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Effect of Oral Contraceptive Pills on Oxidative Stress in Diabetic Rats

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ABSTRACT

Background: Oral contraceptive pills (OCs) are now commonly used in millions of women worldwide. Therefore, information on the risks and benefits of therapies is critically important. Several lines of evidence have proved that oral contraceptive pills induce oxidative stress and depletion of serum antioxidants. Oxidative stress plays a major part in the development of pathological condition among which one is diabetes. The aim of this study was to evaluate the effect of oral contraceptive pills on oxidative stress in streptozotocin (STZ)-induced diabetic female rats.
Methods: Adult female Wistar albino rats (n=40) weighing (200-220g) were divided into four groups; control group, COC group: normal rats treated with COC (combined oral contraceptive pills)daily for every 4 days for 32 days by gastric tube, Diabetic group: the animals were injected by STZ at the dose of 60 mg/kg, Diabetic+ COC group: diabetic rats treated with COC as in COC group. At the end of experimental all rats were sacrificed and parameters were measured.
Results: The results indicated that diabetic rats and diabetic+COCs groups induced hyperglycemia and hyperlipidemia (TC, TG, LDL) and increase in liver function enzymes (AST, ALT) associated with oxidative stress markers indicative of lipid peroxidation (MDA) and decreased the antioxidant enzymes (SOD, CAT, GSH) in pancreatic tissues and disturbance in sex hormones (E2, progesterone) as compared to control group. While, COCs group induced increase in MDA,TC, LDL, AST and ALT with decreased in SOD, CAT, HDL, E2 and progesterone as compared to control group. Diabetic+COCs exhibited an increase in glucose, MDA, AST and ALT accompanied by a decrease in SOD, CAT and E2 as compared to diabetic rats.
Conclusion: *These results suggest that diabetic rats consuming oral contraceptive pills may be more susceptible to oxidative stress by enhanced depletion of antioxidant and increased lipid peroxidation.*

Key words: *Diabetes - Oral Contraceptive Pills - Oxidative Stress- Antioxidants - Lipid profile –Female rats.*

INTRODUCTION

The Human body is continuously exposed to different types of agents that results in the production of reactive species called as free radicals (ROS/RNS) which by the transfer of their free, unpaired electron causes the oxidation

of cellular machinery (1). Oxidative stress (OS) is defined as an imbalance between antioxidants and reactive oxygen species (ROS) (2). ROS, which include free radicals: superoxide (O2•-), hydrogen peroxide (H_2O_2), hydroxyl (OH•). In contrast the antioxidants are enzymatic: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GSR) or non- enzymatic: vitamins and minerals such as vitamin C, vitamin E, selenium and zinc. ROS are byproducts of normal cellular metabolism, low and moderate amounts of ROS have beneficial effects on several physiological processes, including the killing of invading pathogens, wound healing, and tissue repair processes (3). Overproduction of free radicals can cause oxidative damage to biomolecules, (lipids, proteins, DNA), eventually leading to many chronic diseases (4,5) such as atherosclerosis (6), cancer (7), osteoarthritis (8), aortic valve stenosis (9), urolithiasis (10), diabetic nephropathy (11).

Nowadays, evidences have been reported that support the role of oxidative stress in the progression of diabetes, which plays an important role during diabetes, including impairment of insulin action and the elevation of the complication incidence (1).

Diabetes mellitus (DM) is a group of metabolic disease characterized by increase blood glucose level resulting from defects in insulin secretion, insulin action, or both (12). International Diabetes Federation (IDF) in 2015,estimated that, 415 million people have diabetes in the world and higher than 35.4 million individuals were diagnosed with DM in the Middle East and North Africa regions; by 2040 this can upward push to 72.1 million, while 3.4 million cases of diabetes in Saudi Arabia in 2015 (13).

In diabetes mellitus, main sources of oxidative stress are mitochondria. During oxidative metabolism in mitochondria, a component of the utilized oxygen is reduced to water, and the remaining oxygen is transformed to oxygen free radical (O_) which is an important ROS that is converted to other reactive species such as ONOO_, OH and H_2O_2 (14). Weak defense system of the body becomes unable to counteract the enhanced ROS generation and as a result condition of Oxidative stress (15,16).

Oral contraceptives (OCs) are medications administered by mouth that prevent pregnancy primarily by inhibiting ovulation. They are one method of birth control and are of two main types i.e. the combined oral contraceptive pill (COC) containing both estrogen and progesterone and progestogen only pill. Their use was first approved in 1960 in the United States of America (17,18).

The percentage of women in worldwide of reproductive age who are using a contraceptive method at a given point in time frame: 2007-2013 is 64%. While in Saudi Arabia is 24% According to world health organization (2007-2013) (19).

Several studies have investigated the effects of oral contraceptives (OCs) on the OS. Some studies showed a significant increase of lipid peroxides in women using oral contraceptive pills (2,20-23) and a decrease of the liposoluble antioxidants coenzyme Q10 and α-tocopherol was shown in women taking the OC compared to a control group not using OC (24).

COCs have traditionally been thought to adversely affect carbohydrate metabolism by increasing insulin resistance and decreasing glucose tolerance. However, low dose COCs do not cause any clinically changes in carbohydrate metabolism (25). Findings in studies utilizing other measures of diabetic control in women taking COCs have been mixed; some studies found an increase in fasting glucose levels (26), while others demonstrated no change in glycemic control or lipid profiles (27).

The aim of this study was to evaluate the effect of the combined oral contraceptive pill (COC) on oxidative stress in streptozotocin (STZ)-induced diabetic female rats.

MATERIAL AND METHODS:

Animals

Forty (40) adult female Wistar albino rats weighing (200-220g) were included in this study, obtained from King Fahad Research Center. The animals were housed in a well-ventilated room maintained under standard condition of light, feeding and temperature. They allowed for adaptation for two weeks before commencement of the experiment. Maintenance of the animals was approved by the animal ethics committee in accordance with the guide for the care and use of animals by King Fahad Research Center.

Drugs

A yasmin® tablet which is a brand of combined oral contraceptive pill containing (0.03 mg ethinyl estradiol and 3 mg drospirenone) manufactured by Bayer pharma AG Germany were used in this study. Twenty one (21) tablets of drug were dissolved in 100 ml of distilled water. The drug was given daily (0.6 mg/kg body weight) by oral gavage syringe for every 4 days for 8 oestrous cycles (32 days) (28).

Induction of Experimental Diabetes

Streptozotocin (STZ) was used to induce diabetes in rats by a single intraperitoneal injection at a dose of 60 mg/kg body weight (29). Three days after Streptozotocin induction, the blood glucose of each animal was measured by using OneTouch Select Analyzer (LifeScan, Inc., UK). Rats were considered diabetic if their fasting blood glucose level were above 200 mg/dl.

Experimental Design

The rats were divided into four groups comprising of 10 animals in each group as follow:

Control groups: normal rats received only water.

COC group: normal rats received COCs daily for every 4 days for 32 days by gastric tube.

Diabetic group: diabetic control (Streptozotocin 60 mg/kg b.wt).

Diabetic+ COC group: diabetic rats received COCs daily for every 4 days for 32 days by gastric tube.

Determination of Estrous Cycle Phases of Rats:

Vaginal secretion of each rat was collected with a plastic pipette filled with 10 µL of normal saline by inserting the tip into the rat vagina. Vaginal fluid was placed on glass slides and left until dry. Then, one drop of Giemsa stain (purple color) was put to each slide and left until dry. After that, each slide was washed and observed under a microscope. Three types of cells could be recognized: round and nucleated ones are epithelial cells; irregular ones without nucleus are the cornified cells; and the little round ones are the leukocytes (30).

Blood Sample Collection

At the end of the experimental period, the animals were fasted for 12 hours and anesthetized using diethyl ether. Blood samples were drawn using standardized laboratory techniques. (5ml) of blood was collected into tubes, then serum was separated by centrifugation at 3000xg for 15 minutes, which then was divided into several Microtiter tubes and stored at -80ºC until analysis was performed. Immediately after blood sampling, the animals were sacrificed by decapitation and the pancreas was dissected out and washed using the ice cold saline solution. Pancreatic tissues were minced and homogenized (10% w/v) in the ice-cold saline and centrifuged at 4,000 rpm for 15 minutes and the supernatant was used for the estimation of various biochemical parameters.

Biochemical analysis:

Blood glucose and serum insulin were measured based on the methods of Trinder (31) and Clark and Hales (32) respectively. Serum total cholesterol (TC), triglycerides (TGs) and high density lipoprotein cholesterol (HDL-C) were evaluated according to the methods of Roeschlau et al., (33), Fossati and Prenape (34) and Lopes-Virella et al., (35) respectively. Serum Low density lipoprotein-cholesterol (LDL-C), was calculated by Friedwald's Formula (36). Serum estradiol and progesterone were assayed by using ELIZA kit according to the manufacturer's instructions (BioCheck, USA). The activity of serum AST and ALT were assayed by the method of Moss and Henderson (37). The pancreatic MDA concentration, SOD and CAT activity were assayed by the methods of Yoshioka et al., (38), Wheeler et al., (39) and Sinha (40) respectively. Furthermore, GSH content was estimated according to the method described by Teare et al. (41).

Statistical Analysis

The data were analyzed using one-way ANOVA; Results were expressed as mean± (SD) followed by LSD to compare between the different groups. Values of $P>0.05$ were considered non-significantly different, while those of $P<0.05$ and $P<0.01$ were considered significant and highly significant, respectively.

RESULTS

The data presented in Table (1) showed that the diabetic rat group has a significant elevation (P=0.000) in blood glucose level accompanied by a significant decrease (P=0.001) in serum insulin level as compared with the control group. In COC group, no significant changes were recorded in fasting blood glucose and insulin levels, compared with the control group. In Diabetic + COC group, a significant increased (P= 0.000) in blood glucose level was observed with non- significant change in insulin level as compared to the diabetic rat group. However, a significant increased (P=0.000) in glucose level accompanied by a significant decreased (P=0.001) in insulin level were noticed in the Diabetic + COC group, compared to COC group.

Table 1 Effect of COC on fasting blood glucose and serum insulin in STZ-induced diabetic rats.

Groups	Fasting blood glucose (mmol/L)	Seruminsulin (µU/ml)
Control	4.94 ± 0.81	8.125 ± 2.125
Diabetic	13.2 ± 6.207 *** ###	5.298 ± 2.04 ** ###
COC	5.33 ± 0.882	8.451 ± 1.403
Diabetic+ COC	21.775 ± 3.945 *** ### ^^^	5.360 ± 1.607 ** ##

Values are the mean of 10 observations ± SD.

Significantly different from control value at P<0.05*, 0.01**, 0.001***

Significantly different from COC value at P<0.05#, 0.01##, 0.001###

Significantly different from Diabetic group value at P<0.05 ^, 0.01^^, 0.001^^^

The effect of combined oral contraceptive on lipid profile in diabetic female rats was assessed. As shown in Table (2), a significant elevation (P=0.000, 0.037, 0.004) was noticed in the level of TC, TG and LDL-C respectively accompanied by a highly significant decrease (P=0.000) in HDL-C in the diabetic rat group when compared to control rats. The combined oral contraceptives produced a significant increase (P=0.000, 0.032) in TC and LDL-C respectively, while no significant change (P=0.089) in TG accompanied by a highly significant decrease (P=0.008) in HDL-C as compared to control group. In contrast, non- significant changes in lipid profile were observed in diabetic rats treated with COC as compared to the diabetic rat group. Diabetic+ COC group had a significant increase (P=0.000, 0.046) in TC and LDL-C respectively, accompanied by a highly significant decrease (P=0.001) in HDL, compared to COC group.

As shown in table (3) it is obvious that the diabetic rat exhibited a highly significant decrease (P=0.000) in SOD, CAT activities and GSH level in pancreatic tissues accompanied by a highly significant increase (P=0.000) in MDA level as compared to control group. COC group showed a significant decrease (P= 0.000, 0.006) in SOD and CAT activities, respectively, while non-significant decrease in GSH accompanied by a significant increase (P=0.043) in MDA levels as compared to control group. Diabetic + COC group showed significant decreased (P=0.011, 0.044) in SOD and CAT respectively, while non-significant decrease in GSH level accompanied by a significant increase (P=0.0012) in MDA level as compared to diabetic group. Furthermore, highly significant decrease (P=0.000) in SOD, CAT and GSH levels accompanied by a highly significant increase (P=0.000) in MDA level were observed in Diabetic + COC group when compared with COC group.

The results in Table (4) showed that the diabetic rat group exhibited a highly significant decrease (P=0. 000) in serum estradiol level with non-significant decrease in progesterone level as compared to control group. In COC group, there was a significant decrease (P=0.000, 0.029) in the levels of serum estradiol and progesterone respectively as compared to control group. Diabetic rats treated with COC, showed a significant decrease (P=0.003, 0.008) in estradiol level with non-significant decrease in progesterone as compared to diabetic group and COC group respectively.

Table 2 The effect of COC on lipid profile in STZ- induced diabetic rats.

Groups	TC (mmol/L)	TG (mmol/L)	LDL-C (mmol/L)	HDL-C (mmol/L)
Control	1.388 ± 0.121	0.423 ± 0.088	0.125 ± 0.044	1.374 ± 0.170
Diabetic	1.825 ± 0.106 *** ###	0.594 ± 0.125 *	0.197 ± 0.068 **	0.893 ± 0.193 ***
COC	1.601 ± 0.079 ***	0.561 ± 0.077	0.177 ± 0.044 *	1.088 ± 0.347 **
Diabetic+COC	1.856 ± 0.096 *** ###	0.678 ± 0.309 **	0.225 ± 0.049 *** #	0.727 ± 0.134 *** ##

Values are the mean of 10 observations ± SD.

Significantly different from control value at P<0.05*, 0.01**, 0.001***

Significantly different from COC value at P<0.05#, 0.01##, 0.001###

Significantly different from Diabetic group value at P<0.05 ^, 0.01^^, 0.001^^^

Table 3 The effect of COC on the antioxidant levels in STZ-induced diabetic rats

Groups	MDA (nmol/g)	SOD (U/gm)	CAT (U/g)	GSH (mmol/g)
Control	57.474 ± 8.184	1051.778 ± 97.430	3.259 ± 0.766	0.939 ± 0.103
Diabetic	174.65 ± 17.552 *** ###	629.996 ± 96.805 *** ###	0.986 ± 0.47 *** ###	0.52 ± 0.12 *** ###
COC	70.1 ± 6.906 *	825. 04 ± 9.907 ***	2.584 ± 0.486 **	0.882 ± 0.209
Diabetic+COC	195.849 ± 17.402 *** ### ^^	530.12 ± 27.141 *** ### ^	0.505 ± 0.121 *** ### ^	0.416 ± 0.066 *** ###

Values are the mean of 10 observations ± SD.

Significantly different from control value at P<0.05*, 0.01**, 0.001***

Significantly different from COC value at P<0.05#, 0.01##, 0.001###

Significantly different from Diabetic group value at P<0.05 ^, 0.01^^, 0.001^^^

Table (5) reveals that injection of rats with STZ, resulted in a highly significant increase (P=0.000) in the levels of AST and ALT as compared with the control group. Treated rats with combined oral contraceptive (COC) showed a significant increase (P=0.000, 0.013) in AST and ALT levels respectively as compared to control group. Moreover, Diabetic + COC group showed a highly significant increase (P=0.000) in AST and ALT levels as compared to all groups (Control, Diabetic and Diabetic+COC).

Table 4 The effect of COC on the hormone levels in STZ-induced diabetic rats.

Groups	Estradiol (pg/ml)	Progesterone (ng/ml)
Control	117.778 ± 8.259	31.796 ± 4.146
Diabetic	85.538 ± 9.723 ***	29.102 ± 3.977
COC	83.857 ± 8.273 ***	28.432 ± 2.860 *
Diabetic+COC	72.430 ± 9.786 *** ## ^^	27.950 ± 1.495 *

Values are the mean of 10 observations ± SD.

Significantly different from control value at P<0.05*, 0.01**, 0.001***

Significantly different from COC value at P<0.05#, 0.01##, 0.001###

Significantly different from Diabetic group value at P<0.05 ^, 0.01^^, 0.001^^^

Table 5 The effect of COC on the liver function in STZ-induced diabetic rats.

Groups	AST (U/L)	ALT (U/L)
Control	20.96± 2.884	5.740 ± 1.211
Diabetic	33.8 ± 5.514 ***	10.002 ± 1.336 *** ##
COC	30.791 ± 5.420 ***	7.665 ± 1.212 *
Diabetic+COC	42.738 ± 5.535 *** ### ^^^	15.800 ± 2.463 *** ### ^^^

Values are the mean of 10 observations ± SD.

Significantly different from control value at P<0.05*, 0.01**, 0.001***

Significantly different from COC value at P<0.05#, 0.01##, 0.001###

Significantly different from Diabetic group value at P<0.05 ^, 0.01^^, 0.001^^^

DISCUSSION

Oxidative stress plays an important role in the progression of diabetes, including impairment of insulin action and elevation of the complication incidence (1). Oral contraceptives (OCs) are now commonly used by millions of women worldwide as a method for preventing pregnancy. OCs contain two active components: estrogen and progestin (42). Both 17-beta Estradiol (E2) and progesterone action on oxidative stress have been recently reviewed (43). The general consensus is estrogen has antioxidant qualities and ergo reduces oxidative stress, notably lipid peroxidation (44). However, when estrogen and progesterone are co-administered, the beneficial effects of estrogen have been reported to be mitigated (45). Indeed, (46-48) noted an increase in oxidative stress following combined oral contraceptive administration in women and rats.

In the present study, the diabetic rat group showed a significant increase in fasting blood glucose concentration accompanied by a significant decrease in serum insulin level when compared with control rats. A similar result was reported by Kumar et al., (49). The alterations in blood glucose and insulin concentrations in the present study are attributed to STZ which is used to induce destruction of β-cells of islets of Langerhans resulting in degranulation and the reduction of insulin secretion as proposed by Zhang and Tan (50) and Kavalali et al., (51). Administration of COC to normal rats induced a slight increase in blood glucose and insulin levels when compared to non-administrated control rats. This result was in agreement to Berenson et al., (52) and Argrawal et al., (53). The

mechanism by which OCs use leads to impaired glucose tolerance is yet to be clarified. Decrease insulin sensitivity appears to be mainly accounted for by estrogenic component and this effect may be modified by progestogenic component (54,55). While, administration of COCs to the diabetic rat group showed a significant increase in glucose level with non-significant change in insulin level compared to the diabetic rat group. This result was not matched to the result of Adeghate (56) who observed that oral steroid contraceptive agents did not significantly alter blood glucose, glycosylated hemoglobin, and plasma insulin levels of STZ-induced diabetic rats when compared to untreated diabetic rats.

In the present study, a significant increase in serum TC, TG and LDL-C and a significantly decrease levels of HDL-C were seen in the diabetic group as compared to control group. These results are in accordance with some recent studies (57-59). Uncontrolled DM leads to increased levels of free fatty acids (FFA), triglycerides and VLDL. Insulin deficiency causes the excessive mobilization of FFA and underutilization of chylomicrons and VLDL leading to hypertriglyceridemia due to esterification of FFA (60-62). Moreover, insulin affects many sites of mammalian lipid metabolism; it stimulates synthesis of fatty acids in liver, adipose tissues and in the intestine. Insulin deficiency has also been reported to increase the cholesterol synthesis and increase the activity of lipoprotein lipase in white adipose (63). The present study showed that normal rats administrated with COCs, showed a significant increase in TC and LDL-C, while no significant increase in TG accompanied by a highly significant decrease in HDL-C as compared to control group. The current results are consistent with previous studies (64-67). Orally administered estrogen increases triglyceride levels as well, conversely in the situation of concurrently improved HDL and lower LDL levels (68). In contrast, the progestin component of COCs counteracts these lipid changes induced by estrogen, which increases levels of LDL and decreases the concentration of HDL and triglyceride (69), by increased hepatic lipase enzyme activity (66). The significant increase in serum total cholesterol in oral contraceptives users' women may be due to impaired lipoprotein metabolism. Administration of COCs to diabetic rat group showed non-significant changes in lipid profile as compared to the diabetic rat group. Our results were in agreement with previous studies (70,27).

In the present study, the diabetic rat group showed a highly significant decrease in SOD, CAT activities and GSH level in pancreatic tissues accompanied by a highly significant increase in MDA level as compared to control group. Similar to the finding in this study, an increase in MDA levels accompanied with a decrease in the activities of SOD, CAT and GSH level has been observed in some tissues of diabetic rats (71-73). Evidence suggests that oxidative cellular injury caused by free radicals contributes to the development of diabetes. Most of the tissue damage is considered to be mediated by free radicals, which attack the membrane via the peroxidation of unsaturated fatty acids (74). Lipid peroxidation is a characteristic of diabetes mellitus. The increase in lipid peroxidation presents a close relationship with the high glycemic levels and oxidative stress in diabetes mellitus (75-77). The decreased activities of SOD and CAT may be due to glycation of these enzymes, which occurred at persistently elevated blood glucose levels (78), Pigeolet et al., (79) have reported the partial inactivation of these enzyme activities by hydroxyl radicals and hydrogen peroxide. The decreased activity of SOD and CAT could also be due to their decreased protein expression levels in the diabetic condition as reported by Sindhu et al., (80). The depletion of GSH level in diabetic rats might be due to its utilization to alleviate the oxidative stress in diabetes (81). In the present study, normal rats administrated with COCs group, showed a significant decrease in SOD and CAT activities and non-significant decrease in GSH with concomitant significant increase in MDA levels as compared to control group. The significant increase in MDA level in this study is consistent with Kowalska and Milnerowicz (23). This probably reflects the increase in lipid oxidation due to either an increase in the production of free oxidative radicals, or a decrease in the antioxidant defense mechanisms, or both (82). Moreover, Al-Gazally et al., (65) observed that the change in the serum copper concentration, due to the estrogen component of OCs, is certainly the major event leading to the increased level of malondialdehyde. One of the major findings of the present study was the dramatic and a significant decrease in SOD, CAT activities and GSH level in rats administrated oral contraceptive pill, this finding is in agreement with those Jendryczko et al., (83). Fallah et al., (84) suggested that OCPs may stimulate or reduce the activities of GPx and SOD enzymes, respectively. This may be due to an effect of these pills on bone marrow erythroblast maturation via stimulation or inhibition of the synthesis of new active GPx and SOD molecules or may be a result of the increased frequency of an allele of the GPx and SOD enzymes. The decreased in CAT activity in rats administrated oral contraceptive pill, may be due to enzyme protein oxidation as a result of accumulation of H_2O_2 and other radicals (85). It is plausible that catabolism of exogenous hormones by involving activities of P450 cytochromes (CYPs) provokes increased ROS production (86) and depletion of reduced glutathione (87,82). Diabetic rat treated with COCs group showed a significant increase in MDA level accompanied by a significant decreased in SOD and CAT, while non-significant decrease in GSH level as compared to diabetic group. Combined oral contraceptive pills associated with generation of free radical, leading to a disruption in oxidative status. Further deterioration occurs when other risk factor such as diabetes mellitus that also induce the production of free radicals and promote lipid peroxidation are present (88).

Our results revealed that the diabetic rat group caused significant decrease in serum levels of estradiol (E2) while non-significant decrease in progesterone when compared with control rats. The significant decrease in estradiol and a decrease in progesterone were in agreement with khowailed et al., (89). The decreased estradiol levels in diabetes may be due to the reduced aromatase expression and/or interference with its functional activity, this ovarian aromatase activity has been shown to be insulin dependent (90). Evidence from animal model and clinical studies suggests that oxidative stress plays a role in the etiology of adverse reproductive events in both women and men (91). The present study showed that, administrated rats with COCs group, exhibited a significant decrease in serum estradiol and progesterone levels as compared to control group. Our results were in agreement with previous studies (92-94). Combined hormonal contraceptives inhibit the natural production of estrogen and progesterone hormones. Hormonal contraceptives alter the hypothalamicpituitary-ovarian feedback loop, preventing the maturation of the ovarian follicle, precluding ovulation (95), and inhibiting the rise in estrogen (96). Diabetic rat treated with COCs group exhibited a significant decrease in estradiol level with non-significant decrease in progesterone compared to diabetic group. In this trend, Skouby et al., (97) observed a significant decrease in progesterone and non-significant decrease in estradiol among insulin-dependent diabetic women using combined oral contraceptive.

Our results revealed that the diabetic rat group caused a significant elevation in serum AST and ALT activities when compared with control rats. Our results were in agreement with previous studies (98,99). The present study showed that administration of combined oral contraceptive pills to normal rat group induced a significant increase in AST and ALT activities when compared to non-administrated control rats. These results were coincided with Ekhato et al., (100). Estrogens have long been known to cause intrahepatic cholestasis in susceptible women during pregnancy, after administration of oral contraceptives, or during postmenopausal hormone replacement therapy. Estrogen receptor alpha-mediated repression of hepatic transporters and alterations of bile acid biosynthesis may contribute to the development of the estrogen-induced hepatotoxicity (101). All hormonal contraceptives contain progestogens. The liver processes progestogens and estrogens differently because liver cells have estrogen receptors but no progestogen receptors (102,103). Diabetic rat treated with COCs group showed a highly significant increase in AST and ALT levels as compared to the diabetic rat group. Combined oral contraceptive induced significant increase in AST and ALT levels in users (104). Further deterioration occurs when other risk factor such as diabetes that also induces an increase in the markers of liver injury (AST and ALT) in STZ-induced diabetic rats (99).

Based on the above promising results, it can be concluded that diabetic rats consuming oral contraceptive pills may be more susceptible to oxidative stress by enhanced depletion of antioxidant and increased lipid peroxidation.

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