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Toxic Effects of Cyclophosphamide on Male Mice Fertility Development and Body Indicators

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Abstract

Chemotherapy is considered one of the crucial strategies for treating cancer. However, a variety of temporary and permanent side effects have been reported due to the use of these chemical compounds. The current study was designed to highlight the influence of cyclophosphamide (CYP) administration on reproductive development capability and fertility in male mice. Thirty pre-mature male mice were divided into three groups; (1) control groups (2) one group injected interperitoneally with 150 mg CYP, and (3) one group with 250 mg CYP. Injections were administered for four weeks. The results indicate a significant reduction in body and organs weights in treated groups when compared with the control group. Similarly, the reproductive capabilities were also decreased in a increasing trend. Zoospermia was detected in treated groups with CYP, and this reduction was more noticable in the 150 CYP group. Luteinizing hormone levels increased significantly in both treated groups while testosterone levels declined considerably in the CYP-treated groups. These findings imply the risks of using this drug in cancer remediation during the maturation period of mice fertility. More investigations are required to detect whether these CYP-associated consequences are reversible or permanent after treatment discontinuation.

Keywords: Cyclophosphamide; Mice fertility; Reproductive ability; Sperm abnormality; Sexual hormones

1. Introduction

Chemotherapy is considered one of the vital steps in cancer treatment via administration of certain classes of chemicals called anti-cancer drugs (Alam *et al.,* 2018). It is well known that these materials are characterize by their distribution capability through the body via the bloodstream; in turn, this characteristic increases the probability of reaching the cancerous tissues throughout the body (Sutradhar and Amin, 2014). In general, the mechanism of action of these materials is focused on growth suppression and killing tumor cells; however, each group of these chemicals involves distinctive mechanism of action (Dasari and Tchounwou, 2014; Meistrich, 2013).

Cyclophosphamide (CYP) is used to treat several types of cancer, such as leukemia, breast, and ovarian in addition to some solid tumors (Gouspillou *et al.,* 2015; Slater *et al.,* 2015). This drug is a nitrogenous mustard belonging to the cytotoxic group of agents (Singh *et al.,* 2018). Primarily, CYP is an inactive chemical compound and requires inactivation by the cytochrome P450 oxidative enzyme (Vredenburg *et al.,* 2015). The main mechanism of action of this material depends on production of alkylating agents whose main role in repressing tumor growth was characterized to be formation of active electrophiles that actively integrate with the DNA structure (Giraud *et al.,* 2010).

Although CYP is utilized to treat many cancers, a broad spectrum of adverse consequences can be observed in other physiological and structural components in humans (Bhat *et al.,* 2018; Zhang *et al.,* 2019). Most of these impairments result from the same mechanism action of this drug in target cancerous cells (Xu *et al.,* 2015). Hematopoietic toxicity, nausea, and vomiting are the most common side effects of this drug (Lee *et al.,* 2019). Kanno *et al.* (2009) and Zhang *et al.* (2021) revealed that this drug causes a reduction in body mass and kidney weights. Furthermore, CYP can induce hepatotoxicity and neurotoxicity (Raza and Alghasham, 2011). Immunosuppressive effects of CYP were also detected by Huyan *et al.* (2011) who found that low and high doses of this drug could lead to a reduction in white blood cells in general and lymphocyte number in a dose-dependent manner.

To date, several studies examined the deleterious effects of this drug on cancer patients' fertility (Onaolapo *et al.,* 2018). Pavin *et al.* (2018) found that the standard treatment dose of CYP suppresses the formation of steroid hormones from the gonads as a result of histopathological changes in testes. Similarly, the spermatogenesis process was destroyed at different stages after seven days after a single dose owing to the direct effect of this drug on the testicular tissue or the indirect side effect of the CYP on the sexual hormonal balance (Al-Niwehee and Al-Rudaini, 2019; Smart *et al.,* 2018). Teratogenic effects of chemotherapy were reported as a result of testicular functions abnormality (Vassilakopoulou *et al.,* 2015). It was noticed that CYP has the capability of inducing congenital malformations in the new generation (Rengasamy, 2017).

This investigation sought to evaluate the effect of two different CYP doses of on the reproductive proficiency and sexual hormone levels of male laboratory mice during their sexual maturation period. Furthermore, this study tried to define the concurrent toxic effects of CYP on body mass and liver and kidney weights.

2. Materials and methods

2.1 Study design

Thirty pre-mature Swiss male mice (Balb/C) *Mus musculus* L. were provided by the Animal House/ Biology Department/ College of Education for Pure Science/ University of Basrah were used as animal model in current study. Mice age was five weeks. All mice were housed individually in plastic cages covered by stainless-steel nets. Wood shavings were autoclaved and used as bedding material, and water and standard food pellets were provide ad libitum. Animal house temperature was ranged between 22 and 23 ^{o}C, and the light cycle was 12:12 hr light/dark.

2.2 Cyclophosphamide administration

The inter-peritoneal injection route was used to administrate the drug. Two doses of CYP were prepared (150 and 250 mg/kg). The animals were divided into three groups, each one consisting of ten animals:

1) Control group: the animals were injected with 0.1 ml normal saline.
2) First treatment group (A): the animals were injected with 0.1 ml of 150 mg/kg of CYP.
3) Second treatment group (B): the animals were injected with 0.1ml of 250 mg/kg of CYP.

The CYP administration lasted for four weeks as that timeframe of administration is adequate to allow the animal to reach the maturation period under normal conditions.

2.3 Body, liver, kidney, and testicular weight measurements

Body weight was measured before CYP administration when the animal age was three weeks after which this measurement was repeated for each animal after completing thje course of drug administration, that is, when the animal was postulated to be an adult. Body weight development was calculated by subtracting the initial weight (five weeks age) from the final weight (adult age) while the liver, kidney, and testicular weights were examined at the end of the CYP administration.

2.4 Reproductive capability

To examine the effect of CYP on the animals' reproductive capability, two females were housed with each treated male in separate cages with the standard measurements. The number of pregnant females after a week of housing, number of births, and the sum of weights of births for each male were recorded.

2.5 Sperm abnormality

Animals were sacrificed and then dissected. Afterwards, the epididymis was removed. A sperm suspension was prepared from each epididymis cauda after mincing them in physiological saline. One drop of each suspension was used to count sperm number using a hemocytometer chamber. Smears from the suspensions were prepared on glass slidesnafter which each slide was stained with drops of 1% eosin. The slides were air-dried. All slides were examined under a light microscope with oil immersion under the magnification power 100X. Morphological abnormalities for head, middle piece, and tail for one hundred sperm cell on each slide were recorded (Raghuvanshi *et al.,* 2012).

2.6 Hormone Assay

Blood samples were collected using the heart puncture technique for which 1 ml of blood was obtained from each animal and placed in heparinized tubes. All samples were centrifuged (3500 rpm for 10 min), serum was withdrawn and kept in refrigerator (– 4 ºC) until used for further analyses.

2.7 LH Assay

Luteinizing hormone was determined by using an enzyme-linked immunosorbent assay (ELISA) kit (Monobind, USA). This method uses the biotin-treptavidin system, which interacts with the high infinity of the internal surface.

2.8 Testosterone Assay

Testosterone levels were measured using an ELISA kit (Monobind, USA). The principle of this method can summarized as a competitive reaction between sampling hormone and hormone-enzyme conjugate measures the number of testosterone anti-bodies that cover tube's internal surface.

2.9 Statistical Analysis

Statistical Package for Social Science, version 23 (SPSS, V.23) was used for both descriptive and inferential analysis. Data distribution was tested. Data with a Guassian distribution (body and organs weights, and hormones level) were analyzed using a one-way analysis of variance (ANOVA) and applying Tukey's honestly significant difference (HSD) for pairwise comparisons. Reproductive capability data followed a non-normal distribution; therefore, Kruskal–Wallis (*K*) and Mann – Whitney (*U*) tests were applied. A p value ≤ 0.05 (95% Confidence interval) consider statistically significant.

3. Results and discussion

The administration of CYP for four weeks to male mice before they reached the adult stage led to a significant decrease in adult body weight when compared with the control group, $ANOVA = 30.913,\ p \leq 0.05$. Pairwise differences between the three groups using Tukey HSD showed that the highest body weight was in control group (27.87 ± 1.57 g), while the body weights in both treated groups decreased considerably (23.48 ± 2.61 and 21.19 ± 1.36 g, respectively). During maturation, weight gain significantly decreased ($p \leq 0.05$). The animal group that received 250 mg of CYP showed a lower weight gain than the group that received 150 mg of the same drug (7.13 ± 1.53 g versus 4.92 ± 0.98 g, respectively).

The body weight increased in all groups from the age of five weeks until the cessation of CYP administration (Table 1). Weight gain (weight D) differed substantially between the groups, ($ANOVA = 89.536,\ p \leq 0.05$). A multi-comparison est revealed that the group that achieved the highest weight was the control group (11.29 ± 0.82 g), whereas the lowest wright gain was found in the group that received 250 mg of CYP (4.92 ± 0.98 g) aqs shown in table 1.

Test weight decreased significantly after CYP administration in adult male mice (*ANOVA = 159.722, p ≤ 0.05*) as shown in table 1. The average testicular weight in the control group was 0.27 ± 0.013 g, while testicular weights decreased markedly in both the 150 and 250 mg CYP groups (0.11 ± 0.019 and 0.09 ± 0.021 g, respectively). The gonadostomatic index (GSI) varied noticeably among the groups (*ANOVA= 67.688, p ≤ 0.05)*. The highest value of the GSI was detected in the control group (0.89 ± 0.077), and this index decreased with increasing the CYP does administration to reach to 0.42 ± 0.083 in 250 mg group.

Figure 1 clearly reveals that CYP administration caused a significant reduction in both liver and kidney weights *(ANOVA = 23.360, p ≤ 0.05* and *ANOVA = 18.908, p ≤ 0.05,* respectively).

The highest liver weight was found in the control group (1.59 ± 0.196 g), and this weight decreased markedly in in both treatment groups; however the lowest weight was measured in group that received 250 mg of CYP (1.03 ± 0.211 g). Similarly, the kidney weight followed the same trend. Kidney weight decreased considerably in the 150 and 250 mg groups (0.20 ± 0.026 and 0.16 ± 0.026 g, respectively) when compared with that in the control group (0.25 ± 0.399 g).

A chi-squared test revealed that the proportion of pregnant females varied significantly among the treatment groups (Figure 2). The highest percentage of pregnant females was detected in the control group (80%). This percentage declined dramatically to reach 45% in females that had received 150 mg of CYP and 30% in females that were treated with 250 mg while the highest pregnancy failure was found in the female group that received 250 mg of CYP and the lowest was found in the control group.

Table 1. CYP effect on male mice body and testis weights (g) after four weeks of administration (n=10)

Groups	Weight B (g) (mean ± SD)	Weight A (g) (mean ± SD)	Weight D (g) (mean ± SD)	Testis (g) (mean ± SD)	GSI % (mean ± SD)
Control	16.58 ± 0.94	27.87 ± 1.57	11.29 ± 0.82	0.25 ± 0.013	0.89 ± 0.077
150 mg	16.35 ± 1.48	23.48 ± 2.61**	7.13 ± 1.53**▲	0.11 ± 0.019**	0.46 ± 0.077**
250 mg	16.27 ± 0.98	21.19 ± 1.36**	4.92 ± 0.98**▲	0.09 ± 0.021**	0.42 ± 0.083**

**significant difference at the probability of p ≤ 0.05; ▲significant difference between 150 mg and 250 mg groups at the probability of p ≤ 0.05; Weigh B = Body weight (g) before CYP administration; Weight A= Body weight (g) after CYP administration; Weight D = Weight (g) gaining from age 5 weeks to the end of CYP administration; GSI= Gonado-Stomatic Index = (testes weight/body weight)*100

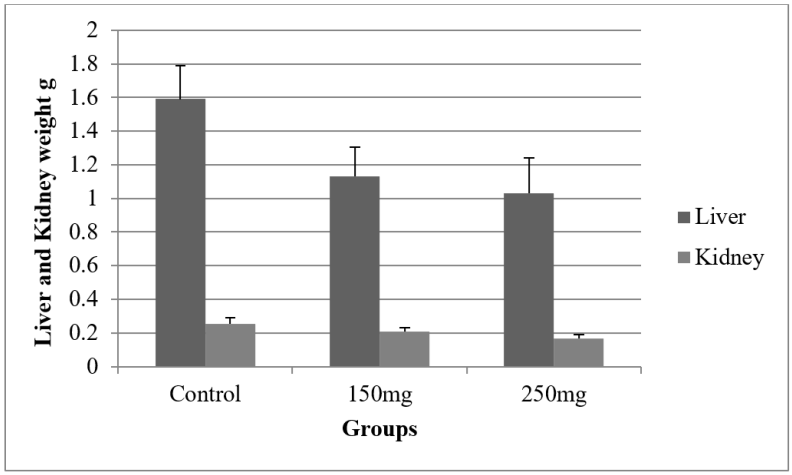
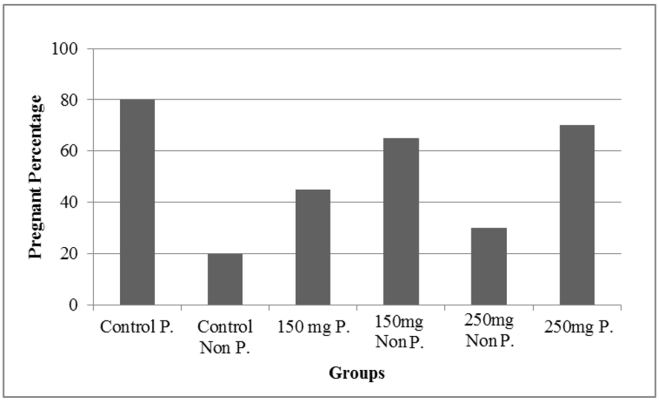


Figure 1. CYP administration effect on the liver and Kidney weights after four weeks of administration

Non-parametric analysis (Kruskul–Wallis test (K) showed a significant decline in the number of births in both treated groups (K = 21.118, $p \leq$ 0.05) as shown in table 2. Pairwise analysis (U) between each two groups revealed that the number of births in the control group was significantly higher than in the 150 and 250 mg groups (U = 23.500; $p \leq 0.05$ and $U = 4.000$; $p \leq 0.05$, respectively), while no evident difference was detected between the groups that received the CYP.

In terms of new birth weights, the inferential statistical analysis indicated to the remarkable significant differences between the groups (K = 119.742, $p \leq 0.05$) as shown in figure 3. The box plot clearly reveals that the birth weights declined significantly, and the lowest weights were recorded in mice group that received 250 mg of CYP (median = 0.533 [0.41 – 0.62]), while the highest were found in the control group (median = 0.853 [0.656 – 1.311]).

Inferential statistical analysis revealed a high significant difference between the three groups in total sperm counts and the proportion of sperm morphological abnormality, $p \leq 0.05$ (Table 3). It is obvious that the total number of sperm declined in the groups treated with CYP and the lowest sperm count number was recorded in group which received 150 mg of CYP (46.583 ± 11.261), while the highest sperm number was detected in control group (77.931 ± 16.297). In contrast, the percentage of sperm morphological abnormalities were high in the treated groups when compared with the control group. Furthermore, five sperm abnormalities (amorphous, lack of hook, banana shape, fusion head with mid piece, and lack of head) were detected in all groups (Figure 4). However, the lowest proportion of sperm abnormalities was observed in the control group, while it was obvious that the highest percentage of abnormalities occurred in group that was injected with 150 mg of CYP. They differed significantly from other groups at $p \leq 0.05$ and $p \leq$ 0.05, respectively (Table 3).



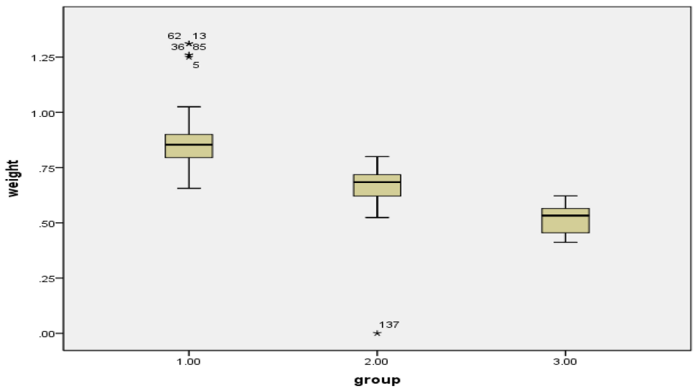
The significant probability is $p \leq 0.05$.; Control P. = Pregnant females in control group; Control Non P. = Non pregnant females in control group; P. = Pregnant females; Non P. = Non pregnant females

Figure 2. CYP administration effect on the percentage of pregnancy in female mice after four weeks of treatments

Table 2. CYP effect on the number of births of treated males after four weeks of administration.

Groups	Median (minimum-maximum)
Control	8* (5 - 10)
150mg	5*▲ (0 - 6)
250mg	0*▲ (0 - 6)

*significant difference at the probability of $p \leq 0.05$; ▲significant difference between 150mg and 250 mg groups at the probability of $p \leq 0.05$



1 = control group; 2 = group received 150 mg of CYP; 3 = group received 250 mg of CYP

Figure 3. CYP administration effects on birth weights of mice after four weeks of treatment

Table 3. CYP administration effects on sperm count and morphology after four weeks of treatments

Groups	Sperm count million/ml	Abnormal Sperm %				
		Amorphous %	Lack of hook %	Banana shape %	Fusion head with mid piece %	Lack of head %
Control	77.931 ± 16.297*	0.04 ± 0.002	0.01 ± 0.002	0.14 ± 0.002	0.01 ± 0.005	0.03 ± 0.006
150 mg	46.583 ± 11.261*▲	3.23 ± 0.012*	0.23 ± 0.004*	0.25 ± 0.001*▲	0.32 ± 0.0.006*	0.21 ± 0.018*▲
250 mg	58.601 ± 18.327*▲	2.21 ± 0.062*	0.21 ± 0.0017*	0.19 ± 0.004*▲	0.30 ± 0.013*	0.13 ± 0.002*▲

*significant difference at the probability of $p \leq 0.05$; ▲ significant difference between 150 mg and 250 mg groups at the probability of $p \leq 0.05$

It is apparent that the CYP administration induced notable differences in LH and testosterone levels among treated and control groups (table 4). Both hormones differed significantly among the groups (*ANOVA = 39.879, $p \leq 0.05$* and *ANOVA = 29.816, $p \leq 0.05$,* respectively). LH hormone levels showed a significant increase in the groups that were given CYP when compared with the control group. The lowest level of LH was detected in the control group (2.91 ± 0.501 ng) while the highest level was found in the control group (4.98 ± 0.480 ng). Testosterone hormone showed a significant decreased in treated groups compared with control. A pairwise analysis accurately defined that the control group showed the highest level of testosterone (6.38 ± 0.608 ng), while this level was reduced considerably to 5.25 ± 0.701 ng in animal group which received 150 mg of CYP and the lowest level was 3.84 ± 1.36 ng in the group that received 250 mg.

Clinically, CYP at different doses is described to treat different types of cancer; thus, the current work sought to emphasize the negative consequences of administration of this medicine on some body and reproductive parameters during the maturation period.

The reduction in mice weight is considered to be one of the most obvious consequences of toxic compounds (Aston *et al.,* 2017). It was clear from the current findings that the weight of male mice decreased considerably after exposure to different doses of CYP. This result agrees with those from a study by Kanno et al. who detected a significant variation in body mass after six weeks of CYP administration. The possible explanation for this output is the integration of CYP administration with food intake. Khorwal *et al.* (2017) stated the loss of appetite and reduction in food intake were due to degenerative disorders in the liver parenchyma and also mentioned that these changes relatively correlated with the CYP dose.

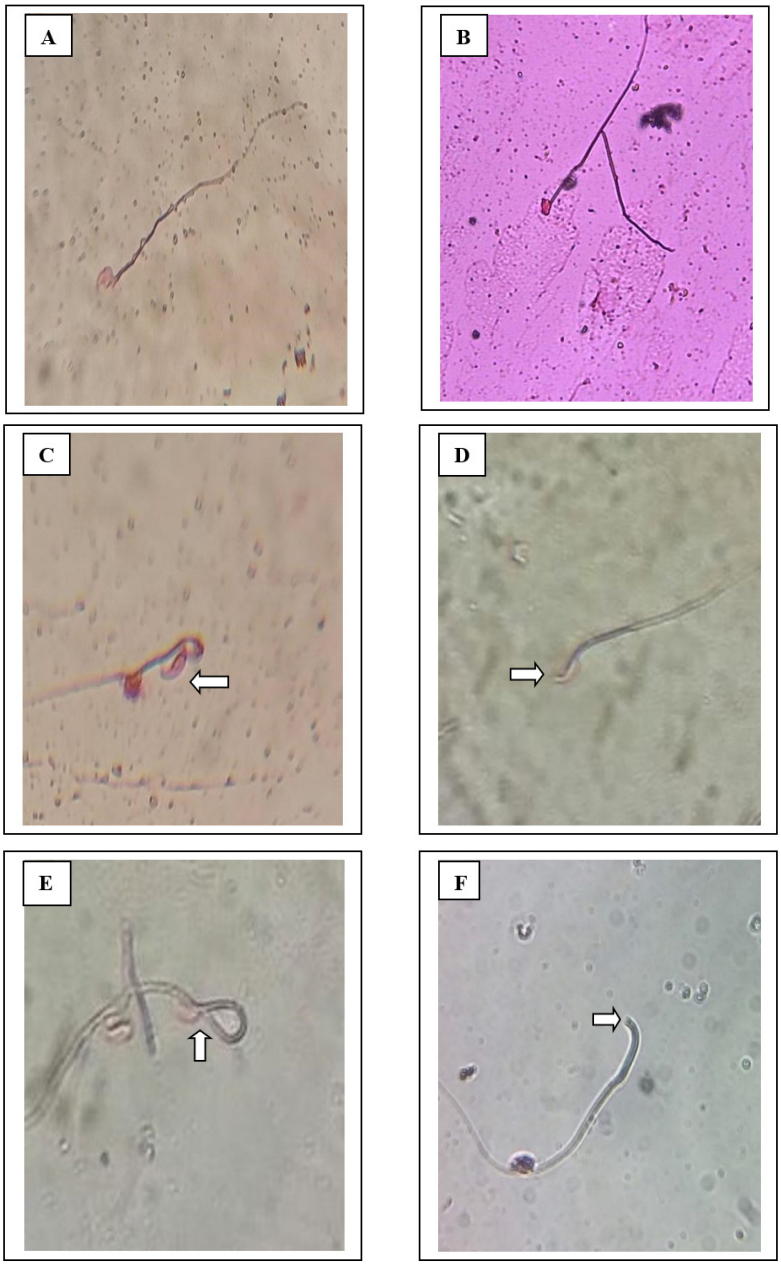


Figure 4. Sperm abnormalities: (A) Normal (B) Amorphous (C) Lack of hook (D) Banana shape (E) Fusion head with mid piece (F) Lack of head

Table 4. CYP effect on the LH and testosterone levels of treated males after four weeks of administration

Groups	Luteinizing Hormone (ng) (mean ± SD)	Testosterone (ng) (mean ± SD)
Control	2.91 ± 0.501	6.38 ± 0.608
150 mg	3.42 ± 0.281*▲	5.25 ± 0.701*▲
250 mg	4.98 ± 0.480*▲	3.84 ± 1.36*▲

*significant difference at the probability of $p \leq 0.05$; ▲significant difference between 150 mg and 250 mg groups at the probability of $p \leq 0.05$

Similarly, testicular weights followed the same trend as body weight. This measurement exhibited a noticeable decrease in groups treated with CYP, and the lowest weight was detected in the group that received 250 mg of the drug. Sujayraj *et al.* (2016) presented similar results concerning CYP toxic actions on testicular weight and GSI parameters. These changes may have been related to the histological destruction which can be induced by CYP after taking into account two essential characteristics of this drug: (1) it is a non-specific medication, which may reach and effect any part of the patient and (2) it has potency as a cytotoxic compound (Drumond *et al.,* 2011). The hepatotoxic property of cyclophosphamide is well-defined foremost as a side effect that can be induced by formation of two metabolites: (1) 4-hydroxycyclophosphamide and (2) acrolein in hepatocytes via cytochrome P450 (Oyagbemi *et al.,* 2016). That finding provides evidence for the low liver weights in groups treated with CYP. Kidney weights in all treated groups showed a significant decreased when compared with the control group. This result is consistent with those from previous studies, such as Rehman *et al.* (2012) and Kanno *et al.* (2009), who reported that CYP administration promotes kidney weight loss. This loss in this organ's weight is derived from CYP's nephrotoxic effect (Rehman *et al.,* 2012).

Reproductive capability parameters showed a severe disturbance under the effect of CYP. Chi-squared and other non-parametric analytical tests showed a significant reduction in the number of pregnant females, births, and the birth weights. These results are consistent with previous outcomes (Elangovan *et al.,* 2017). The feasible reasons of these findings include the interaction of CYP with the spermatogenesis process, a consequences that leads to a decline in the number and activity of sperm (azoospermia or oligospermia) in addition to an increase in the rate of sperm malformation. Both directions can be attributed to the physiological and cytogenetic adverse effects of CYP (Sujayraj *et al.,* 2016). Increasing the prospect of infertility with long-term exposure or administration of CYP can be attributed to two fundamental points: (1) in mice, the half-life values ($t_{1/2}$) of CYP is about 0.52 h after a single intraperitoneal injection, which gives time for CYP and its metabolites to induce toxic actions on the testes (Sujayraj *et al.,* 2016). Secondly, long term administration increases the exposure probability of testicular tissue to CYP, a process that prolongs the drug's influence on spermatogenesis layers via tissue or DNA destruction (Zhu *et al.,* 2017). This point was supported by current findings of sperm count and abnormalities indicated a considerable reduction in sperm number in animals that jad been injected with CYP; concurrently, a clear increase in sperm morphological abnormalities was recorded in treated groups.

Furthermore, current results showed impairments in sexual hormone levels. It is well-recognizsed that sexual hormones, such as testosterone, LH, and follicle stimulating hormone (FSH) determine the fate of germ cells, so depressed levels of these hormonal secretions can induce apoptosis (Arnaud *et al.,* 2017). That process also explains the decline in reproductive capability.

The abovementioned review reveals the impact of CYPon reproductive capabilities while it also highlights the effects of CYP on sexual maturation. The present hormonal disturbance is driven duringthr administration period, and this conclusion is supported by previous studies that report the effects of CYP on the gonadotropin releasing hormone, which plays the key role in sexual maturation (Onaolapo *et al.,* 2018). Nayak *et al.* (2016) stated that the long-term exposure to CYP led to oligospermia as a result of impaired spermatogenesis.

4. Conclusion

CYP can cause infertility for those patients who already received this drug before sexual maturation via CYP's induction of many histological, genetic, and physiological adverse effects. More details are still essential for obtaining a comprehensive picture about this medication. Immunohistochemistry during different stages of administration to detect the changes in the sexual hormone receptors may shed light on several physiological impairments. Moreover, evaluations as to whether these consequences are reversible or permanent are imperative.

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References

Alam A, Farooq U, Singh R, Dubey VP, Kumar S, Kumari R, Naik KK, Tripathi BD, Dhar KL.Chemotherapy Treatment and Strategy Schemes: A Review. Journal of Toxicology 2018; 2(5): 555600.

Al-Niwehee NA, Al-Rudaini AT. Effect of Cyclophosphamide Treatment During the Embryonic Period on Fertility of Adult Male Mice. Iraqi Journal of Science 2019; 60(4): 706-714.

Arnaud L, Nordin A, Lundholm H, Svenungsson E, Hellbacher E, Wikner J, Zickert A, Gunnarsson I. Effect of Corticosteroids and Cyclophosphamide on Sex Hormone Profiles in Male Patients With Systemic Lupus Erythematosus or Systemic Sclerosis. Arthritis Rheumatol 2017 June; 69(6): 1272-1279.

Aston WJ, Hope DE, Nowak AK, Robinson BW, Lake RA, Lesterhuis WJ. A systematic investigation of the maximum tolerated dose of cytotoxic chemotherapy with and without supportive care in mice. BMC Cancer. 2017 Oct 16;17(1):684.

Bhat N, Kalthur SG, Padmashali S, Monappa V. Toxic Effects of Different Doses of Cyclophosphamide on Liver and Kidney Tissue in Swiss Albino Mice: A Histopathological Study. Ethiop J Health Sci. 2018 Nov; 28(6): 711-716.

Dasari S, Tchounwou PB. Cisplatin in cancer therapy: molecular mechanisms of action. Eur J Pharmacol. 2014 Oct 5; 740: 364-78.

Drumond AL, Weng CC, Wang G, Chiarini-Garcia H, Eras-Garcia L, Meistrich ML. Effects of multiple doses of cyclophosphamide on mouse testes: accessing the germ cells lost, and the functional damage of stem cells. Reprod Toxicol. 2011 Dec; 32(4): 395-406.

Elangovan N, Chiou TJ, Tzeng WF, Chu ST. Cyclophosphamide treatment causes impairment of sperm and its fertilizing ability in mice. Toxicology. 2006 May 1; 222(1-2): 60-70.

Giraud B, Hebert G, Deroussent A, Veal GJ, Vassal G, Paci A. Oxazaphosphorines: new therapeutic strategies for an old class of drugs. Expert Opin Drug Metab Toxicol. 2010 Aug; 6(8): 919-38.

Gouspillou G, Scheede-Bergdahl C, Spendiff S, Vuda M, Meehan B, Mlynarski H, Archer-Lahlou E, Sgarioto N, Purves-Smith FM, Konokhova Y, Rak J, Chevalier S, Taivassalo T, Hepple RT, Jagoe RT. Anthracycline-containing chemotherapy causes long-term impairment of mitochondrial respiration and increased reactive oxygen species release in skeletal muscle. Sci Rep. 2015 Mar 3; 5: 8717.

Huyan XH, Lin YP, Gao T, Chen RY, Fan YM. Immunosuppressive effect of cyclophosphamide on white blood cells and lymphocyte subpopulations from peripheral blood of Balb/c mice. Int Immunopharmacol. 2011 Sep; 11(9): 1293-7.

Kanno T, Sensiate L, de Paula N, Salles M. Toxic effects of different doses of cyclophosphamide on the reproductive parameters of male mice. Brazilian Journal of Pharmaceutical Sciences. 2009; 45(2): 313-319.

Khorwal G, Chauhan R, Nagar M. Effect of cyclophosphamide on liver in albino rats: A comparative dose dependent histomorphological study. International Journal of Biomedical and Advance Research 2017; 8(03): 102-107.

Lee CK, Scott C, Lindeman GJ, Hamilton A, Lieschke E, Gibbs E, Asher R, Badger H, Paterson R, Macnab L, Kwan EM, Francis PA, Boyle F, Friedlander M. Phase 1 trial of olaparib and oral cyclophosphamide in BRCA breast cancer, recurrent BRCA ovarian cancer, non-BRCA triple-negative breast cancer, and non-BRCA ovarian cancer. Br J Cancer. 2019 Feb; 120(3): 279-285.

Meistrich ML. Effects of chemotherapy and radiotherapy on spermatogenesis in humans. Fertil Steril. 2013 Nov; 100(5): 1180-6.

Nayak G, Vadinkar A, Nair S, Kalthur SG, D'Souza AS, Shetty PK, Mutalik S, Shetty MM, Kalthur G, Adiga SK. Sperm abnormalities induced by pre-pubertal exposure to cyclophosphamide are effectively mitigated by Moringa oleifera leaf extract. Andrologia. 2016 Mar; 48(2): 125-36.

Onaolapo AY, Oladipo BP, Onaolapo OJ. Cyclophosphamide-induced male subfertility in mice: An assessment of the potential benefits of Maca supplement. Andrologia. 2018 Apr; 50(3).

Oyagbemi AA, Omobowale OT, Asenuga ER, Akinleye AS, Ogunsanwo RO, Saba AB. Cyclophosphamide-induced Hepatotoxicity in Wistar Rats: The Modulatory Role of Gallic Acid as a Hepatoprotective and Chemopreventive Phytochemical. Int J Prev Med. 2016 Mar 1; 7:51.

Pavin NF, Izaguirry AP, Soares MB, Spiazzi CC, Mendez ASL, Leivas FG, Dos Santos Brum D, Cibin FWS. *Tribulus terrestris* Protects against Male Reproductive Damage Induced by Cyclophosphamide in Mice. Oxid Med Cell Longev. 2018 Aug 28; 2018: 5758191.

Raghuvanshi P, Mathur P, Sethi R, Bhatnagar P. Evaluation of the Changes in Sperm Morphology, Sperm Count and Gonadotrophic Ratio of Swiss Albino Male Mice Fed Continuously With Microwave Exposed Food. Advances in Bioresearch. 2012; 3(4): 95 – 99.

Raza M, Alghasham AA. Desferrioxamine protects against toxic damage to liver and kidney induced by cyclophosphamide. Int J Health Sci (Qassim). 2011 Jul; 5(2 Suppl 1): 15-7.

Rehman MU, Tahir M, Ali F, Qamar W, Lateef A, Khan R, Quaiyoom A, Oday-O-Hamiza, Sultana S. Cyclophosphamide-induced nephrotoxicity, genotoxicity, and damage in kidney genomic DNA of Swiss albino mice: the protective effect of Ellagic acid. Mol Cell Biochem. 2012 Jun; 365(1-2): 119-27.

Rengasamy P. Congenital Malformations Attributed to Prenatal Exposure to Cyclophosphamide. Anticancer Agents Med Chem. 2017; 17(9): 1211-1227.

Singh RK, Kumar S, Prasad DN, Bhardwaj TR. Therapeutic journery of nitrogen mustard as alkylating anticancer agents: Historic to future perspectives. Eur J Med Chem. 2018 May 10; 151: 401-433.

Slater ME, Steinberger J, Ross JA, Kelly AS, Chow EJ, Koves IH, Hoffmeister P, Sinaiko AR, Petryk A, Moran A, Lee J, Chow LS, Baker KS. Physical Activity, Fitness, and Cardiometabolic Risk Factors in Adult Survivors of Childhood Cancer with a History of Hematopoietic Cell Transplantation. Biol Blood Marrow Transplant. 2015 Jul; 21(7): 1278-83.

Smart E, Lopes F, Rice S, Nagy B, Anderson RA, Mitchell RT, Spears N. Chemotherapy drugs cyclophosphamide, cisplatin and doxorubicin induce germ cell loss in an in vitro model of the prepubertal testis. Sci Rep. 2018 Jan 29; 8(1): 1773.

Sujayraj RS, Naik P, Vishma BL. Cyclophosphamide Induced Testicular Toxicity – A Comparison Between Acute and Subchronic Doses. IJSR – Intrnational Journal of Scintific Research. 2016; 5(4): 211-214.

Sutradhar KB, Amin MdL. Nanotechnology in cancer drug delivery and selective targeting. ISRN Nanotechnology. 2014; 2014: 12.

Vassilakopoulou M, Boostandoost E, Papaxoinis G, de La Motte Rouge T, Khayat D, Psyrri A. Anticancer treatment and fertility: Effect of therapeutic modalities on reproductive system and functions. Crit Rev Oncol Hematol. 2016 Jan; 97: 328-34.

Vredenburg G, den Braver-Sewradj S, van Vugt-Lussenburg BM, Vermeulen NP, Commandeur JN, Vos JC. Activation of the anticancer drugs cyclophosphamide and ifosfamide by cytochrome P450 BM3 mutants. Toxicol Lett. 2015 Jan 5; 232(1): 182-92.

Xu X, Zhang X. Effects of cyclophosphamide on immune system and gut microbiota in mice. Microbiol Res. 2015 Feb; 171: 97-106.

Zhang Y, Li Y, Luo W, Tang Y, Wang J, Yang R, Gao WQ. Histological, cellular and behavioural analyses of effects of chemotherapeutic agent cyclophosphamide in the developing cerebellum. Cell Prolif. 2019 May; 52(3): e12608.

Zhang Z, Pan T, Liu C, Shan X, Xu Z, Hong H, Lin H, Chen J, Sun H. Cyclophosphamide induced physiological and biochemical changes in mice with an emphasis on sensitivity analysis. Ecotoxicol Environ Saf. 2021 Mar 15; 211: 111889.

Zhu B, Zheng YF, Zhang YY, Cao YS, Zhang L, Li XG, Liu T, Jiao ZZ, Wang Q, Zhao ZG. Protective effect of L-carnitine in cyclophosphamide-induced germ cell apoptosis. J Zhejiang Univ Sci B. 2015 Sep; 16(9): 780-7.