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THE REPRODUCTIVE AND GENOTOXIC EFFECTS OF CADMIUM AND THE PROTECTIVE ROLE OF SELENIUM AND ZINC IN ALBINO RAT

(With 5 Tables and 9 Figures)

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التأثير السام للكادميوم على التكاثر و التركيب الجيني والدور الوقائي للسلينيوم والزنك في الفئران البيضاء

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تم في هذه الدراسة تعريض فئران التجارب البيضاء البالغة بعد تقسيمها إلى خمس مجموعات إلى كل من كلوريد الكادميوم وخليط من كلوريد الكادميوم + سلينيت الصوديوم و كلوريد الكادميوم + سلفات الزنك و سلينيت الصوديوم و سلفات الزنك لمدة شهرين. تم تقدير وزن الفئران وأعضائها التناسلية و الغدد الجنسية، حركه و تركيز و نسبه حياه الحيوانات المنوية، تركيز أنزيم الفوسفاتيز الحمضي في المصل ، الكفاءة التناسلية للذكور كما تم فحص الأعضاء التناسليه هستوباثولوجيا. كما تم عمل اختبار لقياس التأثير الطفري للكادميوم. أظهرت النتائج عدم وجود أي تغير معنوي في أوزان الخصى و الحويصلات المنوية وغدة البروستاتة وكذلك في وزن جسم الفئران. حدوث نقص معنوي في نسبة حركة الحيوانات المنوية، نقص معنوي في نسبة الحيوانات المنوية الحية ، انخفاض تركيز الحيوانات المنوية بالبربخ * زيادة معنوية في نشاط أنزيم الفوسفاتيز الحمضي الخاص بالبروستاتا. أحدث العلاج بالسيلينوم والزنك تحسنا ملحوظا في النتائج بينما لم يسفر العلاج بكل من السلينيوم أو الزنك بمفردهما عن أى تغير معنوي في النتائج.أيدت نتائج الفحص الهستوباثولوجي للخصى و البروستاتا النتائج السابقه. أثر الكادميوم تأثيرا معنويا على الكفاءة التناسلية للذكور متمثلة في نقص نسبة حمل الإناث ونقص نسبة الأجنة وزيادة نسبة أماكن زرع وإمتصاص الأجنة. كما أظهرت النتائج أن كلوريد الكادميوم قد أحدث تأثيرا طفريا معنويا حيث استدل على ذلك من زيادة خلايا الدم الحمراء المتعددة الصبغة و المحتوية على نويات بالمقارنة بالمجموعة الضابطة السلبية ، بينما لم يظهر الفحص الميكروسكوبي لخلايا المجموعات المعالجة بكلوريد الكادميوم والسلينيوم وكلوريد الكادميوم والزنك أية تغيرات فى خلايا الدم الحمراء عند مقارنتها بالمجموعة الضابطة كذلك لم تظهر

الفحص الميكروسكوبي لخلايا المجموعة المعالجة بالسلينيوم بمفرده أو الزنك بمفـــرده آيـــة تغيرات معنوية بالمقارنة مع المجموعة الضابطة .

SUMMARY

In albino rats exposed to $Cdcl_2$, $Cdcl_2$ and sodium selenite, Cdcl2 and zinc sulfate, sodium selenite, zinc sulfate for two months. The cadmium toxicity on male genital system, its genotoxicity and the protective role of zinc and selenium were investigated. The body, sexual organs and accessories weight, sperm motility, sperm concentration, live sperms percentage, acid phosphatase concentration in serum, mating performance, histopathological changes in testis and accessory sexual organs, also the cadmium genotoxicity using micronucleus test were evaluated. Our results revealed, nosignificant changes in body weight as well as in testes and accessory sexual organs weight in Cd treated rats and other experimental groups. There were significant reductions in sperm mass motility, sperm cell concentration and percentages of live sperm as well as a significant elevation in prostatic acid phosphatase activity were recorded in Cd treated group. Reduction of pregnancy percent, reduction of the number of obtained foeti, numbers of implantation and resorption sites were increased. A decrease in the average body weight of obtained foeti females and offspring's from intoxicated males were recorded. The administration of selenium or zinc in association with cadmium induces remarkable protective effect against the male reproductive toxicity of cadmium. Selenium and zinc alone have no remarkable toxic effect in comparison with other experimental or control groups. The previous mentioned results were confirmed by histopathological findings. In the micronucleus test a significant potential mutagenic effect for cadmium was determined. The role of zinc or selenium in protection against cadmium mutagenic effect in comparison with cadmium exposed group was recorded. Selenium or zinc alone had no significant mutagenic effect in comparison with the control group.

Key words: *Cadmium, Selenium, Zinc, Rat..*

INTRODUCTION

The rapid expansion of industrial technology has introduced into our environment increasing quantities of pollutant (Schroeder and Balassa, 1961). Metals are one of the very most important pollutants,

which induce hazard effect among human and animals. Cadmium is a widespread toxic environmental and industrial pollutant, which induces severe alterations in the tissues of laboratory animals and in humans. Moreover, cadmium exposure may lead to carcinogenesis (Waalkes et al., 1994 & 1991). Numerous studies have shown that prolonged exposure to some metals causes chronic or persistent neurologic syndrome, immunosuppressive effects, malignant tumors, teratogenic action, abortion and reproductive failure. (Cannon and Kimbrough, 1979, Nafstad et al., 1983. Sayai et al., 1991; Dalton et al., 1996; El-Tohamy et al., 1997; Latino et al., 1997. Liao et al., 1997; Privezentsev 1997; Richter et al.,1997; Saplakoglu et al., 1997 and Corpas and Antonio, 1998).

Germ cell (sperm-ovum) mutagens may exert their effects through decreased fertility, birth defects, spontaneous abortion, or through changes that may not become evident for several subsequent generations. Such hidden mutagenic effects remain essentially undetectable except when expressed as a gross lesion or malformation. Monitoring of male populations may prove particularly important in that the spermatogenic cycle is continuous in adult and therefore posses continuous opportunities for genetic damage to be expressed as damaged chromosomes (Williams and Burson, 1985).

Concerning the importance of the protective effect of zinc or other metals against acute and chronic cadmium administration, the present study aims to reveal the long term toxic effect of cadmium on the male reproductive system and mutagenic potential in albino rats. Also, some trials were adopted to evaluate the protective effect of zinc or selenium against deleterious effects of cadmium toxicity.

MATERIALS and METHODS

1- Experiment I (Male fertility & protection):

165 apparently healthy adult albino wister rats weighing 70-180 g, supplied by Breeding Unit of the Egyptian Organization for the Biological and Vaccine production were fed on a commercially prepared diet and had free access to tap water continuously available throughout the study. Animals were classified into unequal six groups, 35 rats in the first three groups and 20 rats in the other groups. Rats were exposed to 10 mg cadmium chloride/L in drinking water, (group I), 10 mg cadmium chloride + 1 mg sodium selenite/L, (group II), 10 mg cadmium chloride + 10 mg zinc sulfate/L (group III), 1 mg sodium selenite/ L (group IV),

10 mg zinc sulfate/L (group V), while rats in group VI were given only tap water. This experiment was lasted for 60 days, where the animals were weighed and five animals were sacrificed every two weeks from each group. Serum samples were taken for determination of acid phosphatase concentration according to Moss, (1984). Spermatozoa were obtained from different males by maceration of the epididymis and vasa deferentia, were examined for progressive motility, concentration of sperm cells and percentage of live and dead sperms according to (Blom, 1950, Bearden and Fuquay 1980). Testis, seminal vesicles and prostate gland were calculated as a percentage of body weight g/100 g. Testis, seminal vesicles and prostate gland were used for histopathological examination (Carleton et al., 1967). At the 30, 45 and 60 days from the beginning of the experiment, five mature treated males were taken from the first three groups. Each male from each group was housed with 2 healthy mature female in estrous and zero days of pregnancy were recorded. One pregnant dam from each cage was taken and scarified before the normal delivery (20^{th} days) for detection of any abnormalities. While other dam was left until natural delivery and the foeti weighted and examined for detection of any abnormalities. Also number of foeti in each cages were recorded.

2- The genotoxic effect experiment (Micro-nucleus test):

35 rats were classified into equal seven groups, and intubated with 43.53 mg cadmium chloride /kg B.wt. (½ LD_{50}) (G I). After Sahar Srour (1995), 43.53 mg of cadmium chloride +1 mg sodium selenite /kg B.wt. (GII), 43.53 mg cadmium chloride + 10 mg zinc sulfate/kg B.wt. (GIII), 1 mg sodium selenite/kg B.wt. (GIV), 10 mg zinc sulfate/kg B.wt. (GV), rats of the sixth group receive nothing and were kept as control group while rats of group VII were administered (ethylmethan, sulphonate) at a dose of 250 mg/kg via I/P injection (Control +ve). The animals were scarified 30 hrs after administration of tested substances. 1-1.5-ml fetal calf serum (FCS) into a centrifuge tube, flushed out the bone marrow. The tubes were centrifuged with a speed of 1000 r.p.m. for 5 minutes, then all the supernatant was removed and the pallet was suspended very well with three dropS of FCS, then smeared on a clean slide after drying. The slides were fixed in absolute methyle alcohol for 4-6 hours and finally stained with Wright stain for 15 minutes, then with Gimesa stain for 5 minutes where the mature erythrocytes were stained pink while immature erythrocytes ones (polychromatic erythrocytes "PCE") were stained blue. 1000 PCEs were scored per animal for the

presence of micronuclei (MN). Those micronuclei represented condensed chromosome or chromated fragment that are left behind during anaphase stage of the cell. So, the presence of micronuclei (MN) can be taken as an indication of the existence of chromosomal aberration. 1000 polychromatic erythrocytes per animals were scored.

RESULTS

1- Male fertility study:

There were no significant changes in body weight as well as in testes and accessory sexual organs weight in Cd treated rats and other experimental groups (Table, 1). Significant reduction in sperm mass motility, sperm cell concentration and percentage of live sperm as well as a significant elevation in prostatic acid phosphatase activity was recorded in Cd treated group. The administration of selenium or zinc in association with cadmium induces remarkable protective effect against the male reproductive toxicity of cadmium. This was obvious by elevation of previous mentioned parameter and significant decrease in acid phosphatase activity. Selenium and zinc alone have no remarkable toxic effect in comparison with other experimental or control groups. (Tables 2 & 3). Histopathological examination of testes and accessory organs in cadmium treated group revealed that lesions in testes allover the experimental period in form of degenerative changes in spermatogonial cell in most of the seminiferous tubules with appearance of sertoli cell as predominant cell while in prostate, this lesions appear as edema in the interaciner connective tissue stroma which found infiltrated by mononuclear leucocytic inflammatory cells in association with dilatation in the blood vessels. In selenium and cadmium group histopathological lesions appear as homogenous eosinophillic material in between the seminiferous tubules of the affected testes while in the accessory organ the histopathological lesions appear in the form of liquifactive necrosis surrounded by dead and living neutrophils was replaced some of the prostatic tissue. (Figs., 1, 2, 3, 4, 5 & 6). Zinc in association with cadmium had no histopathological finding observed in both testicular tissue and accessories allover the experimental period. Selenium or zinc alone has no remarkable histopathological findings in comparison with other experimental or control group. In serial mating experiment, the results revealed that cadmium affect the fertility of

treated male rats at 30, 45 and 60 days of experiments. This effect were represented by reduction of pregnancy percent, reduction of the number of obtained foeti, number of implantation and resorption sites were increased and lastly the average body weight of obtained foeti was decreased. Selenium or zinc in association with cadmium improves the reproductive potentiality of treated male. This provement was reflected in the increasment of pregnancy percent, elevation of number and weight of obtained foeti. No cases of resorption sites were recorded (Table 4).

2- The genotoxic effect experiment (Micro-nucleus test):

The obtained results revealed that cadmium exerts a significant potential mutagenic effect as indicated by increased frequency of induced micronuclei in polychromatic erythrocytes in comparison to the negative control, (Table 5). Microscopical examination of bone marrow of cadmium treated rats revealed also high frequencies of micronucleated polychromatic erythrocyte (PCECM), PCEs containing one, two, three microbuclei were recorded and scored as a mutagenic evident but in general those PCEs containing only one small micronucleus in their cytoplasm of individual cell were the most prevalent PCEs recorded in our experiment. Regarding to the groups treated with cadmium and selenium and those treated with cadmium and zinc the obtained result revealed that no remarkable mutagenic effect in comparison with control group. Selenium or zinc alone had no significant mutagenic effect in comparison with the control group (Fig. 7,8&9).

DISCUSSION

This study was undertaken to investigate mainly serious toxic effects of cadmium, with paying special interest to the male reproductive toxic effect, mutagenic as well as the cytotoxic effects that may arise as a consequence of long-term exposure to such harmful chemical. In the same time some trials were adopted by using selenium and zinc for minimizing the toxic effect of cadmium or in other wise for protection against the toxicity of cadmium.

Concerning the data obtained in male fertility experiment, our results of male fertility experiment revealed that cadmium did not produce any significant changes in the weights of treated rats testes or accessory sex glands (seminal vesicles and prostates) as compared to their control group organs weights, along the different periods of the experiment. Regarding the spermatozoal examination in male rats treated

with Cd Cl_2 in dose level of 10 mg/l drinking water for 60 successive days, it was shown that cadmium induce significant decrease in sperm concentration, mass motility and percentage of live sperms. In the same time, the activity of prostatic acid phosphotase in Cd treated rats showed significant elevation. Effects of acute cadmium exposure on the male reproductive system in the adult rat are well documented (Gunn et al., 1961; Saksena et al., 1977; Aoki and Hoffer, 1978; Saksena and Lau 1979). The lower limit for testicular effects has been reported by Gunn et al. (1966) to be approximately 4 μ mol (0.44 mg) cd/kg.

Our results regarding the marked reproductive effect of cadmium toxicity on mature male rats were correlates with those recorded by Laskey et al. (1984) who observed that the weights of the testes, seminal vesicles and epididyamides were reduced at least 40 to 50% in group of male rats receiving 16 or 33 μmol Cd/kg, while vas deferens sperm concentration and HCG-stimulated serum testosterone concentration were essentially zero. Also significant depressions in sperm concentration and HCO-stimulated serum testosterone concentration were found in animals receiving the two lowest doses (1.6 and 7.4 μmol Cd/kg) although no changes in tissue weights were observed in these animals. Our data are in agreement with those of Saksena et al. (1977), at doses of greater than 9 μmol Cd/kg, vas deferens sperm concentration were markedly reduced. Saksena and Lau (1979) reported similar observation, which indicated that Cd toxicity resulted in 50% reduction in sperm concentration 35 days post exposure. This observation was supported by the results of serial mating with control mature female rats, which denote marked lesser in reproductive potentiality of treated male rats (Table, 2). The marked significant reduction in sperm cell concentration may also be attributed to the reduction in meiotic index of the testicular cells which might be due to the usage of Cd access the blood testes barrier (BTB) and gain access to the germ cells in seminiferous tubules (Sugawara et al., 1986; Dixon and Lee 1973) reported that the BTB appeared to be represent an important aspect in the consideration of reproductive and mutagenic effects of environmental chemicals.

The marked significant elevation in the prostatic acid phosphatase activity in Cd treated male rats may be attributed to carcinoma of the prostate Murray et al., (1988), revealed that the prostatic fraction of acid phosphatase may be increased in the serum in case of carcinoma of the prostate particularly if the cancer spread beyond

the capsule of the gland or has metastasized. This result was in accordance with the observation of Waalkes et al. (1989) and (Waalkes and Rehm, 1992). They reported that oral cadmium exposure induce proliferative lesion of rat ventral prostate and when rat were fed cadmium mixed with diets adequate in zinc an increase in the overall incidence of prostatic proliferative lesions occurred in rat fed cadmium (25-200 ppm) and in zinc adequate diets compared to the rats fed cadmium in zinc deficient diet or in control. Also our data was confirmed by the present histopathological examination of prostate of Cd treated rats, which revealed monocular leucocytic inflammatory cells infiltration was observed in the interacine stroma. Hypoplastic activation of the epithelial cell lining the acini with polyp's formation was seen.

Our data revealed that cadmium in a concentration of 10 mg/l drinking water for 60 successive days produce characteristic histopathological changes in the testes and accessory gland of treated rats. Several investigators recorded that exposure to Cd salts causes a variety of severe toxicity in experimental animals. In mice testicular damage was in the form of widely and severely hemorrhage necrosis. Degenerative changes occurred in seminiferous tubules. Atrophy, edema, hemorrhage and sperm were no longer found (Sugawara and Sugawara, 1984; Sugawara et al., 1986 and Sugawara et al., 1989). In rats massive haemorrhagic testicular necrosis and degenerative changes were recorded after Cd intoxication (Parizek and Zahor, 1958 and Parizek et al., 1968).

The susceptibility of testes to the toxic action of Cd may be attributed to what mentioned by Sugawara and Sugawara (1984) who recorded that two mechanisms have been proposed to account for testicular injury caused by the injection of Cd (1) a circulatory failure due to vascular damage and (2) a direct toxic effect of Cd on spermatogenic cells. Several investigators studied the vascular damage in testicular tissue by Cd. (Gunn et al., 1963) found initial vascular damage occurred within the 7 several hours following exposure. A dose of 30 µmol Cd/kg destroyed the veins of the pampiniform plexus. By 6 hours following 30 µmol Cd/kg b. wt. Timm and Schulz (1966) reported visible granules of cadmium in both the interstitial tissues and the leydig cells and by 18 to 24 hours, there was extensive vascular damage with an acute dose of 8 µmol Cd/kg b. wt. there was minimal hemorrhagic necrosis with most of the non necrotic damage to the seminiferous tubules rather than to the interstitial tissue (Meek, 1959; Mason et al.,

1964; Kar and Das, 1960). Kar and Das (1960) also reported that even though there were no apparent degenerative changes in the interstitial tissue at this dose, accessory sex organs were reduced in size. Six days following a dose of 6 µmol cd/kg b. wt. Gunn et al. (1966) observed some edema with no apparent histological differences in either the germinal or interstitial elements.

Concerning the genotoxic study, our results revealed that cadmium induce mutagenic effect through the routine cytogenetic screening which was represented by bone marrow tests Adler et al. (1991). The statistical analysis of the obtained data revealed the significant mutagenic potentiality of cadmium in acute study (single-dose treatment of ½ LD_{50}) by inducing micronuclei in bone marrow polychromatic erythrocytes. Concerning the mutagenic effect of Cd in bone of treated rats, our data was closely correlated with the results obtained by (Bassendowsk *and Zawadzka* 1987) who reported that single and repeated administration of mixture of $Pbcl_2$, ASO and CdSo was evident to increase the frequency of micronucleus and chromosomal aberrations in bone marrow cells. Our results regarding the significant induction of micronucleus in bone cells of treated rats are inagreement with those reported by Privezenter et al. (1997), who shown that $Cdcl_2$ induce nucloid relaxation in peripheral blood lymphocytes after one hour incubation in vitro, while single injection of $Cdcl_2$ (1 mg Cd/kg b.wt) induced DNA damage in PBL, spleen ocytes and thymocytes. Also the same dose induced an increase in the frequency of micronucleated bone marrow erthrocytes in mice. The present data was nearly similar to those observation of Hurna et al. (1997) who mentioned that micronucleus assay was used for genotoxicity testing of Cd by using V79 cells (Chinese hamster lung fibroblasts) which treated by various concentration of Cd. The results revealed an increase in micronucleus frequency with various Cd concentrations. Nearly similar data was recorded by Privezentsev (1997) who noted that $Cdcl_2$ in concentration of 5 mg/l drinking water for 32 days failed to induce cytogenetic damages in mouse bone marrow cells, but 1 mg Cd/kg b. wt. when injected with chronic gamma- irradiation leads to an increase in the number of micronucleus. Unfortunately, no consensus on how cadmium induces genetic damage has yet emerged. At least three different hypotheses regarding this metal's mode of action currently exist (1) cadmium may interact directly with chromation to induce strand breakage, crosslinking or conformational changes in DNA (2) cadmium may act indirectly, by inhibiting various proteins involved in DNA

repair (3) cadmium may act by catalyzing cellular redox reactions whose by products subsequently produce strand breaks, cross-links, or covalent adducts in DNA (Jacobson and Turner, 1980; Waalkes and Poirier, 1985). More recently, other experiments were conducted by Muller et al. (1991); Rossman et al. (1992) indicated that additional factors, such as metal protein-complex formation may be required in order to produce significant levels of DNA damage in vivo.

Ochi et al. (1983) and Ochi and Ohsawa (1985) suggested that an active oxygen species may be ultimately responsible for the metal's genotoxic effects. Also Snyder (1988) revealed that cadmium induced chromosomal damage by stimulating the production of hydrogen peroxide which, in turn, forms highly reactive hydroxyl radicals in the presence of intracellular iron or copper. It has recently been demonstrated that extremely low doses of cadmium can stimulate DNA replication (Lohmann and Beyersmann, 1993) and/or prevent apoptosis (VonZglinicki et al., 1992). In light of these results, and previous experimental data, it is tempting to speculate that low doses of cadmium may achieve geneotoxicity by compromising the cell's ability to cope with spontaneously occurring DNA damage. At high doses, however, cadmium may act by damaging DNA directly, or by stimulating the production of reactive intermediates which subsequently attack the genetic material. Among the heavy metals, cadmium deserves especial importance as a potentially toxic element, and it has now become a ubiquitous concomitant for the human environment and is labeled as dissipated element (Foulkerson et al., 1973).

Concerning the mechanism by which Cd-induced its adverse effects, it was suggested that the increase in Cd-bound to non metallothionein (MT) fraction both the cytoplasmic and particulate fraction of tissues (Sato and Nagai, 1982) is responsible for the damage following chronic exposure to cadmium. It may be postulated that Cd-toxicity results from an alteration in the normal metabolism of lipid (Wada et al., 1982 and Petering et al., 1984) and involvement of oxidation of membrane lipids has been suggested as the key factor in process of injury in organ following Cd-exposure (Hussain et al., 1987). Furthermore, it has been suggested that the toxicity of Cd may result from cellular membrane damage during uptake of Cd-complex into renal cells by pinocytosis (Cherian, 1982). Lastly, the possible involvement of lysosomes and degradation of Cd-MT to generate Cd ions was suggested to cause direct toxicity in the tubules (Suzuki and Cherian, 1987, Foulkes and Blanck, 1991) and induce DNA damage (Snyder, 1988).

On the other hand, it is clear, from the obtained data in the present study, that the selenium and zinc supplementation provided a marked protective effect against the cadmium male reproductive and genotoxic effect of cadmium. Recent studies has been found that Cd cause oxidative damage in different tissues by increasing lipid peroxidation and by inhibiting certain enzyme responsible for deactivation of oxygen species (Shukla and Singhal, 1984; Hussain et al., 1987 and Sole et al., 1990).

Considerable attention has been paid towards the development of safe and effective chelation and protection therapy in management of cadmium poisoning. Optimal intake of different nutrients like minerals (Groten et al., 1991) and vitamins in the diet can favorably affect the Cd-toxicity (Nagyova et al., 1994). However, the free radical scavengers (antioxidant) such ascorbic acid, tocopherol and selenium present in the tissue are known to protect against oxidative damage and signs of Cd-toxicity (Shukla et al., 1988; Fariss, 1991 and Hudecova and Ginter, 1992). On the other hand, among the protective agents used zinc has been shown to modulate both the toxicity (Jacobs et al., 1983; Herkovits and Perez-Coll, 1989 and Coogan et al., 1992) and carcinogenicity (Waalkes et al., 1989) associated with cadmium exposure.

Although the exact mechanism of zinc protection is unknown. It is reasonable to assume that zinc stimulating MT plays a role in this protection, at least at the level of sequestered cadmium away from nucleic materials (Yoshikawa and Chta, 1982). However, it has been suggested that the thiolate clusters in MT are particularly efficient at scavenging hydroxyl free radicals (Thormally and Vasak, 1985 and Coppen et al., 1985). MT has been localized within the cell nuclues, in addition to be found in cytosol (Banerjee et al., 1982). Nuclear MT may stimulate both to generate radicals due to Cd-binding, as well as Scavenge those radicals formed (Coogan et al., 1992). Further, the reduction of the toxic effects of Cd by zinc may be attributed to altered subcellular distribution of Cd (Goering and Klaassen, 1984). Lastly, Cd replaces Zn in Zn-thionein synthesized after Zn pretreatment (Tanaka et al., 1977). Our results are inagreement with the previous findings indicating that zinc has been shown to reverse Cd-induced tissue damage (Cheng, 1988) and diminish some toxic effects of cadmium such as hepatotoxicity and renal toxicity (Sato and Nagai, 1989, Wafaa El-Kholy, 1996 and Nagwa El-Mossalamy et al., 1996). Regarding the role-played by the antioxidant in protection against toxicity of cadmium, Manca et al., (1991) suggested that lipid peroxidation (LPO) is an early

and sensitive reaction to Cd-exposure. It is known that ascorbic acid (vit C), tocopherol (vit E) and selenium plays some role in the antioxidant mechanism against LPO (Hudecova and Ginter, 1992 and Shiraishi et al., 1993). The present study revealed that the intake of selenium and zinc in drinking water of Cd treated male rats improve the reproductive status to large extent. They induce significant increase in the sperm concentration, mass motility and percentage of live sperm. Also both selenium and zinc caused significant reduction in prostatic acid phosphatase of male Cd treated rats. In the same time no remarkable histopathological alterations were seen in male rats treated with the protective agents, selenium and zinc with Cd in the drinking water. From studies of the protective effect of several compounds including Zn on testicular damage induced by Cd (Gunn et al., 1986), it has been shown that Se potently prevented this adverse effect (Mason et al., 1964). Also Gunn et al. (1986) revealed that the testicular injury caused by the Cd injection was prevented by the administration of bimolar Se. The preventive effect of Se was not to the depression of Cd uptake into this organ, but the association of Cd with high-molecular-weight proteins in the organ cytoplasm, as in the form of a - Cd-Se complex (Chen et al., 1974). In study conducted by Whanger et al. (1980) to investigate the mechanism of selenium and cadmium interaction, selenium was shown to divert the binding of cadmium from low molecular weight (Mw) proteins to higher MW ones in the testes of rats. The mechanism involved in the protection by Se against Cd toxicity in rat were investigated by Chen et al. (1975). They reported that Se was found to significantly increase the Cd content in the blood and the testis while decreasing that in the liver and kidney. Se diverted almost all the Cd in the soluble fraction of the testis from low-molecular-weight (Mw) proteins to larger ones. Since the soluble fraction was the major subcellular Cd-binding component, the diversion of Cd by Se appears to be a mechanism involved in the protection by this element against the Cd-induced testicular injury. Sugawara et al. (1986) reported that the testicular dysfunction caused by cadmium is prevented by the simultaneous injection of certain other elements (Se and Zn). Se compounds are classified in this group. The metalloid has several important functions. It is located at the catalytic site of glutathione peroxidase, an enzyme that metabolized H_2O_2 (Rotruck et al., 1973). Se itself, as is vitamin E, is an antioxidant, and its function relates to the membrane integrity (Zalkin et al., 1960). In addition the testicular uptake of Cd was stimulated by the injection of Se together with Cd. Sugawara

et al. (1986) recorded that the proportion of Cd bound to the metallthionein (MT) like protein did not increase greatly in the testicular cystol, although the uptake of Cd into the testis was stimulated by the injection of Se together with Cd. In regard to the MT-like protein, Nordberg (1971) proposed that the protein be involved in the protection of the testes against repeated Cd exposure. However, Wong and Klaassen (1980) reported that there was no relation between the induction of MT-like protein and male reproduction.

Regarding the protective effect of zinc against testicular damage of Cd, Whanger et al. (1980) stated that the ability of zinc to prevent cadmium injury of testes is apparently due to competitive mechanisms Zinc act antagonistically to Cd, appear to be related to lipid peroxidation (Wills, 1965 and Sullivan et al., 1980). In animals treated with Cd, the behavior and activity of zinc should discussed in relation to metallothionein (MT) induction. Also Chavapil et al. (1992) reported that one of various possible mechanisms which Zn stabilized a variety of biomembranes both in vitro and in vivo was related to the inhibition of membrane lipid peroxidation.

Gunn and Gauld (1970) mentioned that the rodent testis is extremely sensitive to cadmium, relatively low doses of Cd can rapidly induce severe testicular hemorrhage necrosis. Recent work also indicates that oral Cd exposure can result in interstitial cell tumors in the rats (Waalkes and Rehm, 1992). Certain treatments modify cadmium carcinogenicity, including administration of zinc, which prevents Cd-induced injection site and testicular tumors (Waalkes et al., 1989). Zinc can clearly have an important impact on cadmium carcinogenesis. In several tissues, including the lung, testes and at the injective site, zinc treatment ameliorates the effects of cadmium (Gunn et al., 1963, 1964; Waalkes et al., 1989 and Oldiges et al., 1989). The marked genotoxic effect exerted by cadmium in the treated rats was counteracted by administration of both selenium and zinc. In 1983, Ochi et al., were the first investigators to demonstrate the soluble cadmium could induce DNA damage in eukaryotic cells. Using the alkaline elution techniques, they showed that $Cdcl_2$ exposure led to increase production of single strand breaks in the DNA of V79 hamster cells (Ochi et al., 1983). Furthermore, marked reduction in the induction of strand breakage was observed when $CdCl_2$ was administered under anaerobic conditions, or when superoxide dismutase (SOD) was added to the culture medium. These results suggested that an active oxygen species might be ultimately responsible for the metal's genotoxic effects. Later, the same

investigators showed that the incidence of chromosomal aberrations was dramatically reduced in V79 cells received catalase, D-mannitol or the antioxidant butylated hydroxytoluene (BHT), prior to Cd exposure (Ochi and Ohsawa, 1985). Also Nocentini (1987) used a variety of experimental techniques to demonstrate that micromolar concentrations of Cd could inhibit replication and transcription of the DNA template in normal and UV-damaged mammalian cells. In the same time, the author discovered that zinc could countered such effects if it was administered at concentration that were five to ten times higher than the concentration of Cd. This is noteworthy given the ability of zinc to inhibit the chronic carcinogenic effects of Cd in the rat or mouse (Gunn et al., 1963, 1964; and Waalkes et al., 1989). In other study conducted to Coogan et al. (1992) shown that the induction of metallothionein can lead to a marked reduction in the incidence of Cd-induced DNA strand breakage. Hochadel et al. (1994) reported that metallothionein is a good example of one cell-specific factor which may play an important role in modulating the genotoxic effects of cadmium.

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Table (1) : Mean value of body weight and accessory sexual organs weight gm in treated and control rat groups

Parameters	Body weight						Testes weight						Seminel vesicle						Prostat weight					
Gr	G1	G2	G3	G4	G5	G6	G1	G2	G3	G4	G5	G6	G1	G2	G3	G4	G5	G6	G1	G2	G3	G4	G5	G6
2 weeks	183.3 ± 3.43	186 ± 6.66	195 ± 2.88	193.3 ± 3.04	195 ± 3.19	190 ± 5.77	1.20 ± 0.68	1.29 ± 0.80	1.30 ± 0.09	1.262 ± 0.14	1.18 ± 0.07	1.21 ± 0.04	1.19 ± 0.11	0.99 ± 0.04	1.11 ± 0.04	1.17 ± 0.08	1.03 ± 0.19	1.12 ± 0.17	0.32 ± 0.04	0.53 ± 0.01	0.375 ± 0.09	0.41 ± 0.12	0.44 ± 0.26	0.41 ± 0.15
4 weeks	206 ± 2.58	215 ± 3.4	206.6 ± 3.33	216.6 ± 3.04	213.3 ± 2.99	200.3 ± 4.48	1.36 ± 0.02	1.32 ± 0.12	1.34 ± 0.06	1.39 ± 0.18	1.41 ± 0.08	1.37 ± 0.07	1.41 ± 0.13	1.46 ± 0.05	1.2 ± 0.29	1.44 ± 0.06	1.75 ± 0.13	1.38 ± 0.15	0.39 ± 0.25	0.435 ± 0.13	0.340 ± 0.07	0.47 ± 0.31	0.53 ± 0.21	0.60 ± 0.09
6 weeks	216.6 ± 8.82	246.6 ± 3.15	240 ± 2.51	230 ± 5.17	240 ± 0.66	240 ± 5.77	1.441 ± 0.05	1.516 ± 0.06	1.436 ± 0.15	1.54 ± 0.34	1.55 ± 0.06	1.45 ± 0.07	1.33 ± 0.23	1.39 ± 5.7	1.37 ± 0.17	1.39 ± 0.11	1.61 ± 0.08	1.26 ± 0.44	0.47 ± 0.16	0.53 ± 0.25	0.41 ± 0.45	0.50 ± 0.15	0.51 ± 0.27	0.46 ± 0.11
8 weeks	236.6 ± 3.21	253.3 ± 3.32	255 ± 7.63	253 ± 3.16	260 ± 5.46	256.6 ± 3.35	1.466 ± 0.16	1.495 ± 0.04	1.5 ± 0.03	1.42 ± 0.41	1.50 ± 0.13	1.56 ± 0.90	1.37 ± 0.18	1.45 ± 0.24	1.38 ± 0.16	1.49 ± 0.28	1.38 ± 0.14	1.37 ± 0.13	0.42 ± 0.14	0.497 ± 0.21	0.520 ± 0.31	0.48 ± 0.11	0.43 ± 0.43	0.49 ± 0.19

Table (2) : Mean values of sperm cell concentrationx 10^6 / Epid, mass motility and live sperm percentage in treated and control raf groups

Time	Parameter	Group (I)	Group (II)	Group (III)	Group (IV)	Group (V)	Group (VI)
2 weeks	Sperm cell conc.	48.66 ± 3.52	54.33 ± 2.90	42.66 ± 0.88	48.01 ± 1.52	55.33 ± 3.84	53.5 ± 1.52
	Mass motility %	65 ± 2.88	70.5 ± 5.01	68.33 ± 4.40	69 ± 3.78	72.66 ± 1.45	75 ± 2.8
	Live sperm %	79.40 ± 1.16	75.16 ± 0.353	78.14 ± 2.02	76.5 ± 1.58	75.12 ± 1.33	83.15 ± 1.66
4 weeks	Sperm cell conc.	38.66 ± 2.33	45.33 ± 5.04	44.83 ± 2.71	48.6 ± 0.88	53.33 ± 2.02	55.1 ± 1.15
	Massmotility %	56 ± 2.9	61.16 ± 1.88	63.3 ± 1.2	71.66 ± 1.6	68.33 ± 4.40	70.66 ± 4.45
	Live sperm %	66.8 ± 2.91	70.14 ± 1.14	76.4 ± 4.12	78.01 ± 2.27	79.44 ± 2.88	79.24 ± 1.41
6 weeks	Sperm cell conc.	18.1 ± 0.57	49.33 ± 2.02	48.66 ± 4.37	52.33 ± 2.18	54.33 ± 5.81	59.03 ± 2.33
	Mass motility %	31.66 ± 1.66	65.72 ± 0.31	65.35 ± 3.39	75 ± 1.93	71.6 ± 1.66	75.66 ± 2.33
	Live sperm %	60.31 ± 3.29	75 ± 1.883	80.33 ± 1.4	22.11 ± 3.11	81.52 ± 3.27	78.43 ± 2.12
8 weeks	Sperm cell conc.	12.33 ± 0.66	53.33 ± 2.30	53.66 ± 0.88	54.3 ± 1.3	61.33 ± 0.58	63.41 ± 2.33
	Mass motility %	20.33 ± 1.66	68.1 ± 0.23	70.66 ± 3.48	75 ± 2.88	75 ± 3.1	78.33 ± 2.03
	Live sperm %	55.2 ± 6.13	78.03 ± 1.05	81.5 ± 3.15	79.4 ± 4.07	83.06 ± 3.25	80.60 ± 2.45

For sperm cell conc., the LSD at 5% is 12.28 & at 1% is 16.82 – The F- values is 6.577 with 5/15 degree of freedom – The prop> F is 0.0011
For mass motility %, the LSD at 5% is 10.97 & at 1% is 15.04 – The F- values is 15.500 with 5/18 degree freedom – The prop> F is 0.0001
For live sperm %, the LSD at 5% is 9.34 and at 1% is 14.04

Table (3) : Mean values of prostatic acid phosphorus activity µ/l in treated and control rat groups.

Time	Group (I)	Group (II)	Group (III)	Group (IV)	Group (V)	Group (VI)
2 weeks	2.98 ± 0.43	2.33 ± 0.28	2.41 ± 0.28	2.49 ± 0.64	2.51 ± 0.39	2.44 ± 0.20
4 weeks	3.52 ± 0.57	2.89 ± 0.42	2.70 ± 0.42	2.61 ± 0.58	2.64 ± 0.25	2.58 ± 0.178
6 weeks	4.53 ± 0.117	3.01 ± 0.68	2.81 ± 0.68	2.59 ± 0.42	2.78 ± 0.44	2.49 ± 0.35
8 weeks	5.57 ± 0.56	3.14 ± 0.27	2.65 ± 0.27	2.72 ± 0.52	2.82 ± 0.69	2.615 ± 0.56

The LSD at 5% is 0.78
The LSD at 1% is 1.07

Table (4) Showing serial mating of treated experimental male rats in differet groups with non treated female rats.

Item	Cadmium Group			Cadmium + Selenium			Cadmium + Zinc		
	30 days	45 days	60 days	30 days	45 days	60 days	30 days	45 days	60 days
No. of male rats	5	5	5	5	5	5	5	5	5
No. of female rats	10	10	10	10	10	10	10	10	10
No. of pregnant female rats	10	5	2	7	5	7	8	10	7
Pregnancy %	100	50	20	70	50	70	80	100	70
No. of foeti	75	25	14	54	20	35	50	53	46
Implantation sites	5	6	2	1	-	2	-	-	2
Resorption sites	2	-	-	-	-	-	-	4	-
Dead foeti	-	-	-	-	-	-		-	-
Average b.wt of foeti	5.72	4.72	3.85	4.705	5.336	6.136	5.16	6.30	5.88

Table (5) : Showing the number of micronuclei in polychromatic erythrocytes in bone marrow of treated and control rat groups

Groups	No. of Identi	Total No. of	Micronucleated PCE (M) cells				Total No. of PCE	Mean values
	Tocti	examine cell	1	2	3	> 3	(M) cells	±SE
Group (I)	1	1000	29	25	3	0	57	60.2 ± 1.39
	2	1000	35	16	6	4	61	
	3	1000	39	18	8	0	65	
	4	1000	43	5	7	5	60	
	5	1000	33	15	7	3	58	
	Total	5000	179	79	31	12	301	
Group (II)	1	1000	2	2	0	0	10	8.8 ± 0.3
	2	1000	5	2	1	0	8	
	3	1000	6	3	0	0	9	
	4	1000	3	4	1	0	8	
	5	1000	6	1	2	0	9	
	Total	5000	28	12	4	0	44	
Group (III)	1	1000	6	2	0	0	8	8.2 ± 0.37
	2	1000	5	4	0	0	9	
	3	1000	5	3	1	0	9	
	4	1000	4	2	1	1	8	
	5	1000	4	3	0	0	7	
	Total	5000	24	14	2	1	41	
Group (IV)	1	1000	5	3	0	0	8	8.8 ± 0.51
	2	1000	6	4	0	1	10	
	3	1000	3	3	1	0	7	
	4	1000	6	2	1	0	8	
	5	1000	6	1	0	0	9	
	Total	5000	26	13	2	1	44	
Group (V)	1	1000	6	2	0	0	8	7 ± 0.45
	2	1000	4	3	0	0	7	
	3	1000	5	1	0	0	6	
	4	1000	5	3	0	0	8	
	5	1000	3	2	1	0	6	
	Total	5000	23	11	1	0	35	
Group (VI)	1	1000	3	0	0	0	3	4.6 ± 0.83
	2	1000	5	1	0	0	6	
	3	1000	2	0	0	0	2	
	4	1000	7	0	0	0	7	
	5	1000	5	1	0	0	5	
	Total	5000	22	2	0	0	23	
Group (VII)	1	1000	52	15	8	4	79	82.6 ± 3.03
	2	1000	46	17	4	4	71	
	3	1000	56	20	8	2	86	
	4	1000	58	21	6	3	88	
	5	1000	67	13	6	3	89	
	Total	5000	279	86	32	16	413	

LSD at 5% is 4.24 and at 1% is 5.71 Prop > f < 0.0001 SE=Standarderror

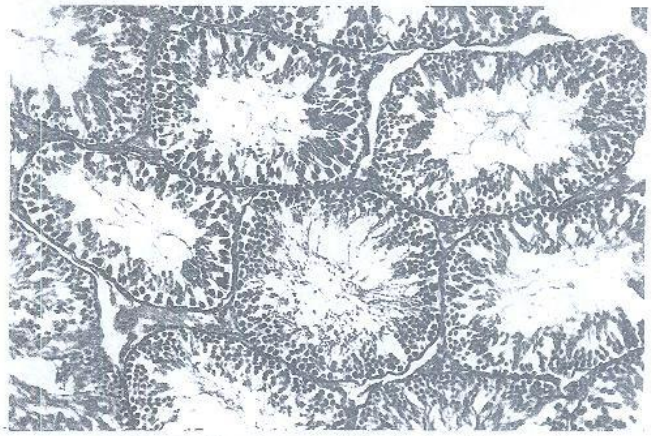

Fig.(1):Testes of rat treated by cadmium for 45 days showing mild degenerative change in semineferous tubules with appearance of multiple sertoli cells as predominent cells. H & E x 40

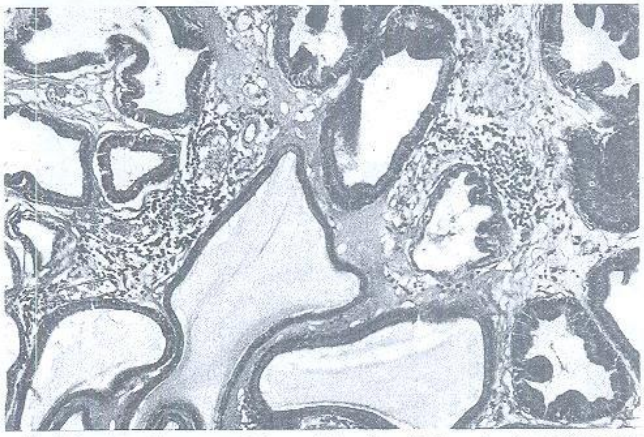

Fig.(2):Prostate of rat treated by cadmium for 45 days showing oedema with mononuclear leucocytic inflammatory cells infiltration and hyperermic blood vessels in the interaciner connective tissue stroma. H & E x 40

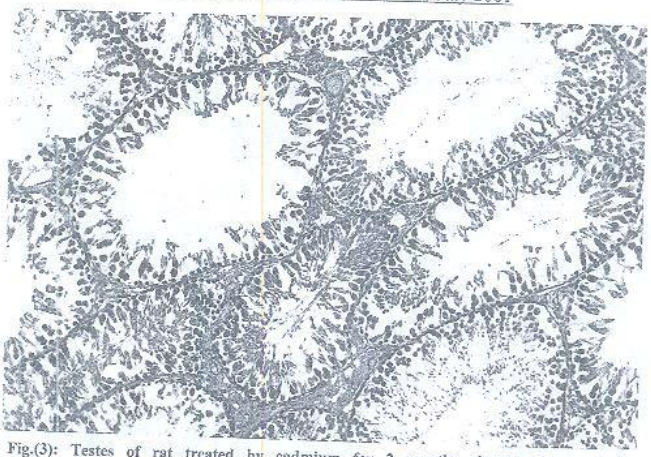

Fig.(3): Testes of rat treated by cadmium for 2 months showing degenerated semineferous tubules with appearance of sertoli cells as the predominnet one. H & E x 40

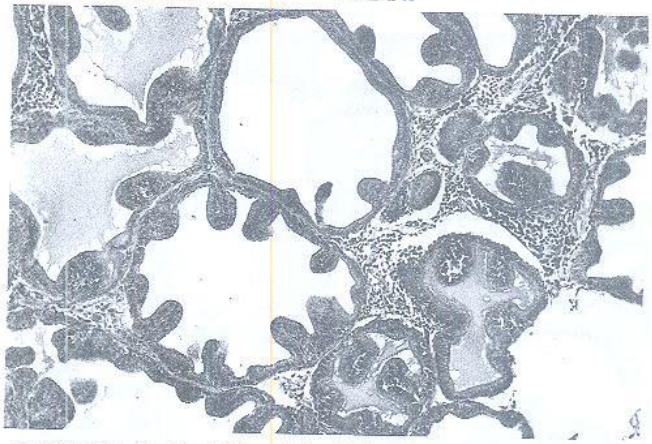

Fig.(4):Prostate of rat treated by cadmium for 2 months showing hyperplasia of the epithelial cells linning the acini with polyps formation in association with mononuclear leucocytic inflammatory cells infiltration in the interaciner stroma. H & E x 40

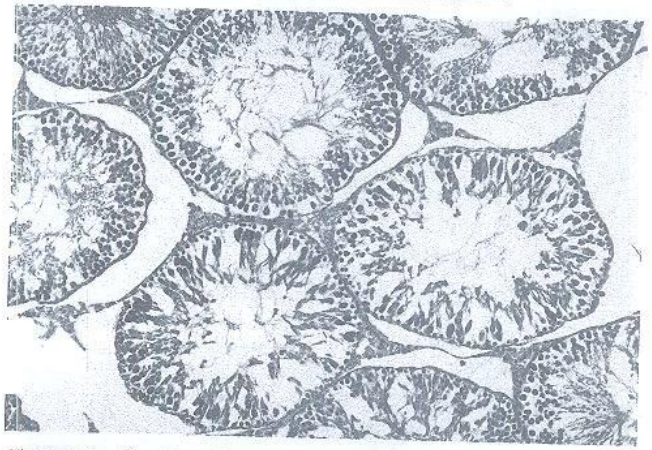

Fig.(5):Testes of rat treated by cadmium and selenium for 45 days showing degenarated seminiferous tubules with appearance of multiple cells of sertoli. H & E x 40

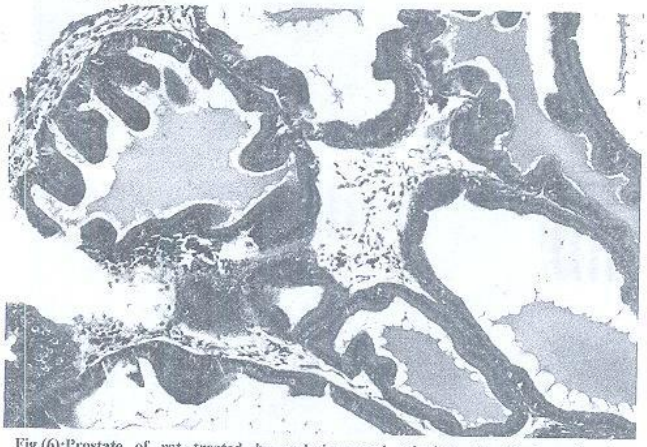

Fig.(6):Prostate of rat treated by cadmium and selenium for 45 days showing hyperplasia of the epithelial cells linning the acini with polyps formation in association with mononuclear leucocytic inflammatory cells infiltration in between the acini. H & E x 40

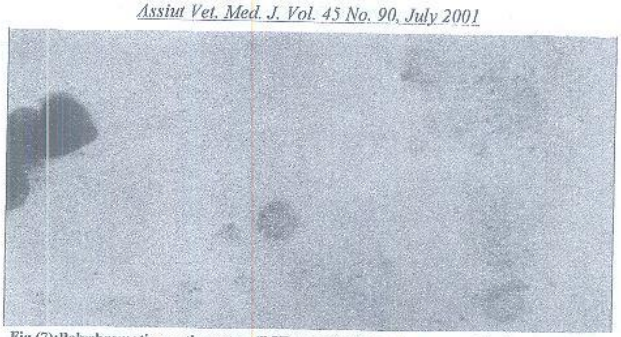

Fig.(7):Polychromatic erythrocytes (PCEs) containing only one small micronucleus in their cytoplasm in bone marrow cells of cadmium chloride treated rats

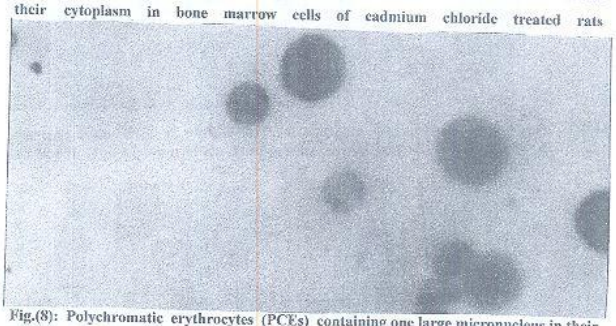

Fig.(8): Polychromatic erythrocytes (PCEs) containing one large micronucleus in their cytoplasm in bone marrow cells of cadmium chloride treated rats.

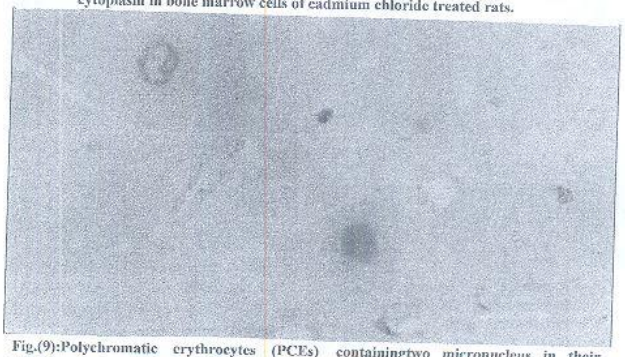

Fig.(9):Polychromatic erythrocytes (PCEs) containingtwo micronucleus in their cytoplasm in bone marrow cells of cadmium chloride treated rats.