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Parenteral Toxicity of Medroxyprogesterone Acetate

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Abstract: Background: Depot Medroxyprogesterone acetate (Depo-Provera®; DMPA) is a long term contraceptive used throughout the world. DMPA exerts its effects by blocking ovulation and inducing endometrial atrophy. Objective: This study designed to investigate the parenteral effect of DMPA on the adult female rats. Design and setting: Eighty healthy adult female rats (*Sprague-Dawley*) were randomly assigned into two major groups; each one divided into four minor groups injected weekly with DMPA doses (Vehicle 0; 2.7; 5.4 and 10.8 mg/kg/day) for four or six weeks, rats were observed for body weights, viability and death, at the end of experiment animals were sacrificed for further and biochemical investigation. Heart blood was drawn and sera were separated for assessment of liver function test, lipid profile, obesity and oxidative stress markers were assessed. Assessment of significant difference between the treated and control groups were carried out using SPSSv12 software. Results: DMPA doses induced marked body weight gain elevations in liver function tests both ALT & AST and decrease in the activity of SOD, GSH-Px, NPSH and increase in production of TBARS. These alterations were statistically significant ($P \leq 0.01$) as well as dose and time dependant. Conclusion: The findings of our study shed more light on the long term effects of DMPA and support the claims that this progestational hormone derivative, while being a contraceptive, may induce harmful health alterations. Thus, special care should be exercised for women use this medication. Cardiovascular, hepatic markers as well as body weight should be evaluated periodically.

Key words: Depo-Provera®, Female Rats, Liver Functions, Obesity, Oxidative Stress.

INTRODUCTION

Hormonal contraceptives have proven to be the most effective and safe contraceptives in history. Contraception dates back as far as ancient Egypt and Greece Kapu and Kumar (2008). After World War II, the increase in world population was alarming and birth control pill was developed for contraception. Since the discovery that progestational steroids compounds could inhibit ovulation Chang *et al.* (1956), several million women have used different types of synthetic progestins to prevent conception.

Currently, worldwide, more than 90 million women in 130 countries depend on injections of long acting depot medroxyprogesterone acetate (DMPA; Depo-Provera®) to avoid unwanted pregnancies FDA (2005). Weight increase is a common concern for women initiating the use of hormonal contraceptives, especially depot medroxyprogesterone acetate (DMPA), and weight increase is a frequent reason for discontinuation. However, there is controversy regarding the relationship between the use of DMPA and weight increase (WHO, 1981 and Taneepanichskul *et al.* (1999). Body weight gain and subsequent obesity, which has been reported by many authors whom assessed the obesity markers adiponectin and leptin during DMPA use. Adiponectin and leptin are members of the adipose secreted proteins termed adipocytokines or adipokines. Leptin and adiponectin were involved in the development of obesity, and although it is now recognized as a hormone that is produced by several tissues, adipose tissue is the principal site of leptin production and the major determinant of the concentration of circulating hormone (Meier and Gressner, 2004; Haluzik, 2005; Schondorf *et al.,* 2005 and Tworoger *et al.,* 2007). Leptin levels increase proportionally with fat mass, whereas adiponectin levels decrease with weight gain (Carmina *et al.,* 2005 and Glintborg *et al.,* 2006).

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Transaminases as ALT and AST are very imprtant markers for liver injury, ALT is a more specific indication of liver disease, whereas AST elevations may be secondary to damage of other organs (Giboney, 2005; Heidelbaugh and Bruderly, 2006; Hoefs *et al.*, 2006; Navarro and Senior, 2006 and Pritchett, 2009). The elevations of aspartate and alanine aminotransferases were observed combined with hepatocellular damage in response to estrogen and progesterone treatment (Fakhry *et al.,* 1988; Faddah *et al.,* 2005 and Taheri *et al.,* 2006). Also, both long-term and short-term users of DMPA were reported with alterations in carbohydrate metabolism and liver malfunction (Mukherjea *et al.,* 1981; Virutamasen *et al.,* 1986 and Ikekpeazu *et al.,* 2009).

It was established that the stressful condition leads to the excessive generation of free radicals, which results in oxidative stress (Khadija *et al*., 2009). In contrast, excessive generation and/or inadequate removal of free radicals results in destructive and irreversible damage to the cell (Lopaczyski and Zeisel, 2001). Free-radical–mediated damage is involved in aging and in the genesis of many chronic diseases such as cancer, cardiovascular diseases, diabetes, and inflammatory diseases (Steinberg, 1992; Niki, 2001 and Young and Woodside, 2001). Elevation of free radical levels may induce a pronounced impairment of the cellular metabolism and significant damage of tissues. The organism is naturally protected against this excessive free-radical attack by enzymatic and chemical detoxification systems (Moller *et al.,* 1996 and Lehucher-Michel *et al.,* 2001). During last decade several reports demonstrated a significant increase in blood lipid peroxides responsible for increased platelet aggregation in rats after oral contraceptive administration (Horwitt *et al.,* 1975; Prasad *et al.,* 1975; Yeung, 1976; Arab *et al.,* 1982; Ciavatti *et al.,* 1989; Palan *et al.,* 1989; Ciavatti and Renaud, 1991; Kose *et al.,* 1993; Sissan *et al.,* 1995 and Berg *et al.,* 1997). While, a little reports revealed significant increased activity of antioxidative enzymes, namely catalase and glutathione peroxidase (GPx) following course of a combined oral contraceptive (ethinylestradiol 20 mg and desogestrel 150 mg) in young women as reported by (Capel *et al.,* 1981; Massafra *et al.,* 1993 and Pincemail *et al.,* 2007). So, in this study we decided to shed some of the light on the potential toxicity of DMPA on body weight, liver enzymes and some parameters of oxidative stress; using adult female rats (*Sprague- Dawley*).

MATERIALS AND METHODS

This study was carried out using healthy eighty adult female Sprague-Dawley rats, 2 months old, from central farm for experimental animals of Vaccera, Giza, Egypt. They were housed in 12-hrs dark and 12 -hrs light and fed a standard rodent pellet diet to acclimate for two weeks then divided into two major groups of 40 rats. The 40 rats were divided into four minor groups (10 rats each yield 8 groups), the first one of them considered as control group and the last three groups are treated groups. These animals were injected weekly intramuscularly with DMPA doses (Vehicle 0; 2.7; 5.4 and 10.8 mg/kg/day) for four or six weeks. These doses were converted from human dose 150 mg, two-and three folds to rat's dose by using multiplication factors for dose conversion between different species by Paget and Barnes (1964). Depo-Provera® was received from one of the family planning private clinics in Cairo. It also sold in Egypt for the contraception use in sterile Depot-aquause solution for intramuscular injection as used in this study is manufactured by The Upjohn Company (Kalamazoo, Michigan, U.S.A.) Methods: This study was conducted in accordance with the U.S. Environmental Protection Agency TSCA Test Guidelines (U.S. EPA, 1985). Rats were observed day after day for body weights, viability and death. After four or six weeks all female rats were euthanized via carbon dioxide inhalation then sacrificed. Blood was drawn from the heart and sera were separated for assessment of liver function test and obesity markers. Also, oxidative stress markers were assessed in liver homogenate. Liver, uterus and ovaries weights were recorded and liver were freezed for oxidative stress assay as well as blood was drawn from heart, centrifugated and sera were freezed for biochemical assay.

Serum Biochemical Assay: Adiponectin was measured using competitive immunoenzymatic quantitative colorimetric method using kits supplied by Dima diagnostics company (Goettingen, Germany) according to the method of Suominen (2004). While, quantitative measurement of leptin in serum was performed using ELISA kit (DRG Diagnostics, Marburg, Germany), according to the method of Vincent and Phoon (2003). The Adiponectin/Leptin ELISA Kit (enzyme-linked immunosorbent assay-ELISA) based on the sandwich principle. A sandwich complex is formed as a result of antigen-antibody reaction and an anti rabbit peroxidase conjugate is added for detection of the bound Leptin and the intensity of color developed is proportional to the concentration of Leptin in the sample. The reaction is stopped by addition of acidic solution, and absorbance of the resulting yellow product is measured spectrophotometrically at 450 nm. The ALT and AST activities

were determined using Diamond Diagnostics kit (Egypt), according to Zilva *et al.* (1988). The principle of this method is transferring of amino groups forming a blue color at a rate proportional to the ALT / AST concentration of the sample. The resultant color in the reaction is measured by reflectance photometry. All assays were run three times in duplicate with standards.

Oxidative Stress Assay: Estimation of oxidative stress biomarkers was carried out using liver homogenate, Liver from control and treated rats were homogenized in ice-cold 0.9% saline to get 10% homogenate. Lipid peroxidation products of the liver homogenate were determined as thiobarbituric acid-reactive substances (TBARS) according to the method of Uchiyama and Mihara (1978). The thiobarbituric acid method was used to quantitate MDA-reactive products. Thiobarbituric acid (TBA) and MDA react to form a schiff base adduct under high temperature/acidic conditions to produce a chromogenic /fluorescent product that can be easily measured employing various analytical techniques such as spectrophotometric or fluorometric methods. Superoxide dismutase activity (SOD) was assayed according to the method of Misra and Fridovich (1972). This method employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5phenyl-tetrazolium chloride (INT) to form a red formazan dye. SOD activity was then measured by the degree of inhibition of this reaction. The activity of liver glutathione peroxidase (GSH-Px) was assayed by using method of Paglia and Valentine (1967) which based on antigen-antibody reaction, which is terminated by the addition of acid and the color change is measured spectrophotometrically at a wavelength of 450nm. Concentration of all nonprotein sulfhydryls (NPSH) were assessed according to the method of Sedlak and Lindsay (1968) this assay based on the Ellman's method, 5,5¢dithiobis-(2-nitrobenzoic acid) is reduced by nonprotein sulfhydryls groups present in TCA extract to 2-nitro-5 mercaptobenzoic acid. This product is characteristic because of its yellow color. For the estimation of NPSH, 50 lL of TCA extract and 100 lL of 6 mM 5,5¢-dithiobis (2-nitrobenzoic acid) (DTBN) were added in succession to 850 lL of 0.2 M phosphate buffer (pH 8.2) and the absorbance was measured at 412 nm.

Statistical Analysis: The statistical analysis of the obtained data was done according to Baily (1994) and the analysis was revised by SPSSv12 for windows (2003).

RESULTS AND DISCUSSION

Maternal Exposure to DMPA:

A- Maternal Body Weight:

Body weights were recorded before the experiment and day after day during four and six weeks of treatment and comparable between the control and DMPA exposure groups, the results showed that DMPA induced body weight gain among all treated groups this increase in the body weight reached maximally (~ + 45.11%) and these changes in the body weights were dose and time dependant and statistically significant ($P \leq 0.01$) (Table 1).

B- Liver Weights and Functions:

DMPA doses to the female rats induced significant increase in the liver weight of the treated rat reached maximally (~ + 36.40 %). Referring to assessed liver enzymes alanine transaminase (ALT) and asparate transaminase (AST) in sera of treated groups, DMPA doses induced increase in these enzymes reached maximally three to four-folds when compared to the control groups GI and GV (Tables 2 and 3). These increases were statistically significant ($P \leq 0.01$) and also were dose and time dependant.

C- Obesity Markers:

The concentrations of adiponectin in the DMPA treated groups showed clear decrease for serum adiponectin levels which reached maximally (~ -35.53%). Moreover, for total serum levels of leptin showed significant increase reached maximally (~ + 248.64%). *(Table1).* These changes were dose and time dependant and statistically significant ($P \leq 0.01$).

D- Oxidative Stress Biomarkers:

DMPA doses significantly induced decrease in the activity of SOD (~ -47.67 %), GSH-Px (~ -45.91 %), NPSH (~ -46.79 %) and increase in TBARS (~ + 86.61 %). These decreases and increases were statistically significant ($P \leq 0.01$) when compared to the control groups (GI and GV) and also were dose and time dependant (Tables 2 and 3).

Table 1: DMPA doses induced body weight gain (gm) and alterations in obesity markers levels both Adiponectin and Leptin in the sera of the female rats.

Groups	After Four Weeks			
	GI	GII	GIII	GIV
Dose	Control	2.7 mg / day	5.4mg / day	10.8 mg/day
Body Weight (gm)	196.20 + 1.68	228.90* + 4.30	237.59* + 1.17	245.10* + 1.52
Weight Gain (%)	--	0.1666	0.2109	0.2492
Adiponectin (ng/ml)	17.21 + 0.65	14.95* + 0.55	13.81* + 0.36	11.87* + 0.61
Change (%)	--	- 13.13 %	- 19.75 %	- 31.02 %
Leptin (ng/ml)	3.32 + 0.19	6.11* + 0.37	8.37* + 0.15	10.98* + 0.59
Change (%)	--	0.4566	1.521	2.3072
Groups	After Six Weeks			
	GV	GVI	GVII	GVIII
Dose	Control	2.7 mg / day	5.4mg / day	10.8 mg/day
Body Weight (gm)	195.58 + 2.17	248.89* + 2.55	267.31* + 1.70	283.81* + 1.39
Weight gain (%)	--	0.2725	0.3667	0.4511
Adiponectin (ng/ml)	17.59 + 0.67	14.40* + 0.16	13.59* + 0.21	11.34* + 0.11
Change (%)	--	- 18.13 %	- 22.74 %	- 35.53 %
Leptin (ng/ml)	3.31 + 0.21	6.45* + 0.22	9.03* + 0.50	11.54* + 0.95
Change (%)	--	0.9486	1.728	2.4864

Data expressed as mean + SD. Where: SD = Standard Deviation. % = Percentage of change from control. * = Significant. (+/-) = Increased / Decreased from control.

Table 2: DMPA induced liver weight gain (gm/100 gm body weight), alterations in liver functions and oxidative stress markers of the female rats.

Groups	After Four Weeks			
	GI	GII	GIII	GIV
Dose	Control	2.7 mg / day	5.4mg / day	10.8 mg/day
Liver Weight	4.41 + 0.13	4.81* + 0.07	5.32* + 0.11	5.89* + 0.18
Liver Weight Gain (%)	--	+ 9.07 %	+ 20.63 %	+ 33.56 %
ALT (µ/L)	15.30 + 2.54	40.39* + 0.69	45.41* + 2.10	68.42* + 3.96
Change (%)	--	+ 163.98 %	+ 196.79 %	+ 347.18 %
AST (µ/L)	11.81 + 1.03	37.30* + 4.44	52.91* + 1.66	63.11* + 2.33
Change (%)	--	+ 215.83 %	+ 380.01 %	+ 434.37 %
SOD (U/mg Protein)	26.14 + 1.51	17.21* + 1.79	15.23* + 1.16	13.92* + 1.22
Change (%)	--	- 34.16 %	- 41.73 %	- 46.74 %
GSH-PX (U/mg Protein)	22.19 + 1.23	18.12* + 1.19	16. 32* + 2.61	15.54* + 1.82
Change (%)	--	- 18.34 %	- 26.45 %	- 29.96 %
NPSH (nmol/mg Protein)	12.52 + 1.62	10.12* + 1.71	9.41* + 1.21	8.31* + 1.15
Change (%)	--	- 19.16 %	- 24.84 %	- 27.12 %
TBARS (nmol/mg Protein)	39.35 + 1.76	50.19* + 2.18	61.45* + 1.26	70.51* + 2.32
Change (%)	--	+ 27.54 %	+ 56.16 %	+ 79.18 %

Data expressed as mean + SD. Where: SD = Standard Deviation. % = Percentage of change from control.
* = Significant. (+ /-) = Increased / Decreased from control.

Table 3: DMPA induced liver weight gain (gm/100 gm body weight), alterations in liver functions and oxidative stress markers of the female rats.

Groups	After Six Weeks			
	GV	GVI	GVII	GVIII
Dose	Control	2.7 mg / day	5.4mg / day	10.8 mg/day
Liver Weight	4.45 + 0.10	5.02* + 0.16	5.63* + 0.09	6.07* + 0.18
Liver Weight Gain (%)	--	+ 12.80 %	+ 26.51 %	+ 36.40
ALT (µ/L)	15.10 + 3.21	42.41* + 2.01	50.41* + 3.40	83.51* + 4.54
Change (%)	--	+ 180.86 %	+ 233.84 %	+ 453.04 %
AST (µ/L)	12.01 + 0.81	42.21* + 1.87	54.98* + 1.82	73.21* + 2.34
Change (%)	--	+ 251.45 %	+ 357.78 %	+ 509.57 %
SOD (U/mg Protein)	25.84 + 1.27	17.13* + 1.28	15.17* + 1.18	13.52* + 1.31
Change (%)	--	- 33.70 %	- 41.29 %	- 47.67 %
GSH-PX (U/mg Protein)	23.61 + 1.93	18.19* + 1.12	15.98* + 1.14	12.77* + 1.16
Change (%)	--	- 22.95 %	- 30.41 %	- 45.91 %
NPSH (nmol/mg Protein)	12.95 + 1.20	9.65* + 1.12	8.24* + 1.53	6.89* + 1.25
Change (%)	--	- 25.48 %	- 36.37 %	- 46.79 %
TBARS(nmol/mg Protein)	40.41 + 1.50	52.61* + 2.54	63.20* + 1.87	75.41* + 0.96
Change (%)	--	+ 36.62 %	+ 56.39 %	+ 86.61 %

Data expressed as mean + SD. Where: SD = Standard Deviation. % = Percentage of change from control.
* = Significant. (+ /-) = Increased / Decreased from control.

Discussion:

Injectable hormonal contraception with long-acting steroidal preparations has become an important method of family planning methods. The most intensively studied and widely used formulation is depot Medroxyprogesterone acetate, a long-acting progestagen, now marketed as a contraceptive in more than 130 developed and developing countries and used by more than 90 million women. Depo-Provera® is the most widely used long-term reversible contraceptive in the US and is used throughout the world. Although there have been anecdotal reports that most hormonal contraceptives are associated with little or no effect on body weight (Yela *et al.,* 2006). Some studies have failed to find that DMPA is associated with significant weight gain (Taneepanichskul *et al.,* 1999). But there are a lot of scientific reports published concerning the deleterious health consequences of overweight and obesity during DMPA administration and some women attribute their weight gain to such use (Speroff and Andolsek, 2003). Also, it was noted that the product labeling for DMPA notes a tendency for women to gain weight during DMPA use: an average of 5.4 pounds by 1 year of use, 8.1 pounds by 2 years, 13.8 pounds by 4 years, and 16.5 pounds by 6 years (Espey *et al.,* 2000). The obtained results are in agreement with Bakry *et al.* (2008) and Bakry and Abdullah (2009) they reported body weight gain in the female rats treated with DMPA (2.7 mg/rat or 5.4 mg/rat) for ten and fifteen days. Similar observations were reported (Moore *et al.,* 1995; Mainwaring *et al.,* 1995; Khoiny, 1996; Polaneczky and Liblanc, 1998; Bahamondes *et al.,* 1998; Risser *et al.,* 1999; Bahamondes, *et al.,* 2001; Mangan *et al.,* 2002; Shadoan *et al.,* 2003; Zukoski *et al.*, 2004; Mia *et al.,* 2004 Andrea *et al.,* 2004 and Le *et al.,* 2009). Concerning the reasons of why DMPA use leads to weight increase? Because of its anabolic effects and fluid retention (Tanner, 1959 and Garn, 1962) and this increase in fluids could depend on modifications on the hypothalamic appetite control center associated with the use of (DMPA) (Leiman, 1972). However, another study was attributed weight increase depends on fat deposition, higher appetite, and dietary ingestion (Amatayakulte *et al.,* 1980). In this study DMPA doses showed significant increase in serum levels of leptin and decrease in serum adiponectin levels. During the past years substantial research efforts have addressed the role of the adipokines adiponectin and leptin in the pathogenesis of the metabolic complications of abdominal adiposity and obesity (Trujillo and Scherer, 2006) .The physiological roles of leptin include the regulation of adipose tissue homeostasis, mostly by modulating appetite and food intake (Casanueva and Dieguez, 1999), and also modulates reproductive function serving as a marker of the adipose tissue energy depots (Moschos *et al.,* 2002).

Human obesity is characterized by resistance to the actions of leptin in several target tissues and the development of compensatory hyperleptinemia (Trujillo and Scherer, 2006). Adiponectin exhibits anti-inflammatory and insulin-sensitizing effects and its serum levels are decreased in abdominal adiposity, in obesity and in disorders of glucose tolerance (Schulze *et al*., 2005 and Luque-Ramírez *et al.,* 2008).

Recently, a significant decrease in serum leptin concentrations was found following bilateral ovariectomized in normal women Messinis *et al*. (1999) and, although treatment with estradiol was without any effect, the addition of progesterone prevented this decrease, suggesting that progesterone plays a role in the control of leptin secretion Messinis *et al*. (2000). This also supported by Messinis *et al.* (2001) who were the first to show an increase in serum leptin concentrations in normal women during treatment with exogenous estradiol and progesterone. Since, the increase in the serum leptin levels is related to the body mass and BMI; and our data reported significant body weight gain of the female rats. So, the increase in serum leptin levels in this study attributed to DMPA doses and indicates a positive relationship with the increase in the body weight. Recent findings have indicated that adiponectin expression is reduced in obese, insulin-resistant rodent models; Adiponectin levels are affected by factors such as gender, aging, and lifestyle; interestingly, female humans and rodents have higher plasma adiponectin levels than males, and females are more sensitive to insulin than males. Adiponectin effects can increase fatty-acid oxidation and energy consumption in part via peroxisome proliferator-activated receptor-α (PPARα) activation (Haluzik, 2005; Ahima, 2006; Kadowaki *et al*., 2006). So, the decrease of plasma adiponectin may accelerate early atherosclerotic vascular damage and reduce various physiologic roles of endothelial cells, including nitric oxide synthesis and supply (Ekmekci and Ekmekci, 2006). This study revealed that DMPA doses induced elevation in serum transaminases (ALT and AST) and oxidative stress biomarkers; thiobarbituric acid-reactive substances (TBARS), superoxide dismutase activity (SOD), glutathione peroxidase (GSH-Px) and nonprotein sulfhydryls (NPSH) in liver homogenate. DMPA doses induced increase in the concentration of both asparate transaminase (AST) and alanine transaminase (ALT). Serum transaminases levels are used to determine their tissue dysfunction or damage in clinical and veterinary studies Folmar *et al.*, (1993). It also considered a sensitive markers measure in evaluating liver function and damage Howanitz and Howanitz (1984). The elevation of aspartate and alanine aminotransferase in the present work are in accordance with Fakhry *et al.* (1988) and Taheri *et al.* (2006) who attributed this increase to

hepatocellular damage induced by estrogen and progesterone. Further, Mukherjea *et al.* (1981) and Virutamasen *et al.* (1986) were reported alterations in carbohydrate metabolism and liver function in long-term users of DMPA, Ikekpeazu *et al.* (2009) reported liver malfunction after short-term use of hormonal contraceptive. Faddah *et al.* (2005) stated that liver functions (AST, ALP and Total bilirubin) were showed activity and significantly elevated in the first year of DMPA administration. In the present study DMPA doses significantly decrease the activity of SOD, GSH-Px, NPSH enzymes and increase in TBARS as a product of lipid peroxidation. A stressful condition leads to the excessive production of the radicals, which results in oxidative stress Khadija *et al*. (2009). Generation of free radicals is an integral feature of normal cellular function. In contrast, excessive generation and/or inadequate removal of free radicals results in destructive and irreversible damage to the cell Lopaczyski and Zeisel (2001). This actually what happen when the rats injected with DMPA doses, whereas, liver plays a central role in the metabolism of progestogens and it is becoming obvious that these substances can act directly or indirectly on the liver to produce a variety of biological effects which have both physiological and pathological significance Hargreaves (1969). Measurement of the function of the antioxidant system may indicate an individual's susceptibility to oxidant induced disease Smart *et al.* (1996). Several studies were carried out to explain the effect of combined oral contraceptives on erythrocyte antioxidant markers like erythrocyte glutathione peroxidase (GSH-PX), erythrocyte catalase (CAT) and erythrocyte superoxide dismutase (SOD) activities (Massafra *et al.,* 1993; Subakir *et al.,* 2000). GSH depletion was considered as an index of oxidative stress Marks *et al.* (1992). In the present work, GSH showed a significant gradual decrease after DMPA administration. These observations are in agreement with those of (Yu, 1994; Faddah *et al.,* 2005) who reported that DMPA administration shifted the oxidative stress towards the oxidative side and decreased the antioxidants including sulfhydryls groups. Reactive oxygen species (ROS) have a great impact on the normal function of biomolecules like nucleic acids, proteins and cell membrane phospholipids, free radicals are generated during stepwise reduction of molecular oxygen (Singh *et al*., 1999). Hallwell and Gutteridge (1999) described several lines of defense against reactive oxygen species in animals. Enzymes with important antioxidant functions include: i) superoxide dismutase (SOD), which catalyses the dismutation of superoxide radical to hydrogen peroxide and water, ii) catalase (CAT), which catalyses the breakdown of hydrogen peroxide to oxygen and water, and iii) glutathione peroxidase (GPX), which facilitates the destruction of both hydrogen peroxide and organic peroxides, reduced glutathione (GSH), a tri-peptide thiol, is an important antioxidant, as well as a co-factor for various antioxidant enzymes Kidd (1997). SOD is the first line of defense against ROS and is active in catalyzing detoxification of superoxide radical Gonzales *et al.* (1984). The hydrogen peroxide generated in this reaction is restored to water in the presence of CAT and GPX. Polyunsaturated fatty acids present in membrane phospholipids are the main target substrates for oxygen radical activity which results in disorganization of cell framework and function Patterson and Leacke (1998). Although oxygen is crucial to a wide range of vital, life-sustaining biological activities, oxygen radicals can disrupt cell membranes, destroy cell enzyme function, alter DNA and cause cell death. Also, High doses of progesterone had an oxidant effect when it stimulated its own receptor in both acute and chronic administration (Borekci *et al.,* 2009; Nazifi *et al.,* 2010).

Conclusion:

The results of this study revealed DMPA induced several alterations in liver functions, oxidative stress markers and body weight gain in the treated female rats. Thus, special care should be exercised for women use this medication. Cardiovascular and hepatic markers as well as body weight should be evaluated periodically.

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