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Comparative Evaluation of Therapeutic Potential of *Apium graveolens* and Aloe Vera on the Reproductive System of Cadmium Treated Male Rats

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Abstract

Efficacy of Aloe vera vs *Apium graveolens* on cadmium treated rats Cadmium (Cd) is a well-known environmental toxicant that is naturally present in air, water and soil. It causes dangerous health problems by generating reactive oxygen species. The reproductive system is most vulnerable to oxidative damage and therefore most affected by Cd. Zinc (Zn) is an essential antioxidant and a chelating agent that is capable of protecting the testis from Cd induced toxicity. *Apium graveolens* commonly known as Celery and Aloe vera are herbal plants rich in antioxidants and they improve various sperm parameters. Male Wistar albino rats were randomly divided into 11 groups. Control received 0.5% Carboxymethyl cellulose in distilled water; the experimental groups such as Cd received 10mg/Kg body weight of CdCl2; Cd+Zn received 10mg/Kg bodyweight of CdCl2+40mg/Kg body weight of ZnCl2; Cd+AV200 received 10mg/Kg bodyweight of CdCl2+200 mg/Kg body weight of Aloe vera; Cd+AV400 received 10mg/Kg body weight of CdCl2+400 mg/Kg body weight of Aloe vera; Cd+AV200+Zn received 10mg/Kg bodyweight of CdCl2+200 mg/Kg body weight of Aloe vera+ 40mg/Kg body weight of ZnCl2 ; Cd +AV400+Zn received 10mg/Kg bodyweight of CdCl2+400 mg/Kg body weight of Aloe vera+ 40mg/Kg body weight of ZnCl2 ; Cd+AG200 received 10mg/Kg bodyweight of CdCl2+200 mg/Kg body weight of *Apium graveolens;* Cd +AG400 received 10mg/Kg body weight of CdCl2+400 mg/Kg body weight of *Apium graveolens;* Cd+AG200+Zn received 10mg/Kg bodyweight of CdCl2+200 mg/Kg body weight of *Apium graveolens*+40mg/Kg body weight of ZnCl2 ; Cd+AG400+Zn received 10mg/Kg bodyweight of CdCl2+400 mg/Kg body weight of *Apium graveolens* +40mg/Kg body weight of ZnCl2 all in 0.5% CMC. The hydroalcoholic extracts of Aloe vera and *Apium graveolens* were used in this experiment. The experiment was conducted for a duration of 56 days. At the end of the experiment blood and tissues were collected and histopathology, sperm analysis, lipid peroxidation and hormone assays were performed. The therapeutic potential of Aloe vera and *Apium graveolens* at two doses (200 and 400 mg/kg body weight) with and without Zn supplementation was evaluated in this experiment. The rats treated with Cd showed severe testicular damages. Zn offered protection from the damages done by cadmium. The hydroalcoholic extracts of Aloe vera and *Apium graveolens* at concentrations of 200 mg/Kg body weight showed better protective effect than 400 mg/kg body weight and the protecting nature was enhanced by zinc supplementation. Comparatively, hydroalcoholic extract of *Apium graveolens* at a dose of 200mg/Kg body weight supplemented with Zn offers the best protection to the testes against damages caused by Cd.

Keywords: Infertility; Spermatogenesis; Cadmium; Zinc; Aloe vera; *Apium graveolens*

Introduction

Infertility is a major health concern in human society. Environmental and occupational exposure to toxic substances such as cadmium (Cd) is one of the most important aetiologies. Cd, a toxic transitional metal element, can cause irreversible damage to various tissues of the body including testis [1]. The World Health Organization (WHO) has listed cadmium poisoning as a major concern for public health [2]. Cadmium accumulates in soil from various human activities. Cadmium is found in nature as cadmium oxide, cadmium sulphide, cadmium carbonate and cadmium chloride. Cd is highly soluble, as compared to other metals, hence readily taken up by plants [3].

Once Cadmium enters the body, it initiates free radical production that causes oxidative damage to lipids, proteins and DNA, and triggers pathological conditions in humans as well as animals [4,5]. Though the basic mechanism underlying how cd induces pathology is not known completely, it is clear that Cd can induce oxidative response in various organs such as reproductive organs especially testis by breaching Blood-Testis Barrier (B-T-B). Cd induces oxidative stress which is dose, duration and tissue dependent [6]. It is by either displacement of redox-active metals, depletion of redox scavengers, inhibition

of anti-oxidant enzymes o r inhibition of th e electron transport chain resulting in mitochondrial damage [7,8].

All organs, tissues, and fluids of the body contain an essential antioxidant trace element the zinc, which is the second most abundant trace element in the body after iron [9]. It plays an important role in cell proliferation, differentiation, normal growth, immune functions, and wound healing. Any plant, whole or part of it, has compounds in it which can be used as a precursor for the synthesis of a useful drug for therapeutic purposes is a plant with medicinal value [10]. Antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens which are found in many of the world's medicinal plant species have great free radical scavenging potential and reduce disease risk [11].

Apium graveolens commonly known as celery, is scented, with solid and fleshy stems growing up to a height of about a meter on an average, belongs to the parsley (Umbelliferae) species of the Apiaceae family [12]. This plant is rich in active compounds such as phytoestrogens, which can be highly effective in the treatment of infertility and reproductive system related problems and antioxidant compounds that increase sperm function and can improve fertility [13].

A study by stated the following findings on Aloe vera. Aloe vera enriches vitamin C and E and thereby enforces its antioxidant role, its extract contains anti-apoptosis factor which stimulates germinal cell division hence increasing primary spermatocytes and stem cells, an increase in testosterone hormone is also observed due to stimulation of Leydig cells by it, Vitamin E in the extract prevents reduction in the number of Leydig and Sertoli cells, improves testis weight, diameter of seminiferous tubule and thickness of the germinal epithelium [14]. Aloe vera is rich in vitamins that include antioxidants like A and C, B (thiamine, niacin, B2 -Riboflavin), B12, and folic acid and it is a source of naturally present antioxidants potentially capable of reducing fat oxidation and oxidative stress [15].

The aim of the present study is to evaluate the protective effects of hydro-alcoholic extract of *Apium graveolens* and Aloe vera with and without zinc supplementation on the reproductive system of cadmium treated rats. Administering Aloe vera and *Apium graveolens* that are rich in antioxidants is expected to prevent the germ cell damage due to Cd. Also, the role of Zn as a chelator and antioxidant is being studied, in this experiment. It is expected to protect testis from cadmium toxicity.

Materials and Methods

Male Wistar albino rats were used for this study and were obtained from Biogen (Bengaluru, India) and kept in the animal house of Siddha Central Research Institute (Chennai, India). The rats weighed 180-200 g and were kept in a controlled condition for 5 days for acclimatization and kept in the same condition until the end of the experiment. The animals were housed in a plastic cage with food and water supply, floored by husk which was replaced every 5 days. After adaptation to the new environment, the rats were randomly divided into 11 groups of 6 animals each **(Table 1).**

Sl No	Groups
1	Control
2	Cd
3	Cd+Zn
4	Cd+AV200
5	Cd+AV400
6	Cd+AV200+Zn
7	Cd+AV400+Zn
8	Cd+AG200
9	Cd+AG400
10	Cd+AG200+Zn
11	Cd+AG400+Zn

Table 1: Grouping of animals. Abbreviations used: Cd-Cadmium, Zn-Zinc, AV- Aloe vera, AG-*Apium graveolens* and CMC- Carboxy Methyl Cellulose.

Preparation o f hydroalcoholic extract o f Apium graveolens and Aloe vera:

10 kg of fresh *Apium graveolens* commonly known as celery were obtained from Conoor, Tamilnadu, India. Plants were certified by a Pharmacognosist before preparing the extract. The whole plant was used for preparing the hydroalcoholic extract. They were thoroughly washed, cut and shade dried. 10 kg of fresh Aloe vera plants were obtained from Amudham Nandavanam Garden in Chennai, India. The plants were certified by a Pharmacognosist. They were washed, cut and shade dried. The coarsely powdered drug was soaked in distilled water and ethanol mixture (1:1 ratio) for 24 hrs. After 24 hrs, the drug was filtered using a filter paper and concentrated under vacuum in a Rotary flash evaporator (Rotavapor R-300). The plant residue was again soaked in hydro alcohol mixture for second time extraction similar to first time extraction. The first and second extracts were combined. The residual moisture was freed by heating over water bath and then the solvent free extract was stored in an air tight glass container for use. The yield of *Apium graveolens* was 377g and Aloe vera was 292g. The extracts were prepared twice with the same amount of raw materials for the entire experiment.

Drug administration:

Group 1: received 1 ml of 0.5% CMC suspension in distilled water.

Group 2: received 10mg/kg body weight of $CdCl_2$ in 0.5% CMC

Group 3: received 10mg/kg body weight of $CdCl_2$ and 40mg/kg body weight of $ZnCl_2$ in 0.5% CMC

Group 4: received 10mg/kg body weight of $CdCl_2$ and 200mg/kg body weight of Aloe vera in 0.5% CMC

Group 5: received 10mg/kg body weight of $CdCl_2$ and 400mg/kg body weight of Aloe vera in 0.5% CMC

Group 6: received 10mg/kg body weight of $CdCl_2$, 40mg/kg body weight of $ZnCl_2$ and 200mg/kg body weight of Aloe vera in 0.5% CMC

Group 7: received 10mg/kg body weight of CdCl2, 40mg/kg body weight of ZnCl2 and 400mg/kg body weight of Aloe vera in 0.5% CMC

Group 8: received 10mg/kg body weight of CdCl2 and 200mg/kg body weight of *Apium graveolens* in 0.5% CMC

Group 9: received 10mg/kg body weight of CdCl2 and 400mg/kg body weight of *Apium graveolens* in 0.5% CMC

Group 10: received 10mg/kg body weight of CdCl2, 40mg/kg body weight of ZnCl2 and 200mg/kg body weight of *Apium graveolens* in 0.5% CMC

Group 11: received 10mg/kg body weight of CdCl2, 40mg/kg body weight of ZnCl2 and 400mg/kg body weight of *Apium graveolens* in 0.5% CMC

Statistical analysis:

Sigma Plot 13.0 Systat software, USA was used for statistical calculations. Statistical differences were determined by one-way ANOVA with All Pairwise Multiple Comparison Procedures using Student-Newman-Keuls Method (P value <0.001).

Results

Concentration of sperm:

The concentration of sperm in Cd treated rats (24.2± 3.1X106/ml) was significantly (<0.001) lower by 3 folds than control. In Cd+Zn treated rats, the concentration (47.8± 3.4X106/ml) was significantly (<0.001) lower by 1.5 folds than control and at the same time significantly (<0.001) higher by 2 folds than the Cd treated rats. In Cd+AV200 treated rats the concentration (62± 3.2X106/ml) was significantly (<0.001) higher by 2.5 folds than Cd treated rats and no significant difference from control group was observed. In Cd+AV400 (51.8±6X106/ml), Cd+AV200+Zn (55.4±3.1X106/ml) and Cd+AV400+Zn (50.9±1.7X106/ml) treated rats the sperm concentration was significantly (<0.001) lower by 1.4, 1.3 and 1.4 folds respectively than control rats but significantly (<0.001) higher by 2.1, 2.3 and 2.1 folds respectively than Cd treated rats. In Cd+AG200 treated rats the sperm concentration (61.4±1.5X106/ml) was significantly (<0.001) higher by 2.5 folds than Cd treated rats, however no significant difference from control group was observed. In Cd+AG400 treated rats the sperm concentration (38.7±2.6X106/ml) was significantly (<0.001) lower by 1.9 folds than control rats but at the same time significantly (<0.001) higher by 1.6 folds than Cd treated rats. In Cd+AG200+Zn (71.1±1.8X106/ml) and Cd+AG400+Zn (50.5±11.6X106/ml) treated rats the sperm concentration was significantly (<0.001) higher by 2.9 and 2.1 folds respectively than Cd treated rats, however no significant difference from control group was observed **(Figure 1).**

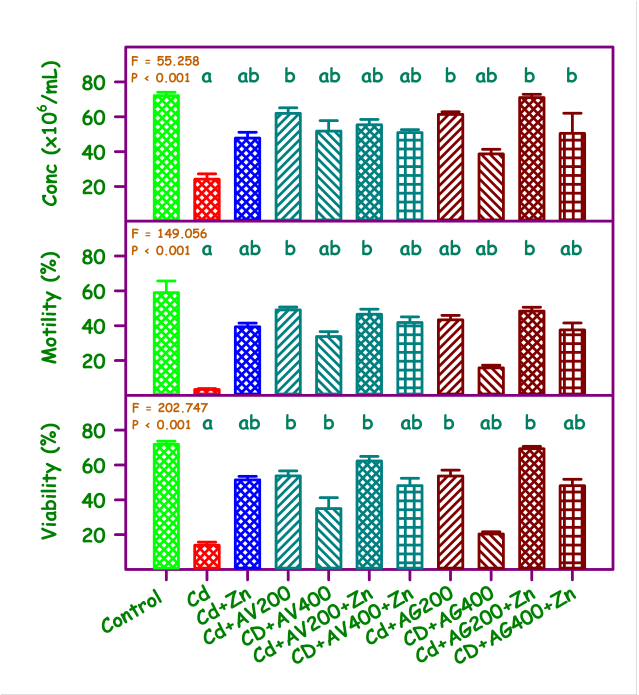

Figure 1: Sperm concentration, Motility and Viability in (Cd, 10 mg/kg, p.o.) toxicity and its protection by zinc (Zn, 40 mg/kg, p.o.), Aloe vera extract (AV, 200 and 400 mg/kg, p.o.), *Apium graveolens* extract (AG, 200 and 400 mg/kg, p.o.) and their combinations. Values are mean+SD (n = 6 each). The 'F' and 'P' values are by one-way ANOVA with Student Newman Keul's multiple comparison test. Significantly different from control group. Significantly different from Cd group.

Motility of sperm:

The motility of sperm in Cd treated rats (3.4± 0.5%) was significantly (<0.001) lower by 17.4 folds than control. In Cd+Zn treated rats, the motility (39.3± 2.2%) was significantly (<0.001) lower by 1.5 folds than control and at the same time significantly (<0.001) higher by 11.2 folds than the Cd treated rats. In Cd +AV200 treated rats the motility (49.0± 1.7%) was significantly (<0.001) higher by 14.4 folds than Cd treated rats and no significant difference from control group was observed. In Cd +AV400 treated rats, the motility (33.8± 2.8%) was significantly (<0.001) lower by 1.7 folds than control but significantly (<0.001) higher by 10 folds than Cd treated rats. In Cd+AV200+Zn the motility (46.5±3%) was significantly (<0.001) higher by 13.7 folds than Cd treated rats and no significant difference from control group was observed. In Cd+AV400+Zn (41.8± 3.2%), Cd+AG200 (43.3± 2.6%) and Cd+AG400 (15.8± 1.5%) treated rats the sperm motility was significantly (<0.001) lower by 1.4, 1.3 and 3.7 folds respectively than control rats but significantly (<0.001) higher by 12.3, 12.7 and 4.6 folds respectively than Cd treated rats. In Cd +AG200+Zn treated rats the sperm motility (48.4± 2.3%) was significantly (<0.001) higher by 14.2 folds than Cd treated rats however no significant difference from control group was observed. In Cd+AG400+Zn treated rats the sperm motility (37.5±4%) was significantly (<0.001) lower by 1.6 folds than control but significantly (<0.001) higher by 11 folds than Cd treated rats.

Viability of sperm:

The viability of sperm in Cd treated rats (13.9± 1.8%) was significantly (<0.001) lower by 5.2 folds than control. In Cd+Zn treated rats, the viability (51.5± 2%) was significantly (<0.001) lower by 1.5 folds than control and at the same time significantly (<0.001) higher by 3.7 folds than the Cd treated rats. In Cd +AV200 (53.8± 2.8%), Cd+AV400 (35.0± 6.3%) and Cd+AV200+Zn (62.4±2.6%) the viability was significantly (<0.001) higher by 3.9, 2.5 and 13.7 folds than Cd treated rats however no significant difference from control group was observed. In Cd+AV400+Zn treated rats, the viability (48.2± 4.2%) was significantly (<0.001) lower by 1.5 folds than control and at the same time significantly (<0.001) higher by 3.5 folds than the Cd treated rats. In Cd +AG200 treated rats the viability (53.7± 3.4%) was significantly (<0.001) higher by 3.9 folds but no significant difference from control was observed. In Cd+AG400 (20.4± 1.2%) treated rats the sperm viability was significantly (<0.001) lower by 3.5 folds than control rats but significantly (<0.001) higher by 1.5 folds than Cd treated rats. In Cd+AG200+Zn treated rats the viability (69.3± 1.4%) was significantly (<0.001) higher by 5 folds but no significant difference from control was observed. In Cd +AG400+Zn (48.1±3.7%) treated rats the sperm viability was significantly (<0.001) lower by 1 .5 folds t han control but significantly (<0.001) higher by 3.5 folds than Cd treated rats.

Lipid peroxidation:

The lipid peroxidation measured in terms of malondialdehyde (MDA) for various groups is as follows. The level of MDA in Cd group (4.06 nmol/ml) was significantly (<0.001) higher by 2.5 folds than the control group but no significant difference from Cd was observed. In Cd+Zn group the level of MDA (3.03 nmol/ml) was significantly (<0.001) higher by 1.9 folds than the control group but significantly (<0.001) lower by 1.3 folds than the Cd treated rats. In Cd+AV200 group, level of MDA (2.89 nmol/ml) was significantly (<0.001) higher by 1.8 folds than the control group but significantly (<0.001) lower by 1.4 folds than the Cd group. In Cd+AV400 group the level of MDA (3.43 nmol/ml) was significantly (<0.001) higher by 2.1 folds than the control group, however no significant difference from Cd was found. The level of MDA in the groups Cd+AV 200+Zn (2.42 nmol/ml) and Cd+AV 400+Zn (2.87 nmol/ml), was significantly (<0.001) higher by 1.5 and 1.8 folds than the control group but significantly (<0.001) lower by 1.7 and 1.4 folds than the Cd treated rats respectively. In Cd+AG 200 group the level of MDA (2.5 nmol/ml) was significantly (<0.001) lower by 1.6 folds than the Cd treated rats. At the same time, no significant difference from control was observed in this group. In Cd+AG 400 group the level of MDA (3.21 nmol/ml) was significantly (<0.001) higher by 1.9 folds than control rats but significantly (<0.001) lower by 1.3 folds than Cd treated rats. In Cd+AG 200+Zn group, the level of MDA (1.92 nmol/ml) was significantly (<0.001) higher by 2.1 folds than Cd group, however no significant difference from contro group was observed. Finally in Cd+AG 400+Zn group, the level of MDA (3.28 nmol/ml) was significantly (<0.001) higher by 2 folds than control group, but no significant diffrence from Cd group was found **(Figure 2)**.

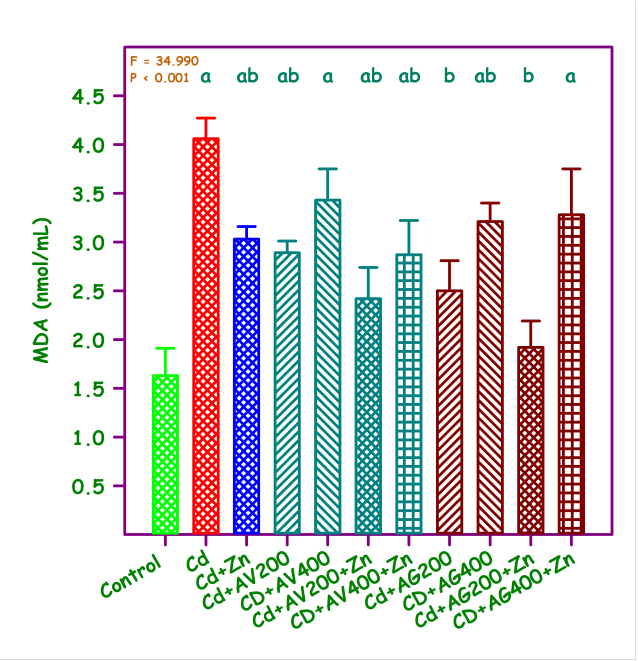

Figure 2: MDA level in LPO expressed in nmol of MDA formed /ml cadmium (Cd, 10 mg/kg, p.o.) toxicity and its protection by zinc (Zn, 40 mg/kg, p.o.), Aloe vera extract (AV, 200 and 400 mg/kg, p.o.), *Apium graveolens* extract (AG, 200 and 400 mg/kg, p.o.) and their combinations. Values are mean +SD (n = 6 each). The 'F' and 'P' values are by one-way ANOVA with Student Newman Keul's multiple comparison test. aSignificantly different from control group. bSignificantly different from Cd group.

Histopathology:

Haematoxylin and Eosin of Testis:

Routine Haematoxylin and Eosin staining was done to observe the changes in the microanatomical features of testis in various experimental groups and compared with control group. There were severe pathological changes in the testis of cadmium chloride treated rats on microscopic examination. The seminiferous epithelium appeared thin due to degeneration of spermatogenic cells, distortion of seminiferous tubules lined by Sertoli cells with increased collagen deposition, incomplete and arrested spermatogenesis, incomplete or loss of spermatogenesis, empty tubules without spermatozoa, thickening of basal membrane with fibrosis, vacuolization of the Sertoli cells and multinucleation of the spermatogenic cells transforming into/becoming multi-nucleated giant cells with fragmented nucleus and shrunken cellular morphology indicating apoptotic pathology. In Cd+Zn treated rats, these pathological changes were reduced and seminiferous tubules appeared near normal hence less damage compared to Cd group was observed. The Cd+AV200 group showed very significant protection unlike Cd+AV400 group which showed damage to the seminiferous epithelium, with slightly increased interstitial space but exhibited better integrity of cellular details compared to cadmium treated groups. The Cd+AV200+Zn showed less damage as the seminiferous tubules and its epithelium appeared to be more intact compared to Cd+AV400+Zn in which some

seminiferous tubules showed some degree of damage and reduced thickness of epithelium with less numbers of germ cells and spermatozoans in the lumen. However, both the groups showed protective effects of Aloe vera and zinc compared to cadmium treated groups. The Cd+AG200 treated rats showed significantly less damage compared to the Cd+AG400. Cd +AG200+Zn treated rats showed the best histological details equivalent to control as evident by the observation under microscope. The seminiferous tubules and its epithelium were intact with various stages of developing germ cells in the epithelium and numerous spermatozoans in the lumen. However, Cd+AG400+Zn showed slightly distorted seminiferous tubule lumen and epithelium with few spermatozoans in the lumen. Hence the rats in the treatment group which received Cd +AG200+Zn showed that the extract was able to preserve the cellular architecture from cadmium induced alterations to a significant extent (Figure 3).

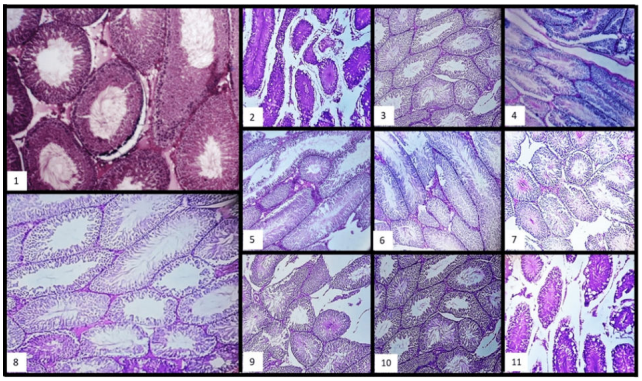

Figure 3: Photomicrograph of H and E stained section of Testis of Control, Cd, Cd+Zn, Cd+AV200, Cd+AV400, Cd+AV200+Zn, Cd +AV400+Zn, Cd+AG200, Cd+AG400, Cd+AG200+Zn and Cd +AG400+Zn at 20X magnification. Free sperms can be seen in the lumen of seminiferous tubules of control rats. Thick seminiferous epithelium is also seen with various stages of spermatogenesis. The interstitial space, thickness of seminiferous epithelium and population of mature sperm in the lumen of seminiferous tubules are compared. 1-Control, 2- Cd, 3-Cd+Zn, 4-Cd+AV200, 5-Cd+AV400, 6-Cd+AV200+Zn, 7-Cd +AV400+Zn, 8-Cd+AG200, 9-Cd+AG400, 10-Cd+AG200+Zn, 11-Cd +AG400+Zn.

Immunohistochemistry of Testis:

Immunohistochemistry of testis from all the groups showed in general, expression of iNOS which was seen as dark spots in the image. It is found to be highest in Cd treated rats and least in Cd +Zn treated rats. In Cd+AG200+Zn, Cd+AV200+Zn the expression was relatively less compared to cadmium (Figure 4).

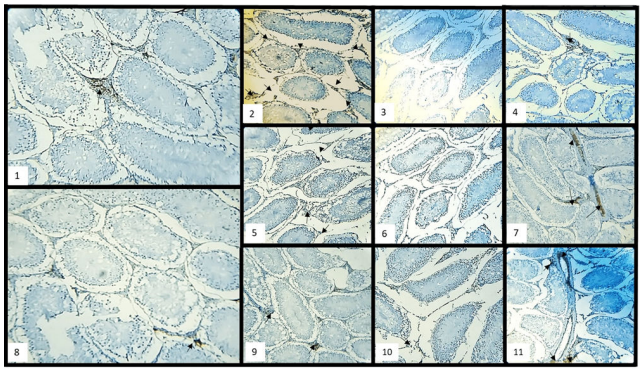

Figure 4: Photomicrograph of Immunohistochemistry of testis of control Cd, Cd+Zn, Cd+AV200, Cd+AV400, Cd+AV200+Zn, Cd +AV400+Zn, Cd+AG200, Cd+AG400, Cd+AG200+Zn and Cd +AG400+Zn for the expression of iNOS seen as dark brown colour for positive protein expression (indicated by black arrow) mostly in the interstitial space between the seminiferous tubules. 1-Control, 2- Cd, 3-Cd+Zn, 4-Cd+AV200, 5-Cd+AV400, 6-Cd+AV200+Zn, 7-Cd+AV400+Zn, 8-Cd+AG200, 9-Cd+AG400, 10-Cd+AG200+Zn, 11-Cd+AG400+Zn.

Quantitative analysis was performed by counting the number of positive cells in microscope fields. To produce numerical data from our immunohistochemical results, an image analysis was performed using Image Pro Plus software (Table 2).

Groups	Relative iNOS expression
Control	4.18
Cd	11.5
Cd+Zn	1.21
Cd+AV200	5.18
Cd+AV400	7.05
Cd+AV200+Zn	5.01
Cd+AV400+Zn	7.12
Cd+AG200	4.54
Cd+AG400	6.16
Cd+AG200+Zn	3.17
Cd+AG400+Zn	6.71

Table 2: Relative expression of iNOS. (Cd, 10 mg/kg, p.o.) toxicity and its protection by zinc (Zn, 40 mg/kg, p.o.), Aloe vera extract (AV, 200 and 400 mg/kg, p.o.), *Apium graveolens* extract (AG, 200 and 400 mg/kg, p.o.) and their combinations.

Hormone analysis

Testosterone Hormone (TST)

In Cd group, the level of TST (1.9 ng/ml) was significantly (<0.001) lower by 2.6 folds than control. In Cd+Zn treatment group, the level (3.4ng/ml) was significantly (<0.001) higher by 1.8 folds than Cd group, however no significant difference from control group was observed. The level of TST in Cd+AV200 (3.9 ng/ml) was significantly higher by 2 folds than Cd group but no significant difference from control group was observed. In Cd +AV400 the level of TST (3.1 ng/ml) was significantly lower by 1.3 folds than control and significantly higher by 1.6 folds than Cd group. In Cd+AV200+Zn treated rats the level of TST (4.2

ng/ml) was significantly (<0.001) higher than Cd group by 2.2 folds, but no significant difference from control group was found. In Cd+AV400+Zn group, the level of TST (2.9 ng/ml) was significantly (<0.001) lower by 1.4 folds than control but significantly (<0.001) higher by 1.5 folds than Cd group. In Cd +AG200 group, TST level (3.9ng/ml) was significantly (<0.001) higher by 2 folds but no significant difference from control group was noted. In Cd+AG400 group, level of TST (3.1ng/ml) was significantly (<0.001) lower by 1.3 folds than control, but at the same time was significantly (<0.001) higher by 1.6 folds than Cd group. In Cd+AG200+Zn and Cd+AG400+Zn the level of TST was 4.2ng/ml and 3.9ng/ml respectively. In both these groups the level of TST was significantly (<0.001) higher than Cd group by 2.2 and 2 folds respectively and no significant difference from control group was found (Figure 5).

Luteinizing Hormone (LH)

In Cd group, the level of LH (22.6 ng/ml) was significantly (<0.001) lower by 2.6 folds than control. In Cd+Zn treatment group, the level (53.8 ng/ml) was significantly (<0.001) higher by 2.4 folds than Cd group, however no significant difference from control group was observed. The level of LH in Cd+AV200 (63.6 ng/ml) was significantly higher by 2.8 folds than Cd group but no significant difference from control group was observed. In Cd +AV400 the level of LH (47.9 ng/ml) was significantly lower by 1.2 folds than control and significantly higher by 2.1 folds than Cd group. In Cd+AV200+Zn treated rats the level of LH (62.7 ng/ml) was significantly (<0.001) higher than Cd group by 2.8 folds, but no significant difference from control group was found. In Cd+AV400+Zn group, the level of LH (54.2 ng/ml) was significantly (<0.001) lower by 1.1 folds than control but significantly (<0.001) higher by 2.4 folds than Cd group. In Cd +AG200 group, LH level (49.4 ng/ml) was significantly (<0.001) lower by 1.2 folds than control group but higher by 2.2 folds than Cd group. In Cd+AG400 group, level of LH (42.1 ng/ml) was significantly (<0.001) lower by 1.4 folds than control, but at the same time it was significantly (<0.001) higher by 1.9 folds than Cd group. In Cd+AG200+Zn group the level of LH (58.0 ng/ml) was significantly (<0.001) higher than Cd group by 2.6 folds, but no significant difference from control group was found. In Cd +AG400+Zn the level of LH (49.6 ng/ml) was significantly (<0.001) lower than control group by 1.2 folds but significantly (<0.001) higher than Cd group by 2.2 folds.

Follicular Stimulating Hormone (FSH)

In Cd group, the level of FSH (22.6 ng/ml) was significantly (<0.001) higher by 2.3 folds than control group. The level of FSH in Cd+Zn (11.8 ng/ml), Cd+AV200 (8.6 ng/ml), Cd+AV400 (11.1 ng/ml), Cd+AV200+Zn (8.1 ng/ml), Cd+AV400+Zn (12.0 ng/ml), Cd+AG200 (8.9 ng/ml), Cd+AG400 (10.5 ng/ml), Cd+AG200+Zn (8.4 ng/ml) and Cd+AG400+Zn (12.4 ng/ml) was significantly (<0.001) lower than Cd by 1.6, 2.3, 1.8, 2.5, 1.7, 2.2, 1.9, 2.4 and 1.6 folds respectively but no significant difference from control group was observed **(Figure 5)**.

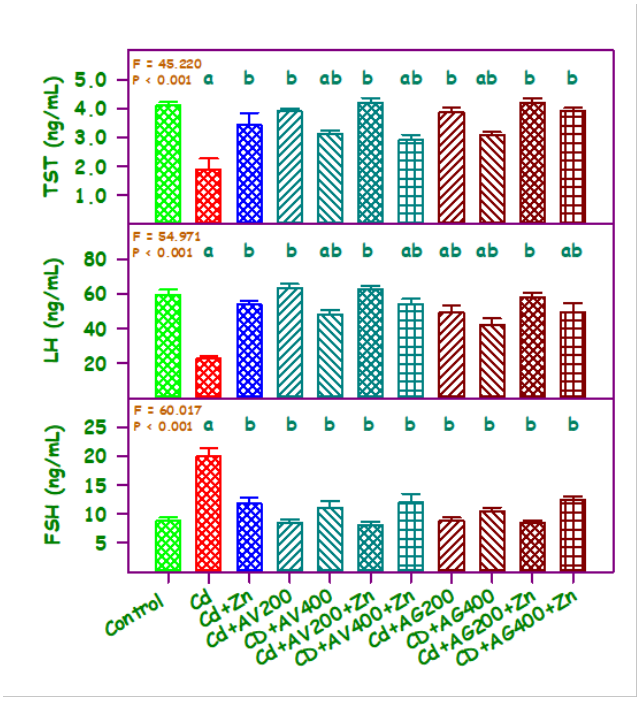

Figure 5: Serum hormone levels in cadmium (Cd, 10 mg/kg, p.o.) toxicity and its protection by zinc (Zn, 40 mg/kg, p.o.), Aloe vera extract (AV, 200 and 400 mg/kg, p.o.), *Apium graveolens* extract (AG, 200 and 400 mg/kg, p.o.) and their combinations. Values are mean+SD (n = 4 each). The 'F' and 'P' values are by one-way ANOVA with Student Newman Keul's multiple comparison test. Significantly different from control group. Significantly different from Cd group.

Discussion

Cd causes a number of metabolic and microanatomical changes, membrane damage, alterations in gene expression and cell death in living organisms [16,17]. Impairment of reproductive capacity by causing severe degeneration of testis, damage to seminiferous tubules and necrosis are some of the consequences of cadmium toxicity in rats [18].

Cadmium causes alteration in androgen status which is indicated by its effect on the weight of the sex organs [19]. In the present study it was found that the testis to body weight ratio decreased and appeared shrunken in Cd treated rats whereas in all the other treatment groups this ratio did not vary significantly. The decrease in organ to body weight ratio could be due to loss of germ cells and interstitial cells. This will render the individual infertile. Like kidney and liver, in testis also Cd administration results in oxidative damage following generation of ROS due to which, antioxidant enzymes such as GPx, SOD and few others show decreased activity with increased LPO [20]. Cadmium induced LPO is at a higher level because the Leydig cell mitochondria and microsomes contain high amount of membrane lipid content. In this study increased LPO was noted in rats treated with Cd and it significantly reduced in treatment groups. In spite of the existence of the blood-testis barrier in testis, oxidative stress can severely damage spermatogenic cells including the basement membrane of the seminiferous tubules due to the high metabolism and cell proliferation in testicular

tissue. Histopathology of testis revealed degeneration of seminiferous epithelium and depletion of germ cells due to this reason. The ROS generated by Cd as a result of oxidative stress, alter the mechanism for binding of receptors, steroidogenesis, and hormone production by disturbing membrane integrity of cells. In the present study the decreased level of LH and Testosterone hormones in Cd treated rats compared to Zn, AV and AG treated ones can be attributed to the fall in total percentage of healthy testosterone producing Leydig cells and increased rate of DNA damage caused due to ROS. The immunohistochemistry of testis showed increased iNOS expression in cadmium treated rats since excessive production of iNOS is an indicative of pathology and inflammatory response.

Fertility is greatly influenced by oxidative stress. Infertile people have a low antioxidant level and high ROS level in their semen. Generally, antioxidants are free radical scavengers which are capable of suppressing or opposing the formation and or their actions of ROS. These include enzymatic (superoxide dismutase, catalase, glutathione peroxidase and reductase) as well as non-enzymatic (vitamin C, zinc, vitamin E, taurine, hypotaurine, and micronutrients) antioxidants. In the present study Aloe vera and *Apium graveolens* were used as source of antioxidants Vitamin C and E and zinc as antioxidant cum chelating agent.

Conclusion

Parízek J observed that Zn could prevent the acute toxicity of cadmium in the testis of rats and mice. In the present study animals treated with Cd+Zn, Cd+AV200+Zn and Cd+AV400+Zn, clearly showed protective effect of Zn over Cd. Zinc plays a major role in protecting the physiological processes such as cellular response to oxidative stress, DNA repair, cell cycle and apoptosis of a cell. It is an important antioxidant and an integral component of the superoxide dismutase enzyme, which can convert superoxide anion into H2O2 and oxygenmolecules. In a study, Cd was administered subcutaneously and its adverse effects were studied. It was found that when Cd was given in combination with Zn simultaneously, the protective effect of Zn was exhibited clearly, and the toxicity caused was less when Cd was administered alone. The present study found similar results. This could be due to antioxidant and chelating properties of Zn. Zn is believed to have a role in production of sperm and viability. The rats treated with Cd+Zn, Cd+AV200+Zn and Cd+AG200+Zn showed increased sperm parameters such as concentration, viability and motility in addition to increased TST and LH levels compared to Cd alone treated rats which supports the argument of protective effect of Zn against Cd as was demonstrated in the present study. The same was reflected in the microanatomical details also. The histological features of testis in Zn treated rats was very well preserved. The present study shows least expression of iNOS in Cd+Zn treated rats as demonstrated by immunohistochemistry studies compared to Cd alone treated rats which clearly demonstrates the protective effect of Zn against Cd.Normal testicular function is restored to some extent by treating the animals with Vit C and Vit E before exposing to Cd because these enzymes can reduce production of ROS in testis. In the present study, the herbal plants used are rich in Vit C and E hence near normal structural and functional integrity was maintained in those groups treated with them. The rats treated with hydroalcoholic extract of *Apium graveolens* showed a significantly good protective effect in the present study. The weight of animals did not show any significant variation as that of Cd treated in which it decreased. Due to its antioxidant and anti-inflammatory properties, celery is able to protect the tissue against harmful effects of free radicals on cells preventing cell death and loss of weight or tissue volume. Previous investigations prove similar results. In the present study Apium graveolens treated rats showed significantly less amount of lipid peroxidation due to its antioxidant properties. The histological features of the testes were very well preserved in Cd+AG200 and CD+AG200+Zn treated rats due to the antioxidant properties of celery. Immunohistochemistry showed less expression of iNOS in Cd+ AG200 with and without Zn. AG 400 was not very effective in protecting the testis from damages done by Cd due to poor dose response. The photomicrographs of IHC testis show less expression of iNOS due to the protection offered by the antioxidants namely apienin and apigenin present in celery. In the evolution and proliferation of germ cells and spermatid differentiation, the level of testosterone is the effective and the most important androgenic hormone and it directly affects Sertoli cells stimulating secretion of testosterone. The hydroalcoholic extract of celery increases testosterone secretion. Antioxidants affect hypothalamic-pituitary-testicular axis there by increase sperm count and fertility. The rats treated with celery showed relatively increased number of germ cells and thick seminiferous epithelium as evident by the histological examinations in the present study. An important parameter to evaluate the function of reproductive system is measuring the blood concentration of reproductive hormones. In the present study, testosterone levels were estimated and it showed significant rise in Cd+AG200+Zn treatment group. Testosterone affects seminiferous tubules and induces spermatogenesis. Similarly, Aloe vera enforces its antioxidant property by reinforcing antioxidant enzymes such as Vitamin C and E by increasing concentration of these vitamins in blood. A significant rise of stem cells and primary spermatocytes compared to control group was demonstrated by a study conducted for a duration of 20 days in which Aloe vera was administered to rats at doses of 100 mg/kg and 200 mg/kg. Anti-apoptosis factor in Aloe vera extract increases stem cells and primary spermatocytes. This by affecting spermatogenesis by stimulating cell division or Leydig cells to secrete testosterone. In the present study the rats were administered with doses of 200mg/Kg and 400mg/Kg of bw showed significant rise in the level of testosterone and LH hormones and also sperm concentration, viability and motility compared to Cd due to the mentioned properties of Aloe vera. However, Cd+AV200 in combination with zinc was found to be more effective than without zinc due to combined actions of antioxidant property of Aloe vera and chelating properties of Zn. AV 400 and AG 400 treatment modality was not very effective with or without Zn, instead it caused some degree of tissue damage as evident by various biochemical, pathological, microanatomical and morphological examinations of the testis tissue and this could be due to poor dose response or the actual reason needs to be explored.

The present study clearly demonstrated that cadmium induces irreversible damage when treated alone and results in reduced fertility potential. Zn protects the testicular tissue from damages caused by cadmium effectively by acting as chelating agent. *Apium graveolens* administered with cadmium offers protection against cadmium toxicity very effectively. Zinc chloride and hydroalcoholic extract of *Apium graveolens* improves morphology, preserves cellular architecture and protects germ cells from apoptosis, spermatogenic cells against free radicals, prevents lipid peroxidation, increases serum levels of testosterone and Luteinizing hormones by virtue of its antioxidant properties slightly better than Aloe vera, though it also has the similar effects. The most effective protection to the rat testis from cadmium induced damage was offered by hydroalcoholic extract of *Apium graveolens* (200mg/Kg body weight), in combination with zinc chloride. Similarly, AV 200 and AG 200 also provide very good protection to the testes from Cd toxicity. However, AV 200 and AG 200 in combination with Zn was more effective due to their combined actions. Cd+Ag200+Zn emerged as the most effective treatment modality for Cd toxicity since it preserves the fertility potential to the greatest extent.

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