Egypt. J. Chem. Environ. Health, 1 (1):94-112 (2015)

Hepatoprotective Effect of Jojoba Oil on DNA Damage and Antioxidant Enzymes Induced by Cadmium in Rats

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Abstract

The present investigation aimed to study the effect of the treated jojoba oil and / or ascorbic acid (vitamin C) in rats exposed to cadmium chloride ($Cdcl_2$) at adose 10 mg/ 100g.b.wt. for 30 successive days . Forty mature rats of an average body weight 150-180 gm were used for the experiments . Fifteen rats were administered cadmium orally at dose 10mg/100gm.b.wt. for 30 successive days (for induced damage of DNA) . All rats divided into 8 equal groups of 5 rats each.Group1:served as control negative. Group2:fed on basal diet and administered cadmium orally for 30 days(control positive).Group3:fed on basal diet mixed with treated jojoba oil in ratio 2.5%. Group4:fed on basal died and administered orally vitamin C. Group5: fed on basal diet mixed with treated jojoba oil in ratio 2.5% and administered vitamin C Goup6(pretreated with cadmium) fed on basal diet mixed with treated J.O. in ratio 2.5% for 30 days. Group7(pretreated with cadmium) fed on basal diet and administered vitamin C orally for 30 days.Group8(pretreated with cadmium) fed on basal diet mixed with treated J.O. in ratio 2.5% and administered vitamin C orally for 30 days.

The obtained results showed that administration of treated jojoba oi land/or vitamin C to cadmium chloride intoxicated rats induced significant increase in the amount of DNA / gm of spleen ,decrease in the percentage of MPCE and increase the frequency of NCE .

Cadmium exposure increased the production of reactive oxygen species (ROS) and altered the levels of oxidative stress related biomarkers of toxicity. Results revealed that cadmium significantly increased catalase enzyme activity and lipid peroxidation concentration (MDA) while reduced glutathione was decreased in rat sera. Furthermore, cadmium exposure were associated with depletion of serum levels of vitamin C, E, A and β- carotene. As compared to the results obtained in control groups the study showed that a lower concentration of serum proteins and albumin were accompanied by decreased globulin alpha 1 and beta along with an increased gamma 2 globulin; and the activity of serum GGT, LDH, ALP and concentrations of cholesterol, and triglyceride,were higher. Moreover, jojoba oil and vitamin C have ameliorated the cadmium-induced toxicity by improving antioxidant markers. The results of this investigation demonstrated that pretreatment with jojoba oil and vitamin C have the

potentials to countermeasure the immunosuppressive, biochemical alteration as well as oxidative damage induced by cadmium in rats.

Introduction

Cadmium (Cd) is a very toxic heavy metal and an important environmental pollutant in the soil, water, air and food**(Benavides et al., 2005).** Relatively large quantities of Cd are found in commercial phosphate fertilizer , thus the increases in soil and plant Cd contents may lead to increases in dietary Cd level. It can be transferred to the food chain and bio-accumulation in human and animals where it has a half – life greater than 20 years **(Andrew etal, 2003 and Jarup, 2002)**.

Cadmium compounds have been classified as a human carcinogen by the International Agency for Research on Cancer (IARC) .It causes the formation of carcinogenesis on human and experimental animals (**Ramesh and Satakopan, 2010).**. Cadmium induces DNA damage in intact cell and affects their repair **(Dally and Hartwig, 1997)**

Prolonged exposure to cadmium results in injury to liver, lung, kidney and testes **(Sultan etal,2004 and Zitkevicius et al., 2011)**.

The toxic effect of Cd is due to its inhibition of liver metabolic enzyme systems containing sulfhydryl groups and uncoupling of oxidative phosphorylation in the mitochondria **(Williams et al., 1999).** This results is appear in increase lipid peroxidation, DNA damage, depletion of sulfhydryls, altered calcium homeostasis, hepatic congestion, ischemia and hypoxia **(Habeebu et al., 1998 and Bharavi et al., 2010).** Cadmium toxicity is associated with several clinical complications, renal dysfunction, bone diseases, hepatic dysfunction **(Jarup et al. 2000).**

The metabolism and excretion of this heavy metal depend on the presence of antioxidants and thiols that cadmium metallothionein-binding **(Simpkins etal,2008 and Patrick,2013) .**

Ascorbic acid is the primary water soluble antioxidant which may have an important role in scavenging free oxygen radicals and in stabilizing the cell membranes, thus maintaining its permeability **(Ki etal, 2013 Ambali et al., 2011; Assia et al., 2012).**

The oil from jojoba plant (Simmondsia chinensis) is the main biological source of wax esters **(Kolscheuer etal, 2006)** in which the unsaturated acids (eicosenoic and oleic) and the unsaturated alcohols (docosenol and eicosenol) are the major acids and alcohols **El-Halawany, (2004)** This oil contains certain quantities of sterols, stanols and different toccopherols **(Tada et al., 2005; El-Mallah et al., 2009).** Jojoba liquid wax reduced nitric oxide (NO) level and tumor necrosis factor- alpha (TNF-alpha).Jojoba

liquid was has effectiveness in combating inflammation in several experimental models **(Habashy etal,2005)**.

Research consistently demonstrates the mixtures made up of smaller amounts of variety of antioxidants are more strongly protective against more conditions than are large amounts of any single antioxidant, therefore the object of this study was to determine the protective effect of both treated jojoba oil and vit.C on the toxicity of cadmium in rats.

Materials and Methods

1. Heavy metal:

Cadmium chloride ($Cdcl_2$) :(99.9% purity) was purchased from sigma chemical Co. It was given at dose 10 mg/100 g.b.wt. for 30 days.

2. Antioxidants:

a. Vitamin C: :(99.9% purity) was purchased from sigma chemical Co. It was given at dose of 1.44 mg/100g.b.wt. for 30 days

b. Jojoba oil : Crude jojoba oil was obtained from National Oil Co. The treated jojoba oil was used at a concentration of 2.5% (Hanan etal,1998) with the basal diet for 30 days**.**

3. Animals:

Forty mature rats of an average body weight of 150-180 were used for the experiments. Rats were left for two weeks for accommodation before starting the experiment. They were fed on standard ration and water supply was given ad-libitum.

Experimental design:

Fifteen rats were administered orally cadmium at dose 10mg/100gm.b.wt. for 30 successive days before starting the experiments to induce damage of DNA(group 6,7,8). After that rats were divided into 8 equal groups of 5 rats each. They were treated as follow:

Group1 : served as control group and fed on basal diets for 30 days.

Group2: fed on basal diets and administered orally cadmium at dose 10 mg/100gm.b.wt. for 30 days(control positive).

Group3: fed on basal diets mixed with treated jojoba oil in ratio 2.5% for 30 days.

Group4: fed on basal diets and administered orally vitamin C at dose 1.44 mg/100gm.b.wt. for 30 days.

Group5: : fed on basal diets mixed with treated jojoba oil in ratio 2.5% and administered orally vitamin C at dose 1.44 mg/100gm.b.wt. for 30 days.

Group6: (pretreated with cadmium) fed on basal diets mixed with treated jojoba oil in ratio 2.5% for 30 days.

Group7: (pretreated with cadmium) fed on basal diets and administered orally vitamin C at dose 1.44 mg/100gm.b.wt. for 30 days.

Group8: (pretreated with cadmium) fed on basal diets mixed with treated jojoba oil in ratio 2.5% and administered orally vitamin C at dose 1.44 mg/100gm.b.wt. for 30 days.

Samples:

- Spleen samples were taken from the sacrificed rats in all experimental groups. Samples were collected in clean dry plastic bags and kept at -20^{o}C for determination of DNA.
- Bone marrow samples were collected from both femurs of rats of each groups at the end of experimental period and used for micronucleus analysis.
- Individual blood samples were obtained at the end of experiment from rats of each group, left to clot, sera were separated and kept at - 40°C for biochemical analysis Then animals were sacrificed and autopsy performed immediately; liver tissue was removed and washed with saline solution, then minced and homogenized (10% w/v) in ice-cold normal saline. The homogenate was centrifuged at 10,000xg for 20 min at 4C° and the resultant supernatant was used for antioxidant assay (Chitra et al., 1999).

Methods:

1- DNA isolation:

The isolation of DNA from spleen of rats according to the following protocol based on the solubility of nucleoprotein in water and the high ionic strength of the solution, these used in the initial extraction.

The tissue at first homogenized in isotonic saline buffered with sodium citrate, pH 7, then protein is removed by treatment with a chloroform/ amyl alcohol mixture and the DNA precipitated with ethanol **(Schwader and Singer,1950).** Estimation of isolated DNA was carried by **(Dische and Schwarz,1973)**.

2- Cytogenetic analysis:

The following protocol established by**(Salamon etal,1980) from each group**, bone marrow cells of five mature rats from each group were extracted with a pin into a clean dry glass slide and homogenized with two drops of fetal calf serum. Cells were smeared on the slide, air dried, fixed in absolute methanol and stained with Giemsa in phosphate buffer pH 6.8. The polychromatic erythrocytes (PCEs,1000/animal) were screened for micronuclei and the changes in mitotic activity **(Hart and Engberg-Pederson,1983 and Al-Bekairi etal,1991)** were assessed on basis of the ratio of polychromatic to normochromatic erythrocytes (PCE/NCE).

3- Serum biochemical studies:

The biochemical assays of serum gamma glutamyle transferase (GGT) and lactic dehydrogenase (LDH) activities were determined according to methods of **(Szase et al., 1976)** , alkaline phosphatase (ALP) activity according to **Tietz,(1996)**, serum urea level according to **Wybenga et al. (1971**), Triglyceride **(Wahlefeld, 1974),** cholesterol **(Watson, 1960)**, high density lipoprotein (HDL), low density lipoprotein (LDL), **(Peace and Kaplan, 1987)**. Vitamin E, A, C and β carotene were performed according to **Henry et al., (1974).** Estimation of serum total protein and electrophoretic pattern were carried out after **SonnenWirth and Jaret, (1980), Davis, (1964)** respectively and calculated according SynGene S. No. 17292*14518 sme*mpcs**.**

- Catalase activity; lipid peroxidation as malonaldehyde (MDA) and reduced glutathione (GSH) in homogenate liver tissues were determined according to **Aebi (1974); Ohkawa et al., (1979) and Ellman (1959),** respectively.

The obtained data were statistically analyzed using t`test after **Petrie and Watson (1999).**

4- Statistical analysis:

Data were expressed as mean ± S.E. Significant different using student "t" test . **(Snedecor,1982)** .

Results and Discussion

Cadmium is a toxic heavy metal that is widely used in different industries. It promotes oxidative stress and development of serious pathological conditions because of long retention in some tissues. In the present study we investigate the antioxidant effect of both vitamin C and treated jojoba oil on DNA, cytogenicity and biochemical parameters alterations induced by cadmium chloride in rats.

Our study revealed that oral administration of cadmium chloride at a dose of 10 mg/100gm.b.wt. for 30 days exerts genotoxic effect. It induced DNA damage and significantly increased the frequency of micronuclei formation in rat bone marrow cells.

DNA analysis revealed that cadmium chloride induced a significant decrease in the amount of DNA/gm of spleen in the group of rats that given cadmium chloride(G2) compared with control –ve group (Table 1). These findings agreed with the results of **(Hanan etal,2008 and Marina etal,2007)**who reported that cadmium compounds enhance the genotoxicity and inhibit DNA repair processes. **(Hartman and Hartwig,2008 and Snow, 2012)** indicate that cadmium blocks DNA repair by disturbing protein interactions involved in DNA damage recognition.

The groups of rats administered treated jojoba oil and /or vitamin C showed significant increase in the amount of DNA/ gm of spleen as compared with control –ve and control + ve group(administered cadmium).

The in vivo micronucleous assay is one of the most assays to reflect chromosomal aberration and DNA damage **(Celik etal,2005)**. Micronucleous test revealed significant in the percentage of micronucleated polychromatic erythrocytes (MPCEs) in cadmium treated group with an increase in PCE/NCE ratio. These findings are in agreement with **(Hanan etal,2008 and Kasuba and Rozga,2002)** who found significant increase in the frequency of micronuclei in rats and human treated with cadmium chloride

Administration of vitamin C and / or treated jojoba oil in groups pretreated with cadmium chloride , reduce the micronucleus frequencies in polychromatic erythrocytes and PCE/NCE ratio of bone marrow (Table 2).

Ascorbic acid affects oxidative changes which occur in the cells thus protect DNA from damage, reduce the micronuclei formation caused by free radicals(**Yuko etal,2003 Kevin etal,2009).**

Whereas treated jojoba oil helps in the storage and utilization of vitamin A and E in liver **(Hanan et al,1998)** and also act as antioxidant which may explain the reduction in the frequency of micronuclei formation and increase of the spleen DNA.

Treatment with Cd caused significant increase of serum GGT, LDH and ALP activities compared to the control group table ( ) . Cadmium is one of the heavy metals which induce membrane damage **(Şlencu et al., 2014).** It could be attributed to the hepatic damage resulting in increased release and leakage out of these enzymes from the liver cytosol into the blood stream which gives an indication on the hepatotoxic effect of this metal **(Pari and Murugavel, 2005).** In vitro, addition of cadmium (as CdCl2) in the culture medium of mouse hepatocytes significantly enhanced LDH and ALP leakage and increased reactive oxygen species (ROS) production **(Pal et al., 2011).** LDH is an

intracellular enzyme, the increased level of LDH in the serum indicate the cellular damages in the liver **Kim et al., (2001).**

Cadmium be attributed to the decreasing of the antioxidant defence system and elevation of free radical **(Kumar et al 2001).** So the decrease in liver GSH induced by cd administration is accompanied by a remarkable increase of GGTP activity could be explained through the fact that GGTP acts in transferring the γ-glutamyl group of GSH to amino acids through γ-glutamyl cycle **Hultberg, and Hultberg, (2005).**

As a result of cadmium activity one notices also renal tubule damage and then glomerular filtration impairment **(Shibutani et al., 2001).** This may account in our study for the increase of urea concentration in the animals receiving cadmium chloride. serum total cholesterol contents and triglyceride were significantly elevated in this study. The observed increase in plasma cholesterol was associated with an increase in LDL cholesterol fraction concentrations in the cadmium treated rats when compared with control (Table). This might be due to the impairment of liver function caused by the imbalance in antioxidant defense system in Cd intoxicated rats. In agreement with the study conducted by **Skoczyńska (2001)** stated that elevated levels of LDL-C and VLDL-C followed by the decrease in the level of HDL were noticed in Cd administered **Pari and Ramakrishnan (2013)**

Vitamin E and A (Vit E and A) is the primary liposoluble antioxidant, which may have an important role in scavenging free oxygen radicals and in stabilizing the cell membranes, thus maintaining its permeability **(Bjørneboe et al., 1990, Navarro et al., 1999).** Vitamin E and A may also affect oxidative changes which occur in other cell organelles **(Ibrahim et al., 2000).** Vitamin C is a potent scavenger of free oxygen radicals and it has been shown that marginal Vit C deficiency results in intracellular oxidative damage in the animal **(Hudécová and Ginter 1992, Nagyová et al.,1994, Tatara and Ginter 1994).** Our results showed that cadmium intoxication decreased the concentration of Vit C, E, A and b-carotene as compared to control animals (table.). The observed depletion of serum levels of vitamin C, E and A can be explained by impairment of liver function and peroxidative processes caused by cadmium (**Pavlović, 2001).**

The exposure to Cd produced a significant adverse effect on the liver redox status, which is indicated by a significant reduction in reduced glutathione and catalase activity while, significant increase in lipid peroxidase levels in liver tissues table ( ).

A Cd-induced significant increase in lipid peroxidation which is usually the most practical and reliable for detecting and screening for oxidative stress .Also The diminution of glutathione level in cadmium treated animals may be as a result of oxidative stress, which has occurred in cadmium toxicity. In other words the reduced

antioxidant production due to the increased oxygen metabolites and the elevated free radicals, which caused a decrease in the activity of the anti-oxidant defense system **(KARA et al 2005Yang and Wang et al., 2013).** GSH depletion in hepatocyte tissue, has been shown to be an important mechanism in the pathology of experimental liver injury **(Yuan et al 2007).** It has been reported that higher GSH levels help to lower cadmium toxicity through conjugation with the cd.**(Hultberg, and Hultberg, 2005 and Moniuszko-Jakoniuk 2005)**. In addition, the decreased activity of hepatic catalase in cadmium treated animals suggests that either there is an interaction between the accumulated free radicals and the active amino acids of this enzymes **(Das et al., 2001)** or there is a direct binding of the metal to the active sites of the enzyme **(Misra et al., 1990).** these results also could be attributed to the decreasing of the antioxidant defense system and elevation of free radical **(Kumar et al 2001).**

cadmium in rats inducing significantly decreased values in serum total protein, albumin, alpha globulin, and gamma globulin while, increase in beta globulin (table,). The decrease in serum total protein and albumin of Cd-treated rats might be due to changes in protein synthesis and/or metabolism **(Dostal et al., 1989; Das and Dasgupta, 2000).** This result is in agreement with other findings **(Yousuf, 2002 and Hristev et al 2008).**

At the same table ( ) the globulin component showed drop in α1, β2, and γ1 and in all the exposure animals with cadmium, while increase α1, β1 and γ 2globulin as compared with control animals. The results coincided with the tune of total proteins and albumin. This may be attributed to that cadmium causes hepatotoxic, nephrosis, hemorrhages (liver and kidneys) **(Zitkevicius et al., 2011).**

In addition, cadmium has immunosuppressive effect inhibitnearly cellular and humeral immunologic reaction **(Raj, et al., 2011).** The decrease in serum level of globulin might be due to the adverse effect of cadmium on synthesis of total proteins and globulin and cadmium affect on the mechanisms of cell-mediated immunity reducing the immune defenses of the organism **(Vazzana, et al., 2014).**

Treatments with vitamin C and jojoba oil alone or in a combination of both restored and ameliorated these biomarkers. The significant improvement of the glutathione level was noticed when compared with that of Group (cadmium). Thus, the observed normalization of GSH levels, and catalase activities following vitamin C or jojoba treatment could be because these vitamins caused a decline in LPO accompanied by an increase in the activities/ level of catalase, and GSH in liver. In other words these vitamin and jojoba played an action in reducing the levels and accumulation of oxygen reactive species.

Jojoba oil proved many valuable uses, it has a hepatoprotective an anti-inflammatory **(El Shamy et al 2001),** immunostimulant and growth promoter **Youssef, (2005).**also Jojoba protein contains high amounts of most essential amino acids, where methionine was the first limiting amino acids **(Motawe, 2005).**
It is well established that vitamin Ccan protect indispensable molecules in the body, such as protein, lipids, carbohydrates and nucleic acids (DNA and RNA) **(Schneider et al., 2001).**

Vitamin C had protected liver function from cadmium intoxication as indicated by the significant restoration of serum total protein, albumin, serum, GGT, LDH and alkaline phosphatase. vitamins played an action in reducing the levels and accumulation of oxygen reactive species. **Layachi and Kechrid (2012)**. However, vit C plus jojoba oil proved more effective as compared to individual of each.

Finally, the treated jojoba oil has a considerable antioxidant effect in comparable to ascorbic acid. Their combined administrations have more beneficial effect in monitoring the genotoxic effect and biochemical alterations in cadmium intoxicated rats.

Table(1): Effect of treated jojoba oil and/or vitamin C on the amount of DNA mg/gm of spleen in cadmium chloride intoxicated rats.

Groups	Amount of DNA mg/ gm spleen
G1 (C-ve)	0.164± 0.0089
G2 (C+ve)	0.08± 0.03***
G3 (J.O.)	4.7± 0.07***
G4 (Vit.C)	2.27± 0.11***
G5 (J.O.+Vit.C)	10.9± 0.2***
G6 (preteated with cadmium + J.O.)	1.01± 0.00005***
G7 (preteated with cadmium + Vit.C)	2.92± 0.024***
G8 (preteated with cadmium + J.O.+ Vit.C)	5.8± 0.063***

Values represents the mean ± S.E.

***P < 0.001

Table(2): Effect of treated jojoba oil and/or vitamin C on the incidences of MPCE on the relation if PCE / NCE in cadmium chloride intoxicated rats.

Group	PCE	MPCE/1000 PCE± S.E.	NCE screened	PCE/NCE ratio
G1	5000	5.6± 0.32	2183	2.29±0.4
G2	5000	5.76±0.62	2035	1.34±0.23
G3	5000	5.4±1.2	1543	2.17±0.3
G4	5000	4.9±0.35	1789	1.73±0.01
G5	5000	21.04±0.01***	438	11.16±0.2***
G6	5000	15.84±0.2***	1786	1.584±0.2
G7	5000	11.44±0.1***	1699	1.77±0.189
G8	5000	7.44±0.081***	1596	2.036±0.01

Values represents the mean ± S.E.

***P < 0.001

Table(3): Effect of cadmium on some serum biochemical parameter of s of male rats.

Parameter	control	vit C	jj	cd	cd+vitC	cd+jj	CD+C+JJ
GGT u/l	13.73± 0.81	12.16± 0.64	14.76± 0.79	23.88± 1.29	20.22± 2.16	19.91± 2.13	17.79± 4.99
LDH u/l	500.14± 28.54	487.35± 27.1	511.61± 24.21	780.013± 23.43	608.33± 26.26	624.55± 29.97	604.71± 23.54
ALP u/l	223.9± 15.32	234.8± 17.74	242.00± 15.64	333.2± 14.38	282.4± 17.86	297± 18.96	290.8± 15.65
urea mg%	33.64± 3.74	30.14± 0.45	29.57± 0.65	49.56± 2.41	44.2± 1.22	41.11± 1.45	43.54± 1.02
Cholesterol mg/dl	76.48± 7.11	74.19± 5.03	81.13± 2.79	121.34± 5.41	101.9± 8.59	95.53± 8.56	89.43± 17.76
Triglyceride mg/dl	85.39± 7.7	85.47± 7.9	79.99± 4.32	132.5± 12.76	116.00± 11.67	122.2± 14.64	116.86± 11.42
HDL mg/dl	41.27± 5.61	43.75± 2.82	41.76± 2.44	17.78± 0.42	29.15± 1.84	30.44± 1.64	33.26± 1.27
LDL mg/dl	59.34± 6.7	60.094± 3.03	57.75± 4.24	114.28± 9.78	92.35± 7.4	84.88± 7.2	85.44± 6.65

- Results are expressed as means ± SEM (n =5), student 't' test

* P < 0.05

Table (4) : Effect of cadmium on serum vitamin C, E, A and β- carotene of male rats.

Parameter	control	vit C	jj	cd	cd+vitC	cd+jj	CD+C+JJ
vitc (µg/dl)	0.78± 0.08	0.98± 0.11	0.79± 0.08	0.39± 0.08	0.57± 0.017	0.55± 0.11	0.61± 0.15
vit E (µg/dl)	498.65± 17.64	513.57± 14.88	533.64± 13.96	380.26± 17.38	423.36± 15.73	466.68± 17.42	465.43± 13.53
vit A (µg/dl)	47.2± 4.83	44.25± 1.31	53.36± 2.38	30.9± 6.24	34.1± 2.73	38.52± 3.63	41.17± 4.52
B carotene (µg/dl)	27.2± 1.26	28.79± 1.33	31.45± 1.14	18.99± 1.17	22.1± 1.12	25.62± 1.13	25.99v 1.21

Results are expressed as means ± SEM (n =5), student 't' test

*** P < 0.05** **** P < 0.01** ***** P < 0.001**

Table (5) : Effect of cadmium on serum vitamin C, E, A and β- carotene of male rats.

Parameter	control	vit C	jj	cd	cd+vitC	cd+jj	CD+C+JJ
vitc (µg/dl)	0.78± 0.08	0.98± 0.11	0.79± 0.08	0.39± 0.08	0.57± 0.017	0.55± 0.11	0.61± 0.15
vit E (µg/dl)	498.65± 17.64	513.57± 14.88	533.64± 13.96	380.26± 17.38	423.36± 15.73	466.68± 17.42	465.43± 13.53
vit A (µg/dl)	47.2± 4.83	44.25± 1.31	53.36± 2.38	30.9± 6.24	34.1± 2.73	38.52± 3.63	41.17± 4.52
B carotene (µg/dl)	27.2± 1.26	28.79± 1.33	31.45± 1.14	18.99± 1.17	22.1± 1.12	25.62± 1.13	25.99v 1.21

Results are expressed as means ± SEM (n =5), student 't' test

*** P < 0.05** **** P < 0.01** ***** P < 0.001**

Table (6) : Effect of cadmium on some anti-oxidant parameter of liver tissue of male rats.

Parameter	control	vit C	jj	cd	cd+vitC	cd+jj	CD+C+JJ
MDA (nmol/g tissue)	2.41± 0.16	2.44± 0.42	2.43± 0.23	4.15± 0.3	3.31± 0.39	3.08± 0.34	2.9± 0.11
GSH (mg/g tissue)	37.8± 2.89	35.76± 1.52	38.23 2.42	22.45± 2.37	26.55± 2.61	29.45± 1.77	31.34± 3.76
Catalase (m.mol/ g tissue)	0.31± 0.03	0.37±0.06	0.45 0.05	0.2± 0.02	0.27± 0.028	0.28± 0.011	0.28± 0.02

- **Results are expressed as means ± SEM (n =5), student 't' test**
- *** P < 0.05 ** P < 0.01 *** P < 0.001**

Table(7): Effect of cadmium on serum total protein and electrophoretic pattern of male rats

Parameter	control	vit C	jj	cd	cd+vitC	cd+jj	CD+C+JJ
Alb	2.65± 0.1	2.45± 0.09	2.41± 0.05	2.06± 0.11	2.39± 0.06	2.29± 0.07	2.26± 0.09
T.alpha globulin	1.34± 0.03	1.44± 0.05	1.41± 0.03	1.25± 0.03	1.23± 0.04	1.23± 0.03	1.27± 0.03
Alpha1	0.99± 0.12	0.97± 0.11	0.84± 0.09	0.39± 0.08	0.61± 0.07	0.68± 0.07	0.74± 0.04
Alpha2	0.42± 0.06	0.46± 0.04	0.57± 0.05	0.86± 0.06	0.62± 0.05	0.55± 0.04	0.52± 0.05
t. beta globulin	1.21± 0.03	1.27± 0.06	1.22± 0.04	1.38± 0.04	1.05± 0.05	1.20± 0.02	1.25± 0.06
Beta1	0.51± 0.04	0.57± 0.03	0.49± 0.04	0.74± 0.03	0.39± 0.02	0.50± 0.05	0.58± 0.03
Beta2	0.79± 0.04	0.69± 0.03	0.72± 0.04	0.63± 0.03	0.66± 0.02	0.69± 0.05	0.66± 0.03
Gamma globulin	2.26± 0.16	2.19± 0.12	2.04± 0.13	1.62± 0.13	1.75± 0.19	1.80± 0.14	1.88± 0.11
Gamma1	1.98± 0.14	1.96± 0.1	1.77± 0.09	1.12± 0.12	1.46± 0.09	1.48± 0.07	1.49± 0.07
Gamma2	0.28± 0.03	0.23± 0.06	0.26± 0.04	0.39± 0.02	0.29± 0.05	0.31± 0.02	0.39± 0.06
T.globulin	4.80± 0.16	4.91± 0.12	4.68± 0.23	4.26± 0.3	4.03± 0.39	4.24± 0.34	4.41± 0.11
A/G ratio	0.54± 0.02	0.49± 0.03	0.51± 0.02	0.48± 0.04	0.59± 0.02	0.51± 0.07	0.51± 0.06
T. protein	7.47± 0.2	7.36± 0.97	7.09± 0.34	6.32± 0.21	6.42± 0.24	6.44± 0.29	6.67± 0.24

- **Results are expressed as means ± SEM (n =5), student 't' test**

*** P < 0.05** **** P < 0.01** ***** P < 0.001**

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