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Metabolic treatment of syndrome linked with Parkinson's disease and hypothalamus pituitary gonadal hormones by turmeric curcumin in Bisphenol-A induced neuro-testicular dysfunction of wistar rat

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ABSTRACT

The metabolic shift in cholinesterase activity and inhibitor of hypothalamus pituitary gonadal hormones were hypothesized as resultant effect of Parkinson's disease (PD) which is clinically characterized by a movement disorder. This study therefore examined the effect of turmeric curcumin (CUR) on index of PD, acetylcholine esterase activity and disorder of hypothalamus pituitary gonadal hormone (HPGH) in Bisphenol-A induced injury using animal model. Forty adult male albino rats were randomly distributed into five ($n = 8$) groups. Group I: vehicle control (olive oil 0.5 ml), Group II was given 50 mg/kg of BPA only, Group III was given 50 mg/kg BPA + 50 mg/kg curcumin, Group IV was given 50 mg/kg BPA + 100 mg/kg curcumin and Group V was administered 50 mg/kg of curcumin only for 14 days. The study examined the effect of curcumin on acetylcholinesterase (AChE) activity, nitric oxide radical (NO^\cdot) production, HPGH (LH, FSH and testosterone), MDA level, antioxidant enzymes (SOD and CAT), in BPA induced male rat. Sperm parameters were similarly examined. The animals induced with BPA exhibited impairment to striatum, leydig cells and sertoli cells by depleting LH, FSH, testosterone and spermatozoa with reduced AChE activity and significant ($p < 0.05$) alteration in cerebral enzymatic antioxidants. Locomotive activity was impeded followed by the increase of brain NO^\cdot level (marker of pro-inflammation). Therapeutically, CUR promoted hypothalamus–pituitary–testicular hormones via modulation of AChE and locomotive activities, reduction of intracellular NO^\cdot level, prevention of striatum–endocrine injury as well as oxidative damage. Hence, CUR abolished HPGH dysfunction linked with PD mediated by BPA in rat.

1. Introduction

Bisphenol A (BPA), popularly known as 2, 2-bis (4-hydroxyphenol) propane is an organic synthetic compound belonging to diphenylmethane derivatives and bisphenols, with two hydroxyphenyl groups [1]. BPA is industrially exploited in the production of polycarbonate plastics, epoxy resins and food packaging containers [1]. Plastics are used in our daily life in the form of infant feeding bottles, beverage bottles, dental fillings and eyeglass lens [1]. High temperature and acidic or basic conditions quicken hydrolysis of the ester bonds linking BPA monomers to foster the release of BPA into humans and the environment [2]. Approximately, 3 million tons of BPA are manufactured annually while 100 t of BPA enters the food chains [2] and its ingestion can cause cytotoxicity. BPA was known to elicit toxic effects on various biological systems especially the reproductive system due to

its estrogenic property [3]. Some researchers had implicated BPA in endocrine abnormalities [4], carcinogenesis [5], neural and behavioral alterations [6], cardiac and hepatic abnormalities [7].

Parkinson's disease (PD) is a progressive neurodegenerative movement disorder resulting from a selective loss of cholinergic neurons [8]. It begins slowly and becomes progressively more severe in younger and middle-aged particularly in elderly patients [9]. PD is clinically characterized by akinesia, rigidity, bradykinesia, resting tremor, postural instability and sensory-motor integration deficits [10–12]. Progressive loss of cholinergic neurons in the substantia nigra par compacta (SNpc) and subsequent depletion of acetylcholine (ACh) in the striatum, is the primary cause of PD symptoms [13]. Although, several environmental pesticides and genetic factors have been suggested as the major causes of PD, but the etiology of PD remains largely unknown [14]. Recent findings reported that the progressions of PD are associated with

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mitochondrial complex I inhibition or dysfunction [15] and oxidative stress has been reported as the fundamental pathogenesis of PD [16].

Metabolic relationship exists between PD and HPG as well as testicular dysfunction. Research has reported that testicular dysfunction (TD) is very common in Parkinson's patients and in patients with other neurological disorders [17]. TD is the most common distressing and disabling characteristics of Parkinson's disease [15]. PD is a progressive degenerative disease that affects a person's ability to coordinate and control muscle movement [15,17], including testes. Neurological disorders caused by PD affects testicular arousal, reduces sexual satisfaction and functions [16,17]. In PD subjects, there is difficulty in finger and testes movement, immobility in bed, rigidity and tremors [11,12]. All these destructively affect testicular dysfunctions caused by neurodegenerative disorder, and consequently resulted in low levels of HPG hormones particularly LH, FSH and testosterone [17,18]. Furthermore, PD patients are testicularly inactive because of decreased level of serum testosterone that could leads to depression, reduced nocturnal and morning erection, low libido, low sperm count and erectile dysfunctions [8,14]. Recent study showed that low secretion or direct consumption of neurotransmitters indirectly decreased the expression HPG hormones essentially serum testosterone [17,18].

Disorders of hypothalamus pituitary hormone secretion and gonadotropins (GnRH) are used for diagnostic studies in humans and mammals [18,19]. The testosterone deficiency in patients with hypogonadal dysfunctions reduced its response to GnRH while the hypothalamic disorders suggest that pituitary gland had been chronically impaired [20]. Investigation has recognized that idiopathic Parkinson's disease and uncontrollable hike of dopaminergic metabolizing enzymes could influence hypothalamus pituitary hormone secretion [21]. As the anterior pituitary endocrine dysfunction had also been documented in patients with Parkinson's disease [22]

The polyphenolic flavonoid curcumin [1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6-hepta- diene-3, 5-Dione] is a widespread nutraceutical, used globally for medicinal purposes and dietary spices [23]. Curcumin (CUR) is a major yellow-orange pigment extracted from the rhizomes of turmeric (*Curcuma longa*) [24]. Studies have demonstrated that CUR exhibited countless potentials, including antioxidant [25], anti-inflammatory, anticancer [26], spermatogenesis [27], protection of DNA from oxidative damage [28], iron-chelating and neuroprotective activities [29]. Notably, researches involving various experimental models strongly supported the clinical application of CUR in PD [30,31]. Also, it exerts promising antioxidant effects in many cells and animal models of PD through modulating the generation of free radicals and reactive oxygen species (ROS) including malondialdehyde (MDA) [32,33]. But, there have been gaps in reports of the natural therapies against PD in sub-Saharan African populations in recent years, predominantly as the regions become more industrialized [34]. Also, the links between BPA and the development of PD in mammals had not been fully explicated. Keeping this in view, we investigated whether BPA was a potential risk factor in the development of PD and/or inhibitor of hypothalamus pituitary gonadal hormones followed by its curative effect of curcumin commonly found in turmeric ginger.

2. Materials and methods

2.1. Chemicals

Acetylcholineiodide, Bisphenol A (BPA), Curcumin (CUR) were procured from Sigma-Aldrich Inc., (St Louis, MO, USA). All other chemicals and reagents used were of highest purity and standard commercially available

2.2. Animal management

About seven-week-old adult male wistar rats (220–240 g) were procured from the Institute for Advanced Medical Research and

Training (IAMRAT) vivarium of University college Hospital, University of Ibadan and were housed in plastic cages in a ventilated room at $25 \pm 2^\circ\text{C}$ under a 12 h/dark cycle. The animals were acclimatized for at least 1 week before the study and had free access to standard laboratory feed (rat chow feed) and water ad libitum. The study was approved by the committee of University of Ibadan's animal care use and research committee (UI ACUREC-2018) and in accordance with international guidelines.

2.3. Treatment procedure

The treatment procedure and dosage selection was based on previous studies showing adverse effects of BPA (50 mg/kg b.wt) on nervous and reproductive system [35]. The selection of CUR was done on its chemotherapeutic treatment against myriads of ailments and particularly the LD₅₀ [36]. Animals were randomly divided into 5 groups of 8 rats each that the weight difference within and between the groups does not exceed $\pm 8\%$ of the average weight of the total rats.

- Group I (control) served as the untreated control and was given single oral daily dose of 0.5 ml olive oil for 14 days.
- Group II (50 mg/kg BPA only) served as treated or induced group and was administered single daily dose of BPA (50 mg/kg b.wt), dissolved by olive oil for 14 days.
- Group III (50 mg/kg BPA + 50 mg/kg CUR) was co-treated daily with single oral dose of CUR at 50 mg/kg b.wt and oral administration of BPA at 50 mg/kg b.wt dissolved with olive oil for 14 days
- Group IV (50 mg/kg BPA + 100 mg/kg CUR) was co-treated daily with single oral dose of CUR at 100 mg/kg b.wt and oral administration of BPA at 50 mg/kg b.wt dissolved with olive oil for 14 days.
- Group V (50 mg/kg CUR only) received daily single dose of CUR at 50 mg/kg b.wt for 14 days. The treatment lasted for 14 days following the previous method of FAO/WHO [36]. The doses were selected following the previous study (36). High dose (100 mg/kg CUR only) of CUR at 100 mg/kg b.wt was not included because previous study had indicated that relatively low dose (50 mg/kg) of CUR has the potential to provide good health benefits for people that have not been diagnosed with health challenges. Adding 100 mg/kg of CUR will definitely show similar trend. Hence, to avoid repetition, we limited the design to only 50 mg/kg of CUR [36].

On termination of 14th day experiment, rats were fasted overnight and after 24 h, they were restrained on the dissecting board and about 4 ml of whole blood was siphoned by cardiac puncture. Each blood was kept into separate bottles for serum LH, FSH and testosterone count. After blood collection, the animals were sacrificed by cervical dislocation and brain and testes were removed and processed for histopathology and biochemical assays. Also, the epididymis were removed and cleared of adhering connective tissue for sperm analysis

2.4. Assessment of locomotive activity: principal indicator of Parkinson's disease

The cylinder test was used to assess rearing behavior of the rats. For this test, each rat was placed in a clear plastic cylinder (height = 30 cm, diameter = 20 cm) for 5 min. The test was conducted under low light conditions, video-recorded, and during video playback, the numbers of rears were quantified. When placed in a clear cylinder, rats engaged in exploratory behavior, including rearing [15]. To be classified as a rear, the animal had to raise forelimbs above shoulder level and make contact with the cylinder wall with either one or both forelimbs. Removal of both forelimbs from the cylinder wall and contact with the table surface was required before another rear was scored. Thus, the numbers of rearing after 7 and 14 days, respectively were recorded

2.5. Acetylcholinesterase (AChE) activity assay

The activity of AChE was determined by the modified method of Ellman [37] and expressed as $\mu\text{mol AChE/g/min}$.

2.6. Measurement of nitric oxide (NO)

Nitric oxide (NO) was measured using the method of Moshage et al., [38]

2.7. Analysis of hypothalamus pituitary gonadal hormones (LH, FSH and Testosterone)

Luteinizing hormone (LH) and follicle stimulating hormone (FSH) were determined using the LH test kit and followed the method as described by Uotila et al. [39]. Testosterone was determined using the testosterone enzyme immunoassay (EIA) and followed the method described by Chen et al. [40].

2.8. Evaluation of spermatozoa

The epididymal sperm concentration was determined with a hemocytometer using a modified method described by Turk et al. [41]. The percentage of forward progressive sperm motility was estimated using a light microscope with a heated stage as described by Sonmez et al. [42]. Total sperm deformity (TSD) and daily sperm production (DSP) were similarly calculated using the method of Joyce et al. [43]

2.9. Lipid peroxidation

Lipid peroxidation was measured by the method of Varshney and Kale [44]

2.10. Superoxide dismutase (SOD)

Superoxide dismutase (SOD) was determined by the method of Misra and Fridovich [45]

2.11. Catalase activity assay

Catalase activity was determined according to the method of Sinha [46]

2.12. Histopathological examination

The brain and testes were quickly fixed in freshly prepared 10% neutral buffered formalin, processed routinely, and embedded in paraffin. In addition, $4\mu\text{m}$ thick paraffin sections were prepared and stained with hematoxylin and eosin (H&E) for histopathological examination. Sections were examined using a light microscope

2.13. Statistical analysis

Data analysis was done using the Statistical Package for the Social Sciences (SPSS) version 21. The data were subjected to statistical analyses such as mean, standard deviation and one-way analysis of variance (ANOVA). Means that are found to be significantly different indicated at $P < 0.05$ were separated by Duncan Multiple Range Test.

3. Results

3.1. Effect of turmeric CUR on BPA induced locomotive alterations in Parkinson's disease biomarkers (numbers of rearing and AChE)

The precise description of locomotive alterations induced by BPA and its modulatory regulation by co-treatment of CUR are shown in

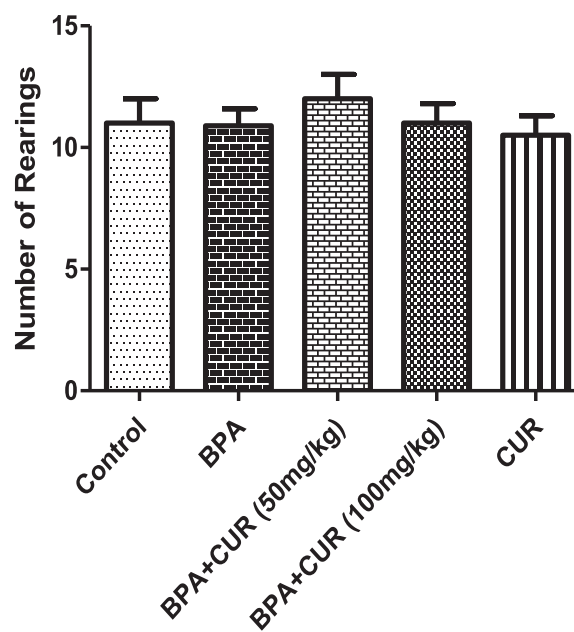


Fig. 1. Effect of BPA on the locomotive activity (indicator of PD) and its attenuation by turmeric curcumin in the rats after one week, shown by the number of rearings. The values were expressed as the means \pm SD ($n = 8$). Bars are not significant ($p > 0.05$) in relation to BPA induced group.

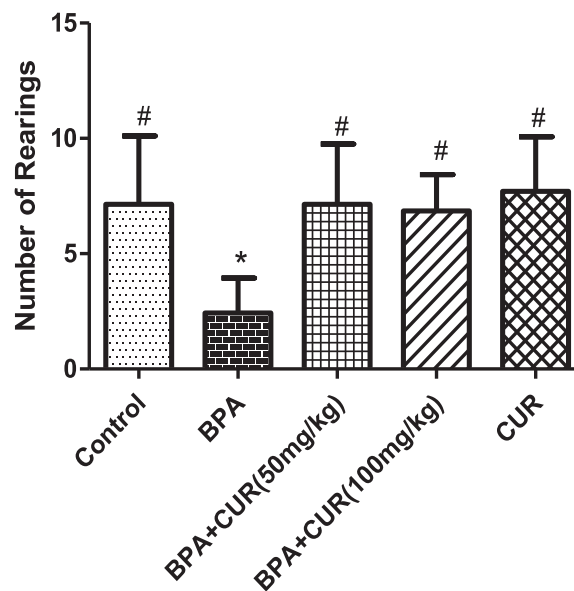


Fig. 2. Effect of BPA on the locomotive activity (indicator of PD) and its attenuation by turmeric curcumin in the rats after two weeks, shown by the number of rearings. The values were expressed as the means \pm SD ($n = 8$). Bar with asterisk (*) is significant in relation to control group, while bars with the number sign (#) are significant in relation to the BPA-treated group ($p < 0.05$).

Figs. 1 and 2. There were no significant locomotive changes observed in the numbers of rearing in all the treatment groups compared with the control after 7 days (Fig. 1). It was 14 days treatment that significantly ($p < 0.05$) depleted the locomotion (indicator of PD) from the control after BPA induced brain toxicity (Fig. 2). However, oral treatment with CUR (50 mg/kg and 100 mg/kg b.wt.) upturned the significant decrease of the numbers of rearing documented for BPA neurotoxicity (Fig. 2). Fig. 3 provided the description of neuronal activity of AChE in different treatment groups. The treatment of BPA significantly ($p < 0.05$) declined the activity of AChE in rat's brain. Conversely, co-treatment of

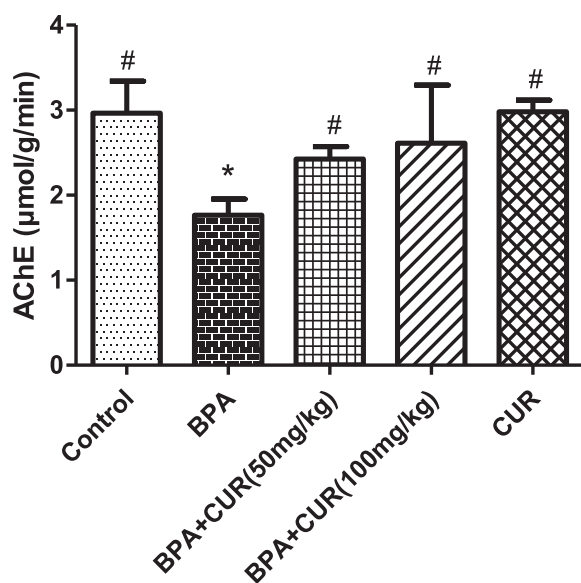


Fig. 3. Effect of turmeric curcumin on acetylcholinesterase (AChE) activity in animal induced with Bisphenol-A. The values were expressed as the means \pm SD (n = 8). Bar with asterisk (*) is significant in relation to the control, while bars with the number sign (#) are significant in relation to the BPA treated group (p < 0.05).

CUR considerably increased the AChE activity both at a dose of 50 mg/kg b.wt and 100 mg/kg b.wt when compared with the control and BPA induced toxicity groups

3.2. Effect of curcumin on cerebral nitric oxide production

The results of NO appraisals across different treatment groups are shown in Fig. 4. The amount of cerebral nitric oxide in the BPA treated group was significantly higher (p < 0.05) than the control animals. Whereas, for both low dose CUR (50 mg/kg b.wt) and high dose CUR (100 mg/kg b.wt) treated rats, cerebral NO was significantly lower than

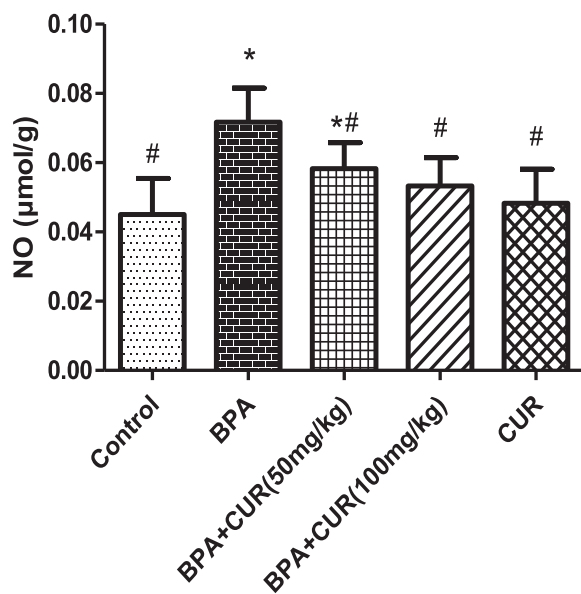


Fig. 4. Effect of turmeric curcumin on Nitric oxide (NO) concentration in animal induced with Bisphenol-A. The values were expressed as the means \pm SD (n = 8). Bar with asterisk (*) is significant in relation to the control, while bars with the number sign (#) are significant in relation to the BPA induced group (p < 0.05).

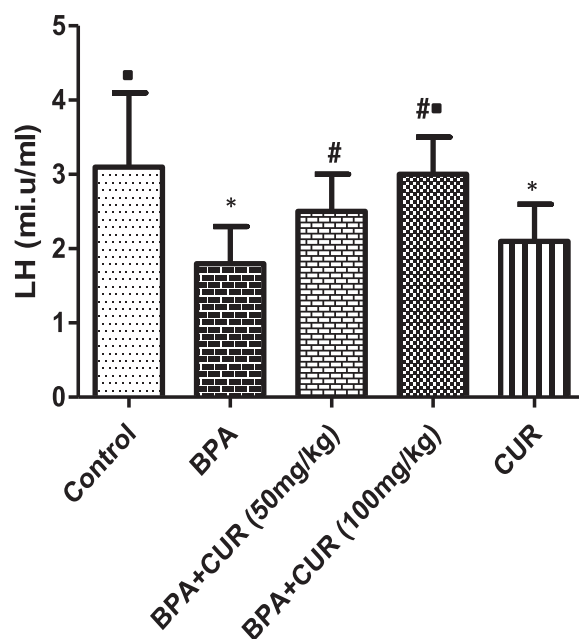


Fig. 5. Effect of curcumin administration on serum luteinizing hormone in animals exposed to BPA for 14 days. Mean \pm S.D, (n = 8). Bars with different asterisks are significantly different (P < 0.05).

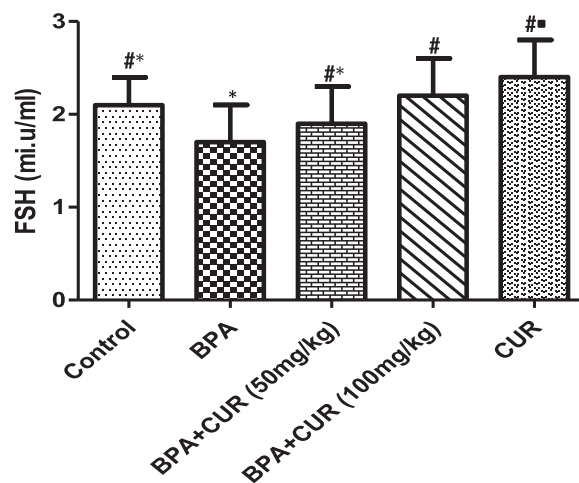


Fig. 6. Effect of curcumin administration on serum follicle stimulating hormone in animals exposed to BPA for 14 days. Mean \pm S.D, (n = 8). Bars with different asterisks are significantly different (P < 0.05).

the toxicant induced group.

3.3. Effect of curcumin on hypothalamus pituitary hormone secretion

Figs. 5–7 presented the depiction of serum level of hypothalamus pituitary hormones (LH, FSH and testosterone) in diverse treatment groups. The administration of BPA remarkably (p < 0.05) depleted the level of LH in serum (Fig. 5). However, co-treatment of CUR significantly elevated the LH levels at doses of 50 mg/kg b.wt and 100 mg/kg b.wt, respectively. There was no significant rise of LH in rats treated with only CUR at 50 mg/kg.b.wt. Similarly, BPA induced diminution in the level of serum FSH when compared with the control rats (Fig. 6). The administration of CUR at high dose (100 mg/kg b.wt) and only treated CUR at 50 mg/kg b.wt showed significant increase in the level of FSH, whereas, low dose of CUR (50 mg/kg b.wt) depicted no significant upsurge in FSH level (Fig. 6). Additionally, BPA treatment significantly (p < 0.05) lowered the level of testosterone (Fig. 7) in

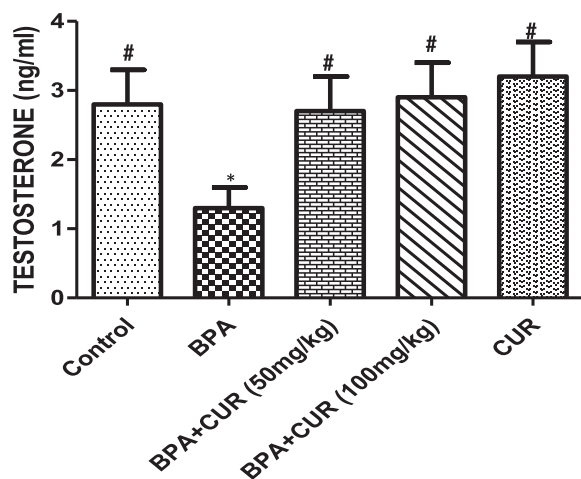


Fig. 7. Effect of curcumin administration on serum testosterone in animals exposed to BPA for 14 days. Mean ± S.D, (n = 8). Bars with different asterisks are significantly different (P < 0.05).

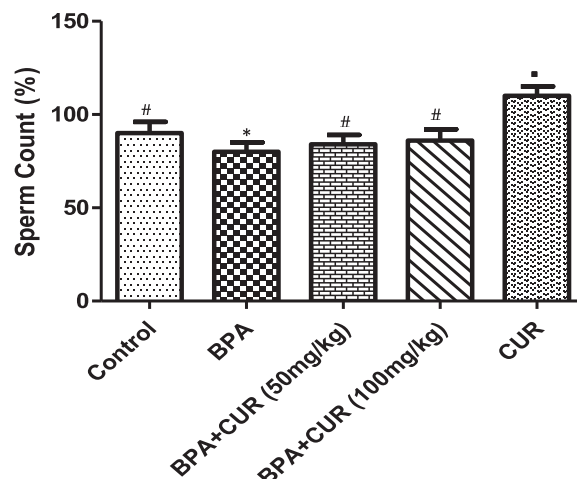


Fig. 10. Effect of curcumin administration on sperm count in animals exposed to BPA for 14 days. Mean ± S.D, (n = 8). Bars with different asterisks are significantly different (P < 0.05).

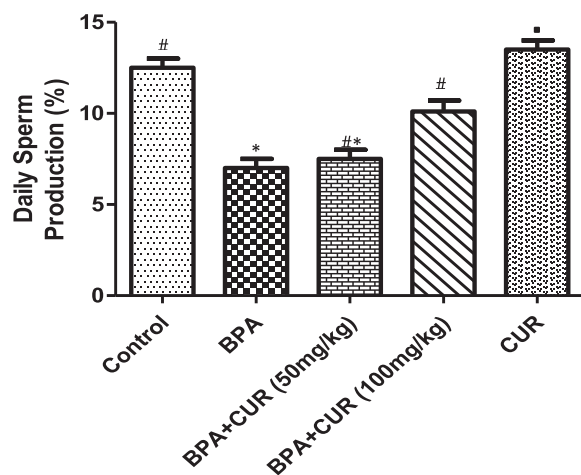


Fig. 8. Effect of curcumin administration on daily sperm production in animals exposed to BPA for 14 days. Mean ± S.D, (n = 8). Bars with different asterisks are significantly different (P < 0.05).

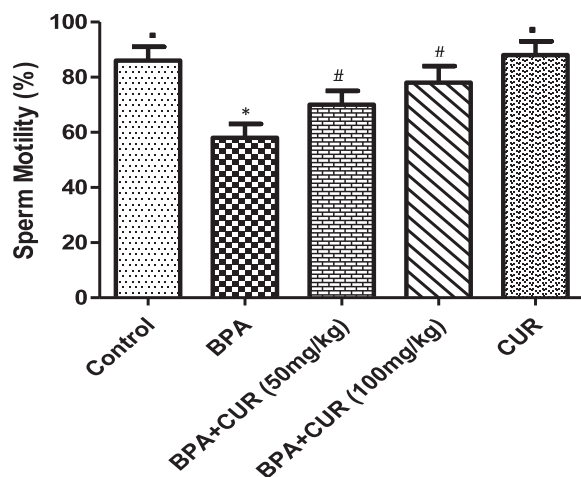


Fig. 9. Effect of curcumin administration on sperm motility in animals exposed to BPA for 14 days. Mean ± S.D, (n = 8). Bars with different asterisks are significantly different (P < 0.05).

relation to the control rats. The administration of CUR at high dose (100 mg/kg b.wt) and only treated CUR at 50 mg/kg b.wt showed significant increase in the level of testosterone, while, low dose of CUR

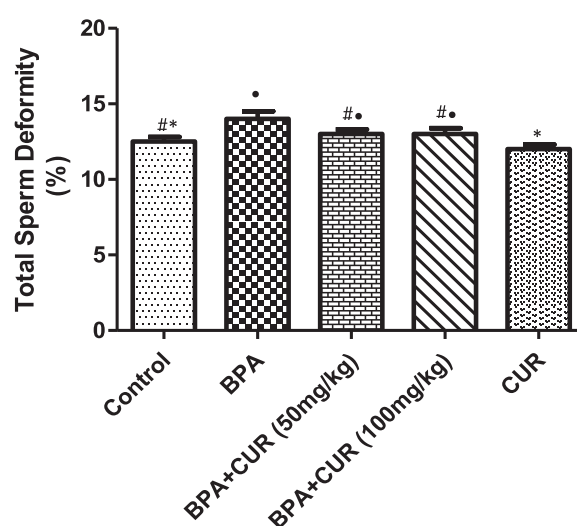


Fig. 11. Effect of curcumin administration on total sperm deformity in animals exposed to BPA for 14 days. Mean ± S.D, (n = 8). Bars with different asterisks are significantly different (P < 0.05).

(50 mg/kg b.wt) portrayed no significant improvement in testosterone level. Lastly, daily sperm production; DSP (Fig. 8), sperm motility; SM (Fig. 9), and sperm count; SC (Fig. 10) were significantly (p < 0.05) reduced with corresponding increase in the total sperm deformity; TSD (Fig. 11) sequel to BPA exposure in relation to the control rats. The administration of CUR at 50 mg/kg b.wt and 100 mg/kg b.wt remarkably reversed the sperm abnormalities by raising the levels of DSP, SM and SC, respectively with resultant reduction in TSD.

3.4. Effect of curcumin on BPA induced alterations in cerebral oxidative stress biomarkers (LPO, SOD and catalase)

Cerebral LPO level revealed the extent of lipid peroxidation and index degree of oxidative stress in the organ. Fig. 12 depicted significant strong augmentation (p < 0.05) of lipid peroxidation (MDA) content in BPA treated group compared to control group. The co-treatment of rats with CUR at both doses (higher and lower) caused the significant reversal to the membrane integrity of the brain. In Fig. 13, the post mitochondrial fraction (PMF) of the brain showed that the activity of superoxide dismutase (SOD) was substantially (P < 0.05) suppressed following the oral treatment with BPA. However, treatment

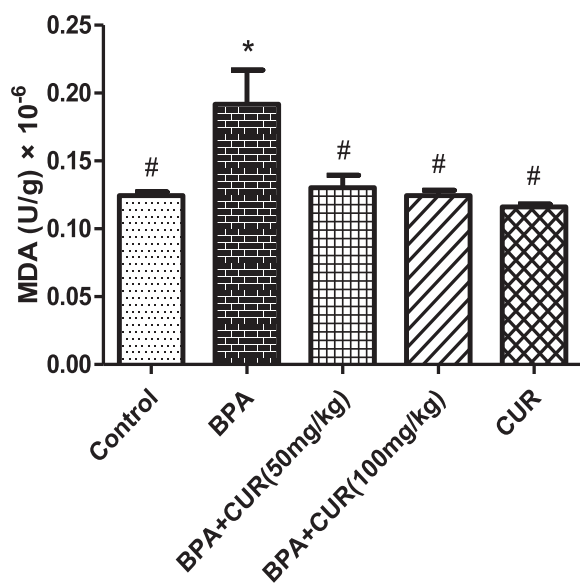


Fig. 12. Effect of turmeric curcumin malonaldehyde (MDA) content in animal induced with Bisphenol-A. The values were expressed as the means ± SD (n = 8). Bar with asterisk (*) is significant in relation to the control, while bars with the number sign (#) are significant in relation to the BPA-treated group (p < 0.05).

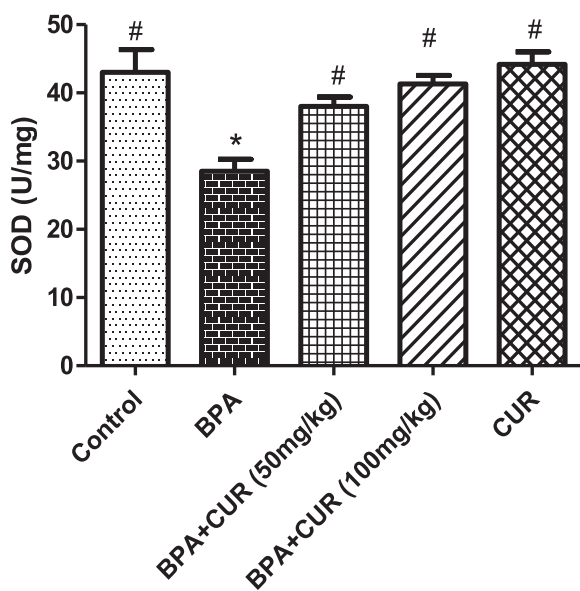


Fig. 13. Effect of turmeric curcumin on superoxide dismutase (SOD) activity in animal induced with Bisphenol-A. The values were expressed as the means ± SD (n = 8). Bar with asterisk (*) is significant in relation to the control, while bars with the number sign (#) are significant in relation to the BPA-treated group (p < 0.05).

of CUR reinstated the normal SOD activity in the brain at 50 mg/kg b.wt and 100 mg/kg b.wt. Fig. 14 described that BPA strongly inhibited catalase enzyme activity in the brain of wistar rats. Administration of turmeric CUR successfully augmented catalase activity both at lower (50 mg/kg b.wt) and higher (100 mg/kg b.wt) doses. Generally, administration of lower dose (50 mg/kg b.wt) of turmeric curcumin alone also showed significant (P < 0.05) difference in the activities of SOD and catalase including MDA content.

3.5. Effect of curcumin on histopathological changes induced by BPA in brain and testes of wistar rats

The control group showed normal histology of the striatum in the

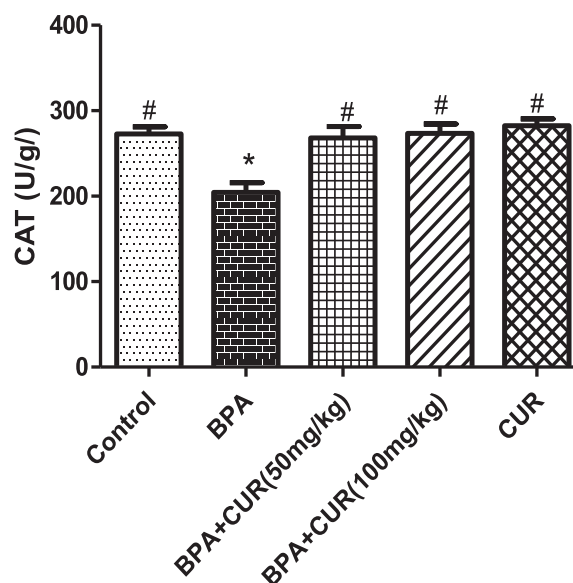


Fig. 14. Effect of turmeric curcumin on catalase (CAT) activity in animal induced with Bisphenol-A. The values were expressed as the means ± SD (n = 8). Bar with asterisk (*) is significant in relation to the control, while bars with the number sign (#) are significant in relation to the BPA treated group (p < 0.05).

brain (Fig. 15). Treatment with BPA (50 mg/kg BPA) caused disseminated congestion in the striatum and neuronal damage especially at microcirculation. The striatum of low dose at 50 mg/kg b.wt of CUR treated group showed some areas of red neurones. The damage to striatum was milder than the toxicant group. High dose of CUR at 100 mg/kg b.wt treated group showed that the striatum have no significant lesion. Also, rats treated with CUR alone at 50 mg/kg b.wt showed normal architecture of the striatum with no significant lesion.

The histopathological evaluation of control testes (Fig. 16) showed normal seminiferous tubules and the seminiferous epithelium consists of normal spermatogonia, spermatocytes, spermatids, spermatozoa and sertoli cells, while the interstitia possessed normal leydig cells. Also, there was active cell division and maturation of the germ cells as evidenced in abundance of terminally differentiated cells (spermatozoa). The leydig cells were similarly normal in control rats. Histopathological examination of BPA induced group depicted that the interstitia containing the leydig cells indicated mild edema resulting in reduced spermatogenesis, depletion of spermatocytes, and spermatogenesis varied from weak to arrest in some seminiferous tubules and impairment of active cell division and maturation of germ cells. Remarkably, both low dose (50 mg/kg b.wt) and high dose (100 mg/kg b.wt) of turmeric curcumin co-treatment as well as CUR treated alone caused restoration of normal seminiferous tubules as the seminiferous epithelium consists of normal spermatogonia, spermatocytes, spermatids, spermatozoa and sertoli cells, while the interstitia contained normal leydig cells. The treatments also displayed active cell division and maturation of the germ cells as evidenced in abundance of terminally differentiated cells (spermatozoa). The leydig cells were likewise restored to normal.

4. Discussion

Parkinson's disease is commonly regarded as a disorder of the central nervous system resulting from the loss of substantia nigra cells in the brain. The substantia nigra cells produce dopamine and several other signaling molecules which are responsible for the coordination of mammalian's locomotion. Activation of these dopaminergic enzymes including AChE may cause dopamine alteration; consequently, prevents neurons to fire well thereby preventing patients to regulate their

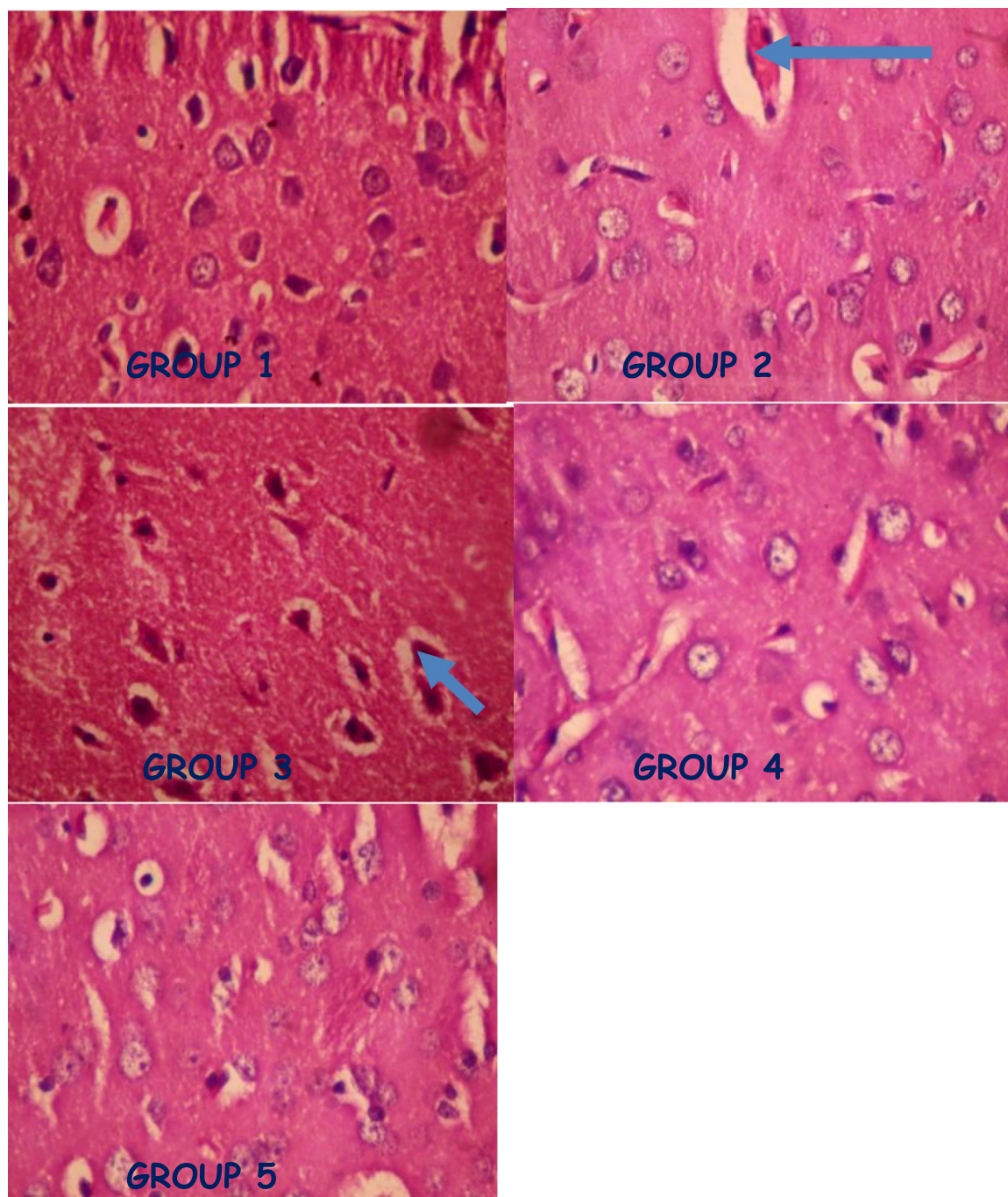


Fig. 15. (X 400): **Group 1** (CONTROL), the striatum showed no significant lesion. **Group 2** (50 mg/kg BPA), the striatum showed disseminated congestion (neuronal damage) especially at microcirculation (Long arrow). **Group 3** (50 mg/kg BPA + 50 mg/kg CUR), the striatum showed area of red neurones (short arrow). **Group 4** (50 mg/kg BPA + 100 mg/kg CUR), the striatum showed no significant lesion. **Group 5** (50 mg/kg CUR), the striatum showed no significant lesion.

movement. Herein, we found that sub-acute exposure to BPA in rats caused alteration in AChE activity and showed decreased locomotion function, which strongly indicated that BPA is risk factor of PD and neuronal damage through mechanistic depletion of AChE. Notably, it can also be suggested that both the dopaminergic and cholinergic systems have been affected. Previously, it has been reported that individuals with PD depicted dopaminergic/cholinergic imbalances originating from the loss of dopamine-producing neurons of the nigrostriatal pathway followed by decreased activity of cortical acetylcholine-degrading enzyme (AChE) [47]. We only checked the activity of AChE because studies have conventionally confirmed that Acetylcholine (ACh) is one of the most important neurotransmitter signaling

molecules involved in the regulation of cognitive functions and neurogenesis [48,49]. Additionally, the significant depletion in the number of locomotive activity after 14 days of BPA exposure compared to 7 days could be linked to the progressive development of Parkinson's disease. Similar study reported that rats (in animal model) tend to be less active in cylinder after repeated exposure to depressant drugs due to impairment of substantia nigra cells and because of their acquaintance with the apparatus [50]. We applied curcumin (50 mg/kg and 100 mg/kg), a polyphenol from the rhizomes of the plant *Curcuma longa* (turmeric) to prevent BPA intoxication in rat. The administration of curcumin significantly reinstated the locomotive and AChE activities. The regaining of these indicative markers amplifies the protection

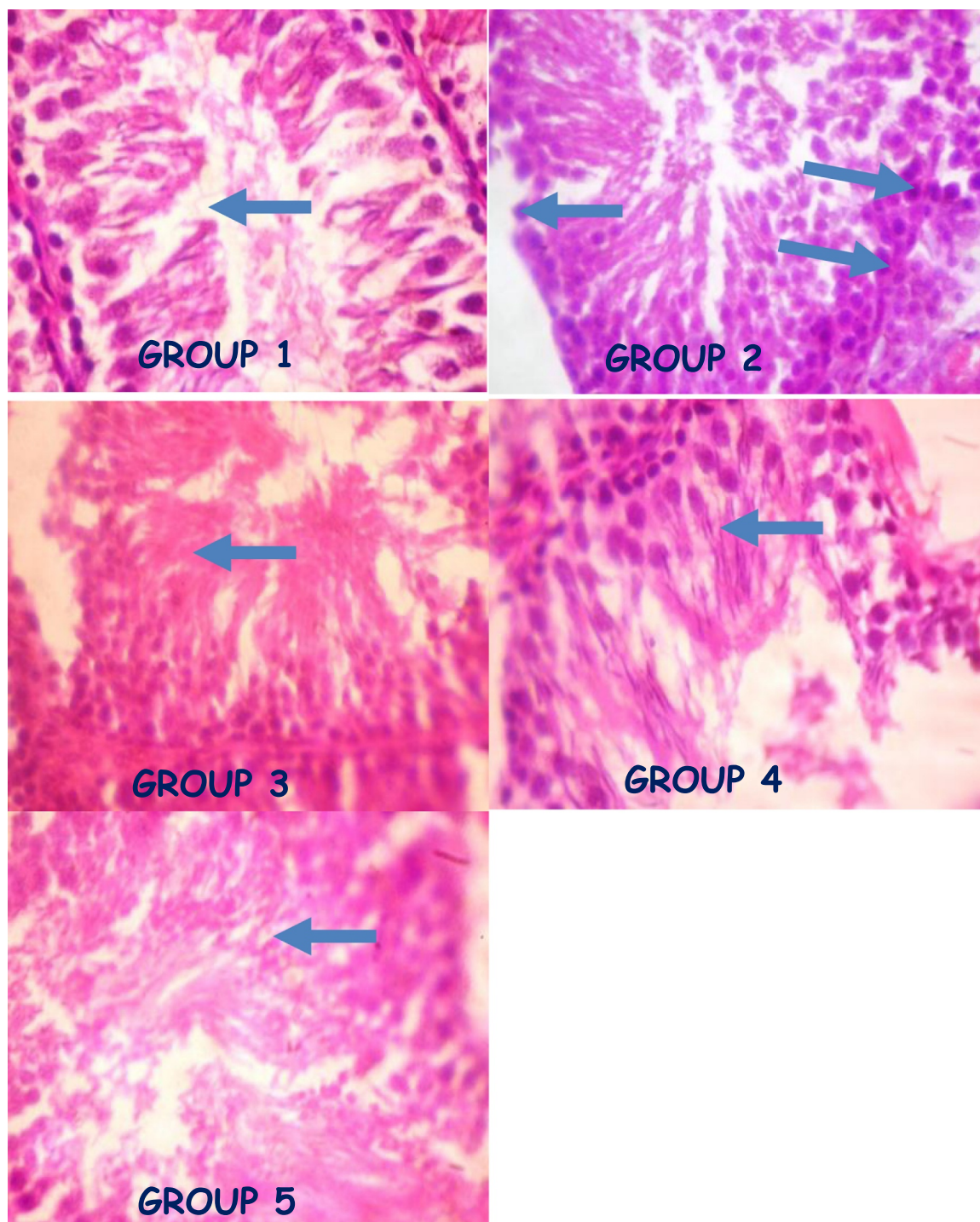


Fig. 16. (X 400): Testicular photomicrograph of **Group 1** (CONTROL) animals showed normal spermatogonia, spermatocytes, spermatids, spermatozoa and sertoli cells, while the interstitia have the leydig cells. Also, there was active cell division and maturation of the germ cells as evidenced in abundance of terminally differentiated cells (spermatozoa) (arrow). The leydig cells were normal; **Group 2** (50 mg/kg BPA), the interstitia harboring the leydig cells showed mild edema (arrows) resulting in reduced spermatogenesis, depletion of spermatocytes, and spermatogenesis varied from weak to arrest in some seminiferous tubules and impairment of active cell division and maturation of germ cells; **Group 3** (50 mg/kg BPA + 50 mg/kg CUR), the seminiferous epithelium consists of normal spermatogonia, spermatocytes, spermatids, spermatozoa and sertoli cells, while the interstitia have the normal leydig cells (arrow). There was also active cell division and maturation of the germ cells as evidenced in abundance of terminally differentiated cells (spermatozoa); **Group 4** (50 mg/kg BPA + 100 mg/kg CUR), the seminiferous epithelium consists of normal spermatogonia, spermatocytes, spermatids, spermatozoa and sertoli cells, while the interstitia have the leydig cells. Also, there was active cell division and maturation of the germ cells as evidenced in abundance of terminally differentiated cells (spermatozoa). The leydig cells were normal (arrow); **Group 5** (50 mg/kg CUR), the seminiferous epithelium consists of normal spermatogonia, spermatocytes, spermatids, spermatozoa and sertoli cells, while the interstitia have the leydig cells. Also, there was active cell division and maturation of the germ cells as evidenced in abundance of terminally differentiated cells (spermatozoa). The leydig cells were normal (arrow).

proffered by CUR therapy against neurodegeneration and Parkinson's disease induced by BPA. Similar to this, earlier studies have shown CUR to be a strong neuroprotection against rotenone sub-chronic induced injury [51,52]. Additionally, some studies in dissimilar experimental models of PD have shown neuroprotective effect of curcumin [24,31] including one study which reported that chronic dietary ingestion of turmeric curcumin provides neuroprotection in toxic mouse model of PD [31].

Nitric oxide (NO) is a signal molecule that involves the pathogenic processes including neurodegenerative disorders. Also, comprehending the pathogenic mechanisms of NO and its role in Parkinson's disease (PD) and other neurodegenerative diseases could be helpful to develop novel neuroprotective therapies for these diseases [53]. Our results showed that BPA toxicity cause significant elevation of neuronal NO level, altering neuronal functions, as afore described by behavioral and morphological alterations in rats [54] on exposure to arsenic. Some studies reported that PD is a neurodegenerative disorder manifested by numerous deformities including inflammation triggered by high level of NO [55,56], mitochondrial dysfunction and heavy metal accumulation in substantia nigra cells [57,58]. Also, high levels of neuronal NO and inducible NO synthase (NOS) were found in substantia nigra of patients and animal models having PD [59,60]. Further studies similar to our finding established that excessive nitric oxide (NO) production via the inducible form of nitric oxide synthase (iNOS) plays a fundamental role in neuronal cell damage [61]. It induces brain glial cells and microvascular endothelium during inflammatory and ischemic conditions [62]. Here, CUR (50 mg/kg and 100 mg/kg); natural polyphenol elicited neuroprotective action on neurodegenerative disorder by depleting the content of cerebral NO in rat assaulted with BPA. The neuroprotective effect could be linked to the fact that CUR possesses the ability to influx brain barrier and directly scavenge pathological absorption of NO. Here, CUR can be regarded as a powerful anti-inflammatory agent of BPA.

Idiopathic Parkinson's disease and uncontrollable hike of dopaminergic metabolizing enzymes could influence hypothalamus pituitary hormone secretion [21]. The anterior pituitary endocrine dysfunction had also been documented in patients with Parkinson's disease [22]. Sub-acute exposure to 50 mg/kg BPA (endocrine disrupting chemical) significantly inhibited the luteinizing hormone-releasing hormone (LHRH), follicle stimulating -releasing hormone (FSRH) and serum testosterone. This action revealed that the suppression of gonadal function may also be attributed to its ability to inhibit hypothalamic LHRH release which acts directly on the testes by inhibiting FSH-stimulated testosterone production. We can say here that the observed pattern of hormonal deviation in rats exposed to BPA (reduced locomotive activity) was a consequence of impairment of hypothalamus pituitary testicular axis. This result was in line with previous studies that disruption of pituitary hormone secretion was due to hypothalamic dysfunction in PD patients [62,63] and dopamine content of the hypothalamus including neuroendocrine hormones (FSH, LH and testosterone) were noticeably abridged in patients with Parkinson's disease [64]. The treatment with CUR (50 mg/kg and 100 mg/kg) potentiated hypothalamus pituitary testicular hormones by triggering FSH, LH and testosterone, thereby showing protection from BPA neuro-testicular toxicity.

Daily sperm production is hormonally driven by brain hormones [65]. The hypothalamus secretes gonadotropin-releasing hormone (GnRH), which acts on the anterior pituitary gland, stimulating the release of follicle stimulating hormone (FSH) and luteinizing hormone (LH). FSH and LH are unrestrained into the bloodstream and act solitarily on the male genitalia to facilitate spermatogenesis within seminiferous tubules (action on FSH) and testosterone assemblage by immediate Leydig cells (action on LH) between the seminiferous tubules. Therefore, any increase in LH, FSH and testosterone will cause male fertility balance to survival. In our study there were increase in daily sperm production (DSP), sperm motility and sperm count in CUR

(50 mg/kg and 100 mg/kg) treated rats, resulting in the physiological spermatogenesis. Activated infertility and sexual dysfunctions are strongly implicated in many men living with Parkinson's disease (PD) [66,67]. Moreover, absolute reduction in total sperm deformity (TSD) or spermatozoa elevation supported the view that CUR protected against decreased libido (sex drive), premature or delayed ejaculation, inability to orgasm and erectile dysfunction, reported as common sexual problems in men with Parkinson's [68]

In recent time, the toxic exposure to BPA has generated a lot of public concern [1]. BPA is capable of crossing blood brain barrier (BBB) [69] and metabolized via hydroxylation, glucuronidation and sulfuration reactions occurring in the liver and extrahepatic cells essentially brain and testes. The water-soluble metabolites of BPA are excreted via the kidney and urine. Consequence upon the metabolic oxidation of BPA by the extrahepatic microsomal CYP-450 enzyme complex system, a highly reactive intermediate, Bisphenol-A-3-4-quinone (BA34Q) is produced [70]. BA34Q directly reacts with glutathione (GSH) initiating depleted GSH. This condition makes BA34Q to bind cerebral proteins forming DNA adducts to elicit lipid peroxidation and antioxidant enzymes reduction, therefore leading to deregulation of hypothalamus pituitary gonadal hormones and cellular injury [70]. Significant elevation of the neuronal tissue levels of MDA (lipid peroxidation) has been linked with xenobiotic induced PD [58] confirming bisphenol-A toxicity. Consistent with these studies [68,71]. BPA exposure in our study led to the decrease in the brain tissue wellness as well as neuronal antioxidant enzymes viz., SOD and catalase. The declined antioxidant enzymes also explained the mechanism of neurotoxicity and PD induced by BPA. It could similarly be associated to free radical generations which were known to have damaging effects on the cells. Turmeric curcumin (*Curcuma longa*), the common nutraceuticals for medicinal and food purposes can scavenge various radicals in both aqueous and organic milieu [23]. The treatment of CUR (50 mg/kg and 100 mg/kg), to rats thus restored the activities of antioxidant enzymes (SOD and CAT) in the brain. The salvage in the activities of these enzymes amplifies the protection elicited by CUR administration to rats against cerebral oxidative stress induced by BPA similar to previous studies [30,31]. Recent studies have demonstrated that CUR is a strong antioxidant depicting potent protection against PD [50,51], mutagenesis [71], carcinogenesis [29,72] of the rat's brain and infertility originated from erectile dysfunction [66].

The striatum is well-known brain component that harbors a group of dopaminergic neurons and acts as a local source of striatal dopamine (DA) [62]. PD can occur when striatum (dopamine-producing neurons) die or damage and showing symptoms including tremor, slowness, stiffness, and balance problems [63]. This study demonstrated that BPA administration causes striatal damage in rat treated. In accordance to our study, mutilation and pathological nigrostriatal dopaminergic terminal loss have been implicated in patients with Parkinson's disease (PD) [8]. Also, histopathological and in vivo studies have demonstrated that the lesion or loss of striatal DA occurs mostly in the caudal putamen and caudate head which was correlated with cognitive or motor decline [13]. Thus, CUR oral administration (50 mg/kg and 100 mg/kg) provided protection to rats from striatum damage by preventing disseminated congestion of the striatum. CUR therefore is suggested as one of the natural antioxidants that can down-regulate brain damage helping as anti-Parkinson's disease mediator. Furthermore, the restoration of striatal damage by CUR could abolish the problem of PD as well as BPA induced neurodegeneration.

Sertoli cells and leydig cells are located in the testes and they control many crucial functions in spermatogenesis [73]. These gonadal injuries are often in Parkinson's disease and other neurodegenerative disorders [74]. Transplantation of functional sertoli cells have been suggested as one of the most alternative treatments for PD in preclinical and clinical studies [75]. The leydig cells showed edema resulting in reduced spermatogenesis, depletion of spermatoocytes, and spermatogenesis varies from weak to arrest in seminiferous tubules and

impairment of active cell division and maturation of germ cells in BPA treated group, consistent with earlier reports [76,77]. The histopathological examination confirmed that swelling or diminished total leydig cell mass was conspicuously noticed in patients with PD. This brought further evidence about the impairment of leydig cells in BPA induced PD. However, CUR (50 mg/kg and 100 mg/kg) co-administration markedly promoted the seminiferous epithelium consists of spermatogonia, spermatocytes, spermatids, spermatozoa and sertoli cells, while the interstitia have the leydig cells. Also, there was active cell division and maturation of the germ cells as evidenced in abundance of terminally differentiated cells (spermatozoa). The leydig cells were similarly normal.

5. Conclusion

Our study found that CUR at doses of 50 mg/kg and 100 mg/kg ameliorated the metabolic targets implicated in parkinson's disease including locomotive decline and dysfunction of hypothalamus pituitary gonadal hormones induced by BPA. CUR administration promoted leydig and sertoli cells as well as striatum that consists dopaminergic neurons including putamen, caudate and substantial nigra. Therefore, we can suggest that CUR (50 mg/kg and 100 mg/kg) co-administration have immense therapeutic potential against BPA induced PD involving dysfunction of hypothalamus pituitary gonadal hormones in rats. Further studies to clarify exact mechanism of action of CUR at molecular level should be examined before starting the clinical test.

Appendix A. Transparency document

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bbrep.2018.12.004.

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