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ZINC AND ALPHA-LIPOIC ACID ALLEVIATE CYPERMETHRIN INDUCED REPRODUCTIVE TOXICITY IN MATURE MALE WISTAR RAT

MADHUBANTI BEPARI, PRALAY MAITY, TUHINA DAS AND SUJATA MAITI CHOUDHURY*

Department of Human Physiology with Community Health, Vidyasagar University, Midnapore, West Bengal, India, Pin-721102.

ABSTRACT

Cypermethrin (α- Cyano-3-Phenoxybenzyl (+ cis, trans) 3-(2, 2-Dichlorovinyl)-2, 2-dimethyl cyclopropane carboxylate) is an active pyrethroid which intensively controls a wide range of pests in agriculture and an ecto-parasiticide in humans and animals. It has moderate carcinogenic, mutagenic, immunosuppressive and hepatotoxic effects. It causes brain and locomotor disorders, polyneuropathy. The present study was conducted to evaluate the adverse effects of cypermethrin on the reproductive system of male Wistar rats, and also to assess the alleviating role of zinc and α-lipoic acid under these toxic conditions. Male Albino Wistar rats received oral cypermethrin treatment at two dose levels(40 mg/kg body wt,80 mg/kg body wt) and combined cypermethrin, zinc (227 mg/l in drinking water), and α-lipoic acid (35 mg/kg body wt) treatments for consecutive 14 days. The effects of the treatments were studied on some reproductive markers in rat testis including antioxidant parameters. Cypermethrin reduced the testicular index, sperm motility of rats of cypermethrin treated groups. Significant increase in seminal fructose concentration, testicular glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), testicular malon-di-aldehyde (MDA) level were seen whereas reduced glutathione (GSH) level and superoxide dismutase (SOD) activity of testis were decreased significantly in a dose-dependent manner compared to the control group. The present study showed that zinc and α-lipoic acid supplementation restored the altered reproductive parameters towards normal levels. The results revealed the beneficial influences of zinc and α-lipoic acid in reducing the harmful effects of cypermethrin on male reproductive system of rat.

Keywords: Cypermethrin; Zinc and α-lipoic acid; Sperm motility; Seminal fructose concentration Testicular glutamate pyruvate transaminase; Antioxidant parameters.

1. INTRODUCTION

Pesticides are widely used agricultural chemicals which are the largest group of poisonous substances being disseminated throughout our environment in spite of their use in public health to control insects, weeds, animals, and vectors of disease (Ecobichon DJ. 1994; Eddleston M et al. 2002). Increasing interest has been seen among health and environmental institutions regarding the potential reproductive effects due to exposure to these agricultural and environmental chemicals (Dalsenter PR et al. 1997). Exposure to such environmental contaminants causes reduction of sperm motility, decreased fertilization ability, producing abnormal sperm in men and wildlife (Alm H et al. 1996). It has been reported that, pesticides with such properties have been shown to cause overproduction of reactive oxygen species (ROS) in both extra- and intracellular spaces, producing infertility in wildlife and human (Sharpe RM and Skakkebaek NE, 1993). The antioxidant system plays an effective role in protecting testes and thus preventing testicular dysfunction

(Oschsendorf FR. 1999). Synthetic pyrethroid insecticides have been used in agriculture, animal husbandry and indoor insect management and account for approximately one-fourth of worldwide insecticide market (Casida JE et al. 1998). Pyrethroids are used widely in an increasing order during the last two decades with the declining use of organophosphate pesticides, which are more acutely toxic to fishes, birds and mammals than pyrethroids (Bateman DN. 2000). Cypermethrin is an active synthetic pyrethroid insecticide is commonly used to control various pests in agriculture, public health and in veterinary practice against ectoparasites (WHO, 1989;Bhunya SP and Pati PC, 1988). It is chemically (+/-) alpha-cyano-(3-phenoxyphenyl) methyl (+)-cis, trans-3-(2, 2-dichloroethenyl)-2, 2-dimethylcyclopropanecarboxylate. (Bhunya SP and Pati PC, 1988). It is highly toxic to bees, fishes, and water insects but has lower toxicity to birds. It has moderate carcinogenic, mutagenic, immunosuppressive and hepatotoxic effects. Low levels of cypermethrin in the aquatic environment produced a significant effect on mature male Atlantic salmon populations through disruption of reproductive functions (Moore A and Waring CP, 2001). Fenvalerate and cypermethrin were reported to impair male rat reproductive function, inducing significant reductions in epididymal sperm count (Song L et al. 2008). However, it is not clear whether fenvalerate and cypermethrin might affect spermatogenesis via interaction with androgens or their receptors. Fenvalerate and cypermethrin directly influence mature rat sperm motility. Cypermethrin induced adverse effects on sperm head shape morphology of mouse as well as clastogenic effects on the root tip cells of *Allium cepa.* Besides generalized toxic effects of cypermethrin, decreased number of implantation sites, number of viable fetuses and weight gain of fetuses in rabbits treated with cypermethrin have been reported (Elbetieha A et al. 2001). Semen ejaculatory volume, sperm motility percentage, mass activity, and concentration of spermatozoa were significantly (P<0.01) decreased by cypermethrin in dwarf goats (Ahmed M et al. 2009).

Moreover, it is observed (Manna et al. 2004) that high dose of cypermethrin caused oedema between seminiferous tubules, in addition to vacuolation and hyalinization in the tubules of rat testis. It has been also reported that the proportion of dead and abnormal sperms were significantly increased in mice after exposure to the cypermethrin like pyrethroid (Bhunya SP and Pati PC, 1988). More recently, much attention has been focused on the possible role of essential trace elements in providing the necessary preventive efficacy with less toxicity and side effects (Xiu YM. 1996; Kang YJ and Zhou Z 2005; Zhou Z et al. 2005). Zinc is a key constituent or cofactor of over 300 mammalian proteins. It is intensively being studied for its protective efficacy in various models of animal toxicity. Zinc is a beneficial agent in reducing the damage arising in increased oxidative stress (Cagen SZ and Klaassen CD, 1979; Cabre M et al. 1999; Zhou Z et al. 2005). Alpha-lipoic acid, a biological antioxidant, shows beneficial effects in oxidative stress because of its synergistic action with other antioxidants (Suzuki YJ et al. 1993). Based on the above facts, the present study was conducted to investigate the combined effects of zinc and α- lipoic acid in the amelioration of the damage inflicted on the reproductive system of the rats intoxicated with cypermethrin.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

Cypermethrin 10% Emulsifiable Concentrate (EC), commercial name (Ustad). Alpha-lipoic acid was purchased from Sigma Aldrich. Indole, Sodium hydroxide(NaOH), Dinitrophenyl hydrazine (DNPH), Thio-barbutaric acid (TBA), Trichloro acetic acid (TCA), Sodium chloride (NaCl), Hydrochloric acid (HCl), Sulfosalicyclic acid(SSA), 2,4,4-dithionitrobenzoic acid(DTNB), Pyrogallol, Tris HCl, Potassium dihydrogen phosphate (KH_2PO_4), Zinc sulphate ($ZnSO_4$) ,Benzoic acid were purchased from Merck Ltd., SRL Pvt. Ltd., Mumbai, India. All other chemicals used were analytical grade and obtained from Merck Ltd., SRL Pvt. Ltd., Mumbai, India.

2.2. Animal care and treatment

The study was conducted on 36 mature Wistar male albino rats weighing 130-150 gm. The animals were housed in polypropylene cages in the departmental animal house at an ambient temperature of 25°±2°C with 12 hrs light-dark cycle and were acclimatized for at least 1 week before prior to different treatments. The animals

were maintained on the standard laboratory feed and water *ad libitum*, throughout the period of experimentation. Thirty six albino mature male rats were randomly divided into six groups, of six animals each. The groups and treatments were designed as:

1. Group I: Control (5 ml /kg body wt.)
2. Group II: Zinc(227 mg/l in drinking water)and α- lipoic acid(35 mg/kg body wt.) control
3. Group III: Cypermethrin-treated (Low dose, 40mg/body wt.) group
4. Group IV: Zinc + α- lipoic acid+ Cypermethrin-treated (Low dose, 40mg/body wt.) group
5. Group V: Cypermethrin-treated (High dose, 80mg/body wt.) group
6. Group VI: Zinc + α- lipoic acid+ Cypermethrin-treated (High dose, 80mg/body wt.) group

After one hour of the treatment of Zinc (227 mg/l in drinking water; Goel A et al. 2006) and α-lipoic acid (35 mg/kg body wt.; Selvakumar E et al. 2005), Cypermethrin was administered at two dose levels. All animals were observed at least once daily to notice whether there was any behavioural change or signs of intoxications. Mortality, morbidity, and food and water consumption were also monitored daily. Animal's weight was taken daily and the dose was adjusted accordingly. Oral treatment at the dose level of 40mg, and 80mg of cypermethrin /kg body weight were done for consecutive 14 days.

2.3. Sample collection

Total body weights of rats in each group were taken before the treatment period and before sacrifice. 24 hrs after the last dose all rats were sacrificed by rapid decapitation. Blood was collected using heparin as anticoagulant (10 UI/ml) from the animals and serum was separated and kept at -20°C for biochemical studies. The testes and other male accessory sex organs (epedidymis and seminal vesicles) were removed immediately and adhering fats were cleaned. Then testicular weights were recorded and stored properly for the biochemical estimation. Then, epididymis and seminal vesicles prepared for fertility evaluation and testicular tissues were taken for the determination of oxidative biomarkers.

2.4. Estimation of Testicular index

Testes of sacrificed male albino rat (Wister) were dissected from its body and all fats were removed from the testes. Then their weights were taken. Testicular index was measured by the following formula:

Testicular index = Testicular weight/Body weight x 100

2.5. Epididymal sperm motility analysis (WHO, 1999)

Assessment of epididymal sperm motility was done by the method of WHO. 1999.

2.6. Epididymal sperm morphology analysis (Laing JA .1979)

This was determined by adding two drops of warm Wells and Awa stain to the semen on a prewarmed slide. Then a uniform smear was made and air-dried. The slide which was stained, immediately examined under the microscope using x400 magnification (Laing, 1979). The types and number of abnormal spermatozoa were calculated from the total number of spermatozoa in the randomly selected five microscopic fields. The numbers of abnormal spermatozoa were expressed as a percentage of the total number of spermatozoa

2.7. Estimation of seminal fructose concentration

Fructose in seminal fluid was measured (Karronen MJ and Malam M, 1995) by taking 0.5 ml tissue homogenate (20mg/ml, as sample), 0.5 ml fructose (0.14 mM and 0.28 mM fructose, as two standards) and 0.5 ml distilled water (as blank) with 0.5 ml indole reagent separately. They were mixed and then 5 ml concentrated HCl was added to each test tube. All test tubes were covered with stoppered and incubated at 50˚c for 20 min., cooled in ice water and then in room temperature. After that the reading was taken at 470 nm.

2.8. Testicular glutamate oxaloacetate transaminase (GOT) and glutamate Pyruvate transaminase (GPT) (Reitman S and Frankel S, 1957)

One ml buffer substrate (GOT/GPT ml) was given in test tube and waited for 5 minutes at 37˚C. After that 0.2 ml tissue homogenate (20 mg/ml) was added to the test tube and incubated at 37˚C for 60 minutes. DNPH (1 ml) solution was mixed to the test tube and waited for 20 minutes. In 0.2 ml working pyruvate standard solution was added to

0.8 ml and 1 ml buffer substrate.and in blank, 1 ml buffer substrate were taken. Distilled water (0.2 ml) and 1 ml DNPH solution were added to each test tube and mixed well and waited for 20 minutes. Mixing 10 ml of 0.4 (N) NaOH solution to all the test tubes, readings were taken at 520 nm. after 10 minutes.

2.9. Estimation of oxidative stress parameters

2.9.1. Testicular Malon dialdehyde (Ohkawa H et al. 1979)

0.5ml of testicular homogenates (50 mg /ml), 0.5 ml normal saline (0.9%), 2 ml TBA TCA mixture were mixed. It was boiled for 10 min and was cooled at room temperature and centrifuged in 4000 r.p.m for 10 min. Reading was taken at 535 nm.

2.9.2. Testicular Reduced Glutathione (Grifth Mindr P. 1998)

200µl of testicular homogenates (50 mg /mL) mixed with sulfosalicylic acid and centrifuged for 10 min at 3000 rpm. The supernatant was added with DTNB and was shaken well. Reading was taken at 412-420nm.

2.9.3 Testicular Superoxide dismutase (Marklund S and Marklund G, 1974)

Testicular homogenates (50 mg /mL) in ice-cold 100 mM Tris-HCl buffer were centrifuged at 10,000 g for 20 min at 4^{o}C. The SOD activities of the supernatants were estimated by measuring the percentage inhibition of the pyragallol autooxidation by SOD (Marklund S and Marklund G, 1974). In a spectrophotometric cuvette, 2 mL of buffer, 100 µl of 2 mM pyragallol and 10 µl of supernatant were poured and the absorbance was noted in a spectrophotometer at 420 nm for 3 min.

2.10. Statistical analysis

The results were expressed as the Mean ± Standard error of mean (SEM). Statistical analysis of the collected data were performed by Analysis of variance (ANOVA) followed by multiple comparison two-tail t-test. The difference was considered significant when $p<0.05$.

3. RESULTS

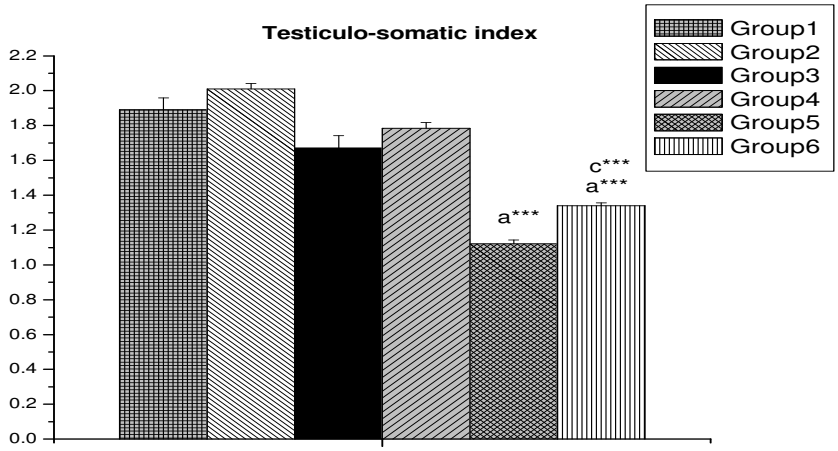

Figure1

shows the effect of Zinc and α- lipoic acid on Testiculo-somatic index in Cypermethrin induced male albino rat (N=6). Results are expressed as Mean±SEM. Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Superscript a, Group-I versus all other groups; Superscript c Group-V versus Group-VI. Asterisks represents the different level of significance (indicates p<0.001).***

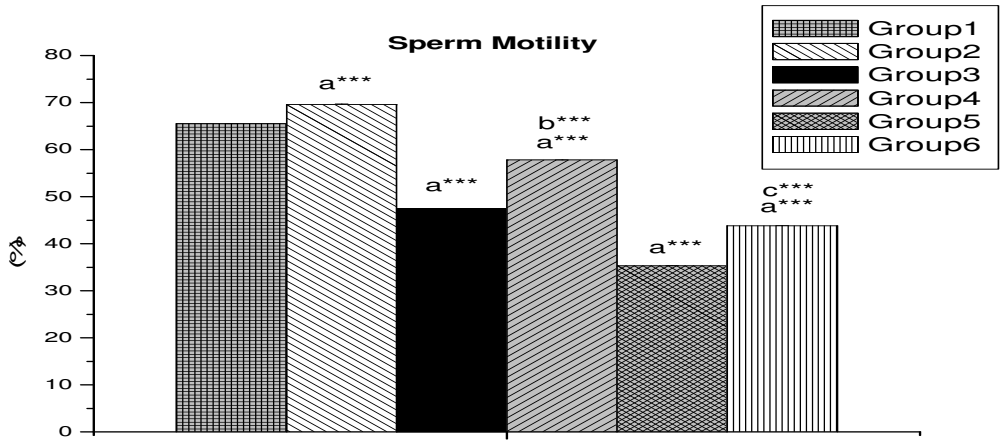

Figure 2

shows the effect of Zinc and α- lipoic acid on Epididymal sperm morphology in Cypermethrin induced male albino rat (N=6). Results are expressed as Mean±SEM. Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Superscript a, Group-I versus all other groups; Superscript b Group-III versus Group-IV; Superscript c Group-V versus Group-VI. Asterisks represents the different level of significance (indicates p<0.001)***

Table 1

shows the effect of Zinc and α- lipoic acid Cypermethrin on Sperm Morphology in Cypermethrin induced male albino rat.

Group	Saline Control (%)	Zn+ Lipoic acid control (%)	Cyp (Low) (%)	Cyp(L)+ Zn+ Lipoic acid (%)	Cyp (High) (%)	Cyp(H)+Zn+ Lipoic acid (%)
Tailless head	4.1±0.2	5.8±0.90	6.1±0.17	4.81±0.15	6.2±0.74	5.2±0.78
Headless tail	4.7±0.5	3.8±0.83	6.3±0.13	4.92±0.16	8.6±0.62	5.1±0.58
Bent tail	5.2±0.32	5.1±0.92	7.1±0.21	6.83±0.14	9.6±0.43	5.6±0.38
Curve tail	6.2±0.3	4.2±0.84	8.4±0.32	6.73±0.26	10.2±1.3	7.9±0.42
Bent mid piece	4.1±0.4	5.7±0.62	6.3±0.26	0.41±0.02	9.83±0.21	6.21±0.23
Coiled tail	0.3±0.02	4.6±0.53.	0.72±0.03	0.41±0.02	1.8±0.09	0.6±0.02
Looped tail	0.12±0.02	0.4±0.06	0.23±0.03	0.19±0.008	1.62±0.12	1.2±0.03
Rudimentary tail	0.23±0.13	0.09±0.006	0.73±0.08	0.31±0.06	2.5±0.72	0.36±0.04
Curve mid piece	6.24±0.72	0.3±0.08	8.21±0.45	6.72±0.32	11.2±0.13	8.2±0.63
Total	**33.9±2.61**	**30.0±4.8^{a***}**	**44.09±1.6^{a***}**	**37.04±1.42^{a***b***}**	**61.55±4.36 ^{a***}**	**40.37±3.48^{a***c***}**

Values represents Mean± SEM (N=6). Results are expressed as Mean±SEM. Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Probability values with different superscripts (a,b,c) differ from each other significantly. Superscript a, Group-I versus all other groups; Superscript b Group-III versus Group-IV; Superscript c Group-V versus Group-VI. Astericks represents the different level of significance (indicates p<0.001).***

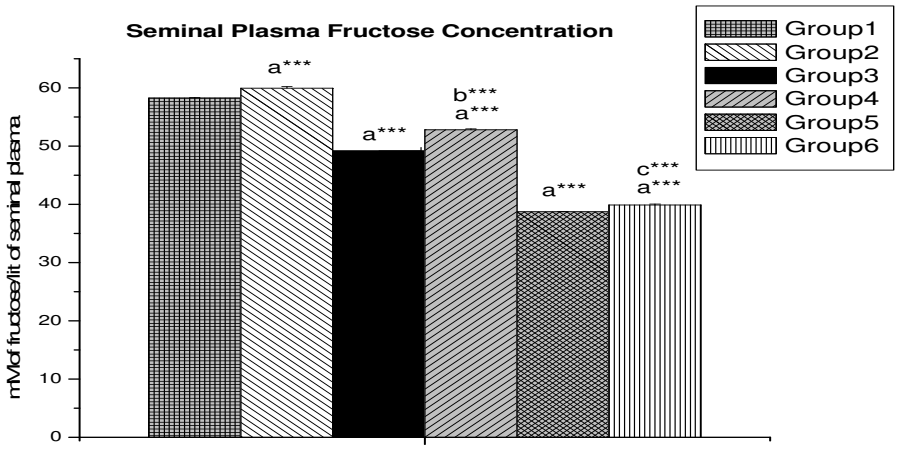

Figure 3

shows the effect of Zinc and α- lipoic acid on Seminal Plasma fructose Concentration in Cypermethrin induced male albino rat (N=6). Results are expressed as Mean±SEM. Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Superscript a, Group-I versus all other groups; Superscript b Group-III versus Group-IV; Superscript c Group-V versus Group-VI. Asterisks represents the different level of significance (indicates p<0.001).***

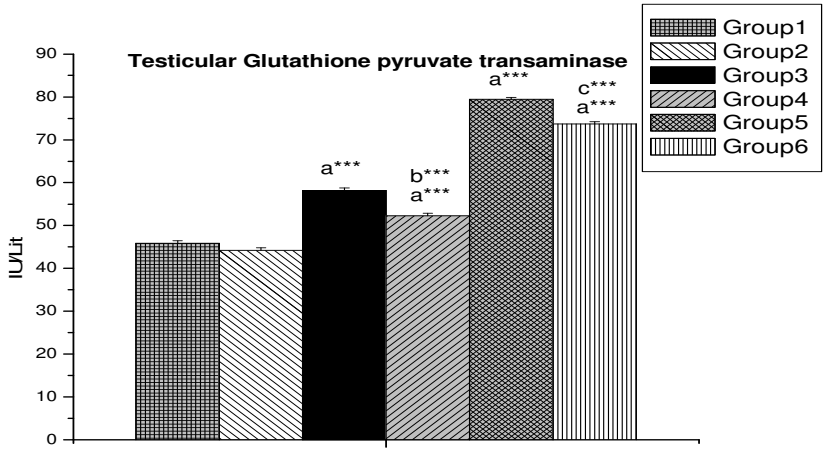

Figure 4

shows the effect of Zinc and α- lipoic acid on Testicular Glutamate Pyruvate Transaminase (GPT) in Cypermethrin induced male albino rat. Results are expressed as Mean±SEM. Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Superscript a, Group-I versus all other groups; Superscript b Group-III versus Group-IV; Superscript c Group-V versus Group-VI. Asterisks represents the different level of significance (indicates p<0.001).***

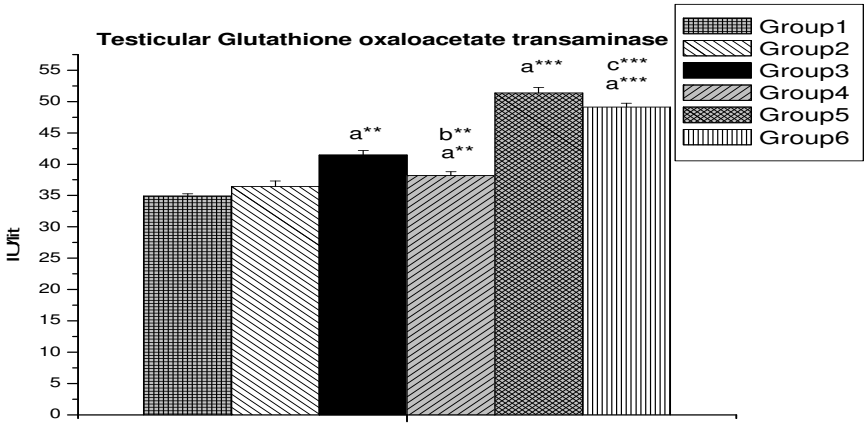

Figure 5

shows the effect of Zinc and α- lipoic acid on Testicular Glutamate Oxaloacetate Transaminase (GOT) in Cypermethrin induced male albino rat. Results are expressed as Mean±SEM. Analysis is done by ANOVA followed by multiple comparison two-tail t-tests Superscript a, Group-I versus all other groups; Superscript b Group-III versus Group-IV; Superscript c Group-V versus Group-VI. Asterisks represents the different level of significance (** indicates p<0.01 , indicates p<0.001).***

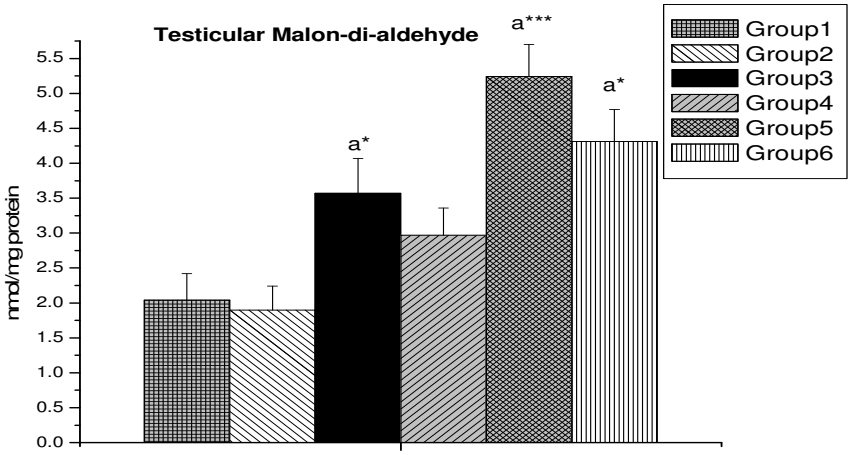

Figure 6

shows the effect of Zinc and α- lipoic acid on Testicular Malon-di-aldehyde in Cypermethrin induced male albino rat. Results are expressed as Mean±SEM. Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Superscript a, Group-I versus all other groups. Asterisks represents the different level of significance (* indicates p<0.05, *** indicates p<0.001).

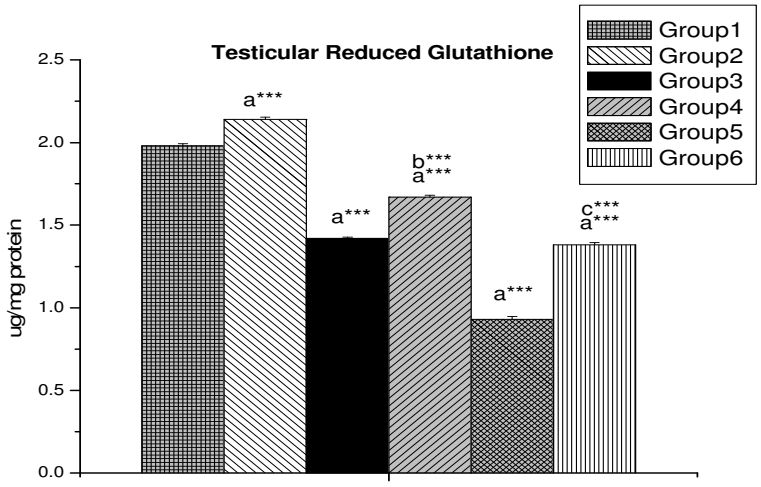

Figure 7

shows the effect of Zinc and α- lipoic acid on Reduced Glutathione in Cypermethrin induced male albino rat. Results are expressed as Mean±SEM. Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Superscript a, Group-I versus all other groups; Superscript b Group-III versus Group-IV; Superscript c Group-V versus Group-VI. Asterisks represents the different level of significance (indicates p<0.001).***

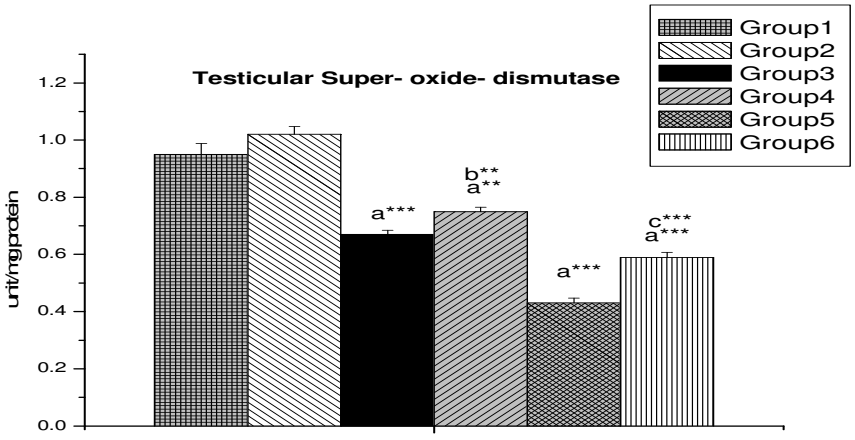

Figure 8

shows the effect of Zinc and α- lipoic acid on Super-oxide dismutase in Cypermethrin induced male albino rat. Results are expressed as Mean±SEM. Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Superscript a, Group-I versus all other groups; Superscript b Group-III versus Group-IV; Superscript c Group-V versus Group-VI. Asterisks represents the different level of significance (** indicates p<0.01, indicates p<0.001).***

Figure 1 shows the effect of zinc and α-lipoic acid on testiculo-somatic index in cypermethrin induced male albino rat. The Testicular index has been decreased significantly ($p<0.001$) in cypermethrin high dose treated group which elevated by the combined treatment of zinc and α-lipoic acid. The effect of zinc and α-lipoic acid on rat epididymal sperm motility in cypermethrin intoxicated male albino rat is shown in Figure 2. In cypermethrin-treated group, epididymal sperm motility decreased significantly ($p<0.001$) compared to the control group. Treatment of zinc and α- lipoic acid decreased the cypermethrin toxicity and restored the normal status of the testicular tissue to a great extent. Table 1 shows the effect of zinc and α-lipoic acid on epididymal sperm morphology of

cypermethrin induced male albino rat. From this table it is observed that abnormal sperms were increased significantly in cypermethrin treated groups which were alleviated by zinc and α- lipoic acid. The effect of zinc and α- lipoic acid on the seminal plasma fructose concentration of cypermethrin induced male albino rat is shown in Figure 3. Seminal vesicular fructose levels were significantly higher in cypermethrin treated group compared to that of control rats. Zinc and α- lipoic acid restored it to a good extent. Testicular glutamate pyruvate transaminase (GPT) (Figure 4) and testicular glutamate oxaloacetate transaminase (GOT) (Figure-5) levels were increased in cypermethrin induced male albino rat in a dose-dependent manner which were altered after the treatment of zinc and α- lipoic acid. In testis increased testicular GPT and GOT levels suggest that cypermethrin causes testicular damage which was prevented by zinc and α- lipoic acid. Figure 6 shows the effect of Zinc and α- lipoic acid on testicular malon-di-aldehyde (MDA) in cypermethrin induced male albino rat. The Testicular MDA level increased significantly ($p<0.05$, $p<0.001$) in cypermethrin-treated groups in a dose dependent manner. Zinc and α- lipoic acid treatment decreased the cypermethrin toxicity. Super-oxide dismutase (SOD), in Cypermethrin induced male albino rat decreased significantly ($p<0.001$) in cypermethrin high dose-treated group compared to the control group (Figure 7). Treatment of zinc and α- lipoic acid decreased the cypermethrin toxicity and restored the oxidative status of the testicular tissue to some extent. Figure 8 shows the effect of zinc and α- lipoic acid on reduced glutathione in cypermethrin induced male albino rat. The Testicular GSH conc. has been decreased significantly ($p<0.001$) in high dose cypermethrin-treated group. GSH conjugation is recognized as a major detoxification mechanism in reproductive tissue. Administration of zinc and α-lipoic acid reduced cypermethrin mediated oxidative stress significantly.

4. DISCUSSION

The present study was conducted to evaluate the adverse effects of cypermethrin on the reproductive system of male Wistar rats, and also to assess the alleviating role of zinc and α-lipoic acid under this toxic condition. Organ weight is a basic benchmark for the toxicological studies (Yavasoglu A et al. 2008). The testicular weight is basically dependent on the mass of the differentiated spermatogenic cells. Decreased number of germ cells, inhibition of spermatogenesis and steroidogenic enzyme activity may be the causes of reduction in testicular weight (Takahashi O and Oishi S, 2001). The testicular index decreased by cypermethrin effectively elevated by the combined treatment of zinc and α-lipoic acid. This may be due to the preventive role of zinc and α-lipoic acid on testicular damage. It was reported (Song L et al. 2008) that cypermethrin decrease sperm motility in vitro. In this study, reduced sperm motility decreased may be due to mitochondrial enzyme activity of the spermatozoa, altered fructose synthesis and secretion by the accessory glands. Zinc and Lipoic acid combination ameliorated the cypermethrin induced reduction in sperm motility. Morphological alteration of sperm cell caused by cypermethrin can be grouped into primary or secondary abnormalities according to the classification by Noarkes DE et al.2004.The only primary sperm abnormality was rudimentary tail abnormality. Some secondary abnormalities i.e. bent mid-piece,curved mid-piece,bent tail,curved tail,normal tail without head,normal head without a tail,looped tail and coiled tail were also found in this study.. The presence of rudimentary tail sperm abnormality was observed higher in cypermethrin treated group rats relative to the control. An aberration in the process of spermatogenesis is the main cause of primary sperm abnormality Hafez ESE. 1987). It was observed that curved mid-piece sperm cell abnormality had the highest occurrence, followed by, curve tail, bent mid piece, bent tail, head less tail, tail less head. Sperm cells which are matured from damaged seminiferous tubules show secondary abnormalities (Thomas MJ and Thomas JA, 2001). But treatment of zinc and α- lipoic acid decrease sperm abnormality significantly ($p<0.001$).It may be due to the combined ameliorative effect of zinc and α- lipoic acid in the reproductive system. Fructose production is a marker of seminal vesicular function due to its inverse relationship with spermatozoa motility. Fructose levels in seminal vesicle homogenates were significantly higher in cypermethrin treated group compared to control rats. Zinc and α- lipoic acid reduced it effectively which indicates the ability of zinc and α-lipoic acid to restore the

normal testicular status. In testis increased testicular GPT and GOT levels suggest that cypermethrin causes testicular damage. Increase of transaminase activity along with the decreased of content of free radical ($O_2^{\cdot}$) scavengers are probably the consequence of cypermethrin induced pathological changes in testis. Oxidative stress defines an imbalance between the formation of reactive oxygen species (ROS) and antioxidative defense mechanisms. During pyrethroid metabolism, reactive oxygen species (ROS) were generated and caused oxidative stress in intoxicated animals (Gupta A et al. 1999). In oxidative stress, lipid peroxidation is occurred due to excessive free radical production and is considered a primary mechanism of cell membrane destruction and cell damage. Malondialdehyde (MDA) is the end product of lipid peroxidation. Cypermethrin increased the testicular MDA; simultaneously it decreased testicular GSH content and SOD activity in this study. But zinc and α-lipoic acid decreased the levels of lipid peroxidation (MDA) in male Wister rats. GSH plays a major role in protecting the cell against oxidative damage by reacting with ROS. In healthy human cells normally ~98% of the total GSH exists in the reduced form while a much smaller fraction (~1%) exists in the oxidized forms – GSSG. (Griffith OW. 1981; Dringen R and Hirrlinger J, 2003; Kennett E et al. 2005). Superoxide dismutase (SOD) is involved in the clearance of superoxide. In the present investigation, decreased levels of SOD level were observed in cypermethrin induced male Wistar rats . In cypermethrin-intoxicated animals the decrease in the activity of superoxide dismutase may be due to the consumption of this enzyme in converting superoxide ($O_2^{\cdot -}$) to hydrogen peroxide (H_2O_2). H_2O_2 is then converted to H_2O by glutathione-S-transferase (GST) and catalase (CAT) enzymes (Mates JM and Sanchez- Jimenez F, 1999). Super oxide dismutase (SOD) protects tissues from oxidative stress and damage by catalyzing the conversion of ($O_2^{\cdot -}$) to H_2O. SOD contains both copper and zinc, zinc is known to induce the production of metallothionein, which is very rich in cysteine and is an excellent scavenger of OH (Prasad AS. 1993).After the treatment of zinc and α-lipoic acid, the activities of the enzyme were significantly increased. The present study demonstrated that zinc and α-lipoic acid decreased the lipid peroxidation and thereby augmented the endogenous antioxidant enzyme in cypermethrin induced male rats and the observed properties may be attributed to the antioxidant properties present in zinc and α-lipoic acid, which is one of the most essential properties of any pesticide antidote. The decreased GSH content and SOD activities and increased MDA level in testis suggest that cypermethrin caused testicular damage. Zinc and α- lipoic acid were used to prevent oxidative damage by interrupting the propagation of the oxidation of polyunsaturated fatty acids. Zinc is not distributed uniformly in tissues; it can show a protective effect especially when administered prior to cypermethrin. From a recent study it has been come to know that when lipoic acid is absent, interaction of other antioxidants are not well, thereby their ability to protect cells are reduced (Packer L et al. 1995). Zinc and lipoic acid tried to normalize the reproductive parameters at a good extent. These observations might also indicate that zinc and lipoic acid has therapeutic effects on cypermethrin -induced male reproductive toxicity.

5. CONCLUSION

From the above discussion it can be concluded that cypermethrin-induced testicular toxicity, lipid peroxidation, oxidative stress in male rats and conjunction supplementation and treatment with zinc and α-lipoic acid has resulted in pronounced ameliorating effect especially at the end of the experiment emphasizing its antioxidant role.

CONFLICT OF INTEREST

Authors declare that there are no conflicts of interests.

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