**INTRODUCTION**

Infertility due to environmental toxicants has alarmingly increased in the past decade and has raised concerns among couples. In about 20% infertility cases, male factor is the primary cause (Wong et al., 2000). A variety of environmental, genetic, behavioral and genotoxic factors result in male infertility by impairment of spermatogenesis at various stages (Toshimori et al., 2004). One of the well-defined causes of impairment of spermatogenesis is exposure to environmental chemicals (Sharpe, 2000).
Chemical element cadmium (Cd), a heavy metal and an environmental pollutant, is released into the environment by various human activities (Henson and Chedrese, 2004). Industrial and environmental exposure to Cd, results in various health problems. It causes various biochemical and physiological alterations in man and lab animals (Santos et al., 2004). Severe degeneration of testicular tissue and associated seminiferous tubule damage and ischemic necrosis results in rats following exposure to Cd (Xu et al., 2005). Cd induces generation of reactive oxidation species (ROS) in testis which is a by-product of O2 metabolism and are free radicals having short life, but accumulation is harmful to the cell.

Oxidative stress affects fertility potential due to depletion of antioxidants by free radicals affecting the normal balance between the two resulting in accelerated tissue damage. Testicular antioxidant defense system plays a major role in protecting the testis from damages caused by oxidative stress due to ROS. Severe impairment of testicular function including germ cell death and inhibition of steroidogenesis results by acute Cd administration in male rats (Ragan and Mast, 1990; Laskey and Phelps, 1991; Yang et al., 2003). Antioxidants are free radical scavengers and they suppress ROS generation and their actions (Fridovich, 1983; Baker et al., 1996).

Zinc (Zn) is a member of the same family as Cd, but is essential in cellular response to DNA repair, oxidative stress and apoptosis (Bagheri-Sereshki et al., 2016). It is an important antioxidant since superoxide dismutase, which is a part of, converts superoxide anion into H2O2+O2 (Ho, 2004). It is important for the physiology of spermatozoa, sperm production and viability (Aitken et al., 1997). In rat and mice testes, Zn provides protection against acute Cd toxicity (Parizek, 1957).

Even though variety of chemical drugs are available for infertility treatment, traditional medicine is gaining popularity due to its less toxicity and adverse effects. Medicinal plant contains active compounds that can be used for therapeutic purposes (Sofowora et al., 2013). Aloe vera, a cactus like perennial herb, showcases its antioxidant role by strengthening Vitamin C and E (Kooshesh et al., 2007).

The present study is to evaluate the protective effects of hydroalcoholic extract of Aloe vera in combination with Zn and without it on the effects of cadmium induced toxicity in the testis of Wistar rats. Aloe vera, is expected exhibit its antioxidant properties and Zn, to act as antioxidant as well as chelating agent thereby prevent testicular damage caused by Cd.

**MATERIALS AND METHODS**

The study was conducted on Wistar albino rats. The rats procured from Biogen (Bengaluru, India) were kept under controlled condition throughout the experiment in the animal house of Siddha Central Research Institute (Chennai, India). After 4 days of acclimatisation, the animals were divided into following groups (Table 1). The experiment was conducted for 56 days. After the completion of the experiment, the rats were sacrificed, blood, testes with epididymis were collected and properly preserved for semen and hormonal analysis.

**Preparation of hydroalcoholic extract of Aloe vera**

Fresh Aloe vera plants were obtained from Amudham Nandavanam Garden in Chennai, India. The plants were certified by a Pharmacognosist. They were washed, cut, shade dried and hydro alcoholic extract (HAE) was prepared by using ethyl alcohol and water. 10 kilos of fresh plants were used and the yield of extract was 292 g.

**Drug administration**

Control: 1 ml of 0.5% carboxymethyl cellulose (CMC).

Cd10: 10mg/Kg body weight of CdCl2 (NICE Chemicals) in 0.5% CMC

Cd10+Zn: 10mg/Kg body weight of CdCl2 and 40mg/Kg bw of ZnCl2 in 0.5% CMC

Cd+AV: 10mg/Kg body weight of CdCl2 and 200mg/Kg bw of HAE of Aloe vera in 0.5% CMC

Group 5: received 10mg/Kg body weight of CdCl2, 40mg/Kg bw of ZnCl2 and 200mg/Kg bw of HAE of Aloe vera in 0.5% CMC.

CdCl2, ZnCl2 and CMC: NICE Chemicals, Sudhagar Biological and Chemicals, Chennai, India.

**RESULTS AND DISCUSSION**

**Sperm concentration**

The concentration of sperm in Cd (24.2±3.1X10⁶/mL) was significantly 3 times lower than control; in Cd+Zn (47.8±3.4X10⁶/mL) was significantly 1.5 times lower than control and significantly 2 times more than Cd; in Cd+AV (62±3.2X10⁶/mL) was significantly 2.5 times higher than Cd but there was no significant difference from control group; in Cd+AV 200+Zn (55.4±3.1X10⁶/mL) it was significantly 1.3 times lower than control and significantly 2.3 times more than Cd.
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Figure 1: Concentration, motility and viability of sperm. Values expressed as mean + SD. 1-Control, 2-Cd, 3-Cd+Zn, 4-Cd+AV, 5-Cd+AV+Zn.

Table 1: Control and various experimental groups. Abbreviations used: Cd- Cadmium, Zn-Zinc, AV- Aloe vera.

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Sperm motility

In Cd, sperm motility (3.4± 0.5%) significantly decreased 17.4 times in comparison to control; in Cd+Zn (39.3± 2.2%) it significantly decreased 15 times compared to control and significantly increased 11.2 times compared to Cd; in Cd+ AV (49.0± 1.7%) it significantly increased 14.4 times than Cd, however any difference that was significant from control could be found. In Cd+AV+Zn (46.5±3%) motility significantly increased 13.7 times compared to Cd but without any significant difference from control group.

Sperm viability

In Cd (13.9 ± 1.8 %), sperm viability significantly decreased 5.2 times compared to control; in Cd+ Zn (51.5± 2%) it decreased significantly 1.5 times compared to control but significantly 3.7 times more than Cd. In groups Cd+AV (53.8± 2.8%) and Cd+ AV 200+Zn (62.4±2.6%) the viability increased significantly 3.9 and 13.7 times respectively in comparison to Cd, but any significant difference could not be observed compared to control in this group (Figure 1).

Testosterone hormone analysis (TST)

In Cd the TST level (1.9 ng/mL) significantly decreased 2.6 times compared to control; in Cd+Zn it (TST level of 3.4ng/mL) significantly increased 1.8 times in comparison to Cd, without any significant difference compared to control. In Cd+ AV, TST level (3.9 ng/mL) significantly increased 2 times compared to Cd, here too no significant difference in comparison to control group; in Cd+ AV +Zn, in comparison to Cd, TST level (4.2 ng/mL) significantly increased 2.2 times. In this group there was no significant difference of values compared to con-
requirements is adequate level of TST. Since for spermatogenesis to take place one of the spermatogenic cells supported by it and this can be due to decreased level of StAR protein and effect of Cd on H-P-T axis. This effect is reflected on the level of hormone by influencing hypothalamic pituitary testicular (HPT) axis or Leydig cells (Parizek, 1957). The StAR protein facilitates delivery of cholesterol, the raw material for steroidogenesis, to cytochrome P450scc on the inner mitochondrial membrane. Another way Cd can readily modify level of hormone by influencing hypothalamic pituitary testicular (HPT) axis or Leydig cells (Lafuente, 2004). In this study, the Cd treated rats showed decreased testosterone and LH levels which could be due to decreased level of StAR protein and effect of Cd on H-P-T axis. This effect is reflected on the sperm concentration, which could be due to reduced spermatogenesis because of decreased TST level, since for spermatogenesis to take place one of the requirements is adequate level of TST.

To counteract the oxidative stress, testis has a number of enzymatic antioxidants such as SOD, catalase and GPx. However, upon exposure to Cd, the levels of these enzymes decrease (Siu et al., 2009; Gupta et al., 2004). Hence it is imperative that enzymatic or non-enzymatic antioxidants may prevent or reduce testicular damage induced by Cd. Zn could be used to prevent the testicular damage by Cd (Parizek, 1957). In the present study, in Cd+AV+Zn treatment groups, Zn clearly exhibited its protective effect due to its antioxidant properties (Hu et al., 2004; Kara et al., 2007; Amara et al., 2008). Furthermore, Zn supplementation prevents increased rate of lipid peroxidation (Shaheen and El-Fattah, 1995). Zn exhibited its protective nature when Cd was co-administered with it, and the toxicity due to Cd was counteracted better by Zn than when Cd was administered alone (Parizek, 1957; Gunn et al., 1961). Similar results were found in the present study. Zn is required for the normal physiology of spermatozoa in addition to its contribution towards sperm production and its viability (Aitken et al., 1997). The Zn treated rats in the present study, showed increased concentration and viability compared to Cd group due to this reason.

Substances rich in antioxidant enzymes such as Vitamin C and E, reduce and or prevent oxidative stress and damages due to Cd in the testis of rats (Acharya et al., 2008). Treating the rat testes with antioxidants like Vitamin C and Vitamin E prior to Cd exposure has shown to restore normal testicular function due to their ability to counter production of excess ROS (Gupta et al., 2004). Aloe vera is one such medicinal plant which has about 75 active compounds that include antioxidant enzymes such as Vitamin C and E (Surjushe et al., 2008). A study observed that hydroalcoholic extract of Aloe vera increased the level of testosterone and LH hormones, and also rise in sperm concentration and motility (Estakhr and Javad, 2011). In this study, rats administered with Cd+AV and Cd+AV+Zn, similar result was observed. This is due to the antioxidant properties of Aloe vera, which by strengthening Vitamin C and E enhances reproductive parameters and improves fertility potential. It stimulates Leydig cells and thereby increases testosterone hormone (Kooshesh et al., 2007). In addition, Aloe vera could have increased intracellular levels of reduced glutathione thus providing protection against Cd (Sharma et al., 2011). Due to the combined actions of hydroalcoholic extract of Aloe vera and Zn, in the treatment group Cd+AV+Zn, the functional integrity was very well maintained. Here Aloe vera is believed to exhibit its antioxidant properties thus offering protection to testis from damages.
due to ROS generated by Cd. Cd, in presence of Zn has to compete for physiological binding sites due to similarity between these ions, and it is understood that Cd is failed by Zn mainly by chelating as well as antioxidant properties of Zn.

**CONCLUSIONS**

Cd induces testicular damage in rat testis. Zn acts as an antioxidant and chelating agent. Thus, co-treatment of Cd with Zn protects testis from damages due to Cd effectively. Combined actions of HAE extract of *Aloe vera* and Zn protects the rat testis from Cd induced alterations very effectively.

**Conflict of Interest**

The authors declare that they have no conflict of interest for this study.

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**REFERENCES**


