercetin administration. Quercetin treatment resulted in restoration of spermatogenesis, reversal of histological damages and marked increase in plasma testosterone concentration along with decrease in estrogen concentration. Similarly, BUN and creatinin levels were reversed when cotreated with quercetin. Lipid profile was also normalized when treated with quercetin. In conclusion, present findings suggest that BPA administration induce toxic effects on testis and epididymis, decrease in germ cell numbers, imbalance plasma

hormonal levels and lipid profile while quercetin amended those toxic effects by restoring normal spermatogenesis, testicular tissue damage and hormonal level. Hence, it was suggested that quercetin can be used as potential therapeutic drug against BPA induced testicular toxicity.

Introduction

It is well accepted that environmental contaminants including synthetic industrial and biomedical chemicals disrupt male reproduction of animals and humans. These chemicals are known to decline the quality as well as quantity of human semen (Colborn *et al*., 1993). Among these chemicals, bisphenol A [BPA, 2, 2-bis (hydroxyphenyl) propane] is produced in one of the largest volume.

Bisphenol A (BPA) is made by acetone and phenol (Figure 1). BPA is a monomer used in the manufacturing of epoxy resins (e.g. food packaging and can-coating), polycarbonate and numerous plastic products (including baby bottles, water storage and supply pipes), dental devices and dental sealants (Vandenberg *et al*., 2009; Fleisch *et al*., 2010). Approximately 3 million tons of BPA is produced annually and almost 100 tons of BPA is entered into environment each year (Vandenberg *et al*., 2009).

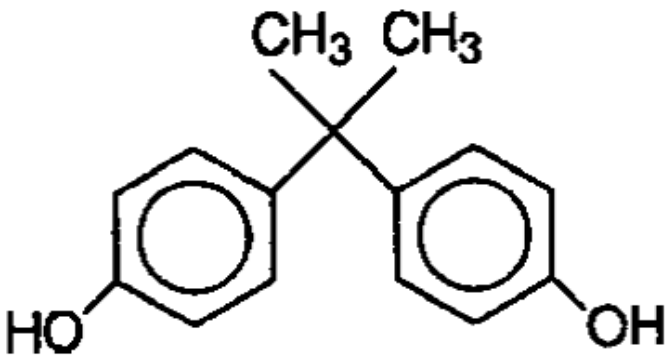

Fig 1. Chemical structure of Bisphenol A

BPA compound may be released from polycarbonate flask during autoclaving (Krishnan *et al*., 1993), from dental sealant into saliva (Chung *et al*., 2012) and from walls of food cans into water or food (Geens *et al*., 2012), reach circulating blood and can cause genotoxic and cytotoxic effects (Verma and Sangai 2009). For example, it is reported that from canned food 10- 20 µg of BPA/can was leached from lacquer lining, while 20- 30 µg of BPA/ml was detected in saliva of those patients who were treated with dental sealant (Brotons *et al*., 1995; Olea *et al*., 1996). Therefore, it is possible that

even low doses of BPA could affect development and reproduction (Talsness *et al*., 2000; Welshons *et al*., 2006).

The potential of BPA to cause adverse effects on human health is highly documented (Rochester 2013; Rezg *et al*., 2014). The detection of BPA in urine has been confirmed in humans (0.3–7.9 µg/day) (Takeuchi and Tsutsumi 2002; Fujimaki *et al*., 2004; Calafat *et al*., 2005). Similarly, BPA has been found in the human blood, serum of pregnant women (1–2 ng/ml), amniotic fluid (8.3–8.7 ng/ml), fetus blood (0.2–9.2 ng/ml), placental tissue (1.0–104.9 ng/g), and milk of lactating mothers (1.1 ng/l) (Ikezuki *et al*., 2002; Schönfelder *et al*., 2002; Ye *et al*., 2005), suggesting that the human fetus is freely exposed to BPA during pre- and postnatal development.

BPA exhibits estrogenic hormone-like properties which can disrupt endocrine system and may lead to negative health effects (Staples *et al*., 1998). It is evident that the early stage of mammalian life is the most sensitive period of the life to BPA effects (vom Saal *et al*., 2007).

BPA shows acute, short-term, and subchronic toxicity (Tyl *et al*., 2002; Tyl 2008). Some studies recognized BPA effect on the kidney, liver, and body weight at doses of 50 mg/kg and higher (Tyl 2008). Many experimental studies revealed possible association between BPA exposure and the development of cancer (e.g. prostate and mammary gland cancer), metabolic complications and disorders of reproductive, neuroendocrine and immune systems (Richter *et al*., 2007; Rochester 2013). Early exposure of rodents to BPA caused increased susceptibility to both mammary and prostate cancer (Prins *et al*., 2007; Soto *et al*., 2008). Other studies have linked high levels of circulating BPA with cardiovascular diseases including obesity, hypertension, diabetes, atherosclerosis and coronary heart diseases (Lang *et al*., 2008; Melzer *et al*., 2010; Lind and Lind 2011).

The deleterious effects of BPA on the adult pancreas have been studied (Alonso-Magdalena *et al*., 2006). A single exposure to low doses of BPA (10 or 100 BPA/kg BW) rapidly increased secretion of plasma insulin levels and decreased glucose levels in male mice. After 4 days of exposure, the mice developed hyperinsulinemia. Effects on

glucose homeostasis and altered pancreatic functions were also observed when similar doses of BPA were given to pregnant mouse dams on days 9–16 of gestation (Alonso-Magdalena *et al*., 2010). BPA exposure also lead to hypercholesterolemia by interrupting adipokines release from adipose tissue, therefore, increasing plasma leptin and decreasing plasma adiponectin levels (Miyazaki *et al*., 2007; Somm *et al*., 2009; Wei *et al*., 2011).

BPA has been reported to induce the conversion of xanthine dehydrogenase into xanthine oxidase in the rat liver thus increasing the reactive oxygen species (ROS) (Sakuma *et al*., 2010). ROS generation and oxidative DNA may be responsible for the harmful side effects of BPA on the human liver. Korkmaz *et al*., (2011) investigated oxidative stress on the kidney tissue of male rats was induced by BPA.

A number of studies have linked BPA exposure to behavioural alterations during gestation and lactation, for example masculinization in female pups (Dessi-Fulgheri *et al*., 2002; Farabollini *et al*., 2002; Ishido *et al*., 2004; Palanza *et al*., 2008). Hence, suggesting the effects of BPA on development of central nervous system (CNS). So far, a few in vivo studies have examined the influence of BPA exposure on brain development during gestation and lactation (Facciolo *et al*., 2002). The effect of BPA on synaptogenesis has been reported in the brains of adult rats and nonhuman primates (Hajszan and Leranth 2010).

Reproductive disorders:

BPA exposure has shown multiple effects on the reproductive system of male and female. Recent epidemiological studies associated the BPA exposure with low semen quality thus suggesting the possible effect of BPA on spermatogenesis by interacting with estrogen receptors (Petersen *et al*., 1998; Li *et al*., 2011). Though mechanism of BPA induced reproductive damage is still ambiguous. But the existing data indicate the involvement of endocrine disruptors interrupting the hypothalamus- pituitary-gonadal axis in BPA induced reproductive toxicity (Ramos *et al*., 2003; Timms *et al*., 2005)

Previous studies reported dose dependent reproductive damage. The exposures of adult male rats to BPA lead to decrease the testicular weight and impaired

spermatogenesis (Taepongsorat *et al*. 2008; Sakaue *et al*. 2001). Spermatogenesis is regulated by Follicular-stimulating hormone (FSH) and testosterone (T) released from Leydig cells, and reduced levels of these hormones can affect spermatogenesis. Subcutaneous administration of adult male rats with a dose of 3 mg BPA/kg/bw per day significantly lower the level of testosterone and elevated the levels of luteinizing hormone (LH) in both serum and testis (Sun *et al*., 2006). At testicular level, oxidative stress caused disruption of steroidogenic activity of Leydig cells (Hales *et al*., 2005). Recently, a group has established the effect of BPA exposure to neonatal male rats leading to aberrant DNA methylation in testis of adult male rats (Doshi *et al*., 2011).

In previous studies, seminiferous tubule development and spermatogenesis were impaired after oral administration of various doses (2 mg to 200 mg/kg) of BPA in male chicks (Furuya *et al*., 2006). In brown trout, the exposure of environmentally significant concentrations of BPA on late prespawning reduced sperm density and swimming velocity (Furuya *et al*., 2006). The possible potential of BPA to cause aneuploidy and DNA adducts was reported in Syrian hamster embryo cells (Tsutsui *et al*., 1998).

Endocrine disruptors:

Endocrine disruptors are defined as the exogenous agents that interfere with natural hormones in the body, maintain homeostasis and the regulation of developmental processes. BPA is well known endocrine disruptor that acts as xenoestrogenic compound even at low doses (D'Cruz *et al*., 2012). Xenoestrogens are small lipophilic molecules that mimic the role of physiological estrogens (Watson *et al*., 2010).

Initially, BPA was known as weak environmental estrogen because of its relative binding affinity for estrogen receptors ER α and ER β which was estimated to be 1000–10,000 fold lower than 17β- estradiol (Kuiper *et al*., 1998). Previous studies proposed that increased male reproductive disorders (low sperm counts, testicular cancer, cryptorchidism and hypospadias) might be due to the increased exposure of developing male fetal to the estrogenic environment (Toppari *et al*., 1996).

Several studies have demonstrated stimulatory effects of BPA on cellular mechanisms at very low concentration even equivalent to estradiol (Alonso-Magdalena

et al., 2005; Zsarnovszky *et al*., 2005; Alonso-Magdalena *et al*., 2008; Hugo *et al*., 2008). Some activities of BPA are attributed to its ability to bind membrane estrogen receptors (Alonso-Magdalena *et al*., 2005; Watson *et al*., 2005; Alonso-Magdalena *et al*., 2008).

Antioxidant System:

Environmental contaminants have been known to generate reactive oxygen species (ROS) in the body including hydroxyl radical and hydrogen peroxide (Ho *et al*., 1998). Reactive oxygen species (ROS) are known to be cytotoxic agents that cause significant oxidative damage to membrane lipids, protein and nucleic acid in the cells (Kabuto *et al*., 2003). ROS is generated in mitochondria from the activity of enzymes such as xanthine, NADPH oxidase and cytochrome P450s (Bánfi *et al*., 2001; Kumagai *et al*., 2002; Zangar *et al*., 2004). Abnormal production of ROS leads to oxidative stress, which harms the spermatozoa through various mechanisms (Sharma and Agarwal 1996). Previous study showed reduction in mitochondrial enzymes in mice testis exposed to BPA (Anjum *et al*., 2011).

A sophisticated system of antioxidants has been developed by testis in order to overcome these risks. Antioxidant enzymes provide the first line of defense against free radicals produced in organisms. Antioxidants stop the electron stealing process by donating one of their own electrons to neutralize ROS. They act as scavengers and prevent cell death and tissue damage that might cause cellular damage and disease (Halliwell 1996). Antioxidant therapy could be significant to inhibit the BPA induced toxicity (Sujatha *et al*., 2001; Latchoumycandane *et al*., 2002).

Flavonoids:

In recent years, several studies have been exploring the therapeutic properties of plant extracts from different parts of medicinal plants. Flavonoids are polyphenolic compounds ubiquitously distributed in natural plants and are used currently as antioxidant additives for different kinds of foods and beverages (Motoyama *et al*., 2009). The dietary intake of flavonoids is evaluated to be 1-2 g/day. Until now, over 8,000 varieties of flavonoids have been reported. Flavonoids occur as glycosides, aglycones (i.e., flavonoids without attached sugar), and methylated derivatives

(Harborne and Goodwin 1988). A variety of structural forms of the flavonoid aglycones occur in plants, all containing fifteen carbon atoms in their basic nucleus: two six-membered rings linked with a three carbon unit (Middleton 1984).

The well documented property of flavonoids is its capacity to act as antioxidant leading to inhibition of oxidative damage mediated diseases. For this reason flavonoid based drugs have been used for treatment of these pathologies (Lu *et al*., 2010; Mirshekar *et al*., 2010). The antioxidant activity exhibited by flavonoids might be due to combination of metal chelation through ortho- dihydroxy phenolic structure along with free radical scavenging properties (Moridani *et al*., 2003). Because of the phenolic structure, these compounds donate hydrogen to inhibit free radical process at three stages i.e. the formation of superoxide ions, hydroxyl radicals generation and formation of lipid radicals (Lien *et al*., 1999; Moridani *et al*., 2003).

Quercetin:

Quercetin (3, 5, 7, 3′, 4′-pentahydroxyflavone) belongs to an extensive class of polyphenolic flavonoid compounds occurring mainly in glycosidic form (Havsteen 1983; Lamson and Brignall 2000). It is present in a variety of human foods including red onions (347 mg/kg), apples (36 mg/kg), cherries, broccoli, grapes, red wine (11 mg/kg) citrus fruits, tea (20 mg/kg) and, at particularly high concentrations, in capers and lovage (180 mg per 100 g) (Hertog *et al*., 1993; Bischoff 2008). It is a powerful bioactive component of the human diet as antioxidant agent (Motoyama *et al*., 2009). Quercetin prevents oxidative damage by scavenging oxygen radicals, providing protection against lipid peroxidation and chelating metal ions (Laughton *et al*., 1991; Inal *et al*., 2002).

Quercetin has many beneficial effects including cardiovascular protection, anticancer and antiallergic activity, anti-inflammatory effects, lowering of blood pressure and improving hyperglycaemia-related diseases (Anjaneyulu and Chopra 2003; Bischoff 2008). Moreover, it has stimulatory effects on sperm quality in adult male rats (Taepongsorat *et al*., 2008).

Pharmacological effects of quercetin are mostly due to its antioxidant activity. One mechanism of quercetin involved scavenging free radicals produced by xanthine/

xanthine oxidase (Dok-Go *et al*., 2003). In addition, quercetin showed peroxyl radical–scavenging activity assessed by measuring the inhibition of hydroperoxidation of methyl linoleate (Ioku *et al*., 1995). Moreover, quercetin is known to inhibit lipid peroxidation mediated Fe^{2+} and Cu^{2+} ions (Ferrali *et al*., 1997; da Silva *et al*., 1998). Negre-Salvayre (1992) revealed that quercetin could decrease oxidative damage to peptides, lipids and DNA (Salvayre 1992).

Because of the multiple pharmacological activities of quercetin for instance suppression of cell proliferation, prevention of platelet aggregation, protection of LDL oxidation and induction of apoptosis, it has been used for clinical therapy (Formica and Regelson 1995). The beneficial effects of quercetin are attributed to different mechanisms, such as reduction of oxidative stress by scavenging free radicals, promoting cellular survival by modulating intracellular signals, or decreasing toxicities of xenobiotics by regulating gene expression (Moskaug *et al*., 2004).

Quercetin has no effect on cortisol production from human adrenal H295R cells stimulated with di-buthylyl cAMP (Ohno *et al*., 2002). An in vivo study showed that intra peritoneal injection of 200 mg quercetin/kg for twice had no effect on fertility in rats (Aravindakshan *et al*., 1985). But daily doses of 50–150 mg quercetin/kg body weight via oral gavage slightly increased prostate weight (Ma *et al*., 2004). Quercetin is found to increase serum testosterone concentration and decrease serum dihydrotestosterone concentration in male rats (Ma *et al*., 2004). The reproductive effects of quercetin in male are still controversial, may be because most of the previous studies have been conducted in vitro. Studies on protective benefits of quercetin against heavy metals and endocrine disruptors have been reported. Quercetin not only protects tissue damage but also improves tissue antioxidant level (Jahan *et al*., 2014; Ciftci *et al*. 2012). Keeping these in view, present experiment was carried out to examine possible protective effects of quercetin on reproductive organs against BPA induced toxicity in adult male rats.

Materials and method

Animals

Twenty adult male Sprague Dawley rats (Rattus norvegicus) weighing 200 ± 10g were obtained from the primate facility of Animal Sciences Department, Quaid-i-Azam University. Animals were randomly assigned into four groups of five rats/ group and housed in separate cages (five rats/ cage). Animals were kept in well ventilated animal room at temperature of 20-25 C^0 and 12 hours dark/12 hours light cycle. The animals were fed with food chaw and tap water ad libitum throughout experiment. Animal handling and all the experimental protocols were assessed and approved by the Animal sciences department ethical committee.

Chemicals

Bisphenol A: Bisphenol A was purchased from Sigma-Aldrich (Germany)

Quercetin: Quercetin in powdered form was purchased from Sigma-Aldrich (Germany).

Bisphenol A preparation:

One gram of Bisphenol A was dissolved in 50ml of 0.9% normal saline, thoroughly mixed and stored at 4^0C in falcon tube wrapped with aluminium foil to protect from light.

Quercetin preparation:

One gram of quercetin was dissolved in 50ml of 0.9% normal saline, vigorously shaked and stored at 4^0C in falcon tube wrapped with aluminium foil.

Experimental design

Twenty adult male rats (weighing 190-250g) were used for this study. The animals were divided into four groups each having five rats. All the doses were given orally for 52 consecutive days.

Group 1: This group served as control group and was provided with oral dose of 0.9% saline by feeding tube.

Group 2: Bisphenol A treated group received 50mg/kg dose of Bispenol A daily via oral gavages (BPA group).

Group 3: Quercetin treated group was given oral gavage of 50mg/kg quercetin (Q group).

Group 4: Bisphenol A+Quercetin treated group was administered with Bisphenol A (50 mg/kg) along with quercetin (50 mg/kg) orally (BPA+Q group).

The dose of Bisphenol A and Quercetin used in this experiment was selected on the bases of previous studies by Kabuto *et al*., 2004 and Farombi *et al*., 2012.

Blood and Tissue collection

The experiment was conducted for 52 days so that the complete spermatogenic cycle within the testis can be considered. At the end of the experiment, animals were weighed and sacrificed by decapitation. Trunk blood was collected directly in heparinized syringes. Blood was centrifuged at 3000 rpm for 15 minutes; plasma was separated and stored at -20 ^{0}C until hormonal analysis and lipid profile determination. Testicular tissues and epididymus were dissected out, washed in saline, weighed and testicular volume was measured. Left testicular tissue and epididymis were fixed in sera for histological studies.

Plasma Creatinine level

Plasma creatinine level was determined by using AMP diagnostic kit (AMEDA labordiagnostik Gmbh, Austria) and analyzed on picco 5 chemistry analyzer.

Principle

In alkaline conditions the reaction of creatinine with picric acid forms a colour complex quantified photometrically at 510 nm.

Reagent composition:

Reagent: R1

Alkaline buffer

Reagent: R2

Picric Acid …….. 40 mmol/L

Surfactant

Standard: STD

Creatinine …….. 2 mg/dL

Organic matrix based primary standard.

Preparation and stability of working reagent:

Bring reagents to room temperature 2 hours before use. Mix 5 volume of R1 with 1 volume of R2.

Stability: 1 month at 15-25 °C when tightly capped.

Samples: Rat blood plasma was used as sample.

Procedure

The reagent was analyzed on chemistry analyzer. Application was as follows

Wavelength	510 nm
Temperature	37 °C
Cuvette	1 cm light path

	Blank	Sample	Standard
Working reagent	1.0 ml	1.0 ml	1.0 ml
Sample	-----	50 µl	-----
Standard	-----	-----	50 µl

Plasma Blood Urea Nitrogen (BUN) level

Plasma blood urea nitrogen level (BUN) was determined by using AMP diagnostic kit (AMEDA labordiagnostik Gmbh, Austria) and were analyzed on picco 5 chemistry analyzer.

Reagent composition:

Reagent: R1

Tris buffer, pH 7.8 …….. 80 mmol/L

α-ketoglutarate …….. 6 mmol/L

Urease …….. 75 KU/mL

Reagent: R2

GLDH …….. 60 KU/mL

NADH …….. 0.32 mmol/L

Standard: STD

Urea 50 mg/dL …….. 8.3 mmol/L

Organic matrix based primary standard.

Preparation and stability of working reagent:

Mix 4 volume of R1 with 1 volume of R2.

Stability: 1 month at 2-8 °C.

Samples: Rat plasma was used as sample.

Procedure:

This reagent was analyzed on chemistry analyzer. Application was as follows

Wavelength	340 nm
Temperature	37 °C
Cuvette	1 cm light path

	Blank	Sample	Standard
Working reagent	1.0 ml	1.0 ml	1.0 ml
Sample	-----	50 µl	-----
Standard	-----	-----	50 µl

Cholesterol level

Plasma cholesterol level was determined by using AMP diagnostic kit (AMEDA labordiagnostik Gmbh, Austria) and was analyzed on picco 5 chemistry analyzer.

Reagent composition:

Reagent: R

PIPES buffer, pH 7.0 …….. 200 mmol/L

Sodium cholate …….. 1 mmol/L

Cholesterol esterase …….. > 250 U/L

Cholesterol oxidase …….. > 250 U/L

Peroxidase …….. >1 KU/L

4-Aminoantipyrine …….. 0.33 mmol/L

Phenol …….. 4 mmol/L

Non-ionic tensioactives …..…… 2 g/L

biocides

Standard: STD

Cholesterol …….. 200 mg/dL

5.18 mmol/L

Organic matrix based primary standard

Preparation and Stability of Working Reagent:

Bring reagent to room temperature 2 hours before use.

Samples: Rat plasma was used for the samples.

Procedure:

This reagent was used on chemistry analyzer. Application was as follows

Wavelength	510 nm
Cuvette	1 cm light path
Temperature	37 °C

	Blank	Sample	Standard
Reagent:	1.0 ml	1.0 ml	1.0 ml
Sample:	-----	10 µl	------
Standard:	-----	-----	10 µl

Triglyceride level

Plasma triglyceride level was determined by using AMP diagnostic kit AMEDA labordiagnostik Gmbh. and were analyzed on picco 5 chemistry analyzer.

Reagents composition:

Reagent: R

Pipes buffer, pH 6.8 …….. 50 mmol/L

LPL …….. > 12 KU/L

GK …….. > 1 KU/L

GOP …….. > 10 KU/L

ATP …….. 2.0 Mmol/L

MG^2 …….. 40mmol/L

POD …….. > 2.5 KU/L

4-AA …….. 0.5 mmol/L

Phenol …….. 3 mmol/l

Non-ionic tensioactivies …….. 2 g/L

Biocides

Standard: STD

Glycerol 2, 26 mmol/L equivalent to 200 mg/dl glycerol trioleate.

Preparation And Stability Of Working Reagent:

Stored at 2-8 ^{0}C and protected from light, the reagent is stable until the expiry date on the label.

Procedure:

This reagent was used on chemistry analyzer. Application is as follows

Wavelength: 500 nm (480- 520)

Temperature: 37 ^{0}C

	Blank	Sample	Standard
Reagent	1.0 ml	1.0 ml	1.0 ml
Sample	-----	10 µl	------
Standard	-----	-----	10 µl

HDL Cholesterol level

Reagents composition:

Reagent: R

Polyethylene glycol …….. 20 % (w/v)

Glycine buffer …….. pH 10.0

Standard: STD

Cholesterol …….. 50 mg/dl

Stability of reagents:

When stored at 15-25 ^{0}C and protected from light, the reagent is stable until the expiry date.

Procedure:

This reagent was used on chemistry analyzer. Application is as follows

Wavelength: 500nm

Temperature: 37 ^{0}C

	Blank	Sample	Standard
Reagent	2.0 ml	1.0 ml	1.0 ml
Sample	-----	10 µl	------
Standard	-----	-----	10 µl

LDL Cholesterol level

LDL cholesterol can be calculated using the following formula:

LDL cholesterol = Total cholesterol- HDL cholesterol- (Triglycerides/5).

Tissue Histology

Testicular and epididymis histology was carried out in order to determine the Bisphenol A induced toxicity and protective effect of quercetin. After removal of testicular and epididymal tissues the following steps were performed.

Fixation

Testis and epididymis were fixed in sera for 4-6 hours. Composition of sera is:

Glacial acetic acid …….. 10ml

Formaldehyde …….. 30ml

Absolute alcohol …….. 60ml

Dehydration

Following fixation, dehydration was carried out at room temperature in the following ascending grades of alcohol

80% Ethanol …….. over night

90% Ethanol …….. 2-4 hours

100% Ethanol …….. 2-4 hours

After dehydration fixed tissues were transferred to the cedar wood oil to become clear and transparent at room temperature.

Embedding

Following steps were taken for embedding.

Benzol 1 …….. 10 minutes (at room temperature)

Benzol 2 …….. 10 minutes (at room temperature)

Benzol + Paraplast (1:1) …… 20 minutes (at 60 C^0)

Paraplast 1 …….. 12 hours (at 60 C^0)

Paraplast 2 …….. 12 hours (at 60 C^0)

Paraplast …….. 12 hours (at 60 C^0)

After embedding, tissues were transferred in a boat containing melted wax. Wax was allowed to solidify after removing bubbles from it. With the help of knife or scalpel paraffin wax blocks were trimmed and mounted on wooden blocks for section cutting.

Microtomy

Paraffin embedded tissues were mounted on wooden blocks and 5 µm thin sections were cut using microtome (Thermo, Shandon finesse 325, UK). The ribbons with tissues were stretched and fixed to previously clean albumenized glass slides on Fischer slide warmer at 60 ^{0}C. These glass slides were placed in incubator overnight for completion of stretching and removal of bubbles any left.

Staining

For staining following method was used to prepare stains.

Preparation of Hematoxylin stain:

For Hematoxylin stain preparation, following chemicals were used.

Hematoxylin …….. 2 g

Ammonium alum …….. 3 g

Sodium iodate …….. 0.24 g

Absolute ethanol …….. 100 ml

Distilled water …….. 100 ml

Glycerol …….. 100 ml

Acetic acid …….. 10 ml

Two gram hematoxylin was dissolved in 100 ml ethanol. 3g alum was dissolved in distilled water in order to prepare ammonium alum solution and then boiled. Ammonium alum solution was added in hematoxylin solution, then glycerol and sodium iodate were added carefully. At the end, acid acid was poured and mixed thoroughly.

Preparation of Eosin Stain

1g of Eosin was dissolved in 100ml of 70% ethanol for Eosin stain preparation.

Staining procedure

Following steps were performed for Hematoxylin and Eosin staining.

Hydration

The slides were deparaffinized in xylene overnight. Paraplast removed tissues were hydrated in descending grades of alcohol.

100% Alcohol ---------- 2-5 minutes at room temperature

90% Alcohol ---------- 2-5 minutes at room temperature

70% Alcohol ---------- 2-5 minutes at room temperature

50% Alcohol ---------- 2-5 minutes at room temperature

30% Alcohol ---------- 2-5 minutes at room temperature

Wash in tap water

Hematoxylin ---------- 2-3 dips

Wash in tap water ---------- 5 to 10 minutes until the tissue were blue in colour.

Dehydration:

30% Alcohol ---------- 3-5 minutes at room temperature

50% Alcohol ---------- 3-5 minutes at room temperature

70% Alcohol ---------- 3-5 minutes at room temperature

90% Alcohol ---------- 3-5 minutes at room temperature

Eosin ---------- 1 dip

90% Alcohol ---------- 1 dip

100% Alcohol ---------- 2-5 minutes at room temperature

Xylene ---------- 5-10 minutes

After staining, slides were mounted with Canada balsam. Cover slips were dipped in xylene and placed on the slides and were placed in incubator overnight. Extra Canada balsam was removed by xylene.

Light Microscopic Study

Five µm thick sections were studied under light microscope (Nikon, 187842, Japan) at 40 magnifications. Slides of all control and treated groups were studied. For histological and morphometrical results of bisphenol A and quercetin, tunica albuginea thickness, seminiferous tubule diameter, seminiferous tubule epithelial height, lumen and interstitial space of testicular tissue while tubular diameter, lumen and epithelial height of epididymal tissue was measured using image J2x software package program.

Microphotography:

Microphotography was done by Leica LB microscope (Germany) equipped with cannon digital camera (Japan).

Quantitative determination of Estradiol concentration

Principle of the Assay

The main principle of ELISA was based on the competitive binding between Estradiol found in test samples and Estradiol-HRP (horseradish peroxidase) conjugate for a constant amount of rabbit anti-estradiol.

Assay Procedure

In order to quantify estradiol concentrations in plasma, 10µl of standards, samples and controls were allotted appropriate number of coated wells. Then 50µl of rabbit anti-Estradiol was added and then 100µl Estradiol-horseradish peroxidase conjugates in all the wells. ELISA plate was mixed for 30 seconds and kept for incubation in water bath

at 37 ^{0}C for 1.5 hours. During this time, a specific amount of HRP-labeled estradiol competes with endogenous estradiol in the standard, specimen or quality specific estradiol antibody during incubation. After incubation, all the wells were washed with distilled water so that unbound estradiol peroxidase conjugate get remove. Then each well was added with a 100µl of tetra-methyl-benzidine (TMB) reagent. Wells were again incubated at room temperature for 20 minutes. After that stop solution of 100µl was added to each well and mixed for a while. The absorbance was read with microtiter well reader at 450 nm. The results were shown in ng/ml.

Quantitative determination of Testosterone concentration

Testosterone concentration in plasma was determined by using Enzyme Linked Immuno Sorbant Assay (ELISA) kits. The ELISA kit was purchased from Amgenix, Burlingame, CA, USA. All samples were quantified in a single assay.

Principle of the Test

The testosterone EIA is based on the principle of competitive binding between testosterone in the specimen and testosterone-horseradish peroxidase (HRP) conjugate for a constant amount of rabbit anti-testosterone.

To determine testosterone concentrations in plasma, tissue 10 µl of standards, specimen and controls were added to desired number of coated wells. Then 100 µl and 50 µl of testosterone-HRP conjugate reagent and rabbit anti-testosterone reagent were added respectively into each well and mixed thoroughly for 30 seconds. All the wells were incubated in water bath at 37 ^{0}C for 1.5 hour. A fixed amount of HRP-labelled testosterone competes with endogenous testosterone in the standard, sample or quality-specific testosterone anti-body during incubation. After incubation all the wells were rinsed and flicks 5 times with distilled water to remove unbound testosterone peroxidase conjugate. Then added 100 µl of TMB-reagent into each well and mix for 10 seconds. Again microwell plate was incubated at 18-25 C^0 for 20 minutes. After incubation 100 µl of stop solution was added to each well and mixed for 30 seconds. Then read the absorbance with microtiter well reader at 450 nm. The results were expressed in ng/ml.

The estimated normal concentration of testosterone in plasma for adult male rat is 3.0 ng/g – 10.0 ng/ml. Sensitivity of the assay, the minimum detectable concentration of the testosterone ELISA assay as measured by 2SD from the mean of zero standard is estimated to be 0.05ng/ml.

Statistical analysis

All the data is shown as Mean ± SEM. One way analysis of variance (ANOVA) followed by tukey's test was used for comparison of different groups using Graph pad prism 5 software. Level of significance was set at $p<0.05$.

Results

Body weight gain:

The mean ± SEM body weight gain of all the experimental groups are shown in Table 1. No significant change in mean body weight gain was observed among all experimental groups.

Testis weight and volume:

Testicular weight and volume of all the experimental groups are shown in Table 2. No significant differences were found when all groups were compared.

Epididymis and accessory organs weight

There was no significant difference in the mean weight of left and right epididymis and seminal vesicle. No significant difference was seen in the mean weight of prostate in BPA treated group as compare to control, quercetin treated and BPA+Q treated group as shown in table 3.

Table 1: Mean ± SEM body weight (g) of control and treated adult male rats after 52 days of treatment.

Group (n=5)	Before Treatment	After Treatment
Control	196 ± 5.43	276.2 ± 12.20
Bisphenol A	196 ± 7.50	247.4 ± 13.41
Quercetin	184.4 ± 2.21	288.6 ± 13.98
Bisphenol A + Quercetin	203.6 ± 3.81	261.6 ± 10.03

Table 2: Mean ± SEM testicular weight (g) and volume (ml) of control and treated groups after 52 days of treatment.

Group (n=5)	Testicular weight (g)		Testicular volume(ml)	
	Left	Right	Left	Right
Control	1.41 ± 0.07	1.43 ± 0.07	1.40 ± 0.60	1.60 ± 0.19
Bisphenol A	124 ± 0.04	1.27 ± 0.05	1.50 ± 0.40	1.40 ± 0.19
Quercetin	1.33 ± 0.04	1.35 ± 0.03	1.30 ± 0.40	1.40 ± 0.19
Bisphenol A+ Quercetin	1.37 ± 0.08	1.36 ± 0.08	1.00 ± 0.10	1.10 ± 0.19

Table 3: Mean ± SEM of epididymis and accessory organs weight of control and treated rats after 52 days of treatment.

Group (n=5)	Epididymus weight (g)		Seminal vesicle weight (g)	Prostate weight (g)
	Left	Right		
Control	0.51 ± 0.03	0.54 ± 0.02	1.33 ± 0.17	0.53 ± 0.02
Bisphenol A	0.55 ± 0.02	0.51 ± 0.02	1.02 ± 0.09	0.65 ± 0.07
Quercetin	0.51 ± 0.02	0.54 ± 0.03	1.17 ± 0.12	0.73 ± 0.05
Bisphenol A+ Quercetin	0.53 ± 0.02	0.53 ± 0.02	1.44 ± 0.08	0.70 ± 0.07

Blood urea nitrogen (BUN):

BUN levels of control, BPA treated, quercetin treated and BPA+Q treated groups are presented in Table 4. A highly significant increase ($p<0.001$) was observed in BPA treated group when compared with control group. There was significant reduction ($p<0.5$) in quercetin treated group as compare to control group. Both quercetin treated and BPA+Q treated groups showed significant decrease ($p<0.01$, $p<0.001$ respectively) when compared with BPA treated group. No significant difference was observed among quercetin treated and BPA treated groups (Fig 2).

Serum Creatinine:

Serum Creatinine levels of control, BPA treated, quercetin treated and BPA+Q treated groups are shown in Table 4. A highly significant elevation ($p<0.001$) in creatinin level was found in BPA treated group as compare to control group. There was no significant difference in quercetin treated and BPA+Q treated group as compare to control group, while, highly significant decrease was observed when compared with BPA treated group ($p<0.01$, $p<0.001$ respectively). No significant difference was found between quercetin treated and BPA+Q treated groups (Fig 3).

Table 4: Mean ± SEM of BUN (mg/dl) and creatinine (mg/dl) concentration in control and treated groups after 52 days of treatment.

Group (n=5)	BUN (mg/dl)	Creatinine (mg/dl)
Control	23.20 ± 1.31	0.48 ± 0.02
Bisphenol A	40.32 ± 1.04^{a***}	0.66 ± 0.02^{a***}
Quercetin	31.63 ± 0.74^{a*b**}	0.49 ± 0.02^{b***}
Bisphenol A+ Quercetin	30.22 ± 1.00^{b***}	0.50 ± 0.01^{b***}

Values are expressed as mean ± SEM

*p< 0.05, **p< 0.01, ***p< 0.001

^{a} = Value vs control

^{b} = Value vs Bisphenol A

^{c} = Value vs Bisphenol A + Quercetin

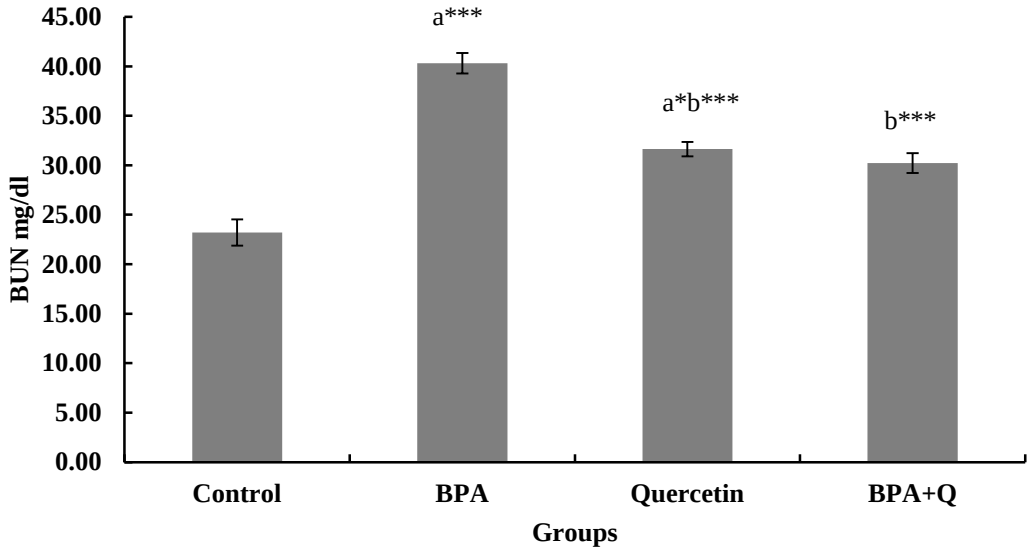

Fig 2: Mean serum BUN (mg/dl) levels of control, BPA, quercetin and BPA + Q treated groups after 52 days of treatment. Data is expressed in Mean ± SEM.

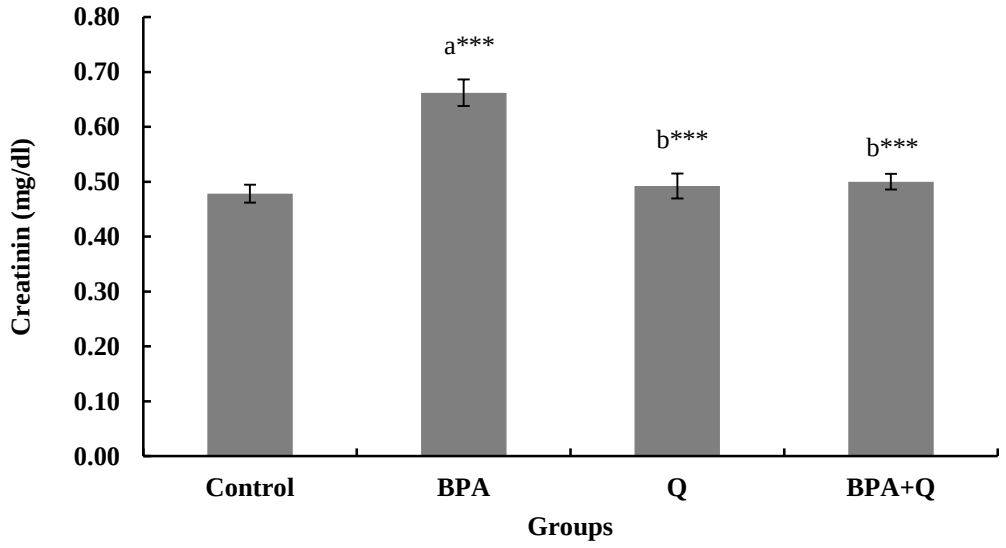

Fig 3: Mean serum Creatinine (mg/dl) levels of control, BPA, quercetin and BPA + Q treated groups after 52 days of treatment. Data is expressed in Mean ± SEM.

Serum Cholesterol

The mean values of serum cholesterol levels of control, BPA treated, quercetin treated and BPA+Q treated groups are presented in Table 5. Serum cholesterol level was significantly increased ($p<0.001$) in BPA treated group as compare to control. Highly significant difference ($p<0.001$) was found in quercetin treated group when compared with control and BPA treated group. Similarly, significant reduction was observed in BPA+Q treated group when compared with control group ($p<0.05$) and BPA treated group ($p<0.001$). No significant difference was found among quercetin treated and BPA+Q treated groups (Fig 4).

Triglyceride

Triglyceride levels were significantly increased ($p<0.001$) in BPA treated group while significantly reduced ($p<0.001$) in quercetin treated group as compare to control (Table 5). Mean level of triglyceride was significantly decreased ($p<0.001$) in quercetin treated and BPA+Q treated group as compare to BPA treated group. Significant difference ($p<0.001$) was observed among quercetin treated and BPA+Q treated groups (Fig 4).

High-Density lipoprotein (HDL)

Mean value of HDL was significantly increased ($p<0.001$) in BPA treated, quercetin treated and BPA+Q treated groups when compared with control (Table 5). Quercetin treatment alone and in combination with BPA significantly increased ($p<0.01$, $p<0.001$) HDL level when compared to BPA treated group. However, no significant difference was observed between quercetin treated and BPA+Q treated groups (Fig 5).

Low-density lipoprotein (LDL)

Mean LDL level was increased significantly ($p<0.001$) in BPA treated, quercetin treated and BPA+Q treated groups when compared with control (Table 5). Significant increase ($P<0.001$) was observed in quercetin treated and BPA+Q treated groups when compared to BPA treated groups. No significant difference was observed among quercetin and BPA+Q groups (Fig 5).

Table 5: Mean ± SEM of serum total cholesterol (mg/dl), triglyceride (mg/dl), HDL (mg/dl) and LDL (mg/dl) of control and treated groups after 52 days of treatment.

Group (n=5)	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Control	43.84 ± 0.89	60.56 ± 0.84	29.52 ± 2.75	4.66 ± 1.05
Bisphenol A	65.38 ± 0.98^{a***}	113.75 ± 2.03^{a***}	57.08 ± 1.79^{a***}	14.45 ± 2.43^{a***}
Quercetin	51.95 ± 1.38^{a**b***}	48.48 ± 0.54^{a***b***}	78.01 ± 1.06^{a***b**}	35.76 ± 1.80^{a***b**}
Bisphenol A+ Quercetin	48.19 ± 0.49^{a*b***}	61.80 ± 1.13^{b***c***}	73.69 ± 1.47^{a***b***}	37.85 ± 1.57^{a***b***}

Values are expressed as mean ± SEM

*p< 0.05, **p< 0.01, ***p< 0.001

^{a} = Value vs control

^{b} = Value vs Bisphenol A

^{c} = Value vs Bisphenol A + Quercetin

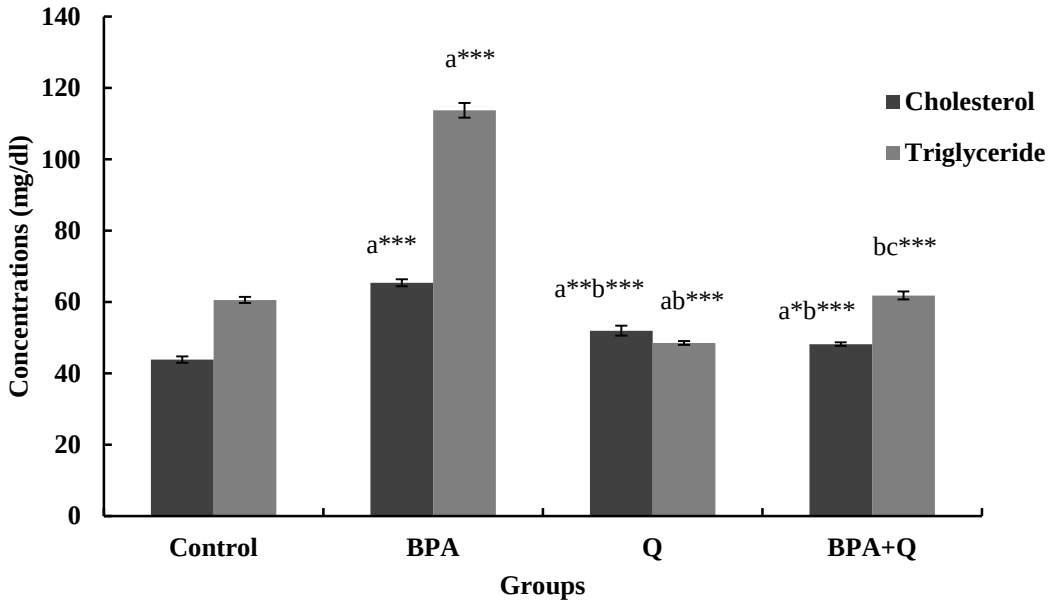

Fig 4: Mean serum Cholesterol (mg/dl) and Triglyceride (mg/dl) levels of control, BPA, quercetin and BPA + Q treated groups after 52 days of treatment. Data is expressed in Mean ± SEM.

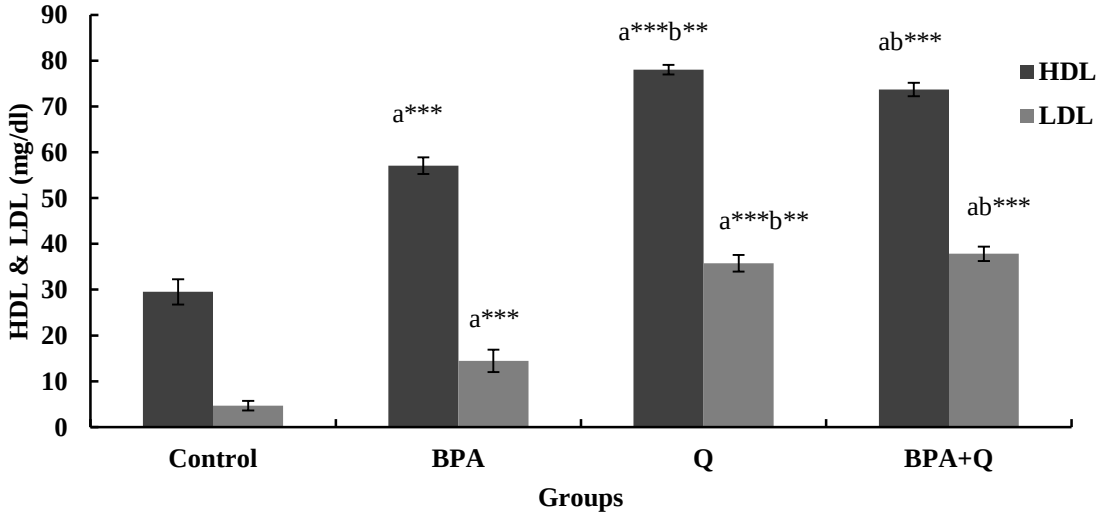

Fig 5: Mean serum HDL (mg/dl) and LDL (mg/dl) levels of control, BPA, quercetin and BPA + Q treated groups after 52 days of tratment. Data is expressed in Mean ± SEM.

Histomorphometric Analysis

The histomorphometric studies of all the experimental groups are presented in Tables 6 and 7. The parameters studied in testis include: seminiferous tubule diameter, tubular lumen diameter, epithelial height, tunica albugenia height and interstitial space. While, epididymal parameters are: ductular diameter, lumen diameter and epithelial height.

Testis:

The histomorphological studies of the testis showed closely arranged seminiferous tubules with normal spermatogenesis in control group. A thick outer layer called tunica albugenia was present covering the seminiferous tubules (Fig 7a). A thick stratified germinal epithelium was present containing proliferating germ cells at different stages of spermatogenesis. Narrow lumen was filled with mature spermatozoa. Interstitial spaces around the seminiferous tubules were filled with Leydig cells (oval, round or irregular shaped) along with large blood vessels (Fig 6a)

BPA exposure caused massive degeneration of tunica albugenia and seminiferous tubules, reduction in tubular diameter along with disruption in normal spermatogenesis as compare to control, quercetin treated and BPA+Q treated groups (Fig 7b). An increase in the interstitial space and wider lumen was also evident along with intense intercellular spaces in germinative epithelium due to loosening of germ cells arrangement along epithelium (Fig 6b)

Histomorphology of testicular section from quercetin treated and BPA+Q treated rats showed major difference in the appearance of seminiferous tubules compared to BPA treated group. Tunica albugenia of both groups was thicker as compared to BPA treated rats (Fig 7c & d). The germinal epithelial length was restored in most of the seminiferous tubules leading to restoration of normal spermatogenesis. Number of spermatogonia, primary spermatocytes, secondary spermatocytes, round and elongated spermatids were also increased as compared to the BPA treated group. Similarly, increase in Leydig cell number caused reduction of interstitial spaces. Luminal diameter was also reduced due to the release of mature spermatids in to the lumen (Fig 6c & d).

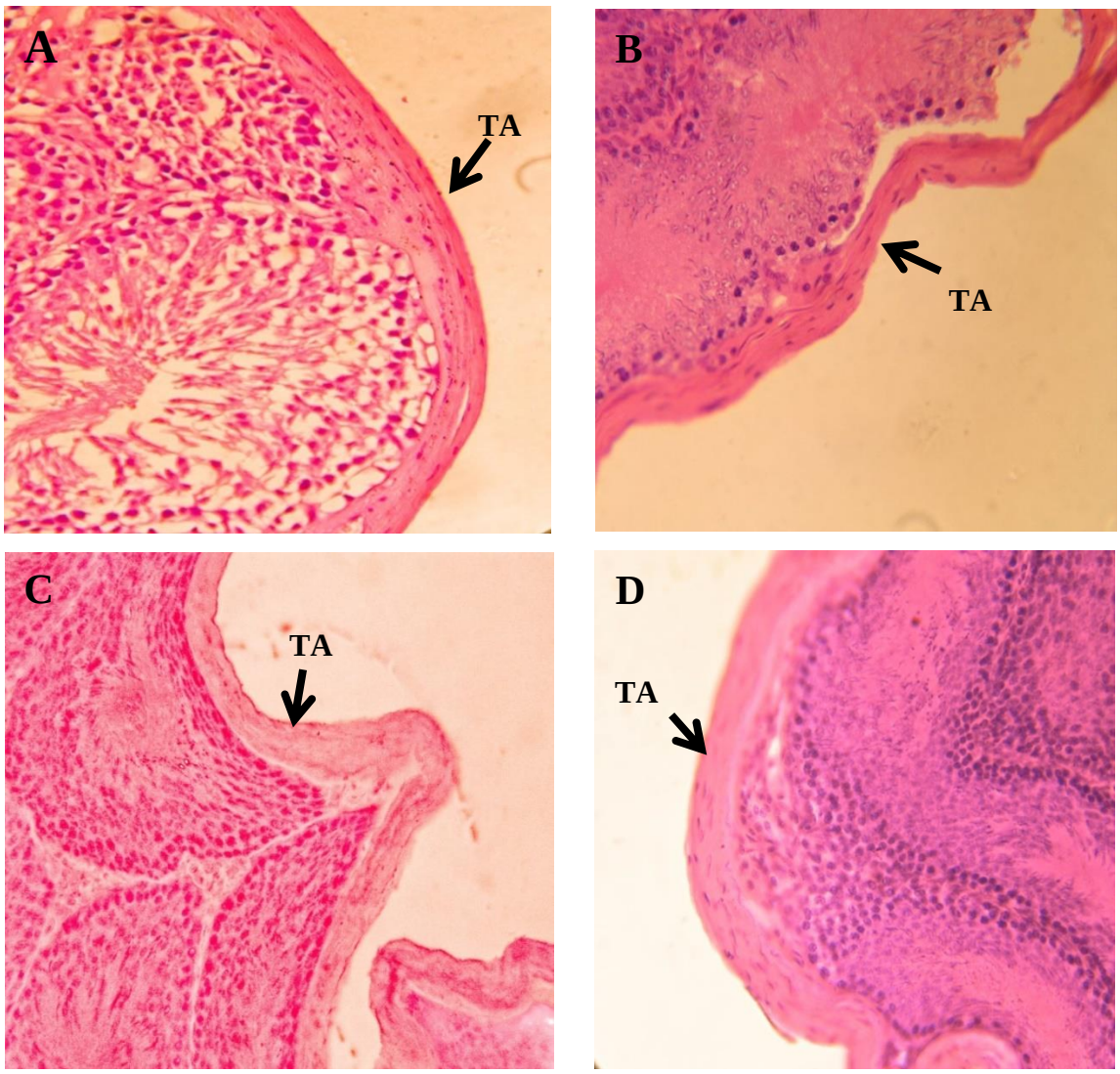

Fig 6: photomicrograph of adult male rat testis (H & E, 40X) from: (A) Control; showing the normal thickness of tunica albuginea (TA), (B) BPA treated; showing a reduction in the tunica albuginea, (C) Quercetin treated; showing increase in the tunica albuginea thickness as compared to BPA treated group alone, (D) BPA+Q; showing increase in tunica albuginea thickness as compared to BPA treated group.

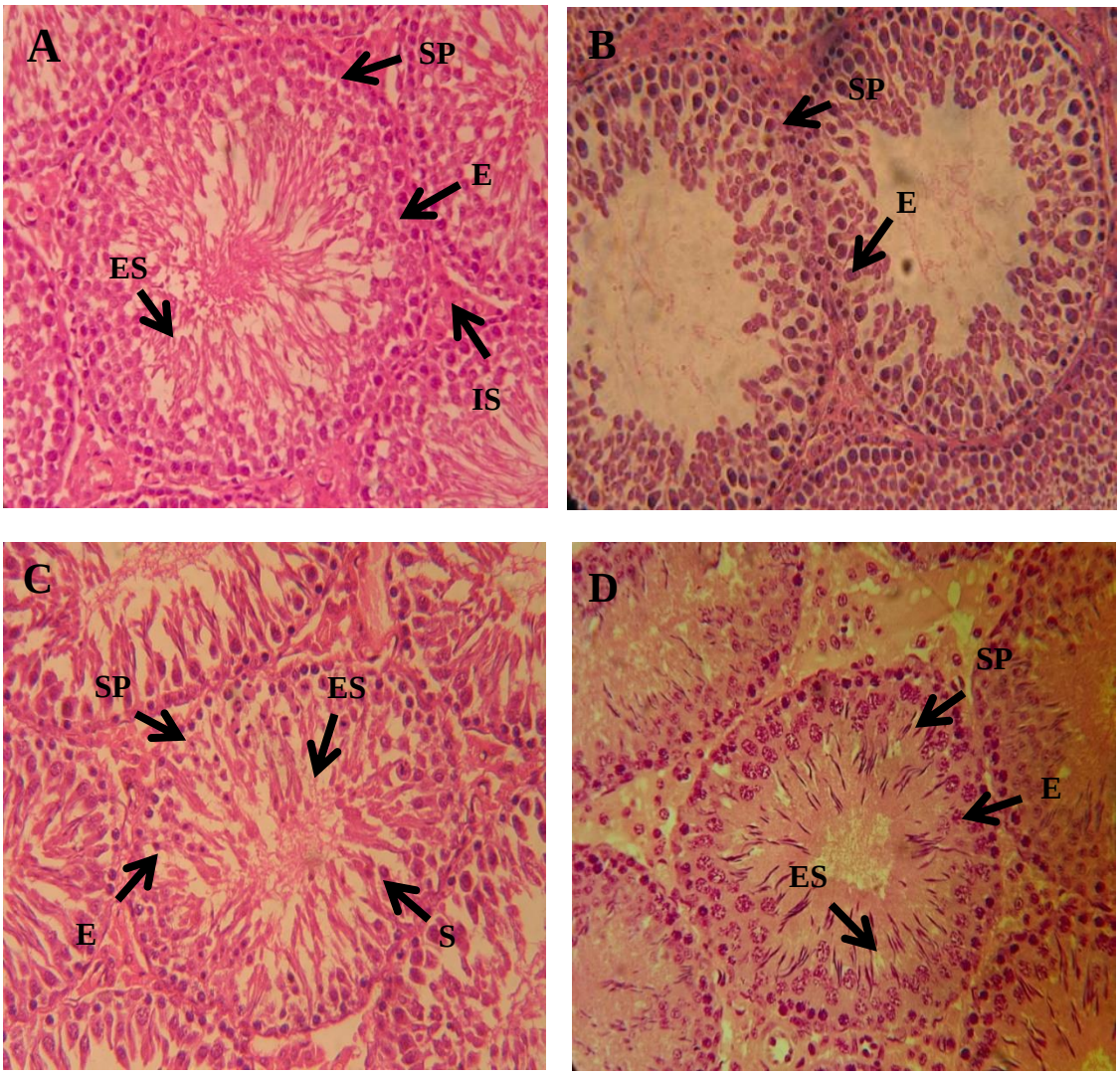

Figure 7: Photomicrograph of seminiferous tubules.(A) Control; showing compact tubules, filled lumen with spermatid, normal germ cell proliferation along epithelium, (B) BPA treated; showing tubules with empty lumen and degenerated epithelial layer with increased interstitial space, (C) Quercetin treated; showing narrow lumen, increased epithelial height and compact tubules with less interstitial space, (D) BPA+Q; showing minimal damage to epithelium, lumen filled with spermatid and less interstitial space. Magnification x40. Spermatogonia (SP), Spermatocytes (SP), Elongated spermatids (ES), Interstitial space (IS), Epithelium (E).

Seminiferous tubule diameter:

A significant reduction ($p<0.001$) in the mean seminiferous tubule diameter was observed in BPA treated and BPA+Q treated group as compared to the control group. Similarly, a highly significant increase was seen in the mean tubular diameters of quercetin treated ($p<0.001$) and BPA+Q treated ($p<0.01$) groups as compared to BPA treated group. No significant difference was noticed among the tubular diameter of quercetin treated and BPA+Q treated groups (Table 6, Fig 8).

Tubular lumen diameter:

Mean tubular lumen diameter in BPA treated and BPA+Q treated groups was increased significantly ($p<0.001$) as compare to control group. There was a slight increase of tubular lumen diameter in quercetin treated group which was significant ($p<0.05$) as compare to control group. Quercetin treatment alone and along with BPA caused significant reduction ($p<0.001$) in tubular lumen diameter as compare to BPA treated group. No significant difference was found between quercetin treated and BPA+Q treated groups (Table 6, Fig 8).

Epithelial height:

Mean epithelial thickness was significantly reduced ($p<0.001$) in BPA treated, quercetin treated and BPA+Q treated groups as compare to control group. Similarly, quercetin treated and BPA+Q treated groups showed significant increase in germinal epithelial height when compared with BPA treated group ($p<0.001$, $p<0.01$ respectively). No significant change was observed between quercetin treated and BPA+Q treated groups (Table 6, Fig 9).

Tunica Albuginea height:

A significant reduction ($p<0.001$) of tunica albuginea height was seen in BPA treated group as compare to control. There was a slight decrease of mean tunica albuginea height in quercetin treated and BPA+Q treated groups which was significant ($p<0.05$) as compared to control. A significant increase ($p<0.001$) of tunica albugenia height was observed in quercetin treated and BPA+Q treated group as compare to BPA

treated group. However, both quercetin treated and BPA+Q treated groups, when compared, did not show significant change in tunica albugenia height (Table 6, Fig 9).

Interstitial space:

A highly significant increase ($p<0.001$) of interstitial spaces between the seminiferous tubules was observed in BPA treated group as compared to control group. Similarly, quercetin treated group showed significant reduction ($p<0.01$) while BPA+Q treated group exhibited no significant difference in interstitial spaces when compared with control. A significant decrease in interstitial spaces was observed in quercetin treated ($p<0.001$) and BPA+Q treated ($p<0.01$) groups when compared with BPA treated group. While no significant difference was seen between quercetin treated and BPA+Q treated groups (Table 6, Fig 9).

Table 6: Mean ± SEM of seminiferous tubule diameter, tubular lumen diameter, seminiferous tubule epithelial height, tunica albugenia height and interstitial space (µm) of testis in control and treated groups after 52 days of treatment.

Group (n=5)	Seminiferous tubule diameter (µm)	Tubular lumen diameter (µm)	Epithelial height (µm)	Tunica albugenia height (µm)	Interstitial space (µm)
Control	227.11 ± 4.20	19.29 ± 0.83	78.89 ± 1.12	28.47 ± 0.62	6.83 ± 0.17
Bisphenol A	167.12 ± 4.29^{a***}	41.31 ± 1.49^{a***}	44.78 ± 0.98^{a***}	19.61 ± 0.47^{a***}	11.28 ± 0.20^{a***}
Quercetin	206.76 ± 2.67^{a**b***}	26.63 ± 0.90^{a*b***}	57.50 ± 0.69^{ab***}	25.11 ± 0.61^{a*b***}	5.62 ± 0.12^{a**b***}
Bisphenol A+ Quercetin	193.28 ± 3.96^{a***b**}	27.50 ± 1.36^{ab***}	54.30 ± 1.06^{a***b**}	26.27 ± 0.42^{a*b***}	6.33 ± 0.17^{b**c*}

Values are expressed as mean ± SEM

*p< 0.05, **p< 0.01, ***p< 0.001

^{a} = Value vs control

^{b} = Value vs Bisphenol A

^{c} = Value vs Bisphenol A + Quercetin

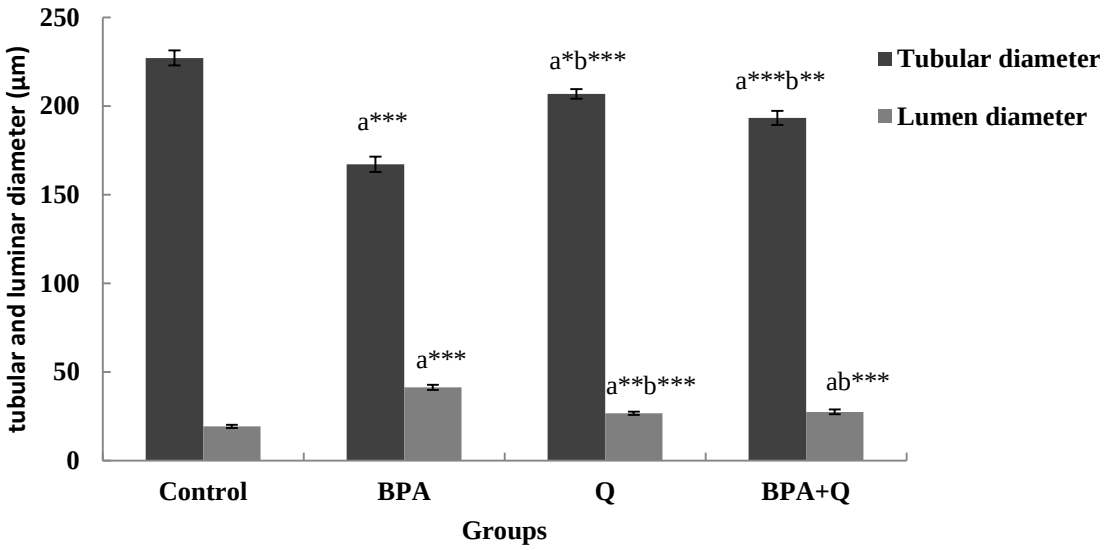

Fig 8: Mean seminiferous tubule diameter and tubular lumen diameter of control, BPA, quercetin and BPA + Q treated groups of adult male rats after 52 days of treatment. Data is expressed as Mean ± SEM.

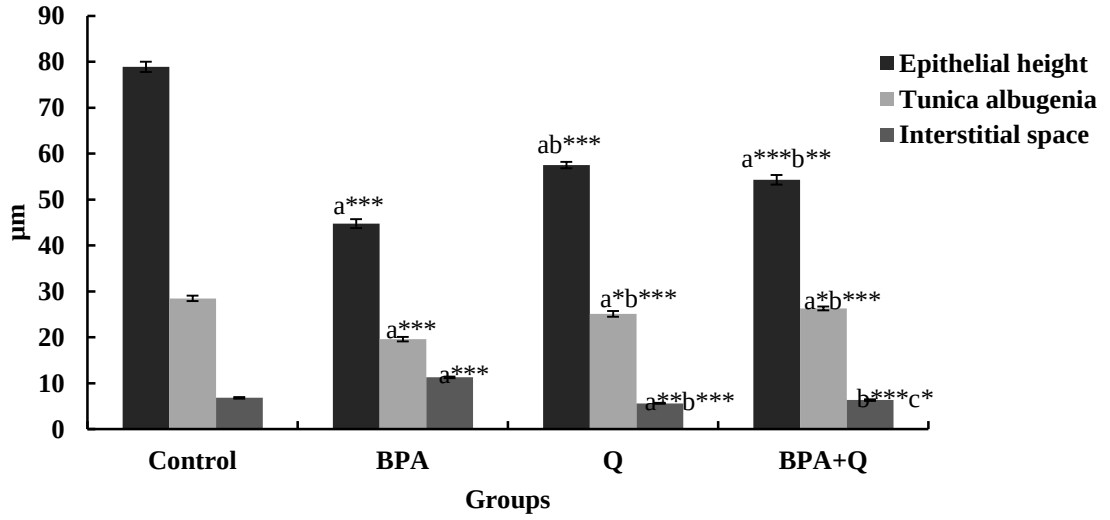

Fig 9: Mean seminiferous tubule epithelial height, tunica albugenia height and interstitial space of control, BPA, quercetin and BPA + Q treated groups of adult male rats after 52 days of treatment. Data is expressed as Mean ± SEM.

Epididymis:

Histomorphometric changes were observed in the caput and cauda of epididymis. The data is given in Table 7.

Caput epididymis:

Histological examination of caput in the control group presented larger diameter, wider lumen and thin pseudostratified epithelium as compare to cauda epididymis. The epididymal tubules were densely arranged and surrounded by stroma. The lumen was filled with large number of spermatozoa (Fig 10a).

Bisphenol A treatment resulted in significant degeneration of epididymal tubules. There was increase in interstitial spaces and reduction in stroma that surrounded the tubules. Number of sperms in the lumen was also significantly reduced in BPA treated rats compared to other groups resulting in the decrease of luminal diameter (Fig 10b).

Histological analysis of caput epididymis in quercetin treated and BPA+Q treated groups showed slight differences from control group. However, noticeable changes were found when both groups were compared with BPA treated group. A significant increase in the tubular diameter as compared to BPA treated group was observed. Sperm concentration in the lumen was restored significantly in quercetin treated and BPA+Q treated group leading to the increase in luminal diameter (Fig 10c)

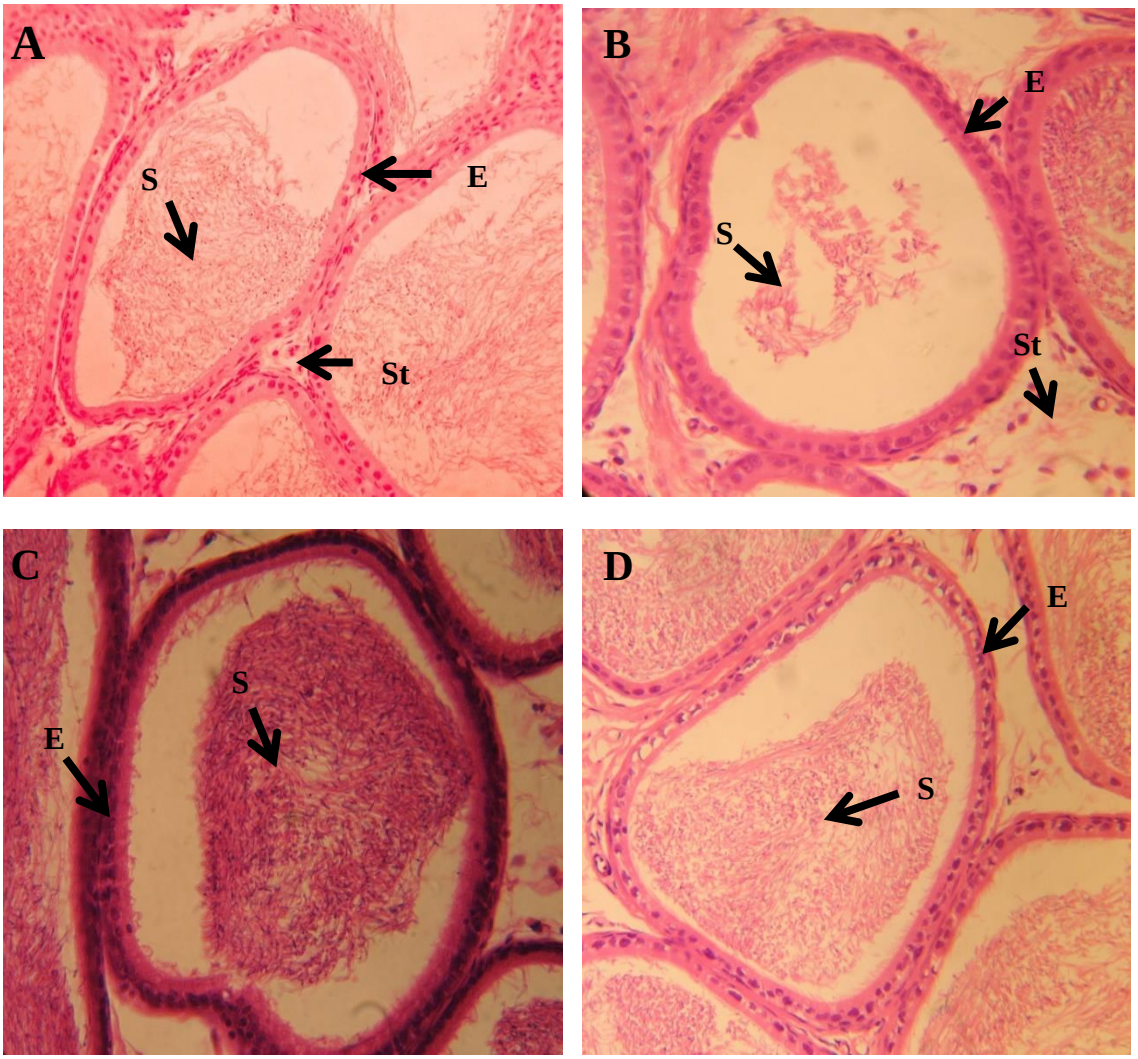

Fig 10: Photomicrograph of adult male rat caput epididymis (H&E, 40X) from: (A) Control group; showing normal morphology of caput epididymis, thin pseudostratified epithelium lined with stereocilia and lumen filled with spermatozoa, (B) BPA treated; showing reduced pseudostratified epithelium with distorted spermatozoa and empty lumen in most of the tubules, (C) Quercetin group; showing no difference fron control group, (D) BPA+Q; showing increase in pdeudostratified epithelium and increased concentration of spermatozoa as compared to treated group. Stroma (St), Epithelium (E), Spermatozoa (S).

Ductular diameter:

A highly significant ($p<0.001$) decrease was observed in the ductular diameter of caput epididymis in BPA treated group as compare to control. A non- significant reduction in the ductular diameter of querctin treated and BPA+Q treated groups was seen when compared to control however both groups showed significant increase ($p<0.05$) when compared to BPA treated group. There was no significant difference between quercetin treated and BPA+Q treated groups groups (Table 7).

Luminal diameter:

There was a highly significant reduction ($p<0.001$) in luminal diameter of BPA treated group as compare to control group. No significant difference in luminal diameter of quercetin treated and BPA+Q treated groups was observed when compared to control but both the groups showed a significant difference ($p<0.05$) in luminal diameter as compare to BPA treated group. However, no significant difference was seen between quercetin treated and BPA+Q treated groups (Table 7).

Epithelial cell height:

A highly significant decrease ($p<0.001$) was observed in the epithelial cell height of BPA treated group as compared to control group. There was a significant reduction in the epithelial cell height of quercetin treated ($p<0.05$) and BP+Q treated ($p<0.001$) groups when compared to control group. However, a highly significant increase of epithelial cell height was observed in quercetin treated ($p<0.001$) and BPA+Q treated ($p<0.05$) groups when compared to BPA treated group. The epithelial cell height of quercetin treated group showed significant difference ($p<0.001$) when compared to BPA+Q treated group as shown in table 7

Cauda epididymis:

The cauda epididymis from control group exhibited normal morphology with compactly arranged tubules. The epididymis tubules were surrounded by stroma. In control group, thick and pseudostratified epithelium was observed lined with stereocilia, and lumen was filled with spermatozoa. The pseudostratified epithelium of epididymis has two kinds of cell: the principle cells which extend from basal lamina to the short lumen while basal cells were located at the basal lamina (Fig 11a).

A significant reduction in ductular diameter, luminar diameter and epithelium height was observed in BPA treated group as compare to control group. The number of spermatozoa in the lumen of cauda epididymis was significantly reduced leading to the narrowing of lumen compared to control (Fig 11b).

Quercetin treated BPA+Q treated groups showed slight differences in histomorphology of cauda epididymus from control group. However, both groups showed obvious changes when compared with BPA treated group. There was significant increase in the ductular diameter and epithelial height as compared to BPA treated group. The increase in number of spermatozoa in the cauda lumen resulted in widening of lumen as compared to BPA treated group (Fig 11c).

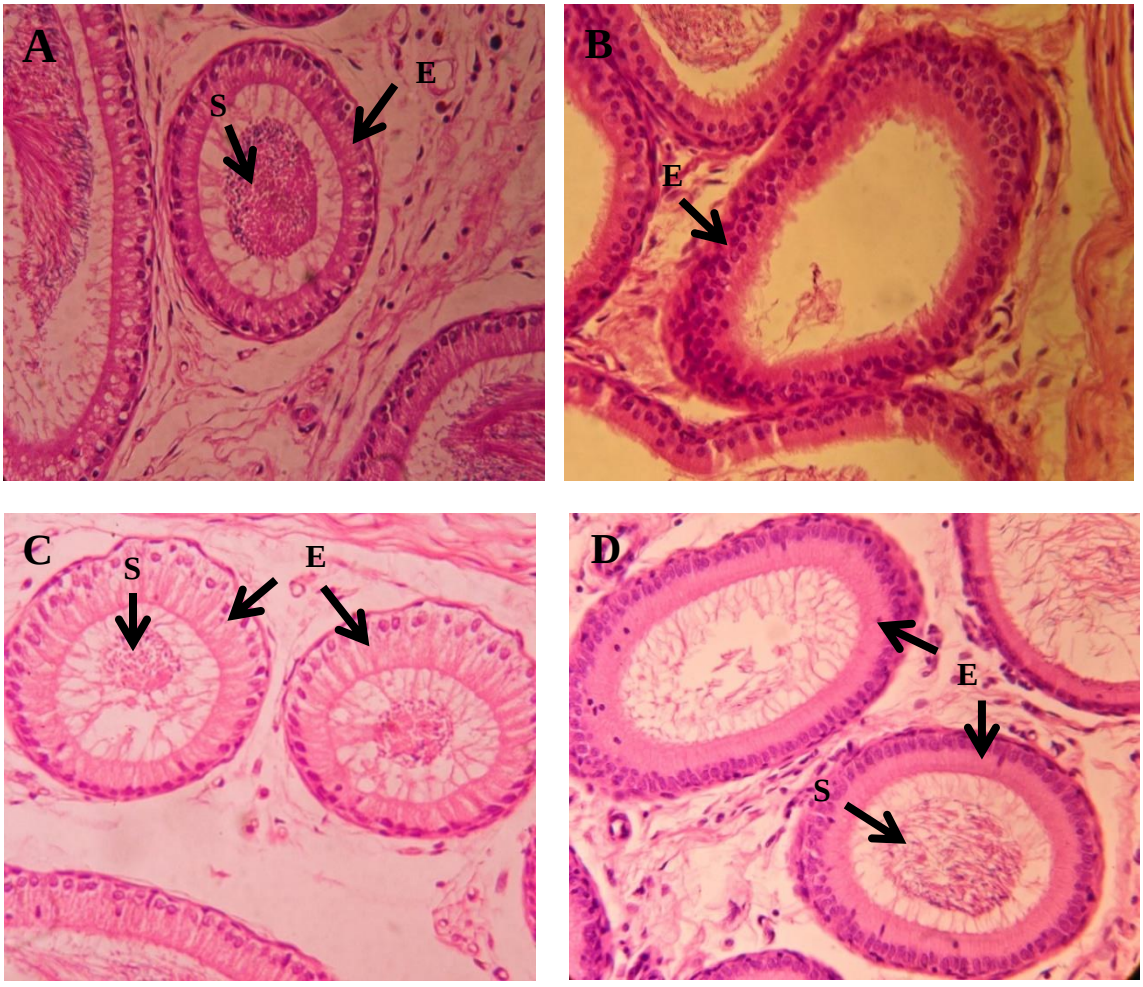

Fig 11: photomicrograph of cross section of cauda epididymis (H&E, 40X) from: (A) Control; showing normal morphology of cauda epididymis showing compactly arranged tubules with thick epithelium, lumen filled with sperm, (B) BPA treated; showing marked changes in structure of tubule with decreased concentration of sperm, (C) Quercetin treated; showing regular arrangement of tubules surrounded by stroma, lumen filled with spermatozoa, (D) BPA+Q; showing increase in epithelium an lumen sperm concentration. Spermatozoa (S), Epithelium (E), Stroma (St).

Ductular diameter:

A highly significant reduction ($p<0.001$) was observed in the mean ductular diameter of BPA treated group as compare to control. There was slight reduction in quercetin treated group while no significant change was seen in BPA+Q treated group when compared to control. Quercetin treated group showed no significant difference in ductular diameter while BPA+Q treated group exhibited significant increase ($p<0.01$) as compare to BPA treated group. No significant difference was observed among quercetin treated and BPA+Q treated groups as presented in table 7.

Lumen diameter:

There was a significant reduction ($p<0.001$) in the mean luminal diameter of BPA treated group as compare to control group. However, when quercetin treated and BPA+Q treated groups were compared with BPA treated group; they showed a significant increase ($p<0.05$) in luminal diameter. There was a slight increase in luminal diameter of quercetin treated group as compare to BPA+Q treated group but that was not significant as presented in table 7.

Epithelial cell height:

Epithelial cell height decreased significantly ($p<0.001$) in BPA treated group as compare to control group. Similarly, quercetin treated and BPA+Q treated group also showed a significant difference in epithelial cell height when compared with control group ($p<0.05$, $p<0.001$). A significant increase ($p<0.01$) was also observed in epithelial cell height of quercetin treated group and BPA+Q treated group ($p<0.001$) when compared to BPA treated group. There was no significant difference in epithelial cell height between quercetin tretaed and BPA+Q treated groups as shown in table 7.

Table 7: Mean ± SEM of ductular diameter (µm), luminal diameter (µm) and epididymis cell height (µm) of epididymis in control and treated groups after 52 days of treatment.

Group (n=5)	Ductular diameter (µm)		Lumen diameter (µm)		Epithelial height (µm)	
	Caput	Cauda	Caput	Cauda	Caput	Cauda
Control	260.47 ± 4.10	239.50 ± 9.68	233.79 ± 5.48	209.47 ± 8.53	31.08 ± 0.35	209.47 ± 8.53
Bisphenol A	$236.91 \pm 5.00^{a**}$	$193.92 \pm 5.66^{a***}$	$205.62 \pm 5.07^{a***}$	$152.95 \pm 5.73^{a***}$	$12.13 \pm 0.19^{a***}$	$152.95 \pm 5.73^{a***}$
Quercetin	$255.34 \pm 5.30^{b**}$	$210.13 \pm 8.52^{a*}$	$221.58 \pm 4.58^{b*}$	$165.19 \pm 5.88^{a***}$	$26.71 \pm 0.61^{a*b***}$	$165.19 \pm 5.88^{a***b**}$
Bisphenol A+ Quercetin	$250.91 \pm 4.06^{b*}$	$215.65 \pm 5.95^{b**}$	$226.29 \pm 4.85^{b*}$	$171.61 \pm 3.98^{a***}$	$19.13 \pm 0.48^{ac***b*}$	$171.61 \pm 3.98^{ab***}$

Values are expressed as mean ± SEM

*p< 0.05, **p< 0.01, ***p< 0.001

[a] = Value vs control

[b] = Value vs Bisphenol A

[c] = Value vs Bisphenol A + Quercetin

Stereological analysis:

The number of different cells was counted in each seminiferous tubule and presented in table 8. The cells counted include: Spermatogonia, Primary spermatocytes, Secondary spermatocytes, Spermatids.

Seminiferous tubule spermatogonia:

A significant reduction ($p<0.001$) in the number of spermatogonia was observed in BPA treated group as compare to control group. There was no significant difference in number of spermatogonia of quercetin treated and BPA+Q treated groups when compared to control, however, both groups showed significant increase ($p<0.001$) in their numbers as compare to BPA treated group. A significant difference ($p<0.001$) was found among BPA treated and BPA+Q treated groups as shown in Fig 12.

Seminiferous tubule primary spermatocytes:

Significant reduction ($p<0.001$) in the number of primary spermatocytes was observed in BPA treated group as compare to control group. There was no significant difference in their numbers in quercetin treated and BPA+Q treated groups when compared to control. Quercetin treated group exhibited a significant difference ($p<0.001$) in primary spermatocytes number when compared to BPA treated group. No significant differences were noticed between quercetin treated and BPA+Q treated groups (Fig 12).

Seminiferous tubule secondary spermatocytes:

There was a significant reduction ($p<0.001$) of secondary spermatocyte numbers in BPA treated group when compared to control. Both, quercetin treated and BPA+Q treated groups showed a non- significant difference in the number of secondary spermatocytes when compared to control while highly significant difference ($p<0.001$) was observed when compared with BPA treated group. A slight difference of significance ($p<0.05$) was seen between quercetin treated and BPA+Q treated groups (Fig 13).

Seminiferous tubule spermatids:

Significant reduction ($p<0.001$) in number of spermatid was observed in BPA treated group when compared with control group. Quercetin treated and BPA+Q treated groups showed no significant difference in their number when compared with control while a significant increase ($p<0.001$) was seen when compared with BPA treated group. There was slight non- significant difference in the number of spermatid between quercetin treated and BPA+Q treated groups (Fig 13).

Table 8: Mean ± SEM number of different cell types in each seminiferous tubule per field of control and treated rats after 52 days of treatment.

Group (n=5)	No. of Spermatogonia	No. of Primary Spermatocytes	No. of Secondary Spermatocytes	No. of Spermatids
Control	60.17 ± 0.93	38.81 ± 0.85	31.56 ± 1.18	93.08 ± 0.97
Bisphenol A	$52.17 \pm 0.82^{a***}$	$34.72 \pm 0.62^{a***}$	$21.86 \pm 0.57^{a***}$	$69.53 \pm 1.46^{a***}$
Quercetin	$63.33 \pm 1.12^{b***}$	$38.97 \pm 0.42^{b***}$	$32.28 \pm 0.54^{b***}$	$93.19 \pm 0.53^{b***}$
Bisphenol A+ Quercetin	$57.72 \pm 0.75^{b***c***}$	36.78 ± 0.91	$29.06 \pm 0.50^{b***c*}$	$87.97 \pm 1.32^{b***}$

Values are expressed as mean ± SEM

*p< 0.05, **p< 0.01, ***p< 0.001

[a] = Value vs control

[b] = Value vs Bisphenol A

[c] = Value vs Bisphenol A + Quercetin

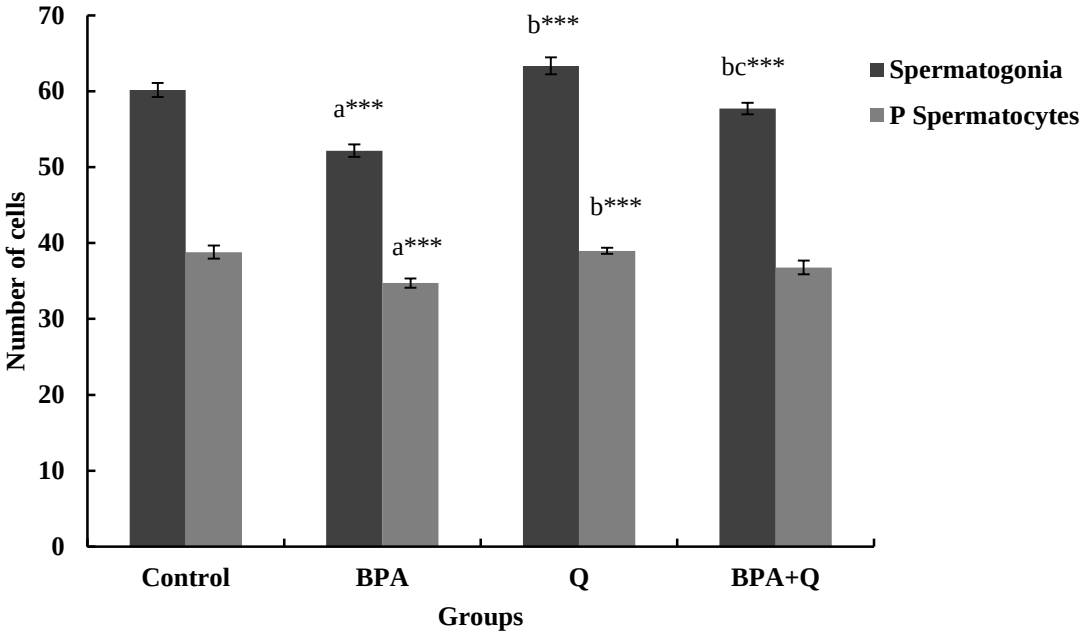

Fig 12: Mean number of spermatogonia and primary spermatocytes in seminiferous tubule of control, BPA, quercetin and BPA + Q treated groups of adult male rat after 52 days of treatment. Values are expressed as Mean ± SEM.

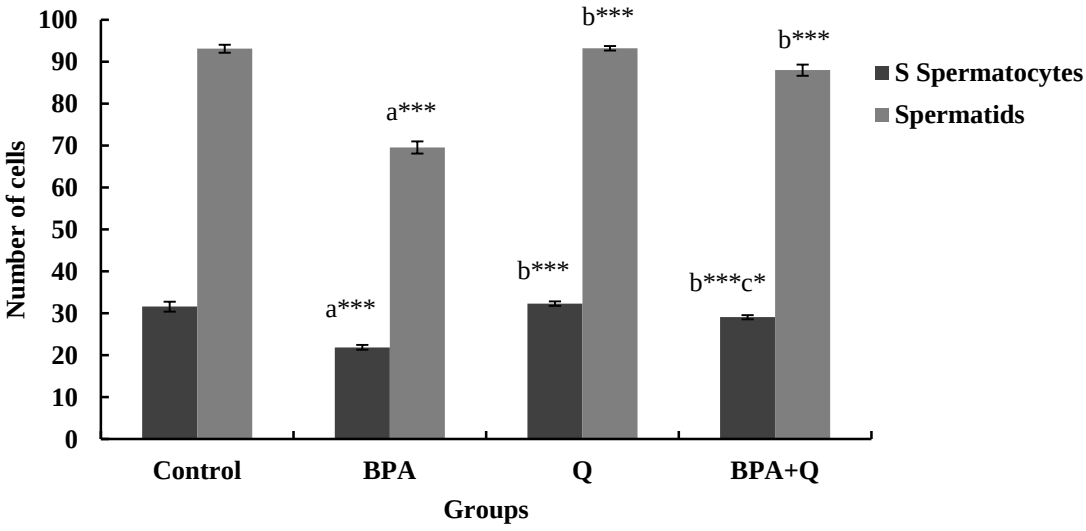

Fig 13: Mean number of secondary spermatocytes and spermatids in seminiferous tubule of control, BPA, quercetin and BPA + Q treated groups of adult male rat after 52 days of treatmnt. Values are expressed as Mean ± SEM.

Hormonal Analysis:

The change in plasma testosterone (ng/dl) and plasma estradiol concentration (ng/dl) in adult male rats after 52 days of treatment is shown in Table 9.

Plasma Testosterone:

Plasma testosterone levels were significantly lowered ($p<0.01$) from 5.78 ± 1.06 ng/ml to 1.67 ± 0.3 ng/ml in BPA treated group as compare to control group. No significant changes were observed in quercetin treated and BPA+Q treated groups when compared with control. However, quercetin alone and in combination with BPA significantly increased ($p<0.05$) plasma testosterone concentration when compared to BPA treated group (Fig 14).

Plasma Estradiol:

A slight decrease in estradiol concentration from 0.86 ± 0.07 ng/ml to 0.78 ± 0.09 ng/ml in BPA treated group when compared to control group was noticed, but that difference was not significant. Similarly, no significant changes were found in quercetin treated and BPA+Q treated groups when compared to control and BPA treated groups (Fig 14).

Table 9: Mean ± SEM plasma testosterone (ng/ml) and plasma estradiol (ng/ml) concentration in control and treated groups after 52 days of treatment.

Group (n=5)	Plasma Testosterone level (ng/ml)	Plasma Estradiol level (ng/ml)
Control	5.78 ± 1.06	0.86 ± 0.07
Bisphenol A	1.44 ± 0.21[a**]	0.78 ± 0.09
Quercetin	3.89 ± 0.26[b*]	0.83 ± 0.09
Bisphenol A+ Quercetin	4.03 ± 0.44[b*]	0.68 ± 0.07

Values are expressed as mean ± SEM

*p< 0.05, **p< 0.01, ***p< 0.001

[a =] Value vs control

[b =] Value vs Bisphenol A

[c =] Value vs Bisphenol A + Quercetin

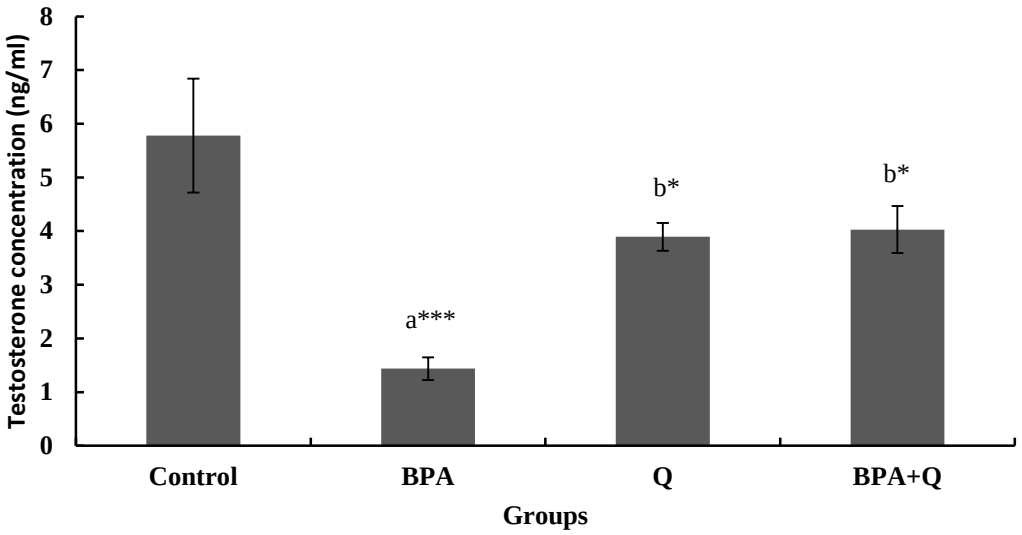

Fig 14: Mean plasma testosterone concentration (ng/ml) in control, BPA, quercetin, BPA + Q treated groups of adult male rats after 52 days of tratment. Data is expressed as Mean ± SEM.

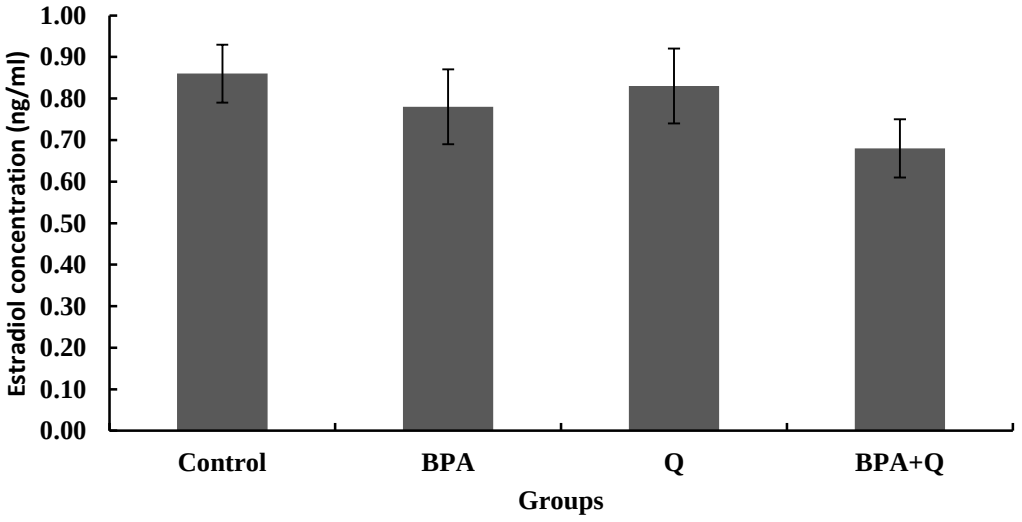

Fig 15: Mean plasma estradiol concentration (ng/ml) in control, BPA, quercetin, BPA + Q treated groups of adult male rats after 52 days of treatment. Data is expressed as Mean ± SEM.

Discussion

The present study was designed to evaluate possible therapeutic effect of quercetin against BPA induced reproductive toxicity and testicular damage in adult male rats. Bisphenol A acts as endocrine disruptor; many studies have revealed its toxic effects on reproduction (Aydoğan and Barlas, 2006; Richter *et al*., 2007). Besides, Bisphenol A is known to cause oxidative stress by disturbing normal redox status in different organs (Hasselberg *et al*., 2004).

In order to induce reproductive toxicity, rats were exposed to BPA at the dose of 50 mg/kg/day for 52 days. The results indicated BPA induced elevation in BUN and creatinine levels, impared spermatogenesis and hormonal imbalance. These effects were amended by oral administration of quercetin at the dose of 50 mg/kg/day for 52 days.

The results of our study indicated no significant change in body weight and reproductive organ weight of BPA treated group as compare to control. These results are similar to previous studies (Wu *et al*., 2013). Similarly, quercetin treatment increased the organ weight but this increase was not significant, this result is in accordance with earlier studies in which 2µg/kg dose of TCDD to rats caused no significant change in organ weights (Ciftci *et al*., 2012). But disagreed with studies reported by Taepongsorat *et al*., (2008) in which no change was observed in body weights of rats when treated with polychlorinated biphenyls. One possible explanation for contradictory results may be difference in amount of dose, time period of experiment and rout of exposure.

As BPA is metabolized in liver and expelled out through kidney, so, there is possibility that BPA can exert toxic effects on these organs. In the present study, administration of BPA at the dose of 50 mg/kg for 52 days caused significant increase in serum blood urea nitrogen levels (BUN) as well as serum creatinine levels. This result is in accordance with previous findings in which BPA was investigated to induce nephrotoxicity by increasing oxidative stress on kidney tissues (Korkmaz *et al*., 2011). Similarly, a study conducted by Helal *et al* (2013) showed significant increase in BUN and creatinine levels when treated with BPA (30 mg/kg/day) for one week. In our

present investigation, treatment with quercetin (50 mg/kg/day) normalized the BUN and creatinine levels.

The result of present study indicated a significant increase in serum total cholesterol, triglyceride and LDL along with reduction of HDL cholesterol in BPA treated group. These results are similar to earlier studies on rabbits, in which administration of intravenous injections of BPA for 12 weeks caused increase in serum total cholesterol and triglycerides (Witting *et al*., 1999). Probably, as BPA has estrogenic activity, so enhanced estrogen significantly affect serum total cholesterol. Another possibility is action of estrogen hormone on lipoproteins that are linked with cholesterol in blood circulation. So, myocardial infractions and arterosclerotic complications may be due to higher estrogenic activity of BPA (Ganong and Ganong 1997). Present data showed that quercetin treatment for 52 days corrected the BPA induced hyperlipidemia. These results are in close agreement with earler studies by Prabu *et al*. (2013) in which quercetin (50 mg/kg/day) treatment amended cadmium induced hypercholesterolemia. This hypolipidemic effect of quercetin is because of its antilipoperoxidative activity and antioxidant effect, which prevents BPA induced oxidative damage by inhibiting oxidation of lipid components in addition to prevention of hepercholestrolemia (Prabu *et al*. 2013).

In present study, BPA exposure for 52 days brought about significant decrease in seminiferous tubule diameter, epithelial height, tunica albugenia height and germ cells, while significant increase was observed in tubular lumen diameter and interstitial spaces thus disturbing normal spermatogenesis. Previously, Gurmeet *et al*. (2014) showed similar results when rats were treated with BPA (100 mg/kg BW). Likewise, a significant decrease of mature spermatids was observed in seminiferous tubules when mouse offspring were exposed to 5μg BPA for 4 weeks (Okada and Kai 2008). Sharpe and Skakkebaek (1993) have indicated that environmental estrogens interfere with spermatogenesis by affecting proliferation and differentiation of Sertoli cells during fetal life (Sharpe and Skakkebaek 1993). Earlier studies show that BPA caused modifications in hypothalamic-pituitary-gonadal axis and reduction in phagocytic functions of Sertoli

cell are perhaps responsible for testicular pathogenicity and spermatozoa toxicity (Vandenberg *et al*., 2009).

It has been reported that Sertoli cells act as central regulator of testicular functions and maintenance of spermatogenesis by interacting with germ cells. So regulation of Sertoli cells proliferation is necessary for normal spermatogenesis. Previous studies showed that FSH stimulates Sertoli cells function as reduction in FSH concentration lead to the decrease in germ cells of spermatogenic cycle (Jin *et al*., 2013).

The spermatogenic cycle initiates with differentiation of spermatogonia for which testosterone action is mendatory. The results of our study showed significantly lower level of plasma testosterone in BPA treated animals. So, it is suggested that the low testosterone level may have caused failure of spermatogenesis and disruption of the seminiferous epithelium. Nanjappa *et al*. (2012) proposed that low plasma testosterone level in BPA treated rats was perhaps due to interference of proliferation and development of Leydig cells in rat. On the other hand, low level of 17β- oestradiol may be the cause of apoptotic degeneration of male germ cells (Pentikäinen *et al*., 2000) that may be the cause of lack of spermatozoa in lumen.

It is generally believed that BPA cause testicular toxicity through oxidative stress and by the production of free radicals. Increased oxidative stress causes damage to the sperm membranes, proteins and DNA that are associated with male fertility (Kalender *et al*., 2005). Excessive production of reactive oxygen species (ROS) could be an indicator for male infertility and causes abnormality in spermatozoa. High concentrations of hydrogen peroxide (H_2O_2) induce lipid peroxidation and can cause reduction in spermatogenesis and cell death. Spermatozoa are highly vulnerable to the damage induced by ROS because of the high content of polyunsaturated fatty acids. To lessen the effects of ROS, spermatozoa are equipped with antioxidant defense systems, which inhibit cellular damage (Turrens, 2003)

The results of our study showed that quercetin amended testicular toxicity induced by BPA that are consistent with previous studies in which quercetin reduced reproductive toxicity induced by TCDD (Ciftci *et al*., 2012). Another study conducted

by Zhang (2005) reported that quercetin prevented oxidative stress induced by polychlorinated biphenyls. Similarly, it was reported that tubular diameter was increased by quercetine treatment in dose dependent manner (Taepongsorat *et al*., 2008). In addition, quercetin inhibited DEPs induced testicular damage (Izawa *et al*., 2008). Quercetin provides protection against oxidative stress with its antioxidant and metal chelating activity. Co-administration of quercetin along with cadmium decreased the production of H_2O_2 and lipid peroxidation (Ola-Mudathir *et al*., 2008). It is assumed that chemical structure of quercetin play important role in free radical scavenging activity. The B ring hydroxyl configuration is an important factor of scavenging ROS because it donates an electron to hydroxyl radicals, thus giving rise to a relatively stable flavonoids radical (Hernández *et al*., 2009; Wolf *et al*., 2010; Fini *et al*., 2012). The antioxidant activity of flavonoids depends upon the arrangement of functional groups around the nuclear structure. The configuration, substitution, and total number of hydroxyl groups significantly influence several mechanisms of antioxidant activity such as radical scavenging and metal ion chelation ability (Zancan *et al*., 2002; Pourcel *et al*., 2006; Agati *et al*., 2011).

The results of histological examination of caput and cauda exhibited significant decrease in tubular diameter, tubular lumen diameter and epithelial height as reported in earlier study in which BPA exposure at low dose lead to complete degeneration of epididymal epithelium along with reduction in spermatozoa of male rats (Chitra *et al*., 2003). This observation may be due to low level of serum testosterone. It was found that quercetine treatment increased tubular diameter, lumen diameter and epithelial height in BPA treated rats. Cotreatment of BPA along with quercetin also revised changes in epididymal structure thus indicating that cotreatment of BPA+Q can prevent epididymal toxicity. These results are in close agreement with previous study in which quercetin corrected TCDD induced epididymal damage (Ciftci *et al*., 2012).

The exposure of rats to BPA for 52 days lead to the significant reduction in plasma testosterone level which is in agreement with previous studies in which reduced testosterone level was observed in rats that were administered subcutaneous injections of BPA for one week (Helal *et al*., 2013). In additional, Akingbemi *et al*. (2004) reported

that BPA disrupt normal testicular functions by acting on pituitary thus reducing the release of LH and Leydig cells steroidogenesis. This reduced serum LH levels along with decreased Leydig cell androgen production lead to the reduction of serum testosterone levels. Another possibility may be the interference of BPA with LH receptor-legend binding leading thus inhibiting testosterone production. Similarly, in vitro study conducted by Akingbemi *et al*. (2004) showed that oral administration of BPA (2.4µg/kg/day) lead to the decreased testosterone production from Leydig cells either by suppressing 17 α-hydroxylase or by enhancing aromatase activity to reduce testosterone production (Kim *et al*., 2010). A recent study proposed that subcutaneous administration of BPA for six weeks lead to reduction in Leydig cells as well as testosterone (Nakamura *et al*., 2010). Therefore, BPA inhibited serum testosterone production via reduced LH release. The treatment with quercetin at the dose of 50 mg/kg/day for 52 days elevated plasma testosterone to normal levels. These results are comparable with previous reports in which quercetin treatment elevated testosterone levels in diabetic rats (Tang *et al*., 2008; Khaki *et al*., 2010). Similarly, quercetin treatment lowered TCDD induced increase in serum testosterone level (Ciftci *et al*., 2012).

The results of our study showed marginal decrease in estradiol level when treated with BPA. Similarly, a recent study showed that BPA caused reduction in estradiol production (Peretz *et al*., 2011). As BPA is estrogenic compound and interacts with estrogen receptors, but this binding to ER is week. Previous studies showed that BPA exhibits estrogen like activities stronger than natural hormone E_2 (Hugo *et al*., 2008) thus elevating estrogen level that is inconsistent with our studies. This conflict may be due to change in amount of dose and period of exposure. Quercetin treatment slightly elevated estrogen level as compare to BPA treated group in our studies. Similar results were reported by Selim *et al*. (2013) where quercetine revised letrozol induced reduction in estradiol level.

Therefore, present study concluded that exposure of Bisphenol A lead to the structural and functional impairments in testes, epididymis and cause hormonal imbalance in rats. BPA has the potential to cause reproductive toxicity and impair

spermatogenesis by producing reactive oxygen species and disturbing natural antioxidant environment. Quercetin treatment ameliorates BPA induced toxic effects by inhibiting reactive oxygen species (ROS) by using its antioxidant properties. So, from our studies, it is suggested that quercetin can be used as potent therapeutic agent against BPA induced reproductive toxicity. Further studies are required to find out exact mechanism played by quercetin as therapeutic agent against toxic effects of environmental pollutants.

References

Agati, G., Biricolti, S. Guidi, L., Ferrini, F., Fini. A., Tattini, M. 2011 The biosynthesis of flavonoids is enhanced similarly by UV radiation and root zone salinity in L. vulgare leaves. J Plant Physiol **168**(3):204-12.

Akingbemi, B.T., Sottas, C.M., Koulova, A.I., Klinefelter, G.R. and Hardy, M.P. 2004 Inhibition of testicular steroidogenesis by the xenoestrogen bisphenol A is associated with reduced pituitary luteinizing hormone secretion and decreased steroidogenic enzyme gene expression in rat Leydig cells. Endocrinology. **145:** 592-603.

Alonso-Magdalena, P., Laribi, O., Ropero, A.B., Fuentes, E., Ripoll, C., Soria, B. and Nadal, A. 2005 Low doses of bisphenol A and diethylstilbestrol impair Ca2+ signals in pancreatic α-cells through a nonclassical membrane estrogen receptor within intact islets of Langerhans. Environ Health Perspect. 969-977.

Alonso-Magdalena, P., Morimoto, S., Ripoll, C., Fuentes, E. and Nadal, A. 2006 The estrogenic effect of bisphenol A disrupts pancreatic β-cell function in vivo and induces insulin resistance. Environ Health Perspect. **114**: 106.

Alonso-Magdalena, P., Ropero, A.B., Carrera, M.P., Cederroth, C.R., Baquie, M., Gauthier, B.R., Nef, S., Stefani, E. and Nadal, A. 2008 Pancreatic insulin content regulation by the estrogen receptor ERα. PloS one **3** (4): e2069.

Alonso-Magdalena, P., Vieira, E., Soriano, S., Menes, L., Burks, D., Quesada, I. and Nadal, A. 2010 Bisphenol A exposure during pregnancy disrupts glucose homeostasis in mothers and adult male offspring. Environ Health Perspect. **118**: 1243-1250.

Anjaneyulu, M. and Chopra, K. 2003 Quercetin, a bioflavonoid, attenuates thermal hyperalgesia in a mouse model of diabetic neuropathic pain. Prog Neuropsychopharmacol Biol Psychiatry **27**: 1001-1005.

Anjum, S., Rahman, S., Kaur, M., Ahmad, F., Rashid, H., Ansari, R.A. and Raisuddin, S. 2011 Melatonin ameliorates bisphenol A-induced biochemical toxicity in testicular mitochondria of mouse. Food Chem Toxicol. **49**: 2849-2854.

Aravindakshan, M., Chauhan, P. and Sundaram, K. 1985 Studies on germinal effects of quercetin, a naturally occurring flavonoid. Mutat Res Lett. **144**: 99-106.

Aydoğan, M. and Barlas, N. 2006 Effects of maternal 4-tert-octylphenol exposure on the reproductive tract of male rats at adulthood. Reprod Toxicol. **22:** 455-460.

Bánfi, B., Molnár, G., Maturana, A., Steger, K., Hegedûs, B., Demaurex, N. and Krause, K.H. 2001 A Ca2+-activated NADPH oxidase in testis, spleen, and lymph nodes. J Biol Chem **276**: 37594-37601.

Bischoff, S.C. 2008 Quercetin: potentials in the prevention and therapy of disease. Curr Opin Clin Nutr Metab Care. **11:** 733-740.

Brotons, J.A., Olea-Serrano, M.F., Villalobos, M., Pedraza, V. and Olea, N. 1995 Xenoestrogens released from lacquer coatings in food cans. Environ Health Perspect. **103**: 608.

Calafat, A.M., Kuklenyik, Z., Reidy, J.A., Caudill, S.P., Ekong, J. and Needham, L.L. 2005 Urinary concentrations of bisphenol A and 4-nonylphenol in a human reference population. Environ Health Perspect. **113** (4): 391-395.

Chitra, K., Rao, K.R. and Mathur, P. 2003 Effect of bisphenol A and co-administration of bisphenol A and vitamin C on epididymis of adult rats: A histological and biochemical study. Asian J Androl. **5:** 203-208.

Chung, S.Y., Kwon, H., Choi, Y.H., Karmaus, W., Merchant, A.T., Song, K.B., Sakong, J., Ha, M., Hong, Y.C. and Kang, D. 2012 Dental composite fillings and bisphenol A among children: a survey in South Korea. Int Dent J. **62**: 65-69.

Ciftci, O., Aydin, M., Ozdemir, I. and Vardi, N. 2012 Quercetin prevents 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin-induced testicular damage in rats. Andrologia. **44:** 164-173.

Colborn, T., vom Saal, F.S. and Soto, A.M. 1993 Developmental effects of endocrine disrupting chemicals in wildlife and humans. Environ Health Perspect. **101**: 378.

D'Cruz, S.C., Jubendradass, R., Jayakanthan, M., Rani, S.J.A. and Mathur, P.P. 2012 Bisphenol A impairs insulin signaling and glucose homeostasis and decreases steroidogenesis in rat testis: An in vivo and in silico study. Food Chem Toxicol. **50**: 1124-1133.

da Silva, E.L., Piskula, M.K., Yamamoto, N., Moon, J.-H. and Terao, J. 1998 Quercetin metabolites inhibit copper ion-induced lipid peroxidation in rat plasma. FEBS Lett. **430**: 405-408.

Dessi-Fulgheri, F., Porrini, S. and Farabollini, F. 2002 Effects of perinatal exposure to bisphenol A on play behavior of female and male juvenile rats. Environ Health Perspect. **110**: 403.

Dok-Go, H., Lee, K.H., Kim, H.J., Lee, E.H., Lee, J., Song, Y.S., Lee, Y.-H., Jin, C., Lee, Y.S. and Cho, J. 2003 Neuroprotective effects of antioxidative flavonoids, quercetin,(+)-dihydroquercetin and quercetin 3-methyl ether, isolated from Opuntia ficus-indica var. saboten. Brain Res. **965**: 130-136.

Doshi, T., Mehta, S.S., Dighe, V., Balasinor, N. and Vanage, G. 2011 Hypermethylation of estrogen receptor promoter region in adult testis of rats exposed neonatally to bisphenol A. Toxicology. **289**: 74-82.

Facciolo, R.M., Alò, R., Madeo, M., Canonaco, M. and Dessì-Fulgheri, F. 2002 Early cerebral activities of the environmental estrogen bisphenol A appear to act via the somatostatin receptor subtype sst (2). Environ Health Perspect. **110**: 397.

Farabollini, F., Porrini, S., Della Seta, D., Bianchi, F. and Dessì-Fulgheri, F. 2002 Effects of perinatal exposure to bisphenol A on sociosexual behavior of female and male rats. Environ Health Perspect. **110**: 409.

Ferrali, M., Signorini, C., Caciotti, B., Sugherini, L., Ciccoli, L., Giachetti, D. and Comporti, M. 1997 Protection against oxidative damage of erythrocyte membrane by the flavonoid quercetin and its relation to iron chelating activity. FEBS Lett. **416**: 123-129.

Fini, A., Guidi, L., Ferrini, F., Brunetti, C., Di Ferdinando, M., Biricolti, S., Pollastri, S., Calamai, L. and Tattini, M. 2012 Drought stress has contrasting effects on antioxidant enzymes activity and phenylpropanoid biosynthesis in Fraxinus ornus leaves: An excess light stress affair? J Plant Physiol. **169:** 929-939.

Fleisch, A.F., Sheffield, P.E., Chinn, C., Edelstein, B.L. and Landrigan, P.J. 2010 Bisphenol A and related compounds in dental materials. Pediatrics. **126**: 760-768.

Formica, J. and Regelson, W. 1995 Review of the biology of quercetin and related bioflavonoids. Food Chem Toxicol. **33**: 1061-1080.

Fujimaki, K., Arakawa, C., Yoshinaga, J., Watanabe, C., Serizawa, S., Imai, H., Shiraishi, H. and Mizumoto, Y. 2004 Estimation of intake level of bisphenol A in Japanese pregnant women based on measurement of urinary excretion level of the metabolite. Nihon eiseigaku zasshi. Jap J Hyg. **59**: 403-408.

Furuya, M., Adachi, K., Kuwahara, S., Ogawa, K. and Tsukamoto, Y. 2006 Inhibition of male chick phenotypes and spermatogenesis by Bisphenol-A. Life Sci. **78**: 1767-1776.

Ganong, W.F. and Ganong, W. 1997 Review of medical physiology. Appleton & Lange Stamford, CT.

Geens, T., Aerts, D., Berthot, C., Bourguignon, J.P., Goeyens, L., Lecomte, P., Maghuin-Rogister, G., Pironnet, A.M., Pussemier, L. and Scippo, M.L. 2012 A

review of dietary and non-dietary exposure to bisphenol-A. Food Chem Toxicol. **50**: 3725-3740.

Gurmeet, K., Rosnah, I., Normadiah, M., Das, S. and Mustafa, A. 2014 Detrimental effects of bisphenol A on development and functions of the male reproductive system in experimental rats. EXCLI. **13.**

Hajszan, T. and Leranth, C. 2010 Bisphenol A interferes with synaptic remodeling. Front Neuroendocrinol. **31**: 519-530.

Hales, D.B., Allen, J.A., Shankara, T., Janus, P., Buck, S., Diemer, T. and Hales, K.H. 2005 Mitochondrial function in Leydig cell steroidogenesis. Ann NY Acad Sci. **1061**: 120-134.

Halliwell, B. 1996 Antioxidants in human health and disease. Annu Rev Nutr. **16**: 33-50.

Harborne, J.B. and Goodwin, T. 1988 The flavonoids: recent advances. Academic Press.

Havsteen, B. 1983. Flavonoids, a class of natural products of high pharmacological potency. Biochem Pharmacol. **32**: 1141-1148.

Hasselberg, L., Meier, S. and Svardal, A. 2004 Effects of alkylphenols on redox status in first spawning Atlantic cod (Gadus morhua). Aquat Toxicol. **69:** 95-105.

Helal, E.G., Badawi, M.M., Soliman, M.G., Abdel-Kawi, N.A., Fadel, H.A. and Abozaid, N.M. 2013 Physiological and Histopathological studies on Bisphenol-A compound as xenoestrogen in male albino rats. EJHM. **50:** 127-136.

Hernández, I., Alegre, L., Van Breusegem, F. and Munné-Bosch, S. 2009 How relevant are flavonoids as antioxidants in plants? Trends Plant Sci. **14:** 125-132.

Hertog, M.G., Hollman, P.C., Katan, M.B. and Kromhout, D. 1993 Intake of potentially anticarcinogenic flavonoids and their determinants in adults in The Netherlands. Natr Cancer. **20** (1); 21-9.

Ho, Y.S., Magnenat, J.L., Gargano, M. and Cao, J. 1998 The nature of antioxidant defense mechanisms: a lesson from transgenic studies. Environ Health Perspect. **106**: 1219.

Hugo, E.R., Brandebourg, T.D., Woo, J.G., Loftus, J., Alexander, J.W. and Ben-Jonathan, N. 2008 Bisphenol A at environmentally relevant doses inhibits adiponectin release from human adipose tissue explants and adipocytes. Environ Health Perspect. **116**: 1642-1647.

Izawa, H., Kohara, M., Aizawa, K., Suganuma, H., Inakuma, T., Watanabe, G., Taya, K. and Sagai, M. 2008 Alleviative effects of quercetin and onion on male reproductive toxicity induced by diesel exhaust particles. Biosci Biotechnol Biochem. **72:** 1235-1241.

Ikezuki, Y., Tsutsumi, O., Takai, Y., Kamei, Y. and Taketani, Y. 2002 Determination of bisphenol A concentrations in human biological fluids reveals significant early prenatal exposure. Hum Reprod. **17**: 2839-2841.

Inal, M., Akgün, A. and Kahraman, A. 2002 Radioprotective effects of exogenous glutathione against whole-body gamma-ray irradiation: Age-and gender-related changes in malondialdehyde levels, superoxide dismutase and catalase activities in rat liver. Methods Find Exp Clin Pharmacol. **24**: 209-212.

Ioku, K., Tsushida, T., Takei, Y., Nakatani, N. and Terao, J. 1995 Antioxidative activity of quercetin and quercetin monoglucosides in solution and phospholipid bilayers. Biochim Biophys Acta. **1234** (1): 99-104.

Ishido, M., Masuo, Y., Kunimoto, M., Oka, S. and Morita, M. 2004 Bisphenol A causes hyperactivity in the rat concomitantly with impairment of tyrosine hydroxylase immunoreactivity. J Neurosci Res. **76**: 423-433.

Jahan, S., Iftikhar, N., Ullah, H., Rukh, G., Hussain, I. 2014 Alleviative effect of quercetin on rat testis against arsenic: a histological and biochemical study. Syst Biol Reprod Med. 1-7.

Jin, P., Wang, X., Chang, F., Bai, Y., Li, Y., Zhou, R. and Chen, L. 2013 Low dose bisphenol A impairs spermatogenesis by suppressing reproductive hormone production and promoting germ cell apoptosis in adult rats. JBR. **27:** 135.

Kabuto, H., Hasuike, S., Minagawa, N. and Shishibori, T. 2003 Effects of bisphenol A on the metabolisms of active oxygen species in mouse tissues. Environ Res. **93**: 31-35.

Kalender, Y., Yel, M. and Kalender, S. 2005 Doxorubicin hepatotoxicity and hepatic free radical metabolism in rats: the effects of vitamin E and catechin. Toxicology. **209:** 39-45.

Khaki, A., Fathiazad, F., Nouri, M., Khaki, A., Maleki, N.A., Khamnei, H.J. and Ahmadi, P. 2010 Beneficial effects of quercetin on sperm parameters in streptozotocin-induced diabetic male rats. Phytother Res. **24:** 1285-1291.

Kim, J.Y., Han, E.H., Kim, H.G., Oh, K.N., Kim, S.K., Lee, K.Y. and Jeong, H.G. 2010 Bisphenol A-induced aromatase activation is mediated by cyclooxygenase-2 up-regulation in rat testicular Leydig cells. Toxicol Lett. **193:** 200-208.

Korkmaz, A., Aydoğan, M., Kolankaya, D. and Barlas, N. 2011 Vitamin C coadministration augments bisphenol A, nonylphenol, and octylphenol induced oxidative damage on kidney of rats. Environ Toxicol. **26:** 325-337.

Krishnan, A.V., Stathis, P., Permuth, S.F., Tokes, L. and Feldman, D. 1993 Bisphenol-A: an estrogenic substance is released from polycarbonate flasks during autoclaving. Endocrinology. **132**: 2279-2286.

Kuiper, G.G., Lemmen, J.G., Carlsson, B., Corton, J.C., Safe, S.H., van der Saag, P.T., van der Burg, B. and Gustafsson, J.A.k. 1998 Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor β. Endocrinology. **139**: 4252-4263.

Kumagai, A., Kodama, H., Kumagai, J., Fukuda, J., Kawamura, K., Tanikawa, H., Sato, N. and Tanaka, T. 2002 Xanthine oxidase inhibitors suppress testicular germ cell apoptosis induced by experimental cryptorchidism. Mol Hum Reprod. **8**: 118-123.

Lamson, D.W. and Brignall, M.S. 2000 Antioxidants and cancer, part 3: quercetin. Alternative medicine review: Clin Ther. **5**: 196-208.

Lang, I.A., Galloway, T.S., Scarlett, A., Henley, W.E., Depledge, M., Wallace, R.B. and Melzer, D. 2008 Association of urinary bisphenol A concentration with medical disorders and laboratory abnormalities in adults. Jama. **300**: 1303-1310.

Latchoumycandane, C., Chitra, K. and Mathur, P. 2002 The effect of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin on the antioxidant system in mitochondrial and microsomal fractions of rat testis. Toxicology. **171**: 127-135.

Laughton, M.J., Evans, P.J., Moroney, M.A., Hoult, J. and Halliwell, B. 1991 Inhibition of mammalian 5-lipoxygenase and cyclo-oxygenase by flavonoids and phenolic dietary additives: relationship to antioxidant activity and to iron ion-reducing ability. Biochem Pharmacol. **42**: 1673-1681.

Li, D.K., Zhou, Z., Miao, M., He, Y., Wang, J., Ferber, J., Herrinton, L.J., Gao, E. and Yuan, W. 2011 Urine bisphenol-A (BPA) level in relation to semen quality. Fertil Seril. **95**: 625-630.

Lien, E.J., Ren, S., Bui, H.-H. and Wang, R. 1999 Quantitative structure-activity relationship analysis of phenolic antioxidants. Free Radic Biol Med. **26**: 285-294.

Lind, P.M. and Lind, L. 2011 Circulating levels of bisphenol A and phthalates are related to carotid atherosclerosis in the elderly. Atherosclerosis. **218**: 207-213.

Lu, Y.X., Zhang, Q., Li, J., Sun, Y.X., Wang, L.Y., Cheng, W.M. and Hu, X.Y. 2010 Antidiabetic effects of total flavonoids from Litsea Coreana leve on fat-fed, streptozotocin-induced type 2 diabetic rats. Am J Chin Med. **38**: 713-725.

Ma, Z., Nguyen, T.H., Huynh, T.H., Do, P.T. and Huynh, H. 2004 Reduction of rat prostate weight by combined quercetin-finasteride treatment is associated with cell cycle deregulation. J Endocrinol. **181**: 493-507.

Melzer, D., Rice, N.E., Lewis, C., Henley, W.E. and Galloway, T.S. 2010 Association of urinary bisphenol a concentration with heart disease: evidence from NHANES 2003/06. PloS one **5**: e8673.

Middleton, E. 1984 The flavonoids, Trends in Pharmaceut Science. **5**: 335-380.

Mirshekar, M., Roghani, M., Khalili, M., Baluchnejadmojarad, T. and Moazzen, S.A. 2010 Chronic oral pelargonidin alleviates streptozotocin-induced diabetic neuropathic hyperalgesia in rat: involvement of oxidative stress. Iran Biomed J . **14**: 33.

Miyazaki, M., Flowers, M.T., Sampath, H., Chu, K., Otzelberger, C., Liu, X. and Ntambi, J.M. 2007 Hepatic stearoyl-CoA desaturase-1 deficiency protects mice from carbohydrate-induced adiposity and hepatic steatosis. Cell Metabolism. **6**: 484-496.

Moridani, M.Y., Pourahmad, J., Bui, H., Siraki, A. and O'Brien, P.J. 2003 Dietary flavonoid iron complexes as cytoprotective superoxide radical scavengers. Free Radic Biol Med. **34**: 243-253.

Moskaug, J.Ø., Carlsen, H., Myhrstad, M. and Blomhoff, R. 2004 Molecular imaging of the biological effects of quercetin and quercetin-rich foods. Mech Ageing Dev. **125**: 315-324.

Motoyama, K., Koyama, H., Moriwaki, M., Emura, K., Okuyama, S., Sato, E., Inoue, M., Shioi, A. and Nishizawa, Y. 2009 Atheroprotective and plaque-stabilizing effects of enzymatically modified isoquercitrin in atherogenic apoE-deficient mice. Nutrition. **25**: 421-427.

Nakamura, D., Yanagiba, Y., Duan, Z., Ito, Y., Okamura, A., Asaeda, N., Tagawa, Y., Li, C., Taya, K. and Zhang, S.Y. 2010 Bisphenol A may cause testosterone reduction by adversely affecting both testis and pituitary systems similar to estradiol. Toxicol Lett. **194:** 16-25.

Nanjappa, M.K., Simon, L. and Akingbemi, B.T. 2012 The industrial chemical bisphenol A (BPA) interferes with proliferative activity and development of steroidogenic capacity in rat Leydig cells. Biol Reprod. **86:** 135.

Nègre-Salvayre, A., Salvayre, R. 1992 Quercetin prevents the cytotoxicity of oxidized LDL on lymphoid cell lines. Free Radic Biol Med**.12**(2):101-6.

Ohno, S., Shinoda, S., Toyoshima, S., Nakazawa, H., Makino, T. and Nakajin, S. 2002 Effects of flavonoid phytochemicals on cortisol production and on activities of steroidogenic enzymes in human adrenocortical H295R cells. J Steroid Biochem Mol Biol. **80**: 355-363.

Okada, A. and Kai, O. 2008 Effects of estradiol-17β and bisphenol A administered chronically to mice throughout pregnancy and lactation on the male pups' reproductive system. Asian J Androl. **10:** 271-276.

Ola-Mudathir, K.F., Suru, S.M., Fafunso, M.A., Obioha, U.E. and Faremi, T.Y. 2008 Protective roles of onion and garlic extracts on cadmium-induced changes in sperm

characteristics and testicular oxidative damage in rats. Food Chem Toxicol. **46**: 3604-3611.

Olea, N., Pulgar, R., Pérez, P., Olea-Serrano, F., Rivas, A., Novillo-Fertrell, A., Pedraza, V., Soto, A.M. and Sonnenschein, C. 1996 Estrogenicity of resin-based composites and sealants used in dentistry. Environ Health Perspect. **104**: 298.

Palanza, P., Gioiosa, L., vom Saal, F.S. and Parmigiani, S. 2008 Effects of developmental exposure to bisphenol A on brain and behavior in mice. Environ Res. **108**: 150-157.

Pentikäinen, V., Erkkilä, K., Suomalainen, L., Parvinen, M. and Dunkel, L. 2000 Estradiol acts as a germ cell survival factor in the human testis in vitro 1. J Clin Endocrinol Metab. **85:** 2057-2067.

Peretz, J., Gupta, R.K., Singh, J., Hernández-Ochoa, I. and Flaws, J.A. 2011 Bisphenol A impairs follicle growth, inhibits steroidogenesis, and down regulates rate-limiting enzymes in the estradiol biosynthesis pathway. Toxicol Sci. **119:** 209-217.

Petersen, D.N., Tkalcevic, G.T., Koza-Taylor, P.H., Turi, T.G. and Brown, T.A. 1998 Identification of estrogen receptor β2, a functional variant of estrogen receptor β expressed in normal rat tissues. Endocrinology. **139**: 1082-1092.

Pourcel, L., Routaboul, J-M., Cheynier, V., Lepiniec, L., Debeaujon, I. (2007) Flavonoid oxidation in plants: from biochemical properties to physiological functions. Trends Plant Sci **12**(1):29-36.

Prabu, S.M., Muthumani, M. and Shagirtha, K. 2013 Quercetin potentially attenuates cadmium induced oxidative stress mediated cardiotoxicity and dyslipidemia in rats. Eur Rev Med Pharmacol sci. **17:** 582-595.

Prins, G.S., Birch, L., Tang, W.Y. and Ho, S.M. 2007 Developmental estrogen exposures predispose to prostate carcinogenesis with aging. Reprod Toxicol. **23**: 374-382.

Ramos, J.G., Varayoud, J., Kass, L., Rodríguez, H., Costabel, L., Muñoz-De-Toro, M. 2003 Bisphenol A induces both transient and permanent histofunctional alterations of the hypothalamic-pituitary-gonadal axis in prenatally exposed male rats. Endocrinology **144**:3206-15.

Rezg, R., El-Fazaa, S., Gharbi, N. and Mornagui, B. 2014 Bisphenol A and human chronic diseases: Current evidences, possible mechanisms, and future perspectives. Environ Int. **64**: 83-90.

Richter, C.A., Birnbaum, L.S., Farabollini, F., Newbold, R.R., Rubin, B.S., Talsness, C.E., Vandenbergh, J.G., Walser-Kuntz, D.R. and vom Saal, F.S. 2007 In vivo effects of bisphenol A in laboratory rodent studies. Reprod Toxicol. **24**: 199-224.

Rochester, J.R. 2013 Bisphenol A and human health: a review of the literature. Reprod Toxicol. **42**: 132-155.

Sakaue, M., Ohsako, S., Ishimura, R., Kurosawa, S., Kurohmaru, M., Hayashi, Y., Aoki, Y., Yonemoto, J. and Tohyama, C. 2001 Bisphenol-A affects spermatogenesis in the adult rat even at a low dose. J Occup Health-English edition- **43**: 185-190.

Sakuma, S., Nakanishi, M., Morinaga, K., Fujitake, M., Wada, S.I. and Fujimoto, Y. 2010 Bisphenol A 3, 4-quinone induces the conversion of xanthine dehydrogenase into oxidase in vitro. Food Chemical Toxicol. **48**: 2217-2222.

Schönfelder, G., Wittfoht, W., Hopp, H., Talsness, C.E., Paul, M. and Chahoud, I. 2002 Parent bisphenol A accumulation in the human maternal-fetal-placental unit. Environ Health Perspect. **110**: A703.

Selim, M.E., Aleisa, N.A. and Daghestani, M.H. 2013 Evaluation of the possible protective role of quercetin on letrozole-induced testicular injury in male albino rats. Ultrastruct Pathol. **37:** 204-217.

Sharma, R.K. and Agarwal, A. 1996 Role of reactive oxygen species in male infertility. Urology. **48**: 835-850.

Sharpe, R.M. and Skakkebaek, N.E. 1993 Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? The Lancet. **341:** 1392-1396.

Somm, E., Schwitzgebel, V.M., Toulotte, A., Cederroth, C.R., Combescure, C., Nef, S., Aubert, M.L. and Hüppi, P.S. 2009 Perinatal exposure to bisphenol a alters early adipogenesis in the rat. Environ Health Perspect. **117:** 1549-1555.

Soto, A.M., Vandenberg, L.N., Maffini, M.V. and Sonnenschein, C. 2008 Does breast cancer start in the womb? Basic Clin Pharmacol Toxicol. **102:** 125-133.

Staples, C.A., Dome, P.B., Klecka, G.M., Oblock, S.T. and Harris, L.R. 1998 A review of the environmental fate, effects, and exposures of bisphenol A. Chemosphere. **36:** 2149-2173.

Sujatha, R., Chitra, K., Latchoumycandane, C. and Mathur, P. 2001 Effect of lindane on testicular antioxidant system and steroidogenic enzymes in adult rats. Asian J Androl. **3:** 135-138.

Sun, H., Xu, L.C., Chen, J.F., Song, L. and Wang, X.R. 2006 Effect of bisphenol A, tetrachlorobisphenol A and pentachlorophenol on the transcriptional activities of androgen receptor-mediated reporter gene. Food Chemical Toxicol. **44:** 1916-1921.

Taepongsorat, L., Tangpraprutgul, P., Kitana, N. and Malaivijitnond, S. 2008 Stimulating effects of quercetin on sperm quality and reproductive organs in adult male rats. Asian J Androl. **10:** 249-258.

Takeuchi, T. and Tsutsumi, O. 2002 Serum bisphenol A concentrations showed gender differences, possibly linked to androgen levels. Biochem Biophys Res Commun. **291**: 76-78.

Talsness, C., Fialkowski, O., Gericke, C., Merker, H.J. and Chahoud, I. 2000 The effects of low and high doses of bisphenol A on the reproductive system of female and male rat offspring. Congenit Anom. **40:** S94-S107.

Tang, X.Y., Zhang, Q., Dai, D.Z., Ying, H.J., Wang, Q.J. and Dai, Y. 2008 Effects of strontium fructose 1, 6-diphosphate on expression of apoptosis-related genes and oxidative stress in testes of diabetic rats. IJU. **15:** 251-256.

Timms, B.G., Howdeshell, K.L., Barton, L., Bradley, S., Richter, C.A., vom Saal, F.S. 2005 Estrogenic chemicals in plastic and oral contraceptives disrupt development of the fetal mouse prostate and urethra. Proc Natl Acad Sci USA **102**:7014-9.

Toppari, J., Larsen, J.C., Christiansen, P., Giwercman, A., Grandjean, P., Guillette Jr, L.J., Jégou, B., Jensen, T.K., Jouannet, P. and Keiding, N. 1996 Male reproductive health and environmental xenoestrogens. Environ Health Perspect. **104:** 741.

Tsutsui, T., Tamura, Y., Yagi, E., Hasegawa, K., Takahashi, M., Maizumi, N., Yamaguchi, F. and Barrett, J.C. 1998 Bisphenol-A induces cellular transformation, aneuploidy and DNA adduct formation in cultured Syrian hamster embryo cells. Int J Cancer. **75:** 290-294.

Turrens, J. F. 2003 Mitochondrial formation of reactive oxygen species. J Physiol. **552**(2): 335-344.

Tyl, R., Myers, C., Marr, M., Thomas, B., Keimowitz, A., Brine, D., Veselica, M., Fail, P., Chang, T. and Seely, J. 2002 Three-generation reproductive toxicity study of dietary bisphenol A in CD Sprague-Dawley rats. Toxicol Sci. **68:** 121-146.

Tyl, R.W. 2008 Commentary to the CERHR expert panel report on bisphenol A. Birth Defects Research Part B: Developmental and Reproductive Toxicology. **83:** 152-152.

Vandenberg, L.N., Maffini, M.V., Sonnenschein, C., Rubin, B.S. and Soto, A.M. 2009 Bisphenol-A and the great divide: a review of controversies in the field of endocrine disruption. Endocr Rev. **30:** 75-95.

Verma, R.J. and Sangai, N.P. 2009 The ameliorative effect of black tea extract and quercetin on bisphenol A-induced cytotoxicity. Acta Pol Pharm. **66:** 41-44.

vom Saal, F.S., Akingbemi, B.T., Belcher, S.M., Birnbaum, L.S., Crain, D.A., Eriksen, M., Farabollini, F., Guillette Jr, L.J., Hauser, R. and Heindel, J.J. 2007 Chapel Hill bisphenol A expert panel consensus statement: integration of mechanisms, effects in animals and potential to impact human health at current levels of exposure. Reprod Toxicol (Elmsford, NY). **24:** 131.

Watson, C.S., Bulayeva, N.N., Wozniak, A.L. and Finnerty, C.C. 2005 Signaling from the membrane via membrane estrogen receptor-α: estrogens, xenoestrogens, and phytoestrogens. Steroids. **70:** 364-371.

Watson, C.S., Jeng, Y.-J. and Kochukov, M.Y. 2010 Nongenomic signaling pathways of estrogen toxicity. Toxicol Sci. **115:** 1-11.

Wei, J., Lin, Y., Li, Y., Ying, C., Chen, J., Song, L., Zhou, Z., Lv, Z., Xia, W. and Chen, X. 2011 Perinatal exposure to bisphenol A at reference dose predisposes offspring to metabolic syndrome in adult rats on a high-fat diet. Endocrinology. **152:** 3049-3061.

Welshons, W.V., Nagel, S.C. and vom Saal, F.S. 2006 Large effects from small exposures. III. Endocrine mechanisms mediating effects of bisphenol A at levels of human exposure. Endocrinology. **147:** s56-s69.

Witting, P., Pettersson, K., Östlund-Lindqvist, A.-M., Westerlund, C., Wågberg, M. and Stocker, R. 1999 Dissociation of atherogenesis from aortic accumulation of lipid

hydro(pero)xides in Watanabe heritable hyperlipidemic rabbits. J Clin Invest. **104:** 213-220.

Wolf, L., Rizzini, L., Stracke, R., Ulm, R. and Rensing, S.A. 2010 The molecular and physiological responses of Physcomitrella patens to ultraviolet-B radiation. Plant Physiol. **153:** 1123-1134.

Wu, H.J., Liu, C., Duan, W.X., Xu, S.C., He, M.D., Chen, C.H., Wang, Y., Zhou, Z., Yu, Z.P. and Zhang, L. 2013 Melatonin ameliorates bisphenol A-induced DNA damage in the germ cells of adult male rats. Mutat Res. **752:** 57-67.

Ye, X., Kuklenyik, Z., Needham, L.L. and Calafat, A.M. 2005 Quantification of urinary conjugates of bisphenol A, 2, 5-dichlorophenol, and 2-hydroxy-4-methoxybenzophenone in humans by online solid phase extraction–high performance liquid chromatography–tandem mass spectrometry. Anal Bioanal Chem. **383:** 638-644.

Zancan, K.C., Marques, M.O., Petenate, A.J., Meireles, M.A.A. 2002 Extraction of ginger (Zingiber officinale Roscoe) oleoresin with CO_2 and co-solvents: a study of the antioxidant action of the extracts. J Supercrit Fluids **24**(1):57-76.

Zangar, R.C., Davydov, D.R. and Verma, S. 2004 Mechanisms that regulate production of reactive oxygen species by cytochrome P450. Toxicol Appl Pharmacol. **199:** 316-331.

Zhang, Y.M.C. 2005 Protective effect of quercetin on Aroclor 1254–induced oxidative damage in cultured chicken spermatogonial cells. Toxicol Sci. **88:** 545-550.

Zsarnovszky, A., Le, H.H., Wang, H.S. and Belcher, S.M. 2005. Ontogeny of rapid estrogen-mediated extracellular signal-regulated kinase signaling in the rat cerebellar cortex: potent nongenomic agonist and endocrine disrupting activity of the xenoestrogen bisphenol A. Endocrinology. **146:** 5388-5396.