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Effect of some antioxidants on the reproductive performance in rats

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The present study was designed to determine the effect of the antioxidants; taurine, ascorbic acid " AA " and beta-mercaptoethanol " β-ME " separately or in combination, on reproductive performance of adult male rats suffering from cadmium chloride "$CdCl_2$" - induced oxidative stress. A total of 180 mature male Albino rats were equally divided into 9 groups; the rats of the 1st were administered distilled water while those of the 2nd group were administered 1/100 LD_{50} of $CdCl_2$. Rats of the remaining groups were administered 1/100 LD_{50} of $CdCl_2$ followed 3 days later by 1 / 50 LD_{50} of either taurine, AA and β-ME or a combination of AA and taurine, AA or β-ME, taurine and β-ME as well as AA, taurine and β-ME, respectively for 2 successive months. The results revealed that application of $CdCl_2$ for 8 successive weeks decreased pituitary and serum levels of gonadotropins (gametogenic hormone"FSH" and interstitial cell stimulating hormone "ICSH") as well as serum testosterone "T" level, altered semen quality, and decreased serum level of total antioxidant capacity with increased serum malondialdhyde "MDA" level. On the other side, application of different antioxidants to $CdCl_2$ induced oxidative stress increased pituitary and serum levels of gonadotropins and serum level of T as well as improved semen quality, increased serum level of total antioxidant capacity and decreased serum level of MDA. In addition, the best improvement in male reproductive performance was achieved after administration of AA and taurine separately or in combination while the least improvement was obtained when β-ME was applied alone.

Male infertility is one of the most important factors resulting in decreased reproductive and productive performances among mammals. In this concern, mammalian spermatozoa membranes are rich in high unsaturated fatty acids that sensitive to oxygen induced damage mediated by lipid peroxidation. The excessive generation of reactive oxygen species " ROS " due to the presence of abnormal spermatozoa, contaminating leukocytes and different types of stress has been identified as one of the defined etiologies for male infertility (Sikka, 1996; Maneesh and Jayalekshmi, 2006). Moreover, the metabolic activities during spermatogenesis itself generate high levels of ROS (Agarwal *et al.,* 2003).

Environmental toxicants are one of most prevalent causes of testicular oxidative stress (Sokol, 1997). Cadmium is the most common environmental toxin that affects male reproductive potency. It is a natural element found in the earth's crust that is usually found as a mineral combined with other elements as oxygen, chlorine and sulfur. Cadmium is also used industrially in the plants of steel, plastic, glass, electrode material of batteries and as components of various alloys (Wilson, 1988). Acharya *et al.,* (2008); Manna *et al.,* (2008) found that Cd increased intratesticular concentration of ROS resulting in elevated levels of lipid peroxidation, protein carbonylation, glutathione disulfide and DNA fragmentation as well as decreased levels of the activities of the antioxidant enzymes. In addition exposure to Cd resulted in a massive testicular germ cell apoptosis (Zhou *et al.,* 1999).

Scientists have developed efficient protective mechanisms (antioxidant defence system) against excessive accumulation of ROS. This system includes enzymes such as catalase "CAT", superoxide dismutase "SOD" and glutathione peroxidase "GPx" / reductase "GR" and numerous non-enzymatic antioxidants such as vitamins (C, E, and A), amino acids including pyruvate, glutathione "GSH", taurine and hypotaurine (Sikka, 2004) and trace elements such as zinc and selenium (Agaya *et al.,* 2005). In this respect, a series of reports has shown that supplementation of different mammalian species with various antioxidants enhanced male reproductive performance due to the scavenging potency of the antioxidants to neutralize the harmful effect of the produced ROS (Sonmez *et*

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al., 2004; Deichsel *et al.,* 2008; Zaki, 2009). However, the majority of the previous studies were implemented under normal conditions without intervention with application of the antioxidant under stress. Therefore, the present study was designed to determine the effect of taurine, ascorbic acid "AA" and beta mercaptoethanol "β-ME" separately or in combination, on reproductive performance of adult male rats suffering from Cd - induced oxidative stress.

Materials and methods

Animals. This study included 180 mature male Albino rats weighing 170 - 190 g obtained from Helwan Farm for the Laboratory Animals (Ministry of Health). Animals were transferred to the Physiology Department, Faculty of Veterinary Medicine, Beni-Suef University where they were left 2 weeks for acclimatization. Throughout the experimental period, rats were kept under constant environmental and hygienic conditions as well as offered food and water ad libitum.

Experimental design. Rats under experiment were equally divided into 9 groups, 20 rats each; the rats of the 1st group (negative control) were administered distilled water while those of the 2nd group (positive control) were administered 1/100 LD_{50} of $CdCl_2$ (Oxford Lab., India). Rats of the remaining groups were administered 1/100 LD_{50} of $CdCl_2$ followed 3 days later by 1/50 LD_{50} of either AA (Oxford Lab., India), taurine (Oxford Lab., India) and β-ME (MERCK-Schuchardt, Germany) or a combination of AA and taurine, AA and β-ME, taurine and β-ME as well as AA, taurine and β-ME, respectively for 2 successive months. In all groups, the administered dose (s) was freshly prepared by dissolving it in 200 µl distilled water and given weekly to each animal through stomach tube for 8 successive weeks. This protocol of administration was previously outlined by Gupta *et al.,* (2004); Acharya *et al.,* (2008).

Sample collection. At the end of the 8th week, individual blood samples were collected from all groups under mild ether anesthesia from the retro-orbital venous plexus at the medial canthus of the eye. Also, the corresponding pituitaries were separated, removed from the posterior part and preserved in acetone for 3 days then weighed and ground in a mortar to be dissolved in phosphate buffer saline "PBS" of pH 6.8 at a rate of 1.0 mg dry anterior pituitary / ml then placed in a sterile labeled vial. Sera and pituitaries, immediately after collection, were preserved in deep freeze at –20°C till the immunological assay of the studied hormones (FSH, ICSH and T). Moreover, serum samples were used for determination of total antioxidant activity (AOA) as well as malondialdehyde level (MDA).

Meantime, corresponding individual semen samples were collected by maceration of epididymis and vasa deferentia to be used for semen evaluation (individual sperm motility, estimation of live/dead % and abnormalities) as mentioned by Narayana *et al.,* (2005).

Estimation of gonadotropins in pituitary and serum samples was performed by means of enzyme linked immunosorbent assay " ELISA " as outlined by Voller et al., (1979). Serum levels of T in the control and treated rats were assayed using the radioimmunoassay technique as described by Jaffe and Behrman, (1974). Determination of T levels was carried out in the Middle Eastern Regional Radioisotope Centre for the Arab Countries, Dokki, using the specific Kits of Diagnostic Products Corporation (Los Angeles, USA). In addition, the AOA serum levels were measured using the protocol described by Koracevic *et al.,* (2001). On the other side, MDA is measured using special kit, MDA kit, (Bio-Diagnostic Company, Egypt).

According to Beardeu and Fuqucy, (1980), progressive forward sperm motility was evaluated by microscopical examination of diluted semen. On the other side, Live/dead percentage of sperms was determined by using eosin nigrosine stain (Bloom, 1950). The sperm cells abnormalities were determined by using alkaline methyl violet according to Blom, (1943). In this concern, sperm abnormalities were classified into primary (giant, dwarf and double head as well as double or coiled tail) and secondary (detached head, bent and/or wavy tail).

Throughout the experimental study, the obtained data were subjected to statistical analysis as outlined by Snedecor and Cochran, (1987) as well as SAS Program (1994) to determine ANOVA.

Results

Pituitary and serum of gametogenic hormone FSH and ICSH levels (i.u) in control and treated rats. It appears from results in Table 1 that pituitary content of FSH among negative and positive controls was 7.31 ± 0.62 and 3.52 ± 0.63 i.u. / mg dry weight, respectively. On the other side, the corresponding serum levels were 4.76 ± 0.48 and 2.31 ± 0.22 i.u. / ml, respectively

Table (1): Pituitary and serum of gametogenic hormone "FSH" and interstitial cell stimulating hormone "ICSH" levels (i.u) in control and treated rats (mean ± SE).

Treatment	FSH		ICSH	
	Pituitary (i.u /mg)	Serum (i.u /ml)	Pituitary (i.u /mg)	Serum (i.u /ml)
Control negative	7.31 ± 0.62^{A}	4.76 ± 0.48^{A}	4.29 ± 0.37^{A}	2.41 ± 0.19^{A}
Control positive	3.52 ± 0.63^{B}	2.31 ± 0.22^{B}	1.67 ± 0.21^{B}	0.72 ± 0.11^{B}
AA with $CdCl_2$	7.25 ± 0.74^{B}	2.81 ± 0.34^{A}	4.22 ± 0.41^{B}	2.26 ± 0.21^{B}
Taurine with $CdCl_2$	7.52 ± 0.61^{B}	3.96 ± 0.56^{B}	4.52 ± 0.52^{B}	2.39 ± 0.17^{B}
β-ME with $CdCl_2$	2.91 ± 0.26^{A}	2.07 ± 0.17^{A}	3.79 ± 0.36^{B}	1.91 ± 0.15^{AB}
AA and Taurine with $CdCl_2$	6.86 ± 0.72^{B}	2.52 ± 0.23^{A}	4.09 ± 0.37^{B}	2.19 ± 0.29^{B}
AA and β-ME with $CdCl_2$	6.98 ± 0.62^{B}	3.07 ± 0.32^{AB}	4.07 ± 0.29^{B}	2.03 ± 0.32^{B}
Taurine and β-ME with $CdCl_2$	7.09 ± 0.59^{B}	4.11 ± 0.47^{B}	4.13 ± 0.36^{B}	2.21 ± 0.17^{B}
AA, taurine and β-ME with $CdCl_2$	7.21 ± 0.53^{B}	4.25 ± 0.51^{B}	4.42 ± 0.47^{B}	2.56 ± 0.26^{B}

SE : standard error

In the same column, treatment that has identical capital letter differ significantly ($p \leq 0.05$) from the corresponding negative or positive controls.

Table (2): Serum testosterone level (ng / ml) in controls and treated rats (mean ± SE).

Treatment	Testosterone level (ng / ml)
Control negative	5.33 ± 0.42^{A}
Control positive	1.05 ± 0.13^{B}
AA with $CdCl_2$	4.62 ± 0.52^{B}
Taurine with $CdCl_2$	5.79 ± 0.61^{B}
β-ME with $CdCl_2$	2.09 ± 0.17^{AB}
Ascorbic and taurine with $CdCl_2$	4.57 ± 0.42^{B}
AA and β-ME with $CdCl_2$	4.31 ± 0.47^{B}
Taurine and β-ME with $CdCl_2$	4.20 ± 0.51^{B}
AA, taurine and β-ME with $CdCl_2$	4.83 ± 0.61^{B}

SE : standard error

In the same column, treatment that has identical capital letter differ significantly ($p \leq 0.05$) from the corresponding negative or positive controls .

which indicate a drastic effect of $CdCl_2$ upon pituitary and serum GH contents. Regarding pituitary ICSH content, it was 4.29 ± 0.37 and 1.67 ± 0.21i.u. / mg dry pituitary weight among negative and positive controls, respectively. On the other side, the corresponding serum levels were 2.41 ± 0.19 and 0.72 ± 0.11 i.u. / ml, respectively which show an inhibitory effect of $CdCl_2$ upon pituitary and serum ICSH contents.

All treatments with different antioxidants improved pituitary content of both hormones except upon using β-ME which failed to restore the normal FSH level. On the other side, combinations of either the 3 antioxidants or taurine alone were successful to regain serum hormones level to their normal pattern.

Serum testosterone level (ng / ml) in controls and treated rats. It appears from Table 2 that serum T level among negative and positive controls was 5.33±0.42 and 1.05±0.13 ng / ml, respectively which clarifies a significant adverse effect of $CdCl_2$ upon serum T levels.

On the other side, all treatments with different antioxidants in combination with $CdCl_2$ improved serum levels of T.

Semen picture of control and treated rats. Results in Table 3 shows that administration of 1/100 LD_{50} $CdCl_2$ for 8 successive weeks in control positive group resulted in alteration of the studied semen parameters as compared with the corresponding control negative ones. Combination between 1/50 LD_{50} of different antioxidants and $CdCl_2$ over the experimental period improved the studied semen parameters as compared with the control positive group.

Taurine alone was found to be the best antioxidant treatment as it overcame the $CdCl_2$ harmful effect and restored the normal profile of the studied semen qualities. The least effective treatment was combination of AA, taurine and β-ME as well as combination of taurine and β-ME.

Table (3): Semen picture of control and treated rats (Mean ± SE).

Treatment	Individual motility %	Live - dead %	Sperm abnormalities		
			Primary	Secondary	Total
Control negative	73.00±0.30	84.80±0.88	3.65±0.15	9.35±0.21	13.00±0.26
Control positive	59.00±0.35	62.20±0.63	8.65±0.32	17.10±0.34	25.75±0.43
Ascorbic acid with cadmium	67.95±0.32	81.30±0.30	4.25±0.19	12.50±0.30	16.75±0.29
Taurine with cadmium	72.65±0.35	83.00±0.68	3.55±0.17	10.25±0.32	13.8±0.32
Betamercaptoethanol with cadmium	69.55±0.44	78.95±0.36	4.20±0.21	9.95±0.31	14.15±0.31
Ascorbic and taurine with cadmium	70.10±0.39	82.90±0.91	4.00±0.22	10.30±0.32	14.30±0.34
Ascorbic and betamercaptoethanol with cadmium	68.50±0.39	83.80±0.48	4.75±0.25	12.70±0.47	17.50±0.34
Taurine and betamercaptoethanol with cadmium	69.35±0.44	77.60±0.64	4.25±0.19	10.90±0.35	15.15±0.38
Ascorbic acid, taurine and betamercaptoethanol with cadmium	66.40±0.31	77.80±0.37	3.95±0.17	10.55±0.31	14.40±0.36

Table (3) continue: Statistical analysis

Groups	Control positive	AA	Taurine	β-ME	AA and taurine	AA and β-ME	Taurine and β-ME	AA, taurine and β-ME
Control negative	$S_{1,2,3}$	$S_{1,2,3}$		$S_{1,2}$	S1	$S_{1,3}$	$S_{1,2,3}$	$S_{1,2,3}$
Control positive		$S_{1,2,3}$	$S_{1,2,3}$	$S_{1,2,3}$	$S_{1,2,3}$	$S_{1,2,3}$	$S_{1,2,3}$	$S_{1,2,3}$
AA			$S_{1,2,3}$	$S_{2,3}$	S_3	S_2	$S_{2,3}$	$S_{2,3}$
Taurine				$S_{1,2}$	S_1	$S_{1,3}$	$S_{1,2,3}$	$S_{1,2}$
β-ME					S_2	$S_{2,3}$	S_3	S_1
AA and taurine						S_3	S_2	$S_{1,2}$
AA and β-ME							$S_{2,3}$	$S_{1,2,3}$
Taurine and β-ME								S_1

S : Significant at $P < 0.05$.
1, 2 and 3 represents individual motility, live-dead % and total abnormalities, respectively

Table (4): Serum levels of total antioxidants, Malondialdehyde in control and treated rats.

Treatment	Total antioxidant (Mmol. / L)	Malondialdehyde (nmol. / ml)	oxidant / antioxidant ratio
Control negative	2.20 ± 0.09 ^{abcef}	2.54±0.07 ^{ab}	1.15
Control positive	1.15 ± 0.09 ^{acbef}	3.75±0.11 ^{acdf}	3.26
AA with $CdCl_2$	3.73 ± 0.10 ^{c}	1.87±0.06 ^{acdef}	0.5
Taurine with $CdCl_2$	3.88 ± 0.07 ^{bd}	1.18±0.09 ^{acdg}	0.3
β-ME with $CdCl_2$	2.99 ± 0.06 ^{acdef}	2.42±0.12 ^{ce}	0.8
AA and taurine with $CdCl_2$	3.96 ± 0.07 ^{a}	2.40±0.14 ^{d}	0.61
AA and β-ME with $CdCl_2$	3.83 ± 0.07 ^{e}	0.85±0.05 ^{aef}	0.22
Taurine and β-ME with $CdCl_2$	3.70 ± 0.09 ^{f}	2.33±0.13 ^{fg}	0.63
AA, taurine and β-ME with $CdCl_2$	3.53 ± 0.08 ^{ade}	2.94±0.15 ^{adef}	0.83

SE : Standard error.
In the same column, data with identical letters differ significantly at least $P < 0.05$.

Serum levels of total antioxidants, Malondialdehyde in control and treated rats. Data presented in Table 4 disclose that administration of 1/100 LD_{50} $CdCl_2$ for 8 successive weeks in control positive group led to remarkable suppression of the serum total AOA as compared with the corresponding levels in the other groups in addition to elevation of MDA level compared with all treated groups.

All applied treatments showed an improvement of the total AOA and reduction of MDA levels as compared with the control negative group. Moreover, taurine alone or combined with AA or β-ME as well as AA alone or combined with β-ME were found to be the best treatments to increase total antioxidant level even more the control negative one.

Discussion

Tables 1, 2 and 3 display levels of pituitary and serum gonadotropins as well as serum T levels in mature male rats subjected to $CdCl_2$ – induced oxidative stress. It appears that administration of $CdCl_2$ for 2 successive months resulted in suppression of FSH and ICSH both in the pituitary gland and in the serum concomitant with a decline of serum T level as compared to the negative control group. In this respect, Lafuente *et al.,* (2003) showed that administration of $CdCl_2$ in drinking water for 30 to 60 days acted as an endocrine disruptor by altering the homeostatic balance of a variety of hormones including pituitary and serum concentrations of gonadotropins and T. Furthermore, Acharya *et al.,* (2008); Manna *et al.,* (2008) found that $CdCl_2$ decreased 3β-HSD and 17β-HSD enzymes responsible for steroid genesis leading to reduction of plasma testosterone level.

Results of the current study showed that application of different antioxidants into rats under stress led to improvement of the reproductive hormonal profiles except in case of pituitary FSH with β-ME treatment as well as serum FSH with β-ME, combination of AA with taurine, AA alone as well as combination of AA and β-ME. Similar pattern is detected in case of ICSH with β-ME.

On the other side, the best restoration of the reproductive hormonal pattern, in comparison with the negative controls, was implemented following application of taurine, combination of the 3 antioxidants and AA alone. In this concern, Miller and Cicero, (1986) found that AA has the ability to increase LHRH in vitro from hypothalamic fragments. Moreover, in vitro research of Karanth *et al.,* (2001) showed that incubation of anterior pituitary from adult male rats with AA for one hour induced significantly high release of both FSH and LH. Regarding taurine, Sloley *et al.,* (1992) documented that injection of goldfish with taurine caused an elevation in serum gonadotropin concentrations.

It is worth mentioning that under stress, taurine alone, AA alone and the combination of the 3 antioxidants were beneficial to protect the pituitary gland hormones and testosterone against $CdCl_2$-induced oxidative stress. However, it is also noticed that β-ME treatment alone has the least protective effect upon pituitary and serum FSH. Therefore, the present study recommends the usage of either taurine alone or AA alone to compensate the harmful effect of oxidative stress upon reproductive hormones. In addition, β-ME alone is not recommended to be supplemented to the animal as it has a weak potential activity against stressors.

In the present study, table 3 displays that administration of 1/100 LD_{50} $CdCl_2$ for 8 successive weeks resulted in alteration of the studied semen parameters (sperm motility, live / dead ratio and sperm abnormalities) as compared with the corresponding negative control. This effect is referred to testicular oxidative stress induced by $CdCl_2$. Previous records showed that $CdCl_2$ increased intracellular concentration of ROS like O_2^{-} $^{-}$, OH^{-} and species that are not radicals in nature (H2O2, NO and $ONOO^{-}$) resulting in elevated levels of lipid peroxidation, protein carbonylation, glutathione disulfide and DNA fragmentation as well as decreased levels of the activities of the antioxidant enzymes, total thiols and reduced glutathione (Acharya *et al.,* 2008; Manna *et al.,* 2008). These results come in accordance with those obtained by Acharya *et al.,* (2008) in mice who reported that even single intraperitoneal injection of $CdCl_2$ (1 mg/kg b.w.) increased sperm abnormalities and decreased sperm count.On the other side, application of different antioxidants (either alone or in combination) with 1/100 LD_{50} $CdCl_2$ improved the studied semen parameters as compared with the control positive group (Table 4) with variable degrees of success being maximal with taurine alone and minimal after combination of the 3 antioxidants. In this respect, Gupta *et al.,* (2004) in rat and Acharya *et al.,* (2008) in mice showed that AA has a protective effect on the testes against Cd toxicity. Narayana *et al.,* (2005) in rat also found that methyl parathion (organophosphorous pesticide) increased the incidence of sperm abnormality. The authors referred these deviations to decreased testicular AA level.

Regarding taurine, it was found to play an important role for preventing changes in membrane permeability induced by oxidative injury leading to stabilization of its activity (Timbrell *et al.,* 1995). In this concern, Manna *et al.,* (2008) found that treating mice with taurine in drinking water has a prophylactic effect on Cd -induced testicular pathophysiology and maintains normal semen quality. On the other side, the antioxidant activity of β-ME comes from its ability to increase the cysteine-mediated GSH synthesis (Zmuda and Friedenson, 1983).

Glutathione is a tripeptide composed of 3 amino acids; glutamate, cysteine and glycine. The sulfur of the cysteine that present as thiol (⁻SH) provides the functional portion of GSH and in the presence of ROS is oxidized to GSSG and thus detoxifies the radicals. GSSG is recycled to GSH by reduced NADPH - dependent GSSG reductase (Jones, 2002). In this respect, Zubkova and Robaire, (2004) showed that GSH depletion by L-buthionine-S,R-sulfoximine " BSO " impaired sperm motility. However, it is observed from the present study that the potential effect of taurine alone, AA alone or their combination is more beneficial than if β-ME is added; a finding that emphasizes the protective activity of taurine and AA.

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تأثير بعض مضادات الأكسدة على الكفاءة التناسلية فى الفئران

تهدف الدراسة الحالية الى معرفة تأثير فيتامين سى او تورين او بيتامركابتوايثانول او خليط من فيتامين سى والتورين او خليط من فيتامين سى والبيتامركابتوايثانول او خليط من التورين والبيتامركابتوايثانول او خليط من فيتامين سى والتورين و البيتامركابتوايثانول على الكفاءة التناسلية لذكور الفئران التي تعرضت لضغوط مؤكسدة بواسطة تناول كلوريد الكادميوم. لهذا الغرض تم تقسيم ١٨٠ من ذكور الفئران الى ٩ مجموعات. المجموعة الاولى تناولت المياه المقطرة اما المجموعة الثانية فتناولت ١٠٠/١ من الجرعة السامة لكلوريد الكادميوم. المجموعات الباقية تناولت ١٠٠/١ من الجرعة السامة لكلوريد الكادميوم ثم بعد ٣ ايام تناولنت ٥٠/١ من الجرعة السامة لكلا من فيتامين سى او تورين او بيتامركابتوايثانول او خليط من فيتامين سى والتورين او خليط من فيتامين سى والبيتامركابتوايثانول او خليط من التورين والبيتامركابتوايثانول او خليط من فيتامين سى والتورين و البيتامركابتوايثانول على الترتيب لمدة شهرين متتابعين. تناول١٠٠/١ كلوريد الكادميوم لمدة ٨ اسابيع متتالية ادى الى نقص مستوى الهرمونات فى الدم وفى الغدة النخامية. ايضا نقص فى جودة المنى ونقص فى مستوى النسبة الكلية لمضادات التأكسد و زيادة نسبة المالون داى ألديهايد فى الدم كما انه دمر الخلايا المنوية. على الجانب الاخر تناول مضادات التأكسد المختلفة مع كلوريد الكادميوم ادى الى زيادة مستوى الهرمونات فى الدم والغدة النخامية. ايضا ادى الى تخسين جودة المنى مع زيادة مستوى الدم من النسبة الكلية لمضادات التأكسد بينما ادى الى تقليل نسة المالون داى ألديهايد. هذه الدراسة ايضا بينت ان العلاج الافضل الذى ادى الى احسن نتيجة هو الفيتامين سى او التورين او المزج بينهما بينما استخدام البيتامركابتوايثانول لم يكن له دور واضح فى تحسين الكفاءة التناسلية.