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Topotecan Induces Spermatotoxicity – An In Vivo Study

KEYWORDS: Topotecan; Sperm abnormality; Sperm count; Motility; Oligoasthenoteratozoospermia; Gonadosomatic index

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ABSTRACT *Topotecan (TPT), an anticancer drug has been evaluated for its spermatotoxicity. Four doses of TPT, viz., 0.25, 0.5, 0.75 and 1.0 mg/kg were administered i.p. to Swiss albino mice and 35-days post-treatment sampling was performed for sperm abnormality, motility, count and gonadosomatic index (GSI). TPT at 0.5 mg/kg onwards induced reproductive toxicity as indicated by the dose-dependent significant increase in the frequency of abnormal sperm accompanied with the decrease in the motility and count. At higher doses of TPT (0.75 and 1.0 mg/kg), there was also a significant reduction in the GSI compared with the solvent control (P<0.05). However, it was not a dose-dependent, indicating that there is no direct correlation between GSI and spermatotoxicity. Thus, TPT treatment needs to be taken care giving the priority to prevent/minimize reproductive toxicity, particularly in case of post-pubertal cancer patients. Various possible mechanisms of TPT-induced spermatotoxicity are discussed.*

1. Introduction:

Topotecan (TPT), which received the U.S. FDA approval in 2007 is used for the treatment of recurrent epithelial ovarian cancer, relapsed small cell lung cancer and cervical cancer. The drug has also been demonstrated the therapeutic activity in hematologic malignancies, colon and cervical cancers and small tumors, particularly non-small cell lung solid tumor [1]. Huang et al. [2] described the usage of TPT as a therapy for Angelman's syndrome, which is under clinical trials for the FDA approval. TPT is a semi-synthetic derivative of camptothecin; a secondary metabolite extracted from the bark of the tree *Camptotheca acuminata* [3]. It acts as a topoisomerase-I inhibitor, and intercalates between DNA bases leading to prevention of DNA replication eventually promoting to cell death/apoptosis [4, 5]. Thus, by virtue of its cytotoxic action, currently it is one of the commonly used agents in cancer chemotherapy. It is well known that a main drawback with most of the chemotherapeutic agents is severe side effects such that not only target cells/tissues, but also normal cells are adversely affected. In case of male cancer patients, a major target for chemotherapy-induced toxicity is the reproductive system, particularly affecting the spermatogenesis [6]. Since TPT is relatively a new drug, reports on its toxicity with special reference to male reproductive system are very scanty. Attia et al. [7] based on male mouse-dominant lethal study reported that TPT is a germ cell mutagen. TPT has been shown to possess binding affinity to DNA-histone complex in chromatin structure [8, 9]. There are also some studies on mutagenicity of TPT in vitro [10, 11] and in vivo [12, 13]. It is already well established that DNA /chromosomal damage is an important mechanism behind the abnormal features of seminal parameters, particularly sperm head deformity [14, 15]. TPT has been demonstrated to induce oxidative stress by in vitro [16] and in vivo experimentations [7, 13]. Oxidative stress/free radical generation is an important factor for chemicals-induced damaging effects on spermatogenesis [17, 18]. Thus, considering the scanty reports on effect of TPT on male reproductive system, its high affinity for DNA and chromatin, and induction of oxidative stress, the present study was taken up to evaluate the TPT induced effect on semen quality (count, motility and morphology), using the Swiss albino mouse as the test system.

2. Material and Methods:

2.1 Chemicals:

Topotecan hydrochloride hydrate ($C_{23}H_{23}N_3O_5 \cdot xHCl \cdot yH_2O$; CAS No. 123948-87-8; Batch No. T2705; Purity, ≥98% -HPLC and enzymatic) purchased from Sigma-Aldrich was used as the test chemical. Cyclophosphamide (Cycloxan, CAS No. 50-18-0; Batch No. KB 9124003 Biochem Pharmaceutical Industries LTD. Mumbai, India, was used as the positive control. Phosphate buffered saline (PBS), Eosin-Y stain was obtained from SRL, India, and all other chemicals and reagents used were of analytical grade.

2.2. Experimental Animals:

Swiss albino mice, *Mus musculus* species (8–10 weeks old; 26±2 g body weight, bred and maintained in the animal house of Biosciences department, Mangalore University were used as the experimental model. Five healthy male mice were used in each treatment and control group. The animals were maintained under absolute hygienic condition in an air-conditioned room at a temperature of 24^{0}C (±1^{0}C) with 12 h light/dark cycle and 50±5% humidity. They were fed with standard mice pellets (Lipton, India) and water *ad libitum*. Care and handling of the animals were according to the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision on Experiments on Animals), India [19]. Experimental animals were sacrificed by cervical dislocation and dissected to remove

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the testes and cauda epididymis to collect the semen for further analysis. The present study was carried out after taking the prior approval from the Institutional Animal Ethics Committee (Ref. No. MU/AZ/99/2013-14/IAEC, dt.2.4.2013).

2.3. Dose and Treatment Schedule:
Four sub-lethal doses of TPT, viz., 0.25, 0.5, 0.75 and 1.0 mg/kg b.wt. were tested for dose response relationship. The selected doses of TPT represent sub-lethal doses, based on the LD_{50} values (Mouse i.p. LD_{50} 40 mg/kg; [20 and [21]. Required concentration of the test chemical was dissolved in distilled water and administered intraperitoneally (i.p.) in 0.2 ml quantity given as a single acute dose, followed by the post treatment sampling time of 35 days.

2.4. Sperm Abnormality Assay:
On 35th day after the treatment with TPT, animals were sacrificed by cervical dislocation, and the testes were dissected out and weighed. Both the cauda epididymis were removed and placed in a watch glass containing 1 ml PBS (pH-7.2). The cauda epididymis was minced thoroughly, and the suspension obtained was filtered through a cheese cloth to remove the tissue debris. The filtered suspension was added with 1% aqueous Eosin Y at 10:1 ratio and kept for 20 minutes for staining. A drop of the sperm suspension was taken on a clean microscope slide, smeared and air dried. The air dried slides were observed under 40X objective of a compound microscope with a green filter. A total of 2000 sperm per animal (500 sperms/slide) was analyzed from each treatment and control groups, to determine the frequency of abnormal sperm in a population of normal ones. spermatozoa with abnormal shape were scored according to the criteria of Wyrobek and Bruce [22]. Individual type and total teratospermia were expressed in terms of percentage.

2.5. Sperm Count:
An aliquot (0.05ml) from the sperm suspension (1ml) was removed before staining and was diluted 40 times with PBS (1:40) and mixed thoroughly. A sample of the diluted sperm suspension was introduced to the Neubaur counting chamber and the total sperm count in 8 squares of 1 mm^2 was determined and multiplied by $5X10^4$ to calculate the number of sperm per epididymis [23].

2.6. Sperm Motility:
Sperm motility was determined by assessing at least five microscopic fields to classify 100 spermatozoa (400 magnifications). The motility was determined by using the basic procedure established for routine human semen analyses and graded fast progressive, slow progressive and no progressive or immotile [24]. The latter three types were taken as the impaired motility. The percentage frequency of motile sperm was determined by subtracting the total number of sperm with impaired motility from the total number of sperm scored for motility.

2.7. Gonado-somatic Index (GSI):
Body weight of the animals on 35^{th} day of the treatment and testes weight collected after dissection were taken. GSI was determined by using the formula: GSI = Testes weight/ Total body weight) x 100, [25].

2.8. Statistical Analysis:
All the values of the results are expressed mean with standard deviation. The results were subjected to statistical analysis for the significance comparing with the solvent control by employing one-way ANOVA with Dunnett's post hoc test.

3. Results and Discussion:
Sperm morphology test, along with the count and motility, has potential use for identifying chemicals that induce spermatogenic dysfunction and perhaps heritable mutations [26]. Oligoasthenoteratozoospermia, a condition representing low sperm count (oligozoospermia), poor sperm movement (asthenozoospermia) and abnormal sperm shape (teratozoospermia) is an indication of male infertility. Although many of the chemotherapeutic agents are known for their testicular toxicity, reports on TPT for its effect on male reproductive system are very scanty. In the present study, the test chemical was evaluated for its effect on certain seminal parameters by 35 days of post-treatment sampling, considering that as studied by Oakberg [27], germ cells that are exposed to chemical agents at late spermatogonial stage take seven weeks to reach the cauda epididymis after undergoing a series of changes to produce the matured sperms. Further, Jameson and De Groot [28] advocated that the total duration of spermatogenesis requires approximately 4.5 cycles in mammals, and for the mouse this is 35 days. Most of the agents induce the testicular toxicity during the initial stage of spermatogenesis as indicated by the results of 35-days post-treatment samplings [29, 30]. In the present study, the i.p. administration of the test chemical induced a dose-dependent increase in the frequency of abnormal sperm (Table 3.1; Fig. 3.1). Among the abnormal spermatozoa, the highest frequency was amorphous followed by hookless, banana and folded type. The exact mechanism(s) of TPT-induced sperm abnormality is not known. However, an attempt has been made here to understand the mechanism of action, based on the previous reports published elsewhere.

TPT by virtue of its mechanism of action as an anticancer drug causes double-stranded DNA damage by interfering in the functioning of topoisomerase I, an enzyme that plays a vital role in DNA replication; as per the fork collision model, lesions are created when the DNA replication fork encounters a TPT-stabilized cleavable complex leading to lethal double-stranded DNA damage [31, 32, 33]. There are many reports indicating that breakage of DNA-double strand leading to its fragmentation is one of the main bases for teratospermia as studied from animal experimentation [34] and human samples [35, 36]. It can be speculated that TPT induces the teratospermia in the initial stage of spermatogenesis, i.e., during meiosis. The second meiotic division is equivalent to mitosis, where DNA replication occurs. Exposure of cells during S-phase of II meiotic division to TPT leads to severe DNA damage by virtue of its basic mechanism of cytotoxic activity. Induction of phosphorylated histone H2AX (γH2AX), a cellular response to double strand breaks (DSBs) is used as an assay to monitor the DNA damage [37]. Using γH2AX as the biomarker through multiparameter laser scanning cytometry, TPT has been shown to form the DSBs during DNA replication [10]. Interestingly, it is noted from a previous report that γH2AX levels are higher in the sperm of male infertility patients than in healthy men [38]. Therefore, measuring the γH2AX levels in sperm in response to TPT exposure would give further insight into the formation of DSBs paving the way to production of abnormal sperm. However, Attia et al [7] advocated from the mouse-dominant lethal study that TPT induces mutation in post-meiotic cells, particularly during early spermatid stage.

Integrity of chromosomes both in terms of structure and

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number, is important in maintaining the morphology of sperm, and any alteration either in structure or number or both affect the shape of the sperm, particularly head part; so much so many of the agents that were reported to induce teratospermia are potential genotoxic by causing chromosomal breakage [14, 39] and damage of spindle fibers [40, 41]. TPT was shown to impart the genotoxicity in bone marrow cells of the Swiss albino mice through micronucleus and chromosomal aberration assays [42]. Bakheet [13] also demonstrated the induction of MN by TPT in bone marrow cells of mice. Attia et al. [43] revealed from their study that TPT-induced MN formation in bone marrow cells was both by clastogenic (47.3%) and aneugenic actions (52.7%). Since TPT is not a target specific drug, through circulation it will reach all types of tissues, including reproductive organs, where it can impart the genotoxicity. Variation in the number of chromosomes, i.e., aneuploidy is a main basis for sperm deformity as indicated by the analysis of the sperm derived from teratozoospermic men, comparing with that of fertile controls [35, 44, 45]. The relationship between the anomalous karyotypes, particularly with structural aberrations and morphologically abnormal spermatozoa has also been proved by two studies carried out on a mouse system [46, 47]. It is noted from these reports that chromosomal aberrations (structural or numerical or both) affect the normal morphology of the spermatozoa. The high frequency of sperm abnormality as observed in the present study may be due to dual action (clastogenic and aneugenic) of TPT affecting the chromosomal integrity. Further studies on aneugenic and clastogenic action of TPT on germ cells would give the plausible explanation for the induction of teratospermia.

By employing the spectroscopic analyses and equilibrium dialysis techniques, Babaei et al. [9] have revealed that TPT has higher affinity to chromatin compared to DNA. The role of protamines in packing the genomic DNA in mature sperm cells, and ratio of histone and protamines are important for sperm shape [48, 49, 50]. TPT may be having affinity to protamines affecting the packing of DNA or upon binding to histone proteins with higher affinity disturb the proper differentiation of sperm cells leading to teratospermia. Studies may be taken up enabling further understanding in this aspect.

Maiorino and Ursini [51] specified that production of Reactive Oxygen Species (ROS) and glutathione depletion in mammalian male germ cells are physiological events that are requisite for the functional maturation and capacitation of spermatozoa. There are many studies proved the direct correlation between oxidative stress and sperm abnormality [52, 53]. Thus, TPT-induced sperm abnormality may be mediated through generation of ROS, which can be justified by earlier studies reporting that TPT is a potent inducer of ROS [13, 16]. Further, lipid peroxidation is a consequence of oxidative stress, which in turn leads to the abnormalities in sperm parameters. There are many studies, which portray the direct link between lipid peroxidation and sperm abnormality [54]. TPT has been shown to induce an increase in lipid peroxidation and a decrease in antioxidant enzyme activities in healthy liver tissue of rabbits [55] and bone marrow of mice [13]. ROS induces peroxidative damage in the sperm plasma membrane leading to both genomic and mitochondrial DNA damage [56]. The similar kind of consequence may happen in the testes during spermatogenesis, and epididymyl sperm leading to abnormal variations in the sperm parameters induced by TPT as observed in the present study. Further studies can be taken up to evaluate the possible role of TPT-induced oxidative stress and lipid peroxidation during spermatogenesis, and in mature spermatozoa stored in cauda epididymis.

In the present study, TPT was also found to affect sperm count and motility as indicated by the dose-dependent decrease in the count (oligospermia) and frequency of motile sperm (asthenospermia) comparing with the concurrent control group (Table 3.2; Fig. 3.1 and 3.2). Reduction in sperm count is an indication of germinal cytotoxicity. Oxidative stress has a direct role in affecting the quality of sperm, including the count and motility [57, 58]. As mentioned above, TPT is a potential inducer of ROS, and it may be one of the main reasons for the significant variation in these two parameters. TPT is known to induce DNA damage, and earlier reports show that DNA damage also affects the motility [38, 59]. Thus, the observed effect of TPT on motility is due to its damaging effect on DNA. Sperm chromosomal analysis of men teratozoospermia and asthenoteratozoospermia showed a 2-3 fold increase in the numerical anomalies compared with the normal control [39]. Ushijima et al. [60] carried out en elegant study and proved that there is a significant increase in the incidence of sex chromosome abnormalities in oligoasthenoteratozoospermia individuals. Since TPT induced the variations in all the three seminal parameters assessed in the present study, there may be a possibility of inducing sex chromosome abnormalities leading oligoasthenoteratozoospermia.

It was observed that the lowest dose of TPT tested, i.e., 0.25mg/kg b.wt. was not found to impart the oligoasthenoteratozoospermia at statistically significant levels, compared with the control groups (Table 3.1 and 3.2). In case of post-pubertal male cancer patients, chemotherapy with certain drugs like cyclophosphamide, mitomycin C, busulfan, etc., their semen samples are collected and cryopreserved for future use in ART, in perspective of severe male reproductive toxicity of those drugs at their therapeutic dose regimens. As far as male reproductive system is considered, compared to those anticancer drugs, whether TPT is safe at its therapeutic doses? The present study poses this question to be addressed by further studies. The results obtained from animal studies, however, cannot directly be extrapolated to humans since species-difference is an important factor affecting the toxicity. Certain chemicals which induce the toxicity at very low doses in one species do not impart the same effect even at a high dose on other animal species, and vice versa [61, 62]. The threshold doses of chemicals to induce a particular effect vary from species to species. As far as the Swiss albino mouse is considered, 0.5 mg/kg b.wt. (or >0.25mg/kg b.wt) of TPT (i.p. treatment) can be regarded as the threshold dose to induce the spermatotoxicity.

Gonadosomatic index (GSI), the ratio between testes weight and body weight, whose significant variation in response to chemical exposure is an indication of testicular toxicity [25]. The test chemical induced a small, but significant decrease in the GSI compared with the vehicle control group at higher two doses (0.75 and 1.0 mg/kg. b.wt; $P < 0.05$), as depicted in the table 3.3 and Fig. 3.4. During the course of time (35 days), there may be recovery of testes weight loss / damage induced by TPT at lower doses. The observed effect at higher doses was not found to be a dose-dependent, and therefore, it can be said that there is no direct correlation between GSI and OAT condition.

As specified earlier, TPT has been demonstrated to induce

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aneuploidy in somatic cells with the higher efficiency than the clastogenicity. Sun et al. [40] proposed in their review papers that teratozoospermia, oligospermia and asthenospermia are markers of abnormal spermatogenesis, which is associated with the elevated sperm aneuploidy. Thus, considering these two aspects in conjunction with the observed effects (oligoasthenoteratozoospermia), the possibility of TPT inducing aneuploidy in germinal cells is high, which can be confirmed by future studies.

Thus, it can be concluded from the present study that TPT has a negative impact on spermatogenesis affecting all the three seminal parameters, viz., morphology, count and motility. The threshold dose required for the effects is 0.5 mg/kg b. wt. Its testicular toxicity has also been noticed by significant reduction in GSI compared with the healthy control. Further studies may be taken up to evaluate its effect on spermatogenesis at the molecular level to understand the mechanism of its testicular toxicity. Cryopreservation of semen from post-pubertal cancer patients treated with TPT may be required as it is there for other chemotherapeutic agents to restore their reproductive capacity.

Table 3.1: Percentage Frequency[A] of Different Types of Abnormal Sperm Induced by TPT at Different Doses and Controls After 35 Days of Treatment:

Dose (Mg/kg b.wt)	Amorphous	Banana shaped	Hookless	Folded	Double headed	Double tailed	Abnormal sperms ±(SD) Total
D.W.	0.88	0.07	0.31	0.06	0.02	0.03	1.37±0.54
TPT - 0.25	0.93	0.09	0.34	0.09	0.04	-	1.49±0.71
TPT - 0.5	1.9	0.72	0.46	0.17	0.07	0.05	3.02±0.98[a]
TPT - 0.75	2.8	0.79	0.57	0.34	0.06	0.07	4.63±0.84[b]
TPT – 1.0	4.9	0.82	0.67	0.39	0.09	0.07	6.94±1.02[c]
CP-50	1.8	0.43	1.49	0.69	0.25	0.16	4.82±0.91[c]

[A] =2000 sperm/animal; 5 animals/group
[a] P<0.05. [b] P<0.01. [c] P<0.001 (Dunnett's post hoc test).

Table 3.2: [A]Effect of Different Doses of TPT on GSI, Sperm Count and Motility After 35 Days of Treatment:

Dose (mg/kg)	Gonadosomatic Index (GSI)			Sperm count/ (x10⁶) ±SD	%Motile sperm[C]
	Body weight (g) Mean± SD	Testes weight (g) Mean±SD	[B]GSI Mean± SD		
D.W.	32.15 ± 3.93	0.228±0.007	0.709±0.07	12.18 ± 2.5	83.11±9.79
TPT - 0.25	31.06 ± 2.12	0.221± 0.005	0.711±0.05	11.86 ± 2.8	79.93±8.16
TPT - 0.5	34.73 ± 3.89	0.244 ± 0.008	0.703±0.06	09.98 ± 2.1[a]	72.14±9.31[a]
TPT - 0.75	30.57 ± 1.12	0.211 ±0.006	0.692±0.07[a]	07.86 ± 1.3[b]	61.33±7.81[b]
TPT – 1.0	31.53 ± 3.29	0.218 ± 0.007	0.693±0.06[a]	06.09 ± 1.6[c]	49.32±7.09[c]
CP-50	29.12 ± 1.6	0.200 ± 0.006	0.687±0.05[b]	08.28 ± 0.9[c]	64.57±8.71[b]

[B] GSI = Testes weight/ Total body weight) x 100
D.W. – Distilled water; TPT – Topotecan; CP-Cyclophosphamide; GSI – Gonadosomatic Index
[C] From 100 total sperm scored
[A] =5 animals/group
[a] P<0.05. [b] P<0.01. [c] P<0.001 (Dunnett's post hoc test).

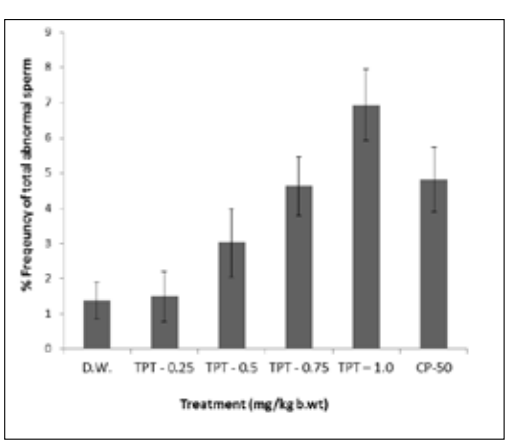

Fig. 3.1: Frequency of Abnormal Spermatozoa Induced by Topotecan and Controls

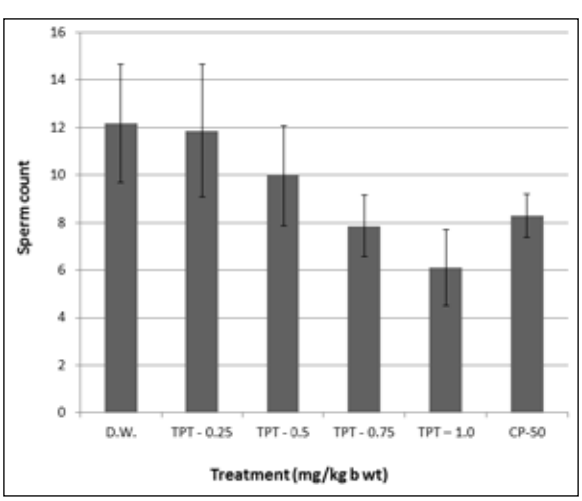

Fig. 3.2: Effect of Topotecan and Controls on Epididamil Sperm Count

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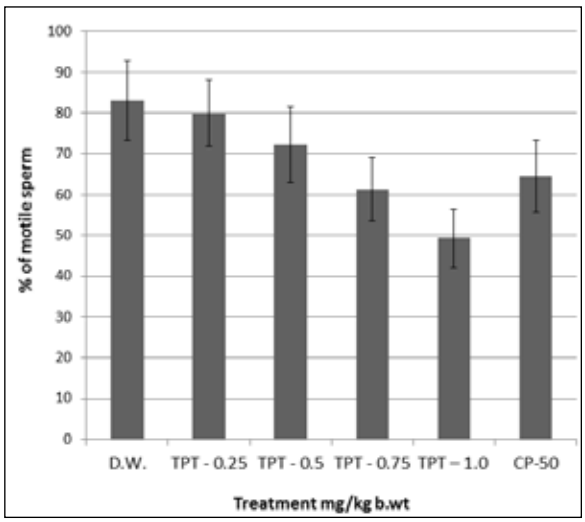

Fig 3.3: Frequency of Motile Spermatozoa in the Presence of Topotecan and Controls

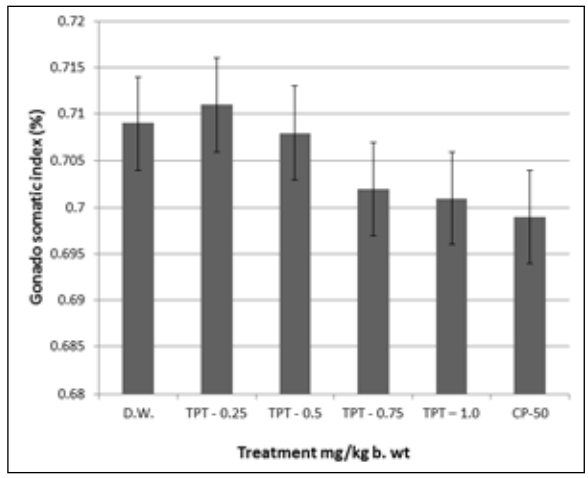

Fig. 3.4: Effect of Topotecan and Controls on Gonadosomatic Index

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REFERENCE

[1] R. Garcia-Carbonero, J. G. Supko, Current perspectives on the clinical experience, pharmacology, and continued development of the camptothecins, Clin. Cancer Res. 8(3) (2002) 641-661. [2] H.S. Huang, J.A. Allen, A.M. Mabb, I.F. King, J. Miriyala, B. Taylor-Blake, N. Sciaky, J.W. Dutton Jr, H.M. Lee, X. Chen, J. Jin, A.S. Bridges, M.J. Zylka, B.L. Roth, B.D. Philpot, Topoisomerase inhibitors unsilence the dormant allele of Ube3a in neurons, Nature. 481(7380) (2011) 185-189. [3] S. Basili, S. Moro, Novel camptothecin derivatives as topoisomerase I inhibitors, Expert Opin. Ther. Pat. 19(5) (2009) 555-574. doi: 10.1517/13543770902773437. [4] B.L. Staker, K. Hjerrild, M.D. Feese, C.A.Behnke, A.B.Burgin Jr, L. Stewart, The mechanism of topoisomerase I poisoning by a camptothecin analog, Proc. Natl. Acad. Sci. 99(24) (2002) 15387–15392. [5] Y. Pommier, E. Leo, H. Zhang, C. Marchand, DNA topoisomerases and their poisoning by anticancer and antibacterial drugs, Chem. Biol.17 (2010) 421-433. [6]M.L.Meistrich, Effects of chemotherapy and radiotherapy on spermatogenesis in humans, Fertil. Steril. 100(5) (2013)1180-1186. [7] S.M. Attia, S.F. Ahmad, M.F. Abd-Ellah, F.M. Hamada, S.A. Bakheet, Germ cell mutagenicity of topoisomerase I inhibitor topotecan detected in the male mouse-dominant lethal study, Food Chem. Toxicol. 62 (2013) 470-474. [8] H. Zhao, P. Rybak, J. Dobrucki, F. Traganos, Z. Darzynkiewicz, Relationship of DNA damage signaling to DNA replication following treatment with DNA topoisomerase inhibitors camptothecin/topotecan, mitoxanthone, or etoposide, Cytometry A. 81 (1) (2012) 45-51. [9] M. Babaei, A. Rabbani-Chadegani, P. Ghadam, Binding of topotecan to chromatin: Insights into cooperative binding and comparison with DNA, Int. J. Biol. Macromol. 80 (2015) 57-63. [10] H. Hashimoto, S. Chatterjee, N.A. Berger. Mutagenic activity of topoisomerase I inhibitors, Clin Cancer Res. 1(4) (1995) 369-376. [11] J.H. Chen, P. Zhang, W.D. Chen, D.D Li, X.Q. Wu, R. Deng, L. Jiao, X. Li, J. Ji, G.K. Feng, J.W. Jiang, X. F. Zhu, ATM-mediated PTEN phosphorylation promotes PTEN nuclear translocation and autophagy in response to DNA-damaging agents in cancer cells, Autophagy. 11(2) (2015) 239-252. doi: 10.1080/15548627.2015.1009767. [12] K.S. Cunha, M.L. Reguly, U. Graf, H.H. Rodrigues de Andrade, Comparison of camptothecin derivatives presently in clinical trials: genotoxic potency and mitotic recombination, Mutagenesis. 17(2) (2002) 141-147. [13] S.A. Bakheet, Assessment of anti-cytogenotoxic effects of quercetin in animals treated with topotecan, Oxid. Med. Cell Longev. 2011; 2011:824597. doi: 10.1155/2011/824597. Epub. 2011 Aug 15. (2011). [14] F. Marchetti, D. Cabreros, A.J. Wyrobek, Laboratory methods for the detection of chromosomal structural aberrations in human and mouse sperm by fluorescence in situ hybridization, Methods. Mol. Biol. 410(2008) 241-271. [15] T. Ryan Schulte, A. Dana Ohl, Mark Sigman, D. Gary Smith, Sperm DNA damage in male infertility: etiologies, assays, and outcomes, J. Assist. Reprod. Genet. 27 (1) (2010) 3–12. [16] M. Timur, S.H. Akbas, T. Ozben, The effect of Topotecan on oxidative stress in MCF-7 human breast cancer cell line, Acta Biochem. Pol. 52(4) (2005) 897-902. [17] P. Kaur, M.P. Bansal, Effect of oxidative stress on the spermatogenic process and hsp70 expressions in mice testes, Indian J. Biochem. Biophys. 40 (2003) 246-251. [18] L. Rato, M.G. Alves, J.E. Cavaco, P.F. Oliveira, High-energy diets: a threat for male fertility? Obes Rev.15(12) (2014) 996-1007. [19] CGLAF (2003) CPCSEA Guidelines for laboratory animal facility, CPCSEA guidelines, Indian. J. Pharmacol. 35 (2003) 257-274. [20] Pfizer Material Safety Data Sheet: Version 2.0, retrieved on 24th Sept. 2015 from: http://www.pfizer.com/files/products/material_safety_data/PZ01399.pdf [21] PubChem, Compound Summary for CID 60700, retrieved on 24th Sept. 2015 from: http://pubchem.ncbi.nlm.nih.gov/compound/topotecan#section=Non-Human-Toxicity-Excerpts [22] A.J. Wyrobek., W. R. Bruce, The induction of sperm shape abnormalities in mice and humans, in: A. Hollaender , F.J. deSerres (Eds.), Chemical Mutagens-Principles and Methods for their Detection, Plenum Press, New York, 1978, pp. 257-285. [23] S.G. Vega, P. Guzman, L. Garcia, J. Espinosa, C. C. de Nava,Sperm shape abnormality and urine mutagenecity in mice treated with niclosamide, Mutat. Res. 204 (1988) 269-276. [24] M. J. Amini, H. Nikzad, A. Taherian, F. Mohammadi, The Effects of vasectomy on epididymal morphology and sperm parameters in adult male Balb/c mice, Int. J. Morphol. 31(4) (2013) 1349-1354. [25] E. Pavlova, D. Dimova, E. Petrova, Y. Gluhcheva, N. Atanassova, Changes in rat testis and sperm count after acute treatment with sodium nitrite, Bulg. J. Agric.Sci. 19 (2) (2013) 186–189. [26] A.J. Wyrobek, L.A. Gordon, J.G. Burkhart, M.W. Francis, R.W. Jr Kapp, G. Letz, H.V. Malling, J.C. Topham, M.D. Whorton, An evaluation of the mouse sperm morphology test and other sperm tests in nonhuman mammals. A report of the U.S. Environmental Protection Agency Gene-Tox Program, Mutat. Res. 115(1) (1983) 1-72. [27] E.F.Oakberg, Duration of spermatogenesis inthe mouse and timing of stages of the cycle of the seminiferous epithelium, Am. J. Anat. 99(3) (1956) 507-516. [28] J. Larry Jameson, D.M. de Kretser, J.C. Marshall, L. J. De Groot, Endocrinology – Adult and Pediatric, Reproductive Endocrinology, sixth ed., Elsevier, Elsevier Health Sciences, Makati City, 2013. [29] S. Padmalatha Rai, K.K. Vijayalaxmi, Tamoxifen citrate induced sperm shape abnormalities in the in vivo mouse, Mutat Res. 492(1-2) (2001)1-6. [30] S. Donya, M. Fawzia, A. Aly, A. Mona, M. Abo-Zeid The Protective Role of Folic Acid, Vitamin B12 and Vitamin C on The Mutagenicity of The Anticancer Drug Ifosfamide, Nature and Science. 8(12) (2010) 164-174. [31] C. Holm, J.M. Covey, D. Kerrigan, Y. Pommier, Differential requirement of DNA replication for the cytotoxicity of DNA topoisomerase I and II inhibitors in Chinese hamster DC3F cells, Cancer Res. 49 (1989) 6365-6368. [32] C.H. Takimoto, S.G. Arbuck, Clinical Status and Optimal Use of Topotecan, Oncology. 11(11) (1997) 1635-1646. [33] Y.H. Hslang, M.G. Lihou, L.F. Liu,Arrest of replication forks by drug-stabilized topoisomerase I-DNA cleavable complexes as a mechanism of cell killing by camptothecin, Cancer Res. 49(1989)5077-5082. [34] B.S. Pukazhenthi, D.E. Wildt, J.G. Howard, The phenomenon and significance of teratosperm in fields, J. Reprod. Fertil. Suppl. 57(2001) 423-433. [35] S. Brahem, M. Mehdi, H. Elghezal, A. Saad, Detection of DNA fragemntation and meiotic segregation in human with isolated teratozoospermia, J. Assist. Reprod. Genet. 28(1) (2011) 41-48. [36] S. Binsaleh, R. Al-Qahtani, K. Madbouly, B. Abu-Rafae, Evaluation of Sperm DNA damage in men from infertile Saudi couples, J. Reprod. Med. 60(3-4) (2015) 135-140. [37] V. Valdiglesias, S. Giunta, M. Fenech, M. Neri, S. Bonassi, γH2AX as a marker of DNA double strand breaks and genomic instability in human population studies, Mutat. Res. 753(1) (2013) 24-40. doi: 10.1016/j.mrrev.2013.02.001. [38] H.Z. Zhong, F.T. Lv, X.L. Deng, Y. Hu, D.N. Xie, B. Lin, Z.N. Mo, F.Q. Lin. Evaluating γH2AX in spermatozoa from male infertility patients, Fertil Steril. 104(3) (2015) 574-581 doi: 10.1016/j.fertnstert.2015.06.004. [39] R.K. Sharma, T. Said, A. Agarwal, Sperm DNA damage and its clinical relevance in assessing reproductive outcome, Asian J Androl. 6(2) (2004) 139-148. [40] F. Sun, E. Ko, R.H. Martin, Is there a relationship between sperm chromosome abnormalities and sperm morphology?, Reprod. Biol. Endocrinol. 2006 Jan 25; 4:1. [41] M. De Braekeleer, M.H. Nguyen, F. Morel, A. Perrin, Genetic aspects of monomorphic teratozoospermia: a review, J. Assist. Reprod. Genet. 32(4) (2015) 615-623. [42] N. Aydemir, R. Bilaloglu, Genotoxicity of two anticancer drugs, gemcitabine and topotecan, in mouse bone marrow in vivo, Mutat. Res. 537(1) (2003) 43-51. [43] S.M. Attia, A.M. Aleisa, S.A. Bakheet, A.A. Al-Yahya, S.S. Al-Rejaie, A.E. Ashour, O.A. Al-Shabanah, Molecular cytogenetic evaluation of the mechanism of micronuclei formation induced by camptothecin, topotecan, and irinotecan, Environ. Mol. Mutagen. 50(2) (2009) 145-151. [44] R. Hristova, E. Ko, C. Greene, A. Rademaker, J. Chernos, R. Martin, Chromosome abnormalities in sperm from infertile men with asthenoteratozoospermia. Biol Reprod. 66(6) (2002) 1781-1783. [45] S.S. Tang, H.Gao, Y. Zhao, S. Ma, Aneuploidy and DNA fragmentation in morphologically abnormal sperm, Int. J. Androl. 33(1) (2010) e163-179. [46] H. Kishikawa, H. Tateno, R. Yanagimachi, Chromosome analysis of BALB/c mouse spermatozoa with normal and abnormal head morphology, Biol Reprod. 61(3) (1999) 809-812. [47] A. Pyle, M.A. Handel, Meiosis in male PL/J mice: a genetic model for gametic aneuploidy, Mol. Reprod. Dev. 64(4) (2003) 471-481. [48] H.J. Clarke, "Nuclear and chromatin composition of mammalian gametes and early embryos", Biochem. Cell. Biol. 70 (10–11) (1992) 856–866. [49] X. Zhang, M. San Gabriel, A. Zini, Sperm nuclear histone to protamine ratio in fertile and infertile men: evidence of heterogeneous subpopulations of spermatozoa in the ejaculate, J. Androl. 27(3)(2006) 414-420. [50] F.G. Iranpour, Impact of Sperm chromatin evaluation on fertilization rate in intracytoplasmic sperm injection. Adv. Biomed. Res 2014 Nov 29; 3:229. doi: 10.4103/2277-9175.145719. eCollection 2014. [51] M. Maiorino, F. Ursini, Oxidative stress, spermatogenesis and fertility, Biol. Chem. 383 (3-4) (2002) 591-597. [52] S.Venkatesh, G. Singh, N.P. Gupta, R. Kumar, M.Deecaraman, R. Dada, Correlation of sperm morphology and oxidative stress in infertile men, Iran J Reproductive Medicine. 7(1) (2009) 29-34. [53] A. Hosseinzadeh Colagar, F. Karimi, S.G, Jorsaraei,Correlation of sperm parameters with semen lipid peroxidation and total antioxidants levels in astheno- and oligoasheno- teratospermic men, Iran Red Crescent Med J.15(9)(2013)780-785. [54] T. Rajesh Kumar, K. Doreswamy, B. Shrilatha, Muralidhara, Oxidative stress associated DNA damage in testis of mice: induction of abnormal sperms and effects on fertility, Mutat. Res. 513(1-2) (2002) 103-111. [55] U. Kisa, O. Caglayan, M. Kacmaz, The effects of topotecan on lipid peroxidation and antioxidant enzyme levels in rabbit liver tissue, Redox. Rep. 10(2) (2005) 79-82. [56] R.J. Aitken, C. Krausz, Oxidative stress, DNA damage and the Y chromosome, Reproduction. 112(4) (2001) 497-506. [57] M. Shiva, A.K. Gautam, Y. Verma, V. Shivgotra H. Doshi, S. Kumar, Association between sperm quality, oxidative stress, and seminal antioxidant activity, Clin. Biochem.4(4) (2011) 319-324. [58] E.C. García-Díaz, L.E. Gómez-Quiroz, E. Arenas-Ríos, A. Aragón-Martínez, J.A. Ibarra-Arias, del Socorro I M. Retana-Márquez, Oxidative status in testis and epididymal sperm parameters after acute and chronic stress by cold-water immersion in the adult rat, Syst. Biol. Reprod. Med. 61(3) (2015)150-160. [59] O. Flaherty C, Redox regulation of mammalian sperm capacitation, Asian. J. Androl. 17(4) (2015) 583-590. [60] C. Ushijima, Y. Kumasako, P.E. Kihaile, K. Hirotsuru, T. Utsunomiya, Analysis of chromosomal abnormalities in human spermatozoa using multi-colour fluorescence in-situ hybridization, Human. Reprod. 15(5) (2000) 1107-1111. [61] J.M. Kleeman, J.R. Olson, R.E. Peterson, Species differences in 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin toxicity and biotransformation in fish, Fundam. Appl. Toxicol. 10(2) (1988) 206-213. [62] V. Sharma, J.H. McNeil, To scale or not to scale: the principles of dose extrapolation, Br J Pharmacol. 157(6) (2009) 907-921.