Toxicology International, *29(4) : 465-473; 2022. DOI: 10.18311/ti/2022/v29i4/29728*

Online ISSN : 0976-5131; Print ISSN : 0971-6580

Toxicology International

Contents available at: www.informaticsjournals.com/index.php/toxi



Potential of *Zingiber officinale* Extract against Chlorpyrifos Toxicity in Caprine Testicular Cells

Priyanka Saraf[1], Harish Panchal[1], Kameshwar Sharma[3], Sanjeev Sharma[2] and Jitender Kumar Bhardwaj[1]*

[1]Reproductive Physiology Laboratory, Department of Zoology, Kurukshetra University, Kurukshetra – 136119, Haryana, India; jkbkuk@gmail.com

[2]Department of Library and Information Science, Kurukshetra University, Kurukshetra – 136119, Haryana, India

[3]Department of Biochemistry, Sri Venkateshvara College, University of Delhi, Delhi – 110021, India

ARTICLE INFO

KEYWORDS: Apoptosis, Chlorpyrifos, Testicular Cells, *Zingiber officinale*

ARTICLE HISTORY

Received: 05.03.2022

Revised: 06.05.2022

Accepted: 06.05.2022

Abstract

Chlorpyrifos (CPF), an organophosphate insecticide, is currently under studies due to its reproductive toxicity in non-targeted organisms. The present study on its efficacy as reproductive toxicant, revealed its effect on testicular cells of caprines. CPF was found to decrease cell viability in testicular cells, induced histo-architectural alterations like loss of tubular interactions and empty luminal spaces in testis; initiated DNA damage associated apoptosis as revealed by MTT, Histology and EB/AO fluorescence assay respectively, at both the doses (10 and 50 µM). CPF mediated toxicity induced oxidative stress in testicular cells as revealed by dose- and time-dependent increase in lipid peroxidation and decline in ferric reducing antioxidant power. However, supplementation of *Zingiber officinale* (ginger) extract reduced the CPF mediated decrease in cell viability, histological defects, incidence of apoptosis and generated oxidative stress parameters; thus, proving its potential as a therapeutic agent against CPF, preventing testicular cells toxicity mediated reproductive disorders.

1. Introduction

Presently toxicity studies are gaining attention due to their large scale ill effects on health and physiology. Pesticides are one of the largest groups of toxicants, causing wide range of acute and chronic diseases. Among these, Organophosphate (OPs) pesticides are raising brows due to its effects on non-target species[1,2]. Studies suggest that these pesticides besides affecting other vital organs, target the reproductive system and is one of the un-noticed sources of infertility in mammals[32,33]. OPs like malathion increases sperm immotility and teratozoospermia[3,4]

*Author for correspondence

whereas diazinon decreases the testicular weight and induces several morphological anomalies in the male gamete[5,6]. Literature reveals Chlorpyrifos (CPF), one of the OPs, resulted in decreased live foetus in mice along with abnormal sperm morphology and decreased sperm count in male mice[7]. Such effects affect the fertility aspect of the individual targeting the germ cells and thus, can be associated with reproductive disorders in the progeny[8]. The scanty information in this field draws attention to assess CPF toxicity at various levels in different mammals and analyse the extent of its efficacy. Caprines like goat are the widely domesticated and socio-economically important animal in India that exhibits a sharp decline in its population over the periods as per 19th livestock census specifically in males with percent decline of 7.79%[9]; thus, forming an important model of study in agricultural land of India with maximum pesticide exposure. Also, pesticides have high potential of inducing oxidative stress[10] and reports suggest the involvement of various pesticides in inducing oxidative stress-mediated cytological changes[11,12,37].Therefore, the present study was designed to investigate the cytological and biochemical changes in testicular cells of *Capra hircus* due to CPF exposure along with ameliorating ability of *Zingiber officinale* or ginger extract against CPF toxicity that will benefit as a natural therapeutic approach in clinical field in near future.

2. Materials and Methods

2.1 Collection of Testicular Tissue

Testes of *Capra hircus* (Jammunapari breed) were collected from slaughter house in and around Kurukshetra and were brought to the laboratory in ice cold normal saline (0.9%) within half an hour. The testes were properly cleaned and decapsulated after which the testis was cut into small pieces with the help of sharp blade.

2.2 *Zingiber officinale* Extract Preparation

To prepare *Zingiber officinale* (ginger) extract, 500 gram of its rhizome were cut into small pieces after washing and peeling it with knife. The pieces were crushed using pestle and mortar to make fine homogenate with 50% ethanol. This homogenate was centrifuged at 2500 rpm for 10 min and then supernatant collected was incubated at 55°-60°C to allow the solvent in the supernatant to completely evaporate. The dried residual part was diluted in culture media to use as *Zingiber officinale* or Ginger Extract (GE).

2.3 *In vitro* Treatment

For *in vitro* culture, the testicular tissue pieces were incubated with commercially available Chlorpyrifos, with percentage purity of 20%, at 10 and 50 µM doses concentration along with 5 and 10 mg/ml G.E., supplemented as an ameliorant, in DMEM (Dulbecco's modified eagle medium) supplemented with 10% FBS and antibiotics (200-unit having concentration of penicillin 100 IU/mL and streptomycin 100 IU/mL) and kept in CO_2 incubator (5% CO_2, 95% humidity, 38°C) for exposure duration of 4 hours and 6 hours.

2.4 Cyto-genotoxicity Analysis

2.4.1 Cell Viability Test using MTT Assay

The testicular cells (10,000 cells/well) were plated in 96 well plate with 100 µL of fresh culture media having different doses of pesticide and ameliorant. The plates were then incubated with 10 µL of MTT stock for 37°C for 2 hours. The reaction was terminated by addition of 200 µL of DMSO for 10 minutes at 37°C. Absorbance was measured using iMarkMicrotitre Plate Reader (Bio-Rad, California, US) at 595 nm.

2.4.2 EB/AO Differential Staining for Apoptosis Detection

Post treatment, the testicular cells (n = 500) were obtained and mixed with equal quantity of ethidium bromide/acridine orange staining solution to be viewed under fluorescence microscope (Olympus, Japan) using 500-525 nm filters for their fluorescence differential staining exhibit green fluorescence in healthy cells while the apoptotic cells displayed red-orange fluorescence[13].

2.4.3 Histo-morphological Analysis

Treated testicular tissues were fixed in bouin's fixative for 24 hours. After washing of fixed tissues, it was dehydrated through graded series of ethanol, embedded in paraffin wax at 60°C and was finally sectioned serially at 5 µm. The trimmed sections were stained with Hematoxylin and Eosin (H and E) stain and mounted with DPX. Observation and photography were done under light microscope[14].

2.5 Biochemical Analysis of Oxidative Stress

2.5.1 TBARS Assay for Estimation of Lipid Peroxidation

The treated testicular tissue was used to prepare the extract and that extract was mixed with Tris-HCl buffer, $FeSO_4$ and ascorbic acid along with double distilled water to make final volume upto 1 mL as per protocol of Beuge and Aust, 1978[15]. The mixture was incubated for 15 minutes at 37°C. Post incubation, TCA and TBA were added. The reaction mixture was incubated for 15 minutes in hot water bath, after cooling centrifuged for 5 minutes at 3000 rpm and eventually absorbance of the supernatant was taken at 532 nm spectrophotometrically.

2.5.2 Ferric Reducing Anti-oxidative Power Analysis

To the reaction mixture, 100 µL of tissue extract, 300 µL of distilled water and 3 mL of working FRAP reagent (freshly prepared) was added after which absorbance was measured immediately at 593 nm at zero minute after vortexing and 4 min after incubation at 37˚C[16].

2.6 Statistical Analysis

The data were expressed as Mean ± Standard Error of Mean (SEM) with all experiments carried out in triplicate using SPSS 16 statistical software (SPSS, Inc., Chicago, IL). Statistical significance was inferred at $p<0.05$.

3. Results

Chlorpyrifos was found to affect the metabolic activity of testicular cells as revealed by MTT assay based on mitochondrial dehydrogenase enzyme activity. CPF led to a significant decline in percent testicular cell viability in dose- and time-dependent manner. The decline observed was more than 35% at 10 µM and 50% at 50 µM concentration of CPF after 4 hours exposure duration that further increased after increasing the exposure duration to 6 hours. However, supplementation of *Zingiber officinale* extract at 5 and 10 mg/mL revealed fewer declines in cell viability in comparison with control and only CPF treated groups (Figure 1). Maximum amelioration was observed after 10 mg/ml G.E. addition.

CPF also enhanced the genotoxicity induced apoptosis in testicular cells as EB/AO stains intercalates DNA at damage sites and indicates DNA fragmentation induced apoptosis. The results revealed that CPF treatment led to increase in red or orange fluorescent apoptotic cells occurrence at both the doses (Figures 2B and 3B) in comparison with control (Figures 2A and 3A), but on accretion of ameliorant *Zingiber officinale* (5 and 10 mg/mL), incidence of occurrence of green fluorescent healthy testicular cells increased [Figures 2(C-D), 3(C-D)] that revealed its mitigating potentials [Figures 2 and 3].

Further, classic histology was performed to assess the cytotoxicity of CPF in testicular cells. The results revealed that CPF-induced degenerative changes in the

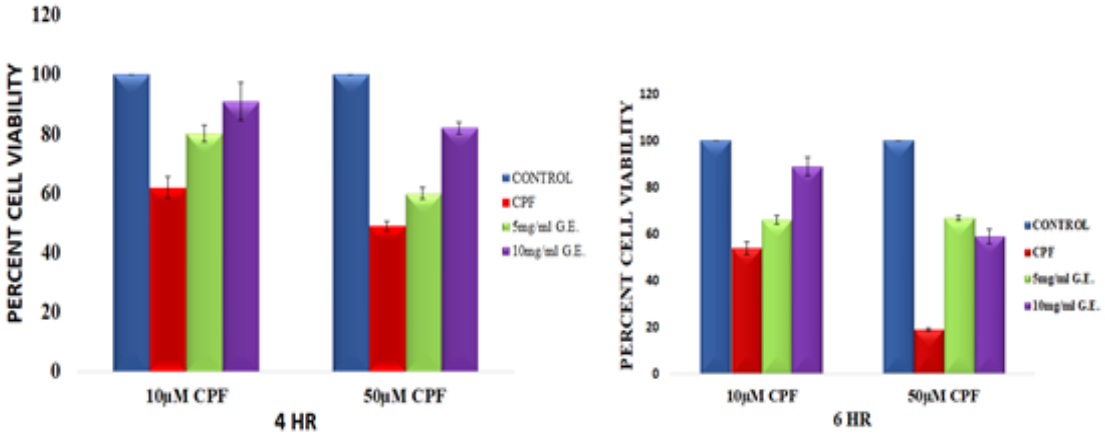


Figure 1. Percentage cell viability of spermatogonial cell after treatment with chlorpyrifos at 10 and 50 µM, with or without ginger extract (5 and 10 mg/ml) supplementation after 4 hours and 6 hours exposure durations, respectively. All the results are expressed as mean ± SE of mean (SEM)

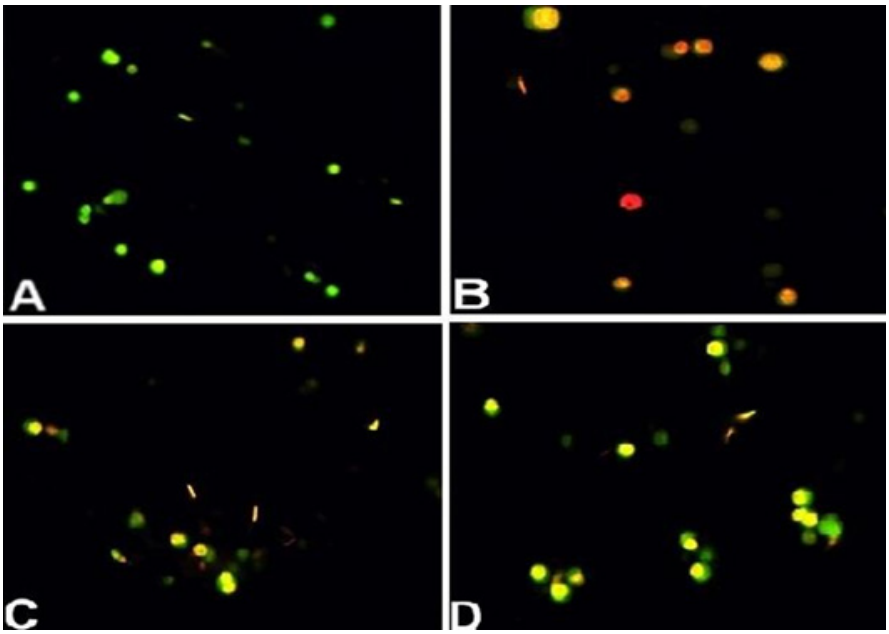


Figure 2. Fluorescent microphotograph of testicular cells treated with CPF at 10 µM **(B-D),** in comparison with control **(A)** and ameliorative action of *Zingiber officinale* extract at 5 mg/ml **(C)** and 10 mg/ml **(D)** after 4 hours exposure duration, stained with EB/AO showing apoptotic cells with red fluorescence and normal testicular cells with green fluorescence at 400X.

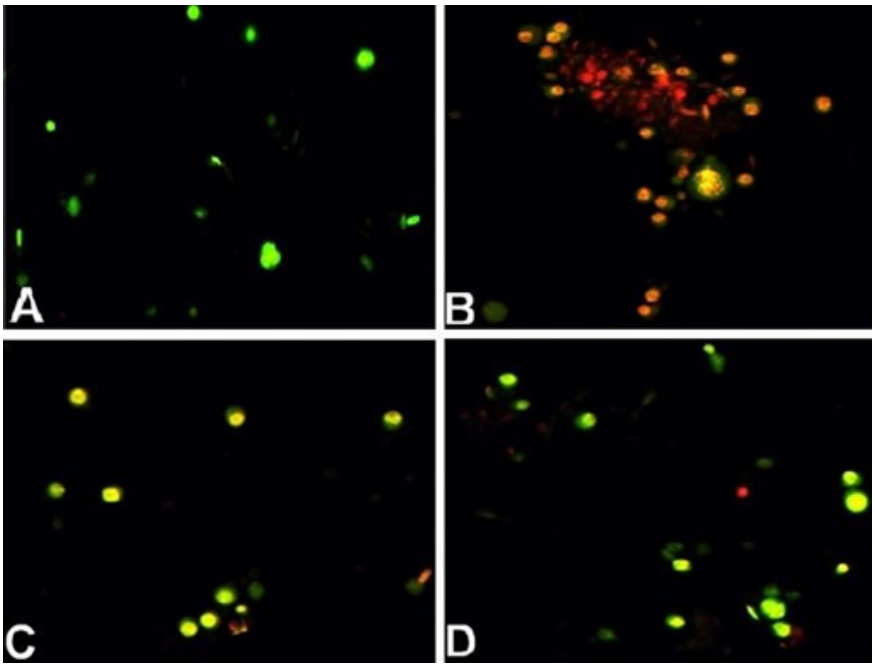


Figure 3. Fluorescent microphotograph of testicular cells treated with CPF at 50 µM **(B-D),** in comparison with control **(A)** and ameliorative action of *Zingiber officinale* extract at 5 mg/ml **(C)** and 10 mg/ml **(D)** after 6 hours exposure duration, stained with EB/AO showing apoptotic cells with red fluorescence and normal testicular cells with green fluorescence at 400X.

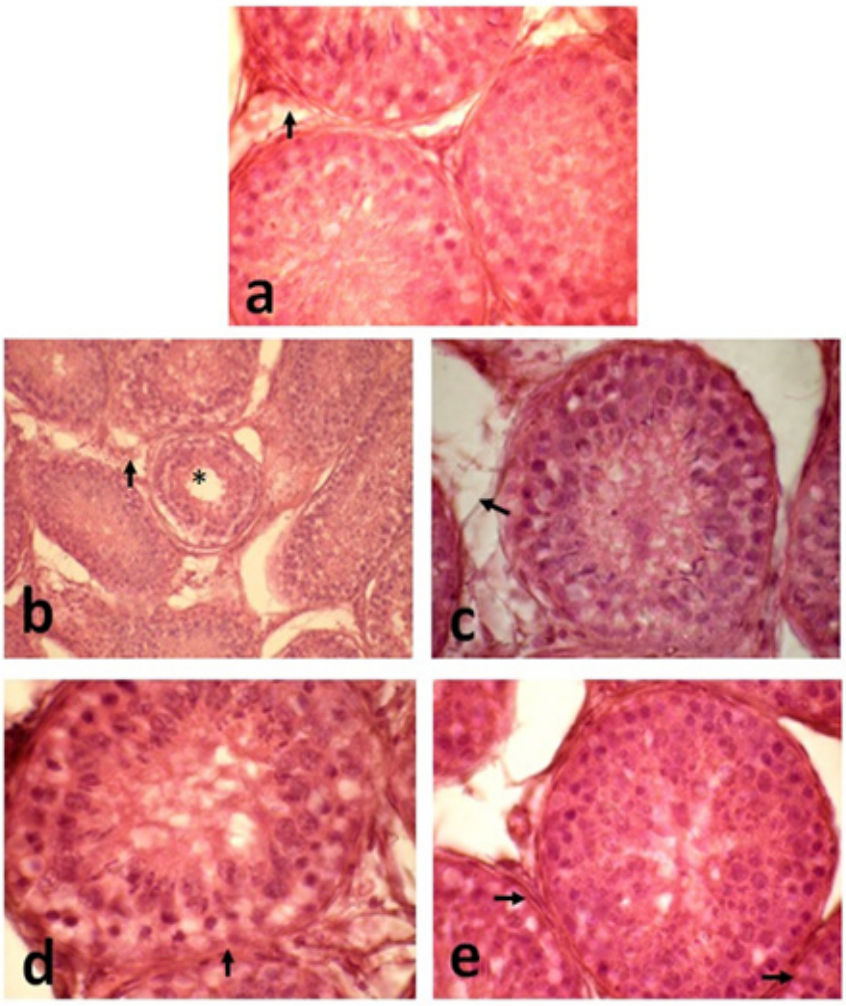


Figure 4. Light micrograph of testicular tissue showing characteristic features of testicular degeneration after treatment with chlorpyrifos at 10 **(b, d)** and 50 µM **(c, e)** with supplementation of *Zingiber officinale* extract at 10 mg/ml (d, 3), after 6 hr exposure duration post hematoxylin and eosin staining. **Arrow** - represents inter cellular interactions; *- represents degenerated luminal spaces.

testicular tissues at both the doses (Figure 4). CPF at 10 µM concentration showed prominent degeneration in inter tubular association and at highest dose of 50 µM, besides this, also caused damaged and empty luminal spaces. Both the features indicate affected testicular cells' development due to CPF toxicity. Supplementation of *Zingiber officinale* extract at 10 mg/mL had mitigating effect at both the doses of CPF as less degenerative changes have been observed.

To analyse the oxidative stress in CPF-induced testicular tissue, TBARS and FRAP assay was performed that assessed the lipid peroxidation and total antioxidant power. As the results revealed, CPF enhanced lipid peroxidation and declined ferric reducing antioxidant power in dose- and time-dependent manner (Figures 5 and 6), respectively. CPF increased the TBARS values in testicular tissue to 1.428 ± 0.12 and 2.022 ± 0.16 units after 10 and 50 µM exposure concentrations, respectively, in comparison with control (0.387 ± 0.006) which however, showed significant

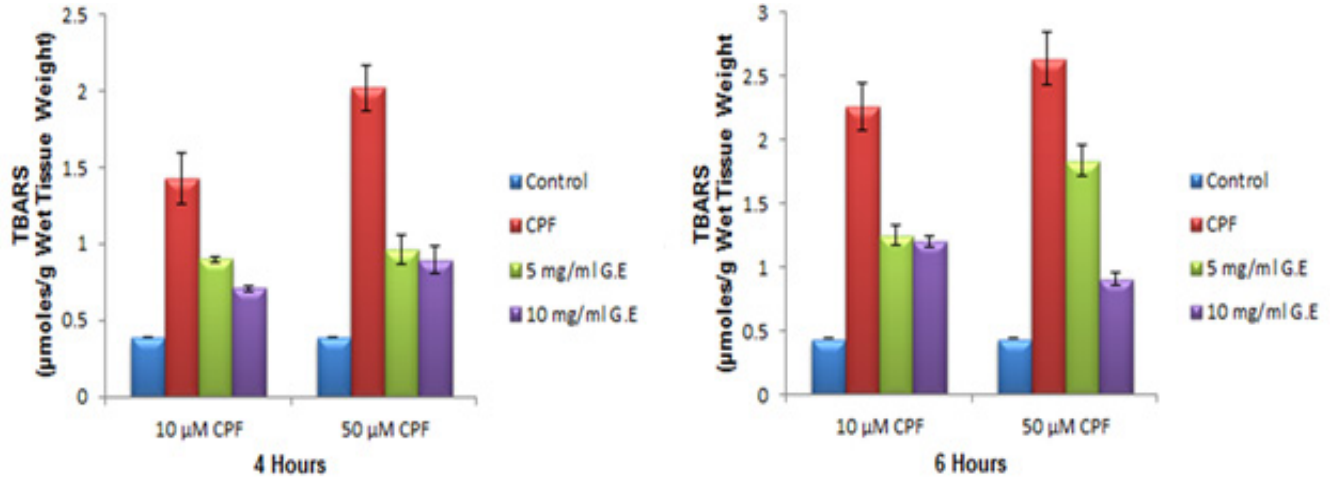


Figure 5. Graph depicting increased Lipid peroxidation (µmoles/g wet tissue weight) observed in treated testicular tissue by TBARS assay after treatment with CPF at 10 and 50 µM, with or without *Zingiber officinale* extract (5 and 10 mg/ml) supplementation, after 4 hours and 6 hours exposure durations, respectively. All the results are expressed as mean ± SE of mean (SEM).

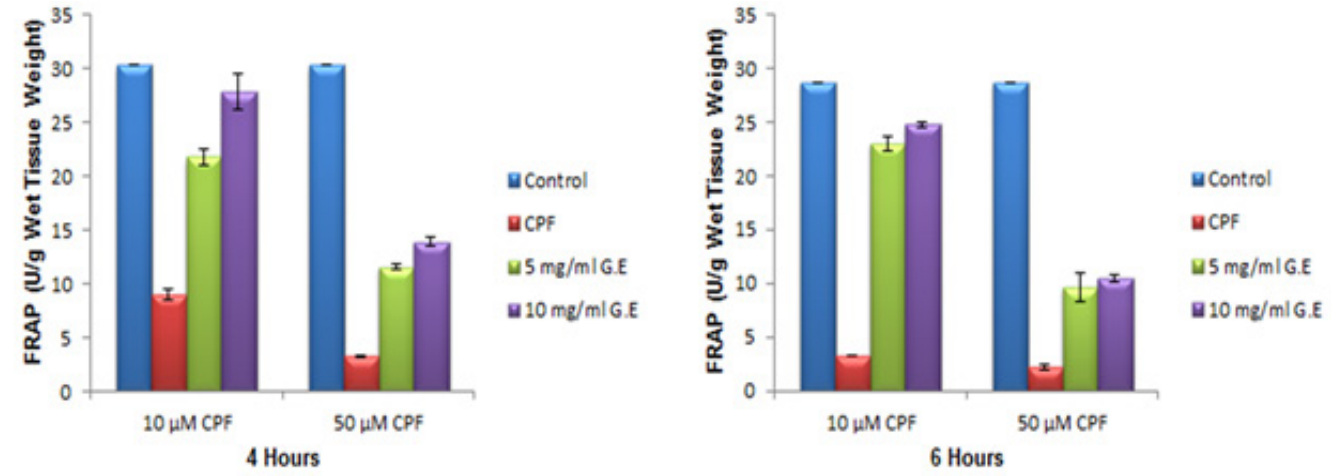


Figure 6. Graph depicting ferric reducing antioxidative power (U/mg protein) in treated testicular tissue after treatment with CPF at 10 and 50 µM, with or without *Zingiber officinale* extract (5 and 10 mg/ml) supplementation, after 4 hours and 6 hours exposure durations, respectively.All the results are expressed as mean ± SE of mean (SEM).

decline after G.E. addition at both the doses after 4 hours exposure duration. Similar trend has been observed after 8 hours treatment. Also, FRAP value too was found to be altered by CPF treatment. It significantly reduced the FRAP activity to 9.00 ± 0.78 and 3.27 ± 0.001 units after 10 and 50 µM exposure concentration in comparison with control (30.36 ± 0.01) after 4 hours duration. GE supplementation at 10 mg/mL concentration elevated the values to 27.88 ± 2.7 and 13.9 ± 0.95, respectively. Though similar trend has been observed after 6 hours treatment; most prominent effects of the extract has been observed against 10 µM CPF at both the durations. All the results show significant differences in comparison with control ($p>0.05$).

4. Discussion

The study revealed that CPF exhibits cytotoxicity in caprine testicular cells bringing various histo-architectural

changes in testis, probably by inducing genotoxicity and oxidative stress. CPF-declined percent cell viability in testicular cells clearly indicates CPF mediated alterations in metabolic activities as MTT assay is based on activity of mitochondrial NADP dehydrogenase activity that in turn affects the metabolic activity of the cell and ATP generation, causing the cell death[17]. Earlier studies on p,p-DDE and Cyclophosphamide exposure have also showed similar decline in cell viability[18,19]. Damaged DNA is the hallmark characteristic of apoptosis and CPF induced increase in incidence of red fluorescence testicular cells, indicating apoptosis positive cells as EB/AO is a differential dye that intercalates DNA at damaged sites to exhibit its fluorescence[20,21]. A number of studies have used this technique to reveal genotoxicity mediated apoptosis against pesticide exposure[22,23,34,35]. Further, CPF induced degeneration in testicular histo-architecture that showed degeneration in inter-tubular association, signifying an altered physiology affecting spermatogonial cells development. Few reports indicate altered intercellular interaction that signifies late apoptotic stages directly affecting inter-cellular or tubular communication[13,24,38]. Empty luminal spaces observed signifies degenerative sperm formation.Results of TBARS and FRAP assay showed that CPF-induced cellular changes may be probably triggered by induced oxidative stress in testicular tissue. CPF treatment increased lipid peroxidation and declined ferric reducing antioxidant activity, indicating oxidative stress mediated mechanism in CPF-induced toxicity. Studies hint the relation between increased oxidative stress and apoptosis induction. Several pesticides have been assessed to reveal apoptosis inducing properties like malathion, methoxychlor where oxidative stress generation is found to be prominent reason[12,36]. Similar result was obtained post malathion treatment in spermatogonial cells, suggesting that increased ROS increases the membrane permeability for apoptosis signalling molecules[25]. Malondialdehyde produced during lipid peroxidation suppress the anti-apoptotic Bcl-2 expression that may also contribute to CPF induced-apoptosis[26]. ROS generated in response of pesticide targets the sugar moiety resulting in the DNA damage, as also observed in present EB/AO assay results, bringing apoptosis within testicular cells[4,27]. The efficacies of CPF were found to be counteracted by *Zingiber officinale* extract showing decrease in oxidative stress and elevation in cell viability percentage. *Zingiber officinale* is antioxidant and anti-inflammatory in nature that contains zingerone, which is an antioxidant and degrades the ROS and increase the level of antioxidant enzymes[28]. Literature also cites that it regulates various signalling pathways like Bax/Bcl2, ROS/NF-κB/COX-2, caspases-3, -9, and p53[29], thus regulating CPF mediated apoptosis in spermatogonial cells. Studies support that antioxidants have huge protective effect against pesticide induced toxicity[8,30,31]. In conclusion, CPF has been found to be effectively causing degenerative changes in testicular tissue proving to be potent reproductive toxicant. However, *Zingiber officinale* extract possesses the anti-oxidative therapeutic potential against CPF-induced toxicity in testicular cells that will prove beneficiary in ameliorating reproductive toxicity issues surfaced due to pesticide treatment in wide and can be used clinically in infertility treatment.

5. Acknowledgements

The authors acknowledge the financial assistance provided by CSIR, New Delhi. The authors are also thankful to Department of Zoology, Kurukshetra University, Kurukshetra, Haryana, for providing lab facilities.

6. References

1. Anderson HR, Vinggaard AM, Rasmussen TH, Gjermandsen IM, Bonefeld-Jørgensen EC. Effects of currently used pesticides in assays for estrogenicity, androgenicity and aromatase activity in vitro. Toxicol Appl Pharmacol. 2002; 179:1–12. PMid: 11884232. https://doi.org/10.1006/taap.2001.9347
2. Bhardwaj JK, Saraf P. Granulosa cell apoptosis by impairing antioxidant defense system and cellular integrity in caprine antral follicles post malathion exposure. Environ Toxicol. 2016; 31(12):1944–54. PMid: 26420608. https://doi.org/10.1002/tox.22195
3. Bustos-Obregon EL, Gonzalez-Hormazabal P. Effect of a single dose of malathion on spermatogenesis in mice. Asian J Androl. 2003; 5(2):105–17.
4. Bhardwaj JK, Saraf P, Kumari P, Mittal M, Kumar V. N-Acetyl-cysteine mediated inhibition of spermatogonial cells apoptosis against malathion exposure in testicular tissue. J Biochem Mol Toxicol, 2018a; 32(4):e22046. PMid: 29457669. https://doi.org/10.1002/jbt.22046
5. Pina-Guzman B, Solis-Heredia MJ, Quintanilla-Vega B. Diazinon alters sperm chromatin structure in mice by phosphorylating nuclear protamines. Toxicol Appl

Pharmacol. 2005; 15:189–98. PMid: 15629194. https://doi.org/10.1016/j.taap.2004.06.028
6. El-Mazoudy RH, Attia AA. Endocrine-disrupting and cytotoxic potential of anticholinesterase insecticide, diazinon in reproductive toxicity of male mice. J Hazard Mate. 2012; 210:111–20. PMid: 22284168. https://doi.org/10.1016/j.jhazmat.2011.12.073
7. Farag AT, Radwan MH, Sorour F, Okazy AE, Agamy S, Khaliek El-Sebae A. Chlorpyrifos induced reproductive toxicity in male mice. Reprod Toxicol. 2010; 29(1):80–5. PMid: 19850121. https://doi.org/10.1016/j.reprotox.2009.10.003
8. Bhardwaj JK, Saraf P. N-acetyl Cysteine-Mediated effective attenuation of methoxychlor-induced granulosa cell apoptosis by counteracting reactive oxygen species generation in caprine ovary. Environ Toxicol. 2017; 32(1):156–66. PMid: 26635070. https://doi.org/10.1002/tox.22221
9. Bhardwaj JK, Kumar V, Saraf P, Kumara P, Mittal M. Current status and changing national scenario of goat population: A review. Agricul Rev. 2018b; 39(2):91–103. https://doi.org/10.18805/ag.-1752
10. Al-Gubory KH. Environmental pollutants and lifestyle factors induce oxidative stress and poor prenatal development. Reprod Biomed Online. 2014; 29:17–31. PMid: 24813750. https://doi.org/10.1016/j.rbmo.2014.03.002
11. Haneia IM, Hasan AA, Reda HE, Wessam MA, Mona IS. Effect of methomyl on fertility, embryotoxicity and physiological parameters in female rat. J Appl Pharm Sci. 2013; 3(12):109–19.
12. Bhardwaj JK, Mittal M, Saraf P, Kumari P. Pesticides induced oxidative stress and female infertility: a review. Toxin Reviews. 2018c; 39(3):1–13. https://doi.org/10.1080/15569543.2018.1474926
13. Bhardwaj JK, Saraf P. Malathion induced granulosa cell apoptosis in caprine antral follicles: An ultrastructural and flow cytometric analysis. Microsc Microanal. 2014; 20(6):1861–8. PMid: 25409908. https://doi.org/10.1017/S1431927614013452
14. Pearse, AGE. Histochemistry. Theoretical and applied, 1968; (2 vols), London: Churchill.
15. Buege JA, Aust SD. Microsomal lipid peroxidation. Meth Enzymol. 1978; 51:302–10. PMid: 672633. https://doi.org/10.1016/S0076-6879(78)52032-6
16. Benzie IFF, Strain JJ. Ferric Reducing Ability of Plasma (FRAP) as a measure of antioxidant power: The FRAP assay. Anal Biochem. 1996; 239:70–6. PMid: 8660627. https://doi.org/10.1006/abio.1996.0292
17. Ricci JE, Munoz-Pinedo C, Fitzgerald P, Bailly-Maitre B, Perkins GA, Yadava N, Scheffler IE, Ellisman MH, Green DR. Disruption of mitochondrial function during Apoptosis is mediated by caspase cleavage of the p75 subunit of Complex 1 of the electron transport chain. Cell. 2004; 117:773–86. PMid: 15186778. https://doi.org/10.1016/j.cell.2004.05.008
18. Tsai-Turton M, Luong BT, Tan Y, Luderer U. Cyclophosphamide-induced apoptosis in COV434 human granulosa cells involves oxidative stress and glutathione depletion. Toxicol Sci. 2007; 98(1):216–30. PMid: 17434952. https://doi.org/10.1093/toxsci/kfm087
19. Shi YQ, Wang Y, Song Y, Li HW, Liu CJ, Wu ZG, Yang K. P,p'-DDE induces testicular apoptosis in prepubertal rats via the Fas/FasL pathway. Toxicol Lett. 2010; 193:79–85. PMid: 20025943. https://doi.org/10.1016/j.toxlet.2009.12.008
20. Nafisi S, Saboury AK, Keramata N, Neaultc JF, Tajmir-Riahic HA. Stability and structural features of DNA intercalation with ethidium bromide, acridine orange and methylene blue. J Mol Struct. 2007; 827:35–43. https://doi.org/10.1016/j.molstruc.2006.05.004
21. Banerjee S, Banerjee S, Saraswat G, Bandyopadhyay SA, Kabir SN. Female reproductive aging is master-planned at the level of ovary. PLoS ONE. 2014; 9:e96210. PMid: 24788203 PMCid: PMC4008600. https://doi.org/10.1371/journal.pone.0096210
22. Li R, Wei M, Shao J. Effects of verapamil on the immediate-early gene expression of bone marrow mesenchymal stem cells stimulated by mechanical strain in vitro. Med Sci Monit Basic Res. 2013; 19:68–75. PMid: 23435320 PMCid: PMC3638684. https://doi.org/10.12659/MSMBR.883790
23. Liu K, Liu P, Liu R, Wu X. Dual AO/EB staining to detect apoptosis in Osteosarcoma cells compared with flow cytometry. Med Sci Monit Basic Res. 2015; 21:15–20. PMid: 25664686 PMCid: PMC4332266. https://doi.org/10.12659/MSMBR.893327
24. Escobar ML, Echeverria OM, Casasa AS, Garcia G, Aguilar SJ, Vazquez-Nin GH. Involvement of pro-apoptotic and pro-autophagic proteins in granulosa cell death. Cell Biol. 2013; 1(1):9–17. https://doi.org/10.11648/j.cb.20130101.12
25. Devine PJ, Perreault SD, Luderer U. Roles of reactive oxygen species and antioxidants in ovarian toxicity. Biol Reprod. 2012; 86(2):27. PMid: 22034525 PMCid: PMC3290661. https://doi.org/10.1095/biolreprod.111.095224
26. Das UN. Essential fatty acids, lipid peroxidation and apoptosis. Prostaglandins Leukot Essent Fatty

Acids. 1999; 61:157–63. PMid: 10582655. https://doi.org/10.1054/plef.1999.0085
27. Mahfouz R, Sharma R, Thiyagarajan A, Kale V, Gupta S, Sabanegh E, Agarwal A. Semen characteristics and sperm DNA fragmentation in infertile men with low and high levels of seminal reactive oxygen species. Fertil Steril. 2010; 94:2141–6. PMid: 20117780. https://doi.org/10.1016/j.fertnstert.2009.12.030
28. Riaz H, Begum A, Raza SA, Khan ZM, Yousaf H, Iariq A. Antimicrobial property and phytochemical study of ginger found in local area of Punjab, Pakistan. Int Curr Pharm J. 2015; 4(7):405–9. https://doi.org/10.3329/icpj.v4i7.23591
29. De Lima, RMT, Dos Reis AC, de Menezes APM, Santos JVO, Filho JWGO, Ferreira JRO, de Alencar MVOB, da Mata AMOF, Khan IN, Islam A, Uddin SJ, Ali ES, Islam MT, Tripathi S, Mishra SK, Mubarak MS, Melo-Cavalcante AAC. Protective and therapeutic potential of ginger (*Zingiber officinale*) extract and [6]-gingerol in cancer: A comprehensive review. Phytother Res. 2018; 32(10):1885–907. PMid: 30009484. https://doi.org/10.1002/ptr.6134
30. Abarikwu SO. Protective effect of quercetin on atrazine-induced oxidative stress in the liver, kidney, brain, and heart of adult Wistar rats. Toxicol Int. 2014; 21:148–55. PMid: 25253923 PMCid: PMC4170555. https://doi.org/10.4103/0971-6580.139794
31. Jain J, Hasan W, Yadav RS, Jat D. Protective Effects of Quercetin against Rotenone Induced Histopathological and Biochemical Alteration in Testes of Mice. Toxicol Int. 2021; 28:57–65.
32. Bhardwaj JK, Kumar V, Saraf P, Panchal H, Rathee V, Sachdeva SN. Efficacy of N-acetyl-L-cysteine against glyphosate induced oxidative stress and apoptosis in testicular germ cells preventing infertility. J Biochem Mol Toxicol. 2021; e22979.
33. Bhardwaj JK, Kumar V, Panchal H, Sachdeva SN. Transmission electron microscopic analysis of glyphosate induced cytotoxicity and its attenuation by N-acetyl-L-cysteine in caprine testicular germ cells in vitro. Ultrastruct Pathol. 2021; 45(6):407–13. PMid: 34698588. https://doi.org/10.1080/01913123.2021.1993400
34. Bhardwaj JK, Kumari P, Saraf P, Yadav AS. Antiapoptotic effects of vitamin C and E against Cypermethrin induced oxidative stress and spermatogonial germ cell apoptosis. J Biochem Mol Toxicol. 2018; 32(8):e22174. PMid: 29975445. https://doi.org/10.1002/jbt.22174
35. Kumar P, Duhan M, Kadyan K, Bhardwaj JK, Saraf P, Mittal M. Multicomponent synthesis of some molecular hybrid containing thiazolepyrazole as apoptosis inducer. Drug Res. 2018; 68:72–9. PMid: 28910831. https://doi.org/10.1055/s-0043-116947
36. Bhardwaj JK, Kumari P, Saraf P, Mittal M, Yadav AS. Male infertility owing to pesticide poisoning and antioxidant induced amelioration: A review. J Cell Tissue Res, 2017; 17(3):6307–14.
37. Phogat A, Singh J, Kumar V, Malik N. Toxicity of the acetamiprid insecticide for mammals: A review. Environ Chem Letters. 2022; 20:1453–78. https://doi.org/10.1007/s10311-021-01353-1
38. Sharma RK, Bhat RA, Goyal AK, Bhardwaj JK. Germ cells apoptosis during spermatogenesis in mammals. J Entom Zoo Stud. 2015; 3(3):506–15.