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Assessment of reproductive disruption in Bisphenol A (BPA) exposed fish, *Heteropneustes fossilis* (Bloch, 1794)

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ABSTRACT
The present study was aimed to investigate the toxic effects of sub lethal concentrations of Bisphenol A (BPA) at concentrations of 1/10th, 1/20th and 1/30th on a freshwater catfish (H.fossilis.) during 28 days of exposure. Significant alterations in gonadal histopathology, gonadal- somatic index (GSI), protein and nucleic acid derived indices (RNA: DNA ratio) were observed in dose dependent manner in fish exposed to fish exposed to BPA. Decrease in the gonadosomatic index (GSI) was evident with increase of sub lethal doses of BPA. The histopathological changes of BPA exposed fish were witnessed in gonads of H.fossilis reflecting their sensitivity to BPA-estrogenic like effects. Histopathological examinations revealed detrimental changes at all the developmental phases. The histopathological examination clearly reveals the impairment of spermatogenesis and lobular structures of testes in exposed fish. The disintegration of the testicular histological organization in BPA treated fish may be due to the result of oxidative damage, which reflected in the degenerative symptoms in testicular histology. Ovaries of Group II, III and IV showed oocytes with abnormalities in shape with disintegrated cytoplasm and higher percentage of follicular atresia in a dose-dependent manner in contrast with control fish. Maximum reduction in DNA, total protein level was observed in the testicular tissue of Group III while RNA levels were greatly reduced in the ovary of Group III exposed fish. The reduction in RNA: DNA ratios were found to be almost analogous in the testis and ovary of exposed fishes (1:0.459 and 1:0.454) respectively. The results demonstrate the deleterious influence of sub-lethal doses of BPA significantly inhibited gonadosomatic index (GSI) and RNA: DNA ratio as evidenced by their histology. We conclude that exposure to sub lethal concentrations of BPA in H.fossilis resulted in a significant gonadal damage possibly due to high free radical production and the disruption in defense mechanism in the gonadal tissue. This study will be beneficial for upcoming research in explaining the detailed effects of BPA in other fish species.
Keywords*: Bisphenol A, DNA: RNA ratio, GSI, Histopathology, H.fossilis, Reproduction*

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INTRODUCTION

BPA like endocrine disrupting chemicals (EDCs) has the capability to compete with endogenous steroid hormones binding receptors to disturb and alter the synthesis of reproductive hormones [1 and 2]. Approximately more than one million pounds of BPA are released annually into the freshwater environment [3]. BPA can enter the body ***via*** different ways such as ingestion, inhalation and dermal absorption and interfere in the endocrine system. Regrettably, like other chemical pollutants, BPA (a synthetic chemical) is also released into the food and environment from the various consumer products (plastic bottles, food and beverage cans) [4, 5 and 6]. Published literature reveal that BPA mimics the function of estrogens and can induce negative effects on reproductive function, immune system, neuroendocrine system and overall health of the fish [7, 8, 9 and 10].

Reproduction is highly energetic process [11] that involves a series of metabolic activities in gonadal tissues which are eventually under the control of nucleic acids [12]. BPA is known to enhance production of reactive oxygen species (ROS) in fish species [13] that can attack a variety of biomolecules like RNA, DNA, lipids and proteins leading to oxidative damages. Since the correct functioning of cells and tissue is the crucial factor for the steadfastness of reproductive function, the study of histopathology and biochemistry of gonads could definitely provide sensitive and correct biomarkers of BPA toxicity. Nucleic acid content (RNA, DNA), protein content, and RNA/DNA ratio were sensitive to toxicant stress [14].

Therefore, the measure of nucleic acid content (DNA and RNA) in gonadal tissues is supposed as an index of the ability of an organism for the synthesis of required proteins. The RNA: DNA ratio is based on the statement that the amount of DNA is constant under varying ecological conditions within the somatic cells while the amount of RNA which openly involved in protein synthesis is identified to vary with age, phase of the life cycle, and stress-state and with changing environmental conditions [15]. Thus, animal in good health status be likely to have higher RNA: DNA ratios than do those in poor condition. Giesy and Graney [16] used RNA: DNA ratios as biomarkers to notice chronic stress. The RNA: DNA ratio is the one of widely-used sensitive and integrative biochemical index by which one can predict status of physiological activity (growth, reproduction, secretion, etc.) of an animal under a specified environmental conditions [17 and 18]. Bulow [19] considered RNA–DNA ratios as markers of growth and metabolism of a fish. RNA is directly involved in protein synthesis and therefore increases in RNA content may reflect active protein (enzyme) synthesis while DNA content is typically constant making the RNA: DNA ratio an indicator of protein synthesis capacity per cell. Hypothetically, the growth rate is directly correlated to protein synthesis, which consecutively is related to the amount of RNA in cells as the quantity of DNA in a cell remains fairly constant, the RNA/DNA ratio would provide a good indication of the rate of protein synthesis and hence growth. Indeed, RNA/DNA ratio has been considered to be a promising indicator of growth in ecotoxicological studies [17].

The estrogenic activity of BPA is believed to be mediated through its binding ability to the oestrogen receptors (ERs) in fish [2]. BPA is known to disrupt oestrogen / androgen ratio [20, 21 and 22], feminization and alterations in gonadal development [8, 23, and 24], induction of vitellogenin production [25, 26 and 27] RNA: DNA ratio, [18, 28 and 29], histopathological alterations in gonads [26, 30, 31 and 32] and oxidative stress [6, 26, 33, and 34]. Most of the detrimental effects of BPA in cells and tissues are mediated by enhanced oxidative stress coupled with genomic and non-genomic mechanisms. Even though, the intracellular reactive oxygen species (ROS) are decisive factors of cellular physiology, their increased levels can directly affect DNA, RNA, and proteins, subsequently affecting the tissue to pathology [35].

Histopathological examination proposes a potent means in the study of reproductive health of fishes [36, 37, 38, 39 and 40]. Gonadal histology, in conjunction with protein, nucleic acids and gonadal-somatic index analysis can provide better evidences on the effects of different environmental stressors on reproductive health. Gonado-somatic index (GSI) is one of the vital parameter and provides a thorough representation on the subject of the fish reproductive strength and status. A number of researchers employed GSI as a tool of indicator of reproductive status of fish and other organisms [41, 42, 43 and 44]. The recently published literature have shown that exposure to BPA reduced GSI index and gonadal maturation in fish and other species [8, 45, 46 and 47]. Fish and other aquatic animals are characteristically exposed to different concentrations of BPA at different stages of their life history [48]. Though a huge amount of toxicological investigations have studied the effects of BPA in rodents [49, 50 and 51], but the potential effects of BPA exposure on fish health have hardly ever been studied.

Therefore, based on the availability and economic importance, the catfish ***H. fossilis*** was selected as test organism. The primary objective of this investigation was to assess the effects of sub lethal concentration of BPA on the reproductive function by examination of histopathological changes in gonads, measuring the protein content, nucleic acids and enzymes in both ovary and testis and measuring serum levels of steroid hormones involved in reproduction and metabolism.

MATERIAL AND METHODS

Experimental animal and Chemical

Healthy adult male and female fish of *H.fossilis* of approximately same weight and length (weight of 25.11 ± 1.13g and total length of 17.88 ± 0.35) were purchased from local commercial fish market in the month of June 2018 as March-June is known as the breeding period for *H.fossilis* [52]. Fish were sexed by peripheral inspection of the urogenital papilla and by also microscopic observation of the gonads. After acclimatization for 15 days to the laboratory condition, the fish were divided into 3 treatments (0.714, 1.428 and 2.142 mg/L of BPA and a control with 20fish (10+ 10 male and female) in each 40L aquarium. The dosage selection of BPA was based on our earlier publications [5 and 6] respectively. All fish were fed with commercial food pellets once a day. Test chemical, Bisphenol-A (BPA) of 99.8% pure was obtained from Chemex Organochem Private Limited, Mulund West, Mumbai, Maharashtra (India). Cleaning, re introducing with freshwater and feeding activities were sustained regularly on every alternative day. All aquaria were placed in similar and natural environmental conditions. After the termination of experiment, on 29th day, four fishes from each treated aquarium were handpicked and dissected for testis and ovary and were immediately washed in 0.75% saline solution, blotted with tissue paper, kept in Teflon tubes, and finally stored at -20^0C for later analysis.

Gonadosomatic index (GSI)

The main and important reproductive parameter namely the gonadosomatic index (GSI) was calculated by the method given by employing the following formula.

Gonado somatic Index (GSI) =Weight of gonad/fish weight×100

Histopathology

Fixed gonadal tissue (testis and ovary) was administered for in graded ethanol series, cleared in xylene and finally embedded in paraffin wax (melting point 60^{o}C). The embedded block were sectioned at 6µ on a rotary microtome, mounted on glass slides, dried and stained with haematoxylin and eosin (H&E). Sections were examined under a light microscope (NIKON ECLIPSE E 400, USA) and photographed by using digital camera attached to the microscope.

Tissue Preparation

After 28 days of the experiment, both the testis and ovary were excised, weighed and soaked with tissue rapidly from the male and female fish respectively and kept in the deep freezer until further use. The tissues were weighed maximum up to 0.1g and homogenized in 2 ml of 0.5 M (pH 7.4) Tris-HCl ice cold buffer by using REMI Lab Homogeniser (RQ-127A/D) equipped with Teflon pestle. The gonadal homogenates were subsequently centrifuged at 9,000 rpm for 25 minutes at 4^{0}C. The supernatant was collected in Teflon tubes and immediately used for the biochemical analysis in all experimental groups including control. Standard procedures used in clinical biochemistry laboratories based on protocol of commercial kits were followed for the determination of all biochemical parameters.

Biochemical estimations

Nucleic acids DNA were estimated by diphenylamine method as described by Mu, Plummer [53]. The estimation of RNA was done by orcinal method described by Jain et al. [54]. Total protein in the both the gonadal tissue was estimated according to method Lowry et al. [55]. RNA: DNA was calculated as the ratio of mean RNA (mg/gm) to mean DNA (mg/gm) of 4 pooled samples per treatment and sex.

Statistical analysis

The collected data are presented as mean ± standard error of the mean. Student t-tests were performed used to evaluate and compare the values of the means from two samples. All the statistical analysis was performed on windows 10 MS-Excel add on software.

RESULTS

The results of GSI, biochemical estimations and gonad histopathology on are presented in the form table and images. The fish, *H.fossilis* exhibited several morphological injuries on body surface, eyes and fins upon exposure to various sub-lethal concentration of BPA.

Table 1. Gonadosomatic index (GSI), nucleic acid derived indices and protein parameters of *H.fossilis* exposed to different sublethal concentrations of BPA for 28 days.

		GSI (%)	DNA (mg/gm)	RNA(mg/gm)	Protein(mg/gm)	RNA:DNA ratio
Control	Male	0.41%	6.79 ± 0.11	15.26± 0.3	5.7 ± 0.27	1: 0.44
	Female	1.16%	6.6± 0.12	14.85± 0.11	6.8 ± 0.21	1:0.44
Group I	Male	0.33%	6.04± 0.1	14.54± 0.2	4.8 ± 0.3	1:0.415
	Female	1.02%	5.09± 0.2	12.7± 0.11	5.9 ± 0.14	1:0.421
Group II	Male	0.26%	5.61± 0.1	11.8± 0.3	4.1± 0.16	1: 0.473
	Female	0.9%	4.9± 0.2	10.9± 0.11	4.8 ± 0.17	1:0.449
Group III	Male	0.19%	5.1± 0.3	11.1 ± 0.13	3.2 ± 0.15	1:0.459
	Female	0.8%	4.5± 0.11	9.9± 0.15	4.1 ± 0.21	1:0.454

Values are expressed as mean ± standard error (***N*** = 6)

The gonadosomati index (GSI)

Published literature reveals that the GSI value of fish is attributed to the consequent changes in the reproductive activities (gonadal development). In the present study, the GSI values of both male and female *H.fossilis* were estimated in all exposed fish and control group and values are expressed as percentage (Table 1). Results revealed higher values of GSI in both the sex of fish of control group which significantly declined (P> 0.01) in all exposed fish in dose dependent manner. The peak value of male GSI (0.41%) was obtained from control fish while least value of male GSI (0.19%) was recorded in the fish of Group III. The results of female GSI also followed similar trend with a peak value of GSI (1.16%) was obtained from control fish while least value of male GSI (0.8%) was recorded in the fish of Group III.

Histopathological analysis

The results of histopathological examination are displayed in Fig 1 &2.

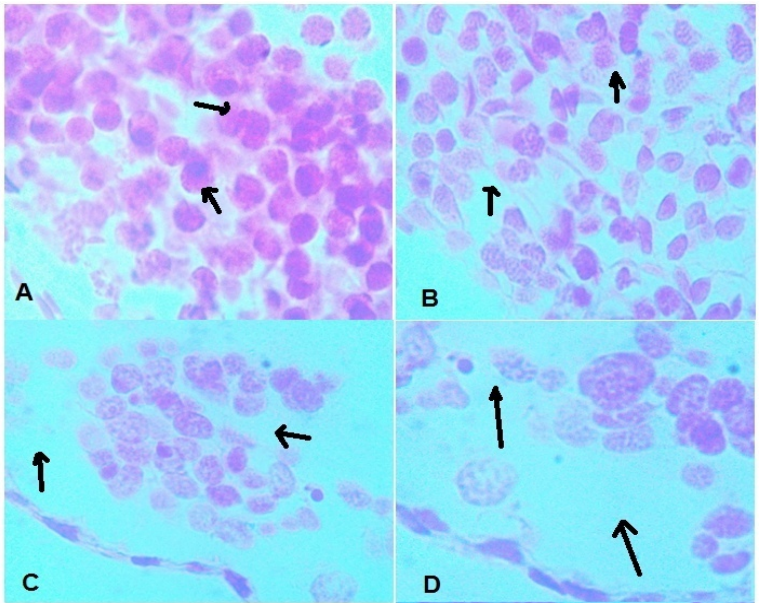

FIG. 1. A-D. 25. Effects of sub lethal concentration of BPA on histopathology of testis of *Heteropneustes fossilis*. A. Control fish show normal structure with seminiferous tubules B. Testis from Group I showing necrosis, nuclear hypertrophy, reduced lumen, degeneration. C. Testis of Group II showing degeneration, presence of melano-macrophage centers, reduced lumen and necrosis D. Histology of testis from Group III showing atrophy, reduced lumen, germ cell syncytia, hypertrophy and vacuolated germ cells (All sections are H&E Stained x400).

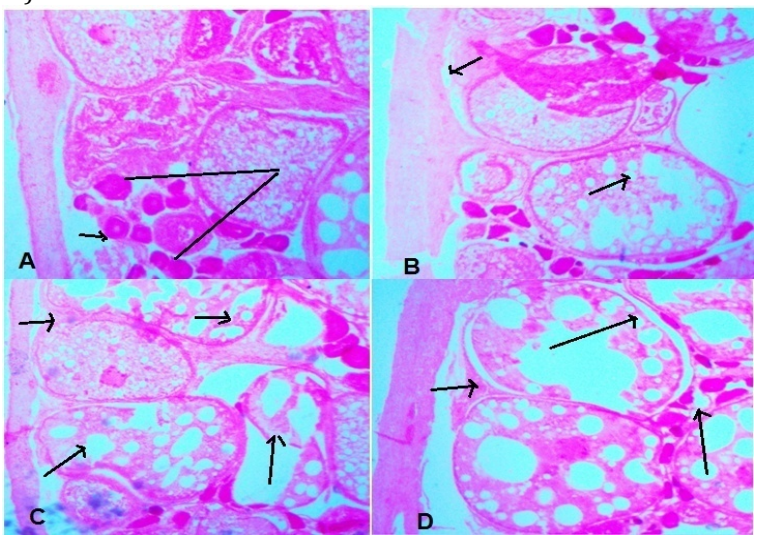

FIG. 2. Effects of sub lethal concentration of BPA on histopathology of ovary of *Heteropneustes fossilis*. A. Control fish show normal structure with oogonia, oocyte with different developmental changes. B. Ovary from Group I showing atretic oocytes, ruptured zona radiata and karyoplasmic clumping necrosis and nuclear hyper trophy C. Histology of ovary from Group II showing cellular degeneration, follicular atresia, broken zona radiata and fibrosis. D. Histology of ovary from Group III showing degeneration, atrophy, germ cell syncytia, hypertrophy and pyknosis and vacuolated oocytes and decreased vitellogenesis (All sections are H&E Stained x400).

The testes of control fish showed usual lobular spermatogonia with different developmental stages of spermatocytes (Fig.1 A) while the testis of Group I exposed to 1/10% of BPA (Fig.1B) revealed mild structural anomalies like necrosis, reduced lumen, and degeneration of spermatogonia. The testis of Group II treated with 1/20% of BPA (Fig.1C) exhibited the presence of melano-macrophage centres (MMC), reduced lumen, necrosis, broken capsule and pyknosis. The testis of Group III treated with 1/30% of BPA (Fig.1D) shown more severe structural anomalies like decreased number of spermatogonia, clumping of spermatocytes, blood congestion, pyknotic nucleus, cellular rupture and atrophy. The shape of the seminiferous tubules was not well defined. The severities of histopathological alterations were elevated in exposed in fish in dose-dependent manner.

Microscopic observations confirmed that all fishes were at similar phase in the commencement of the experimentation. The ovary is a paired organ in the majority of teleost fishes and is composed of ovarian follicles. In the present experiment, the micrograph of ovary of control fish (Fig.2A) exhibited normal structure with primary oocytes of different sizes. The nucleus of the oocytes was filled with multiple nucleoli and the layer of the zona radiata was found to be thin in the growth phase. The ovary of the fish exposed to 1/10% (Group I) (Fig.2B) of BPA exhibited necrosis, vacuolation, reduced lumen, oocyte atresia, ruptured cell wall and decreased vitellogenesis. The ovary of fish exposed to 1/20% of BPA (Group II) (Fig.2C) exhibited many atretic follicles. Karyoplasmic clumping, necrosis, and ruptured zona radiata were also seen in the ovary of fish exposed to 1/20% of BPA. On the other hand, the ovary of fish of group III exposed to of 1/30% of BPA (Fig.2D) exhibited more intense anomalies as compared to Group I and II. Degeneration of oocytes, reduction in number of ovum, structural deformation, atretic follicles, and damaged zona radiata was clearly seen. Reduction in number of matured ovum, higher number of degenerated oocytes and atretic follicles were seen in the ovary of group IV fish. It was clearly noticed that the number of atretic follicles increased with increase of the dose. Thus fish from all experimental groups showed oocytes with irregularities in shape and disintegrated cytoplasm in contrast with control fish. In fact, ovaries of fish exposed to higher doses showed higher percentage of follicular atresia and karyoplasmic clumping compared with control. Microscopic examination clearly revealed that oocytes of various developmental stages get affected differently at various concentrations of BPA treatment. Hence, it becomes apparent that steady sub-lethal doses of BPA can bring substantial alterations in the overall histological structures of the fish *Heteropneustes fossilis.*

Biochemical composition

Changes in the nucleic acid, protein contents and the RNA/DNA ratio of the testes and ovaries are displayed in Table 1. The concentrations of DNA, RNA and protein content of the testes and ovaries of *H.fossilis* during the experimental period (breeding time) in control group did not vary significantly (P> 0.01) but significantly reduced in dose-dependent manner in all BPA exposed fish. Fish exposed to sub-lethal concentrations of BPA revealed a significant dose-dependent decrease in total protein, nucleic acids (DNA and RNA) of both testis and ovary. Maximum reduction in DNA, total protein level was observed in the testicular tissue of Group III while RNA levels were greatly reduced in the ovary of Group III exposed fish. The reduction in RNA: DNA ratios were found to be almost similar in the testis and ovary of exposed fishes (1:0.459 and 1:0.454) respectively. We found a significant negative association was established between RNA/DNA ratio and protein contents of gonads as the increased concentrations of BPA decreased the nucleic acid derived indices and protein content in both the gonadal tissue.

DISCUSSION

Reproductive function has been considered as a reliable and insightful biomarker for the evaluation of the response of organisms to ecological stress. A number of biomarkers are used in the ecotoxicological studies for the assessment of reproductive health status of fish and other organisms. It is well known reality that several EDCs and xenobiotics can affect structure and function of fish gonads [56, 57, 58 and 59]. To our knowledge, the present investigation is the foremost to spotlight on the effects of BPA on reproductive potential on *H.fossilis*. The values various combined biomarkers including GSI, histology of gonads, nucleic acid derived indices (RNA: DNA), and protein were identified to evaluate the reproductive fitness of fish, *H.fossilis* after exposure to sub-lethal concentrations of BPA. Results demonstrated that BPA can disrupt the reproductive function in the both the sexes of *H.fossilis* which could help us to understand the reproductive toxicity of BPA.

In general, the gonadosomatic index (GSI) varies during the reproductive cycle of both male and female species that can be frequently used as biomarker of reproductive health of fish species [60]. In the present study, the GSI values of both male and female *H.fossilis* exposed to various sub lethal concentrations of BPA significantly reduced the GSI values in dose-dependent manner. Published literature evidently confirmed that exposure to xenobiotics and EDCs can induce deterioration in gonads involving reduction in gonadosomatic index (GSI) along with histological anomalies in the gonads. The results of the present study are in agreement with several other studies on different fish species. The study of Hassanin et al. [61] found that fish (*Cyprinus carpio*) exposed to polluted water of Ishizu river with EDCs (nonylphenol, bisphenol A and 17β-estradiol) revealed reduced testicular GSI and delay in the onset of spermatogenesis. Asifa, Chitra [62] found a significant reduction in GSI in the fish *Pseudetroplus maculatus* exposed to Chlordecone. The experiment of Roush, Jeffries [63] confirmed that GSI can be used as an effective index in the screening assays of EDCs. In a long-term study, Saravanan et al. [64] found reduced GSI and endocrine disruption effects of nonylphenol on the reproductive potential in fish species. In contrast, the recent study of Forner-Piquer et al. [24] shown increased GSI in male zebrafish exposed to 20 µg/L of BPA but reduced the percentage of the space occupied by spermatogonia

and altered the reproductive function of both male and female fish. The results thus pointed out that Bisphenol A (BPA) could negatively affect the gonadal structure and function in *H.fossilis,* which could critically deteriorate the reproductive potential of an animal. The reduction in GSI values in BPA exposed fish was perhaps due to inhibition of enzymes synthesis and release of reproductive hormones as in arsenic exposed fish, *Mystus vittatus* [65].

Histopathology proposes an authoritative means in the examination of reproductive vigour of fish and other species. Gonadal histology, in combination with biochemical and hormonal analysis and morphological gonadosomatic index (GSI) can offer clear clarification into the effects of environmental contaminates on reproductive health. The results of histological analysis of both testes and ovaries revealed that fish were in an advanced stage of maturity. Ovaries of control fish exhibited normal histological architecture with all the stages of oogenesis (oogonia, oocytes, perinucleolar oocytes, vitellogenic oocytes and atretic oocytes with a central nucleus). However, the ovaries of fish exposed to BPA showed increased oocyte atresia, hyperplasia/hypertrophy, decreased vitellogenesis, Interstitial fibrosis, egg debris, decreased post-ovulatory follicles. In the histopathological examination, the testes and ovary of the fish exposed to various sub lethal concentrations of BPA inhibited overall reproductive development in *H.fossilis*. Our results are in agreement with the studies of Mihaich et al. [66] Sohoni et al. [67] in fathead minnow, *Pimephales promelas,* Al-Sakran et al. [68] in *Cyprinus carpio*, Lora et al. [69], Yang et al. [70], Maharajan et al. [71] in zebra fish, (*Danio rerio),* Huang et al. [72] in marine fish, *Oryzias melastigma*, Wang et al. [8] in goldfish (*Carassius auratus*), and Yan et al. [47] in Japanese medaka (*Oryzias latipes*). The histopathological examinations of the present study evidently indicate that exposure to BPA did affect the histopathology of testes and ovary of *H.fossilis* like necrosis, nuclear hypertrophy, reduced lumen, degeneration and vacuolated germ cell which may lead to deleterious effects on the reproductive health of the fish. Future investigations from both laboratory and field are required to explain the underlying molecular mechanism for BPA induced reproductive failure in *H.fossilis*. Yang et al. [70] found estrogenic activity of BPB and destruction of the reproductive fitness in dose dependent manner along with the alterations in the histopathology of testis and ovary and alteration of the genes of HPG axis of zebra fish exposed to BPB. The study of Wang et al. [8] shown reduced gonadosomatic index (GSI) and shrunken histopathology, disruption of testes and ovarian maturation through apoptosis in BPA exposed goldfish, (*Carassius auratus***)** along with the reduction of plasma *11-ketotestosterone* and hypothalamic-pituitary-gonad (HPG) axis-related genes *(sgnrh, fshβ **and** lhβ)*. The study of Liu et al. [73] found bioaccumulation, failure of ovarian function, apoptosis and disturbed genetic pathways in the both sexes of seahorse (*Hippocampus erectus*) exposed to BPA at environmentally relevant concentrations.

Nucleic acid-derived indices like RNA: DNA ratio is frequently applied successfully as an indicator of growth, metabolism and health status in ecotoxicological studies [17]. Nucleic acid content (RNA, DNA), protein content, and RNA/DNA ratio were sensitive to toxicant stress [14] and reflect the stress status of fish and other species [74]. In the present study, the RNA: DNA ratios and protein content were correlated with GSI and histopathological studies. Results revealed that nucleic acids, RNA: DNA ratios and protein content of both testes and ovary in BPA treated fish were negatively correlated with GSI and histopathological studies in a dose dependent manner. The RNA: DNA ratio is based on the statement that the amount of DNA is constant under varying ecological conditions within the somatic cells while the amount of RNA which openly involved in protein synthesis is identified to vary with age, phase of the life cycle, and stress-state and with changing environmental conditions [15]. Thus, animal in good health status be likely to have higher RNA: DNA ratios than do those in poor condition. The RNA: DNA ratio is the one of widely-used sensitive and integrative biochemical index by which one can predict status of physiological activity (growth, reproduction, secretion, etc.) of an animal under a specified environmental conditions [17 and 18]. Bulow [19] considered RNA–DNA ratios as markers of growth and metabolism of a fish. As RNA level is known to link openly with protein synthesis, its increase may directly reflect active protein (enzyme) synthesis. Hypothetically, the growth rate is directly correlated to protein synthesis, which consecutively is related to the amount of RNA in cells as the quantity of DNA in a cell remains fairly constant, the RNA/DNA ratio would provide a good indication of the rate of protein synthesis and hence growth. In fact, certain xenobiotics and their metabolites have the potential to interfere and inhibit DNA synthesis and gene expression by altering the chromosomal aberration and replication process which induce mutations and cellular hyper-proliferation. In an experiment, Laing et al. [45] found a significant reduction in DNA methylation in the testes due to the decline in dnmt1 expression in breeding zebrafish (*Danio rerio*) exposed to BPA. The BPA exposure in zebrafish also caused reduced fertilization, potentially through estrogenic mechanisms. Zhang et al. [75] found a significant relationship between BPA concentration and decreased transcription and reduced gene expression in the testis and ovaries of rare minnow, *Gobiocypris rarus.*

The significant reduction in DNA, RNA and protein contents in bisphenol A treated animals in this experiment might be due to BPA DNA adducts formation [76]. During the metabolism, bisphenol A (BPA) is oxidized to bisphenol O-quinone that acts as an intermediate for the binding of DNA to prevent RNA polymerase synthesis and mRNA synthesis. The malfunction in mRNA synthesis resulted in inhibition of protein synthesis which was reflected in histopathological alterations in gonads in the form necrosis and apoptosis [77]. The major biological routes associated like metabolism, cell cycle, immune response, and the development of extracellular matrix development are known as trademarks of tissue injury. Besides, the transcriptomic and proteomic analysis evidently revealed a positive correlation of reduction in nucleic acid and protein content with histopathology [78]. The reduced synthesis of nucleic acids and damage in the DNA proliferation and inhibition of enzyme activities involved in DNA replication and repair mechanisms can affect the final product of gene expression. The reduction of protein content in gonadal tissues, in this experiment, possibly due to their probable consumption for energy and metabolic purposes. As free amino acids which released due to the breakdown of proteins are utilized for glucose synthesis through gluconeogentic pathway, the decreased levels of protein synthesis in fish exposed to toxicants was resulted [79]. The oxidized form of BPA (bisphenol-o-Quinone) can react with DNA to generate DNA adducts in the presence of peroxidase and H_2O_2. Moreover, the vitro analysis shown that BPA affects hepatic DNA adducts DNA damage in spermatocytes and fetal oocytes [80].

CONCLUSION

On the whole, it is concluded that the sub-lethal concentrations of BPA caused considerable disruption to reproduction possibly via estrogenic mechanisms in breeding *H.fossilis*. The ability of BPA to cause reproductive disruption confirmed here raises fear for its toxicity when living beings are exposed to BPA in polluted environments. Notably, BPA might also cause significant modifications in the transcription of genes involved in epigenetic regulation in testis and ovaries notably on dnmt1, which probably happened in combination with a reduction in global DNA methylation. The outcomes this experiment offer confirmation of the negative effects of BPA in a fish model and advocate for the replacement of BPA within consumer products to reduce its concentrations in the environment. These observations underscore that GSI, nucleic acid derivative indexes and histopathological analysis are sensitive and direct biomarkers for the ecotoxicological studies.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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