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Effect of Palm oil supplementation on oxidative stress and fertility in male rats

A Thesis Presented
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List of Abbreviation

BTB	Blood-Testis Barrier
CAT	Catalase
$Cdcl_2$	Cadmium chloride
CDNB	1-chloro-2,4-dinitrobenzene
CVD	Cardiovascular diseases
DTNB	5,5 dithiobi, 2-nitrobenene
FFA_S	Free Fatty acids
FSH	Follicular Stimulating Hormone
GnRH	Gonadotropin releasing Hormone
GSH	Reduced glutathione
GST	Glutathione S-transferase
H_2O_2	Hydrogen peroxide
IARC	International Agency for research on Cancer
LH	Leutenizing Hormone
NO	Nitric Oxide
PKO	Palm Kernel Oil
PO	Palm Oil
PUFA	Poly un saturated fatty acid
ROS	Reactive Oxygen species
RPO	Red Palm Oil
SFA	Saturated Fatty acids

SOD	Superoxide dismutase
TAG$_S$	Triacylglycerols
WHO	World Health Organization

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Introduction

Fertility is defined as the natural capability of gonads to produce healthy off springs. The quantity and quality of the sperm generally gives a significant information about male fertility. Moreover, The contribution of other human factors such as nutrition and endocrinology affect fertility of male ***(Fu and Sinclair, 2000).***

Oxidative stress is produced by the peroxidation and oxidation of many cellular lipids, proteins, carbohydrates and nucleic acids. The detailed chemistry of oxygen radical generation and the countervailing effect of oxygen radical scavengers have been covered by many recent reviews ***(Pryor, 2006; Szabó et al., 2007).***

Essential oils are very important for health as they can be used as antibacterial, antibiotic and antiviral. Also, practioners published reports that these oils are useful in variable diseases as cancer, Alzheimer, labor pain and cardiovascular diseases, etc. ***(Lai et al., 2011).***

Reeves and Weihrauch (1979) defines Palm oil (PO) (**dendê oil**, from Portuguese) as an edible vegetable oil derived from the *Mesocarp* (reddish pulp) of a fruit called oil palms.

The tropical belt of Africa, Southeast Asia and parts of Brazil use Palm oil extensively in commercial food industry in other parts of the world due to its lower cost ***(USDA, 2006)*** with high oxidative stability (saturation) of the refined product when used for frying ***(Matthäus, 2007)***.

PO can be used in both edible and non-edible oil industry. PO, with low amounts of free fatty acids (FFAs), low impurity content and good bleaching, is considered of high quality oil and used in the edible oil industry.

Conversely, low-quality oils are used in non-edible industry such as biofuel, candles, cosmetics and soap production ***(Henson, 2012)***. PO is constituted of more than 95% neutral triacylglycerols (TAGs, or triglycerides) and less than 0.5% FFAs ***(Gunstone, 2011; Dunford, 2012)***.

The exposure to many environmental agents can result in testicular damage. These environmental agents present in many forms such as heavy metals, thermal stress or chemotherapeutic agents. Both heavy metals and thermal stress induce testicular damage. When testicular tissue is exposed to heavy metals or thermal stress leads to oxidative stress with DNA damage and apoptosis ***(Aktas et al., 2012).*** However, The underlying molecular mechanism of cadmium and thermal stress to induce testicular damage remains not fully understood**.**

The uses of cadmium in nature are represented in several forms that causes severe testicular damage as a response of the increase of reactive oxygen species in testicular tissues that lead to imbalances between antioxidant and pre-oxidants initiating chain reaction ***(Aktas et al., 2012; Oguzturk et al., 2012)*.**

Exposure to cadmium leads to many pathological conditions in various organs ***(Ognjanovic et al., 2008).*** Therefore, it is necessary to find the suitable antioxidant agent to overcome the cadmium induced testicular injury.

Cadmium produces harmful effects on spermatogenesis and production of normal sperm ***(Mosher and Pratt, 1991)***.

In case of insufficient nutrients intake, there would be harmful effects on spermatogenesis and production of normal sperm ***(Mosher and Pratt, 1991).***

In contrast, sufficient consumption of natural antioxidants nutrients could protect sperm from oxidative stress and improve male fertility ***(Jedlinska-Krakowska et al., 2006)*.**

Aim of study

This study aimed to elucidate the protective role of palm oil in cadmium induced testicular damage in male rats and investigate its beneficial role in fertility through the following measurements:

1)Evaluation of :

I) Weight of testis to see the effect on fertility.

II)Semen quality, livability and motility.

2) Biochemical analysis (Free radicals and antioxidants):

a) Malondialdehyde (MDA) level.

b) Nitric oxide (NO) level.

c) Catalase (CAT) activity.

d) Reduced glutathione (GSH) activity.

e) Glutathione-S-tansferase (GST) activity.

3)Hormonal analysis:

a) Testosterone hormone.

b) Follicle stimulating hormone (FSH).

c) Luteinizing hormone (LH).

4) Histopathological examination.

Review of Literature

I. Palm Oil:

Indigenous or traditional medicines are widely spread all over the world (around 80%) of people according to World Health Organization (WHO) for health maintenance issues ***(Pantsi et al., 2011).*** Palm oil has a protective effect against oxidative stress ***(Jegede et al., 2015).***

In the last few decades, it is recorded that there is a great relationship between nutrition and health. The essential nutrient of the human diet is fat which is highly consumed in vegetable oils. Fat diet contains high concentration of saturated fatty acids (SFA) which reduces cardiovascular diseases (CVD) ***(Assmann et al., 2014)*** and other diseases resulting from obesity and cancer prevention therapy ***(Berger, 2014).***

There is an increase in demand for vegetable oils worldwide where palm oil produces ten times more oil per unit area in comparison with other oil seed crops. Palm oil has been considered the most important vegetable oil in between other oils and its percentage reaches 32% of global fats and oil production in 2012 ***(Mba et al., 2015)***.

I. i-Sources and origin of Palm oil:

The fruit of the oil Palm tree (Elaeisguinesis) is the main source of Red palm oil (RPO), its origin is tropical Africa, but nowadays it is produced from different parts in the world as West Africa, South America and Southwest Asia ***(Esterhuyse et al., 2005).***

I. ii- Chemical composition of palm oil:

I.ii.i-Fatty Acids:

The main component of Palm oil is triacylglycerol (TAG), the glycerol molecule is esterified with three fatty acids. During extraction process of palm oil from the *mesocarp* of the oil palm fruit, TAG attracts other fat soluble cellular components (sterols, phosphatives, tocopherols, tocotrienols, pigments, monoglycerols, diglycerols and free fatty acids.

Palm oil is consisted of antioxidants, vitamins, phytonutrients and aliphatic acids ***(Sambanthamurthi, 2000)***.

Red palm oil contains both saturated and un saturated fatty acids in equal percentage, so it is balanced oil in its processed and un processed forms ***(Sundram et al., 2003)***.

Palm oil (PO) can be differentiated from palm kernel oil (PKO); the PKO is extracted from the seeds of the palm fruit while PO from the *mesocarp* by different methods as wet or dry processes ***(Mba et al., 2015).***

Chemical and physical differences between PO and PKO were illustrated in (Table I) ***(Mba et al., 2015).***

Table (I): Fatty acid composition of palm oil (PO) and Palm kernel oil (PKO)

Fatty acids	**Palm oil (PO)**	**Palm Kernel oil (PKO)**
Caproic acid (6:0)	-	0.2
Caprylic acid(8:0)	-	3.3
Capric acid (10:0)	-	3.5
Lauric acid (12:0)	0.2	47.8
Myristic acid (14:0)	1.1	16.3
Palmitic acid (16:0)	44.0	8.5
Stearic acid (18:0)	4.5	2.4
Oleic acid (18:1)	39.2	15.4
Linoleic acid (18:2)	10.1	2.4
Linolenic acid (18:3)	0.4	-
Arachidic acid (20:0)	0.1	0.1
Total SFAs	49.9	82.1

I.ii.ii- Carotenes:

There are different types of carotenes in Red palm oil as beta-carotene, alpha-carotene and lycopene; these carotenes are responsible for the red color of palm oil ***(Ng and Choo, 2016)*.**

I. iii-Importance of Palm oil:

Jedlinska-Krakowska et al. (2006) said that balanced diet and sufficient consumption of natural antioxidants can protect sperm from oxidative stress and improve male fertility, While unbalanced diet can cause harmful and unhealthy effects on spermatogenesis and can cause production of abnormal sperm ***(Mosher and Pratt, 1991)*.**

Nutrition affects on fertility directly by enhancing the process of Oocyte and spermatozoa development, Ovulation and fertilization, and indirectly through affecting hormones concentration and its metabolites ***(Wathes et al., 2007)***.

In the past, the main dietary fat used was palm oil as it was thought that it has nutritional and healing properties, while now it is used for treatment of many diseases and maintenance of a good health ***(Khanna et al., 2003)***.

Palm oil is used extensively in different uses such as baked goods, candies, cakes, cheese analogs, chips, chocolate, confectionary fats, cookies, cooking oil, crackers, doughnuts, frozen meals (pancakes, pies, pizza, potatoes), ice cream, industrial frying fats and instant noodles, margarines, microwave popcorn, non-dairy creamers, peanut butter, salad dressings, snacks, soups, supplements/vitamins, vegetable ghee ***(Edem, 2002)*.**

PO is a very important source of edible oil for health due to its composition of alpha carotene, beta carotene, lycopene, vitamin K, CoQ10, squalene, phytosterols, flavonoids, phenolic acids, glycolipids and finally vitamin E which contain four tocotrienols, RPO contains all four tocotrienols, the antioxidant activity of tocotrienols is more 60 times of ordinary vitamin E, this composition of palm oil make it a super antioxidant food ***(Goh et al., 1998)*.**

Vitamin E is a powerful lipophilic antioxidant that is absolutely vital for the maintenance of mammalian spermatogenesis and deficiencies of vitamin E leads to a state of oxidative stress in the testes that disrupts both spermatogenesis and the production of testosterone ***(Gavazza and Catalá, 2006)***.

Alpha and Gamma tocotrienol possess higher antioxidant activities when compared with alpha tocopherol due to their differences in tail structures and also their powerful ability in recycling alpha and gamma tocotrienoxyl in biological membranes compared with alpha tocopherol ***(Packer et al., 2001).***

Vitamin A is an important composition of RPO as it is necessary for reproduction through the synthesis of sex steroids ***(Alais and Linden, 1991),*** spermatogenesis and embryogenesis ***(McArdle and Ashworth, 1999).***

Palm oil has an anti-microbial effects, so it is used on wounds but research does not confirm its effectiveness ***(Ekwenye and Ijeomah, 2005).***

It is thought that saturated fats are associated with cardiovascular risk but palm oil has been proven to be effective against cardiovascular disorders as it contains palmitic acid, the main saturated fat in palm oil, that has a

similar effect on lipid profile as the monounsaturated fat oleic acid is currently recommended ***(Chong and Ng, 1991).***

RPO has a protective role against several diseases associated with oxidative stress as arterial thrombosis and hypertension ***(Narang et al., 2004)*** and is also help to decrease incidence of the consequences of ischemia and reperfusion injury ***(Bester et al., 2006).***

The antioxidant properties of palm oil are exerted mainly against reactive oxygen species (ROS), so by these antioxidant properties palm oil prevent aging, CVD and cancer ***(Sen et al., 2007)***.

Aboua et al. (2009) showed that RPO could inhibit apoptosis in rat sperm and help to reduce oxidative stress in patients with HIV, AIDS and tuberculosis ***(Oguntibeju et al., 2010)***.

Saturated fats have different effects on cholesterol in blood ***(Ng et al., 1991).*** Palm olein is more unsaturated and hypocholestrolemic when compared to coconut oil and animal fats (saturated fats) ***(Chong and Ng, 1991; Ng et al., 1991).***

Rats fed on palm oil enjoy more reproductive ability due to increasing function of their sex hormones as palm oil increase biosynthesis and utilization of proteins, so rats fed on it enjoy ***(Aboua et al., 2012).***

A lot of natural products protect mammalian tissues effectively against many drugs or chemicals induced toxicities ***(Hosseinimehr, 2014).***

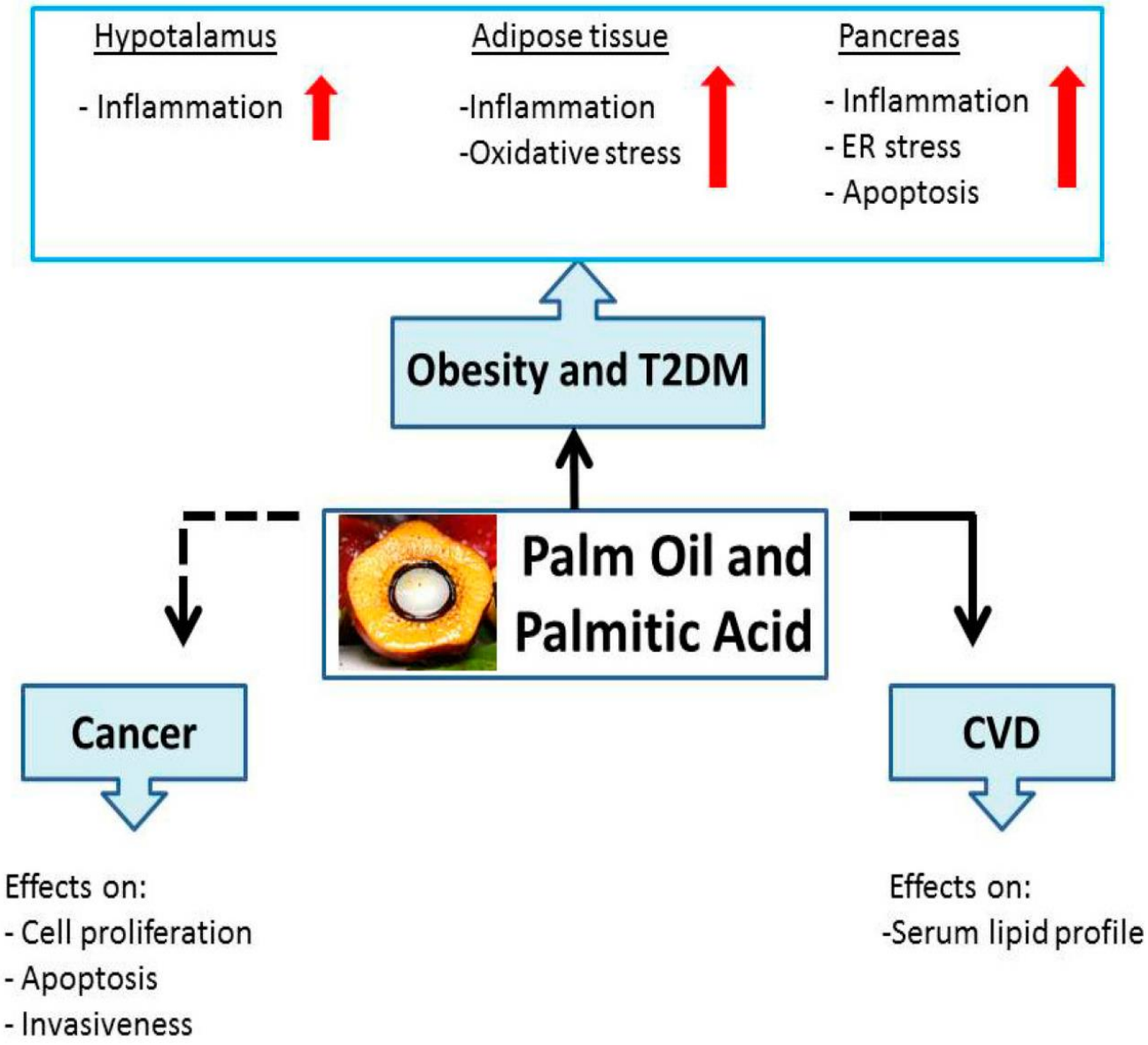


Photo (I) Schematic representation of palm oil and palmitic acid effects on human health *(Mancini et al., 2015).*

II. OXIDATIVE STRESS:

There is relationship between fertility and stress as follow: Increased levels of stress leads to reduced fertility, Couples who wanted to conceive should limit alcohol consumption ***(Homan et al., 2007)***.

Oxidative stress occurs due to an imbalance between the production of reactive oxygen species and ability of biological systems to induce detoxification of the reactive intermediates or repair the resulting damage ***(Bansal and Bilaspuri, 2010),*** to ensure fertilization for male, sperm should be capable of performing its function and adequate number of sperm required ***(Lifeng et al., 2006)***.

When imbalance occurs between the production of reactive oxygen species (ROS) and their removal by antioxidant activity systems , this leads to occurrence of oxidative stress , ROS are Oxygen- based molecules such as: superoxide anion ($O2^-$), hydrogen peroxide (H_2O_2) and hydroxyl ion (OH^- **)** ***(Papa and Skulachev, 1997)***.

Through Catalase enzyme pathway, hydrogen peroxide can be reduced and gives Oxygen and water ***(Mates, 1999; Pryor, 2006).***

However production of hydroxyl ions can occur through occurance of ionizing radiation with water and this is dangerous as it leads to release of other free radicals ***(Cotran et al., 1994).*** The mechanisms of action of many agents on male reproductive functions are not fully understood ***(Barlow et al., 1999).***

There are some molecules in testes that are non-enzymatic antioxidants which protect these testes against oxidative stress caused by any causative agent ***(Kutlubay et al., 2007).***

There is two types of toxicants; direct and indirect toxicants ,direct ones affect testes without endocrine mediation, while indirect toxicants act at anon-germ cell site ***(Mueller et al., 1998),*** this is explained as direct toxicants produce their primary effect directly on testes, epididymis, leydig cells, sertoli cells or spermatozoa.

Then this primary effect lead to disruption of endocrine homeostasis, this occur as the affected testis is under hormonal control and regulatory feedback mechanism**,** while indirect toxicants produce their primary effect on hypothalamic pituitary axis controls or on any site except the testes ***(Tyl, 2002).***

Some chemicals may antagonize action of sex hormones by mimic their action result in stimulation of receptor or blocking or reducing the binding and biological activity of these endogenous hormones and so induce complex disturbances in the homeostatic process, It may also result in Oxidative stress in testis which is induced by a lot of environmental toxicants, Methoxyethanol (used in paints) causes harmful effects on testis as testicular degeneration ***(Hardin, 1983)*** as it causes oxidative stress ***(Syed and Hecht, 1998).***

One of these environmental toxicants is pesticides, exposure to these pesticides can cause testicular damage and increase occurance of apoptosis as it increase testicular oxidative stress ***(Samanta and Chainy, 1997)***

2, 4, 6-trinitrotoluene is a toxicant from petroleum and coal industries cause severe oxidative stress in testis causing apoptosis and germ cell damage ***(Homma-Takeda et al., 2002)***.

Oxidative stress is commonly defined as an imbalance between oxidants and antioxidants at the cellular or individual level. It causes the oxidative modification of cellular macromolecules, cell death by apoptosis or necrosis, and structural tissue damage ***(Lykkesfeldt and Svendsen, 2007)***.

In broilers, oxidative stress may occur as a consequence of nutrition, including the contamination of feed with fungal toxins**,** high environmental temperatures and several pathological conditions, such as increased activity of the immune system (e.g., infection, vaccination), pulmonary hypertension, ascites and coccidiosis ***(Georgieva et al., 2006)***. In testis ***(Marchlewicz et al., 2007)***, It causes decrease in sperm cell concentration in rat testis, decrease sperm motility in epididymis as it increases ROS in epididymis ***(Hsu et al., 1997)***.

Increasing intake of dietary antioxidants may help maintain an adequate antioxidant status and, therefore, the normal physiological function of a living system ***(Record et al., 2001)***.

Jedlinska-Krakowska et al. (2006) said that balanced diet and sufficient consumption of natural antioxidants can protect sperm from oxidative stress and improve male fertility**.**

A lot of natural products protect mammalian tissues effectively against many drugs or chemicals – induced toxicities ***(Hosseinimehr, 2014).*** Antioxidants are very important natural defense mechanisms that are very potent in minimizing the hazard effect of free radicals in mammals ***(Noori, 2012).***

Many antioxidants such as Vit E, Vit C, Zinc, selenium and Melatonin prevented testicular damage induced by Cadmium ***(Amara et al., 2008).***

Both tocotrienol and tocopherol possess ROS scavenging activities that would interrupt the propagation of a free radical chain reaction However, tocotrienols were found to be more potent as an antioxidant in biological membranes compared with tocopherol due to its chromanol nucleus and un saturated isoprenoid side chain ***(Traber and Atkinson, 2007).***

III. Cadmium chloride:

It is well known that there is trace elements that maintain testicular function (Zinc, manganese, and selenium), but also there is heavy metals that affect badly and are very toxic on testicular tissue (lead, mercury, iron, cadmium, cobalt, chromium) ***(Bitner Anderson et al., 1992; Allouche et al., 2009)***.

It is well known that most heavy metals pollute environment that cause harmful effects on health of both human and experimental animal models ***(Kaya et al., 2002).***

Inhalation and ingestion of mercury compounds affect testicular spermatogenic and steroidogenic functions in male animals ***(Fossato da Silva et al., 2011).***

Mercury decreases testosterone level, so it has harmful effect on testicular and accessory gland functions in rat and mice leading to decrease sperm count, motility and morphology ***(Mohamed et al., 1987)***.

Lead as a heavy metal increases lipid per oxidation and decreases capacity of antioxidants, cadmium as a rare heavy metal also does this effect ***(Järup et al., 2000),*** it causes severe human health risk still date, without any physiological function within the human body ***(Godt et al., 2006).***

Cadmium chloride ($cdcl_2$) is a metal salt that is widely used in industries, it is very toxic as it accumulates in body inhibiting some enzymes containing sulfhydryl groups ***(Rhman et al., 2011),*** its formula is $cdcl_2$ as it consists of cadmium and chloride.

Cadmium atomic number is 48 as a symbol cd, it is soft bluish-white metal which is chemically similar to Zinc and Mercury as two stable metals,

it looks like Zinc as it prefers to be in oxidation state +2 in most of its compounds and looks like Mercury in its low melting point compared to transition metals.

Cadmium is also an environmental hazard as it causes general population toxicity in case of long term exposure to cadmium in contaminated food and water and research is ongoing regarding the estrogen mimicry that may induce breast cancer ***(Rhman et al., 2011; Mann, 2012)***.

ATSDR. (2008) said that 0.04 ug/m^3 and less than 1*u*g/L of cd levels in the air and drinking water not threaten health**.** Person take nearly 1ug cd/day in food not only that but additionally smokers who smoke one pack of cigarettes per day absorb 1-3ug cd and absorb the double of this amount of cadmium in case of heavy smoking ***(Waalkes, 2003; ATSDR., 2008)*.**

The rank of Cd between toxicants is 7^{th} in the Priority List of Hazardous Substances of the Agency for Toxic Substances and Disease Registry ***(ATSDR, 2007)***.

Cadmium is classified as a known human carcinogen in 1993 according to International Agency for Research on Cancer ***(IARC, 1993)*.**

Great efforts are done to control the exposure of cd to general public in recent years but cd has along biological half –life nearly 20-40 years in human and accumulates in body ***(WHO, 2000)***. Cadmium causes cancer in various organs as kidney, liver, pancreas and prostate ***(Thompson and Bannigan, 2008)*.**

Cadmium causes many problems on health. Zinc, Lead , copper, and cadmium as heavy elements has many uses in life as manufacture of batteries, pigments, metal alloys, and coating of some cursive metals as well

as an impurity in a lot of used materials ***(Chargui et al., 2011; Liang et al., 2015).***

Testis and ovary as reproductive organs are sensitive to cd toxicity, cd also causes damage to embryos ***(Thompson and Bannigan, 2008)***. There are several sources of cadmium, Cigarette smoke is one of its main sources ***(Lin et al., 2010).***

Human exposed to cadmium through many ways as inhalation of cigarette's smoke and ingestion of contaminated food or water ***(Duruibe et al., 2007; Montinaro et al., 2007).***

Cadmium has toxic effect on mammalian organs as liver ***(Arroyo et al., 2012; Cobbina et al., 2015),*** kidney ***(Jha et al., 2013; Cobbina et al., 2015)*** and other body organs ***(Cedergreen, 2014).***

Accumulation of cadmium occurs primarily in liver where glutathione and metallothionein sequester the majority of intra cellular cd. cd/metallothionein complex is slowly released from the liver in the blood stream ***(Yang and Shu, 2015).***

Cadmium has low molecular weight, so cd/metallothionein is freely filtered in the kidney and reabsorbed from the glomerular filtrate by megalin/cubilin receptors of PCT cells ***(Johri et al., 2010; Chargui et al., 2011).***

Cadmium as a heavy element is carcinogenic and results in various tumors in several organs ***(Cedergreen, 2014).*** Cadmium cause cancer, so it is classified as a human carcinogen by the International Agency for research on Cancer (IARC) ***(IARC, 1993).***

Some genes respond early to cd as c-fos, c-jun, c-myc and others, those are put in the proto-oncogenes group ,so these genes are considered as Immediate early response genes (IEGs) ***(Lin et al., 2010; Arroyo et al., 2012).***

Cadmium affects badly on mammalian cells as it cause induction of reactive oxygen species (ROS) in these mammalian cells ***(Jomova and Valko, 2011).*** Reactive oxygen species (ROS) is the main cause of apoptosis in the cells ***(Gürpınar et al., 2012).***

Many antioxidant molecules (GSH, GST, and SOD) are induced as a result of cd effect. Glutathione(GSH) (the most important antioxidant molecule present in cells and plays an important role in Cadmium detoxification ***(Arini et al., 2015)***.

Therefore Glutathione S-transferase (GST) and isozymes family catalyse GSH ***(Yadav et al., 2014)***, so to protect cells against reactive oxygen species (ROS) , there is several enzymes produced as peroxidases, Catalases and superoxide dismutase (SOD) ***(Esteban et al., 2014),*** Cell proliferation and apoptosis are two contradictive processes that occurred in the cell, since different genes are stimulated and expressed in each process .

There is correlation between cd and cancer incidence ***(Joseph, 2009),*** many studies mentioned the association between cd exposure and apoptotic cells ***(Bautista-Covarrubias et al., 2014; Peng et al., 2015).*** Cadmium affects on genes, many genes respond to cadmium depending on its effects ***(Whitfield et al., 2010).***

There are a lot of genes that are considered as apoptosis markers as caspase gene family that has cysteine proteases activity involved in the

apoptotic pathway ***(Thornberry, 1998; Belzacq et al., 2003).*** Cadmium causes oxidative stress ***(Tremellen, 2008).***

Cadmium can be stored in the body for long time especially in liver and kidney (long Biological half-life). Cadmium induces oxidative stress, so it causes testicular damage as testis is very sensitive to cadmium ***(Parizek, 1957),*** the mechanism of this toxicity caused is not fully understood.

Exposure to cadmium decreases several antioxidant enzymes activities in testes greatly such as Catalase, glutathione peroxidase and Superoxide dismutase , so testes decrease this oxidative stress through activating antioxidant mechanism and expressing these enzymes ***(Gupta et al., 2004)***.

Gonadal deficiency leads to azoospermia and oligospermia, Infertility can be induced by congenital or developmental disorders, androgen receptor disorders, Y chromosome defects, and acquired disorders e.g. (infection, drugs, toxins, and smoking) ***(Dohle, 2010).***

Sperm problems have occurred by several genetic factors as abnormal shape of sperm which cannot penetrate female egg surface ***(Auger, 2010),*** absence of sperm due to testicular failure ***(Dada, 2011)***, low sperm count that lead to decrease the chance of conception ***(Sharpe, 2012).***

Infected or injured testes, multiple sclerosis, diabetes, impotency, ejaculation problems, prostatectomy as functional problems lead to male infertility ***(Mclachlan et al., 2015).***

III. i- <u>The blood -testis barrier (BTB) and cadmium toxicity:</u>

Among all organs, Testis as a reproductive organ is very sensitive to cd toxicity, so acute exposure to cadmium results in testicular edema,

hemorrhage, necrosis, germ cell loss, BTB disruption and also may lead to sterility in various mammalian species as rodents, rabbits, dog, calf, and stallion (in vivo), while in vitro cd causes testicular damage ***(Li and Heindel, 1998).***

Morphological analysis describes endothelial cells disruption of micro vessels , edema and hemorrhage which lead to irreversible injury to mammalian testes ***(Parizek, 1960; Mason et al., 1964),*** this occurs apparently due to vascular system disruption ***(Parizek, 1964),*** this disruption lead to testicular ischemia and necrosis through affecting semineferous epithelium ***(Chiquoine, 1964)***.

The unique vasculature of mammalian testis makes it more sensitive to cadmium toxicity than other organs ***(Mason et al., 1964; Gunn, 1970),*** the vascular system follows mammalian testis in sensitivity to cadmium toxicity ***(Prozialeck et al., 2008),*** while in guinea pigs BTB is sensitive firstly to cd ***(Johnson, 1969)***.

Cadmium affects badly on sperm by reducing sperm count and induction of poor semen quality, so reduces male fertility ***(Benoff, 2000)***. The mechanism of cadmium toxicity is not fully understood, cadmium causes oxidative stress at high levels ***(Tremellen, 2008).***

Cadmium can be stored in liver and kidney for long time as it has long biological half-life, in case of testis cadmium affects it badly causing testicular damage as it induces oxidative stress in testicular tissue which is very sensitive to cadmium ***(Parizek, 1957; WHO, 2000)***.

The most susceptible organs to cadmium toxicity are liver and kidney ***(Abd-El-Baset and El-reheem, 2008)***. According to cadmium absorption

and retention in some tissues as liver, kidney and gastrointestinal tracts, cadmium toxicity is determined ***(Rhman et al., 2011)***.

Cadmium can be used in several industrial processes, its harmful effects are not withstanding, it can be used as a stabilizer in PVC products, neutron- absorber in nuclear power plants , an anti-corrosive agent, a color pigment, and in fabrication of nickel- cadmium batteries and production of phosphate fertilizers ***(Godt et al., 2006).***

There are several anthropogenic factors that lead to increased levels of cadmium in environment as waste incineration and fossil fuel combustion ***(Nair et al., 2013).***

Cadmium concentration in environment has increased unlike other heavy metals as it is in agricultural soils and this due to phosphate fertilizer application, sewage sludge, waste water, and pesticides ***(Limei et al., 2008).***

There are many sources of cadmium as smelting of ores, mining at mines, and its use in making Nickel-cadmium batteries ***(Liao et al., 2005)***.

Cadmium accumulates in the body tissues and organs (kidney, liver, lungs, bones and reproductive organs) in human which make them at high risk for cadmium ***(Alvarez et al., 2006),*** which leads to occurrence of oxidative stress due to induction of Reactive oxygen species (ROS) causing dysfunction and oxidative damage in different tissues ***(Liu et al., 2008)***.

III. ii-Cadmium-induced testicular oxidative stress:

Nowadays, both normal and/or pathophysiological conditions cause testicular oxidative stress which result in male infertility by 50% between infertile men examined to date , this illustrate the importance of cd as an inducer of oxidative stress ***(Tremellen, 2008).***

Although the testis expresses several antioxidants enzymes, such as superoxide dismutase, catalase and glutathione peroxidase to counteract the oxidative stress, their levels are greatly diminished upon cd exposure ***(Gupta et al., 2004)*.**

Therefore, it is reasonable to assume that antioxidant agents (enzymatic and non-enzymatic) may prevent or at least reduce the cd toxicity to the testis. Indeed, ***Parizek (1957)*** was the first to report that Zn could prevent testicular damage induced by cd**,** while ***Gunn et al. (1963)*** observed the prevention of cd-induced Leydig cell tumors by Zn**.**

However, at that time the oxidative stress induced by cd was not established, but the authors were correct when proposing that the prevention of the cd-induced testicular injury by Zn could be due to the similarity between these ions and their 'competition' for the physiological binding sites of Zn. Subsequent studies with substances having antioxidant activities, such as vitamin C, vitamin E, Zn, selenium and melatonin, have also demonstrated that the oxidative stress was associated with the cd-induced testicular damage, as these substances reduced and/or prevented both the oxidative stress and damage in the testes caused by cd ***(Burukoğlu and Bayçu, 2008)***.

III. iii- <u>Cadmium-induced cellular toxicity in testis:</u>

The physicochemical properties of the cd^{+2} ion act as it is similar to Ca^{+2} in ionic radii and to Zn^{+2} in electron configuration, Cd^{+2}**,**Ca^{+2} or Zn^{+2} in crucial physiological processes which are mediated by these ions and this lead to activation or inhibition of many signaling pathways, Oxidative stress induced by cd may be by binding to sulfhydryl groups of proteins and by depleting glutathione ***(Valko et al., 2005)*.**

Cadmium exposure can also result in interstitial cell tumors in the rat testes, indicating probable testicular dysfunction ***(Waalkes and Rehm, 1992).***

Cadmium treatment can also enhance the appearance of chemically induced prostate tumors in rats ***(Shirai et al., 2003)***.

IV- Fertility:

Nutrition affects on fertility directly by enhancing the process of Oocyte and spermatozoa development, Ovulation and fertilization and indirectly through affecting hormones concentration and its metabolites ***(Wathes et al., 2007)*.**

McGary Brougher et al. (2005) said that increased GnRH secretion, Weight of the testes and sperm production associated with diet containing high energy.

In the avian testis, there is a strong effect of FSH, LH and Testosterone on spermatogenesis and steroidogenesis ***(Vizcarra et al., 2010)*.**

The presence of some important fatty acids lead to an increase in GnRH, FSH, LH concentration and quality and morphology of sperm ***(O'Donnell, 1994)*.**

Also Long chain polyunsaturated fatty acids are very important for development of testicular seminiferous tubules, spermatogenesis and other testicular structures ***(Ayala et al., 1977)*.**

IV. i- <u>Anatomical structure of male reproductive system:</u>

Male reproductive system anatomically consists of a Pair of testes, Epididymis, accessory sex organs and accessory glands. The ovoid testes consist of seminiferous tubules, interstitial leydig cells which separate the seminiferous tubules produce testosterone. Testis has exocrine and endocrine function, Exocrine function as it produces sperms and Endocrine function through release of Testosterone.

Testosterone is essential to maintain spermatogenesis and secondary sexual characters and accessory sex organs function.

The spermatozoa are stored in a highly convoluted duct and inside this duct they become capable of fertilization, this duct is the epididymis. A viscous fluid which contains several essential nutrients for the sperms is secreted by the accessory glands ***(Setchell et al., 1994)***.

Curtis and Amann (1981) stated that any alteration of the function of these organs results in impairing the male fertility. The male reproductive tract is made up of the testes, ducts and glands that open in these ducts, the testes are responsible for male reproductive system through producing sperms and male sex hormone (Testosterone) ***(Chiarini-Garcia and Russell, 2001)*** .

The testis are formed physiologically of highly convoluted tubules which called seminiferous tubules. Spermatozoa are formed in these tubules, the rat has 30 tubules ***(Dym, 1976),*** the seminiferous tubules in rat has diameter of 50-100 *u*m ***(Setchell et al., 1994)***.

The main function of the male reproductive system is to produce normal live offspring, this occur when female genitalia delivers viable sperm that fertilizes the viable ova ***(Tyl, 2002; Costa et al., 2005).*** The ejaculate should contain adequate viable sperm count and adequate sperm motility for optimal fertilization.

Tyl (2002)**and** ***Costa et al. (2005)*** showed that production of viable sperm in the testes is controlled genetically (Y chromosome) and hormonally (hypo thalamic pituitary gland axis). Nutritional status, liver metabolism, endogenous hormones, and vascularization also play an important and indirect role. Release of gonadotropin-releasing Hormone (GnRH) from hypothalamus is the first step for regulation of the neuro endocrine system.

Gonadotropins (luteinizing Hormone(LH) and follicle stimulating Hormone (FSH)) are secreted via Anterior pituitary gland. Testosterone is synthetized by interstitial cells of leydig cells which is stimulated by LH which is necessary for spermatogenesis process.

Also, FSH attaches with Sertoli cells to support spermatogenesis ***(O'Donnell, 2001).*** Seminiferous epithelium is composed mainly of Sertoli cells and spermatogenic cells, Spermatogenic cells replicate and differentiate into mature spermatozoa ***(Steinberger and Steinberger, 1971).***

Contraction of seminiferous tubules occur by smooth muscle myoid cells which surround internal layer of non-cellular material which present in these tubules ***(Clermont, 1957)***.

Leydig cells, blood and lymph vessels are present in the interstitial tissue which is between the seminiferous tubules ***(Fawcett et al., 1973; Clark, 1976).*** Hormone is produced by leydig cells ***(Bergh, 1983).***

Chemes (1986) **and** ***Clermont et al. (1987)*** said that function of Sertoli cells acted in nourishment of spermatozoa , fluid secretion , blood testicular barrier formation, phagocytosis and androgen binding protein secretion .

Spermatogonia present inside the seminiferous tubules of the testis is responsible for formation of spermatozoa, this process of formation of spermatozoa is called Spermatogenesis ***(O'Donnell, 2001).***

Formation of mature spermatozoa passes by a complex series of biochemical and morphological transformations; development of germ cell occurs through formation of Spermatogonia, primary spermatocyte, secondary spermatocyte, and spermatid, this step lead to release of

spermatozoa into the lumen and proceeds through the rete testis to the epididymis.

Inside the epididymis the spermatozoa undergo several biochemical changes to be motile and capable of fertilization. Stages of spermatogenesis are not the same in all species, they are 12 stages in mouse, 14 in rat, 6 in the human ***(Clermont, 1972).***

O'Donnell (2001) said that Spermatogonia are differentiated into: type A Spermatogonia, intermediate Spermatogonia (only in rodents), and type B Spermatogonia, type B Spermatogonia is considered to be committed to differentiation.

Clermont (1972) showed that mitotic divisions occur to Spermatogonia before meiosis. After last mitotic division , type B Spermatogonia transformed into primary spermatocyte, then transformed to secondary spermatocyte through the first meiotic division ***(Hess, 1990)*** then undergo second meiotic division to form haploid round spermatid which then differentiated into mature elongated spermatids, this occur during process of spermatogenesis ***(Cheng and Mruk, 2010)***.

The process of spermatogenesis occur within 70 days passing by different stages of development beginning with spermatogonium (germ cell) and end with mature sperm, these mature sperms produced in testes then pass to epididymis and presented in semen and so this mature sperm can fertilize a female egg ***(França et al., 2005) .***

The release of mature spermatids into seminiferous tubule lumen is the end stage of spermatogenesis that is what is called spermiation ***(Cheng and Mruk, 2010).***

Fertilization is the combination of genetic materials of normal mature sperm that contains 23 tightly packed chromosomes and genetic material of a healthy egg ***(Garrett et al., 2003)*.**

During Fertilization, not all sperms can reach the fallopian tube , only one sperm can fertilize the female egg to form zygot (combination of 23 chromosomes from each cell, sperm cell and egg cell), not all sperms reach the fallopian tube as some cannot survive in vaginal acidic media, others cannot penetrate the cervical mucus ***(De Jonge and Barratt, 2006)*.**

IV. ii- Hormonal Regulation of Spermatogenesis:

There are many proteins which are produced by Sertoli cells that are important for development of germ cells, so there is coordinated interaction between Germ cells and Sertoli cells through ligand and receptor – pathway ***(Parvinen, 1982)***.

França et al. (1998) showed that according to the kinetics of rat spermatogenesis, Germ cells and mouse Sertoli cells are developed, Germ cells growth and fate controlled by itself.

Testicular function beside sperm production, hormonal production , these testicular hormones are essential for development and maintenance of secondary sex organs and functions , and also necessary for feedback loop of the hypothalamus and the anterior pituitary to control the secretion of the gonadotropins LH and FSH, The major endocrine regulators of spermatogenesis ***(McLachlan et al., 2002)*.**

Leydig cells secrete androgens (testosterone) through stimulus from LH, Testosterone then stimulates seminiferous tubules to form spermatozoa, while FSH acts on sertoli cells to regulate spermatogenesis, in case of lack of GnRH, LH and FSH can be injected with androgens to stimulate all

stages of spermatogenesis ***(Singh et al., 1995),*** this is an evidence that spermatogenesis requires androgen.

FSH is mainly required during neonatal period during sertoli cell division ***(Krishnamurthy et al., 2000).*** FSH is not essential for spermatogenesis but it is essential for fertility, Negative feedback on hypothalamic GnRH production governed by inhibits control spermatogenesis endocrine regulation ***(Russell and Clermont, 1977)***.

During physical examination of males, any abnormalities or alteration in male characters must be noticed as size and shape of penis , size of breasts, size and position of testis in scrotum ***(Jarow and Sigman, 2010).***

Testes must successfully descend from abdomen into scrotum for maintaining fertility as the temperature in scrotum enhance sperm production in semineferous tubules and normal testicular function ,between semineferous tubules there is leydig cells responsible for production of male sex hormones (Testosterone) ***(Toth, 2004; De Gendt et al., 2005).***

When healthy sperms produced by male testes success to pass to female fallopian tube and penetration of one sperm to a healthy egg, fertilization occur and so implantation of the fertilized egg in uterus, this is called fertility, while in case of any disturbance in the mentioned steps, infertility is the result ***(Jong et al., 2006).***

The organisms live their life through using their energy for growing, reproduction and maintaining vital functions to avoid death ***(Varea and Bernis, 2013).***

There is three types of life history of primates increasing age of sexual maturation , biological reproduction ,and extended life expectancy as follow ,the reproductive age of monkeys is older and they live more than do

prosimians, the reproductive age of apes is older and they are live more than do monkeys and the reproductive age of human is older and they are live more than do apes ***(Last, 2014)*.**

IV. iii-Factors affecting male fertility:

Oxidative stress which can be defined as imbalance between reactive oxygen species and antioxidants in the body affect male fertility as it leads to damage and deformity of the sperm ***(Makker et al., 2009)*.**

There are many factors that can decrease and cause disturbances in fertility as excessive exercise, caffeine and alcohol consumption and smoking ***(Joo et al., 2012)*.** There are various chronic diseases that can cause male infertility as diabetes, hyperthyroidism and hypothyroidism ***(Aboua et al., 2013)*.**

Measuring serum testosterone, FSH, LH levels and joining their results with semen analysis can determine male fertility and infertility analytically ***(Jungwirth et al., 2012)***.

Male fertility and infertility can be diagnosed by semen analysis, in which ejaculated semen sample must be fresh and done in qualified laboratory, samples are examined microscopically to determine sperms shape, movement and number ***(Kliesch, 2014)***.

The control of testicular function is a complex process that requires the functional integrity of the seminiferous tubules and leydig cells with a suitable multi hormonal stimulation, The steroidogenic capacity of leydig cells is essential for spermatogenesis, however, not only hormonal aspects are important in testis physiology, lipid composition is also crucial. Several factors have demonstrated that nutrition is an environmental factor of major importance ***(Simopoulos, 2002)*.**

Other authors have demonstrated that dietary fats can modulate steroidogenic function of mammalian testis ***(Gromadzka-Ostrowska et al., 2002).*** Un balanced diet can cause harmful and unhealthy effects on spermatogenesis and can cause production of abnormal sperm ***(Mosher and Pratt, 1991).***

MATERIALS AND METHODS

Materials

I. Palm Oil: purchased from **Malaysia.**

I. i- Physicochemical properties: ***(Cottrell, 1991; Edem, 1999)***

- **Appearance:** light yellow to orange-red in color depending on the amount of carotenoids present, the level of oxidation by lipoxygenases in bruised fruit stored for various periods before processing and the oxidation catalyzed by iron during processing and bulking**.**
- **Melting point:** 34.2 c^o
- **Relative density**: (50 ◦C/water at 25 ◦C) 0.89–0.92
- **Refractive index:** 1.46 (η)
- **Moisture and impurities**: 0.1 (%)
- **Iodine value**: 47–55.83
- **Saponification value**: (196–208.2) mg KOH/g.
- **Unsaponifiable matter**: 0.01–0.5 (%)

II. Cadmium chloride: was supplied from (Oxford Inc. for laboratory reagents 99.5%, India), which was used for oxidative stress induction in male rats.

II. i- Physicochemical properties: ***(Lide, 2009)***

- **Appearance:** a white crystalline
- **Chemical Structure:** compound of cadmium and chlorine, with the formula $cdcl_2$.

- **Solubility:** (hygroscopic solid) highly soluble in water and slightly soluble in alcohol**.**

III. Chemicals used for semen analysis:

1- Physiological normal saline.

2- Eosin-nigrosin stain**.**

IV. Chemicals used for histopathological studies:

1- Absolute alcohol (El-Gumhoria Co. – Egypt).

2- Canada balsam

3- Formalin (El-Gumhoria Co. – Egypt).

4- Methyl alcohol (El-Gumhoria Co. – Egypt).

5- Paraffin wax (prolabo – England).

6- Xylene (Chemico – Egypt).

Chloroform: purchased from Sigma Aldrich Co., which used before testicular dislocation and slaughter by Euthanization.

V. Experimental animals:

Fourty male white Wistar albino rats used to carry out this study. The rats purchased from the animal house, Faculty of veterinary medicine, Mansoura University. The weight of rats had an average of 180±20 grams. The age of rats ranged from 16 -18 weeks old. The animals were allowed to accommodate to the laboratory conditions, Faculty of veterinary medicine, Mansoura University (12hour light/dark cycle) for7 days before start of the study. The animals maintained in clear stainless cages where their beddings

were changed twice a week. The rats were having free access to solid pellet diet and water ad libitum throughout the study. The animals were given a diet formulation according to national research Council (NRC, Table 1). The stocking density of rats was five rats for each cage.

VI. Diet:

Table (II): The diet formulation:

The diet formulation required for the current study was designed according to the guidelines stated in ***NRC (1995)***.

Food ingredients	Normal diet
Wheat	19.004 k.g
Ground Yellow Corn	46.51 k.g
Soya bean meal (44%protien)	22.945 k.g
Lime stone	0.738 k.g
Di-Calcium Phosphate	1.702 k.g
Fish meal(65% protein)	6 k.g
Salt	1.011 k.g
Mineral and vitamins premix	1 k.g
Lysine	0.26 k.g
Methionine	0.13 k.g
Total Lysine %	0.2%
Total Methionine %	0.5%

VII. Equipment:

1-Electric balance (from Driver Instrument Company, U.S.A).

2-Glass slides, test tubes, stomach tube.

3-Insulin syringes, scissors, forceps.

4-Centrifuge (Shanghai Surgical Instrumental Factory).

5-Microscope (Germany).

6-Spectrophotometer SP50 (Gallen KAMP, U.K).

7-Automatic micropipette with different volumes (U.K)

8-Haemocytometer (Germany).

VIII.Experimental design:

The main objective of this study was to investigate the effect of palm oil supplementation on fertility and oxidative stress on male rats. Oxidative stress was induced in rats by single dose injection of cadmium Chloride

In order to achieve the aim of the present study, the rats were divided into four groups:

- **Group 1 (control group):** consisted of 10 rats, which received a balanced diet including breed, barely, carrot and milk for 30 days.

- **Group 2 (palm oil group):** consisted of 10 rats, each rat received(4ml)palm oil daily ***(Aboua et al., 2012)*** inoculated by stomach tube for 30 days and 0.1% cholate added to diet for digestion of fat ***(Van Nieuwkerk et al., 1996)***

- **Group 3 ($cdcl_2$ group):** consisted of 10 rats, each rat was injected single dose of $cdcl_2$ (2.5 mg/kg intraperitoneally /week**) *(Santos et al., 2004)***.
- **Group 4 (palm oil +$cdcl_2$ group):** consisted of 10 rats, Each received co-operation of (4ml)palm oil daily ***(Aboua et al., 2012)*** given by stomach tube and single dose of $cdcl_2$ (2.5mg/kg intraperitoneally/week) for 30 days ***(Santos et al., 2004)*** and 0.1% cholate added to diet for digestion of fat ***(Van Nieuwkerk et al., 1996)***.

VIII.i- Sacrificing the rats:

Chloroform was used for anaesthetizing the rat's. When the rats became fully unconscious, testicular dislocation was applied.

VIII.ii- Blood sampling:

After the end of the experiment, rats were anaesthetized by chloroform and blood samples were collected from the heart of the post anesthetic rat through inserting the needle of the syringe under the xiphoid process of the sternum until it reached the heart ventricles, then take with a syringe 5-10 ml of blood.

The blood samples taken in tubes as non-heparinized which left in a room temperature till clotting, then centrifuge at 3000 rpm for 15 minutes to these blood samples to separate serum samples which are used to determine:

1) Serum Testosterone hormone.

2) Serum Follicle stimulating hormone (FSH).

3) Serum Luteinizing hormone (LH).

VIII.iii-Dissection of the rats:

After sacrificing, rats were dissected to obtain both testes.

VIII. iv- semen collection:

Spermatozoa were recovered from the tail of epididymis by its squeezing and so determined sperm motility by wet mount method through placing a sample of diluted semen on a pre wormed microscope slide , examining it with a microscope and estimating the fraction of the population that is motile, Also sperm morphology was determined using eosin and nigrosin stain, briefly, a drop of eosin and nigrosin stain was mixed with a drop of sperm suspension were put on a slide and examined under microscope at x400 magnification.

The testes were then separated from epididymis, weighted and stored in 2 parts after washing in normal saline:

1) **The first half was stored in normal saline at -20 for determination of:**

   A) Catalase activity

   B) Reduced glutathione(GSH) concentration

   C) Glutathione –S-Transferase (GST) activity

   D) Nitric Oxide(NO) level

   E) Malondialdehde (MDA) level

2) **The remaining half was immersed in 10% neutral buffered formalin for** histopathological examination for evaluating the spermatogenic status of testes.

II Methods

I-Semen Evaluation:

1) Individual motility:

A drop of epididymal spermatozoa diluted in sodium citrate dehydrate 2.9% solution was put on warm clean glass slide, covered by covered slip, mounted on hot stage (37Cº)phase constract microscope and examined at high power(40X).Individual motility was recorded as the percentage of the forward progressive motility of spermatozoa ***(Zemjanis et al., 1970).***

2) Live Spermatozoa:

Epididymal spermatozoa diluted in sodium citrate dehydrate 2.9% solution were stained with eosin-nigrosin stain. Live spermatozoa were differentiated from dead ones by the resistance of their plasma membranes to eosin stain ***(Öztürkler et al., 2001).***

3) Morphological sperm abnormalities:

Epididymal spermatozoa stained with eosin-nigrosin stain were examined for abnormal head, neck, or tail abnormalities as examined by ***Mancini et al. (2015)***.

II. Biochemical analysis:

II. i-Determination of antioxidant activity in rat testis:

- **Homogenization of tissue:**

Tissue was homogenized according to this technique of ***(Fernandez-Botran et al., 2002)*.** Testis sample was homogenized in ice-cold phosphate buffer saline pH 7 with a ratio of 1:9. After the homogenization process was completed, the homogenate was transferred to centrifuge tube and then centrifuged at 10000 rpm for 20 minutes and aliquots of supernatants were assayed.

1) Determination of Catalase activity:

CAT activity was determined as described by ***Aebi (1984)*.**

The kit was purchased from (Biodiagnostic _Egypt).

Principle:

Catalase reacts with a known quantity of H_2O_2. The reaction is slopped after exactly one minute with catalase inhibitor according to the following equation:

Catalase

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

In the presence of Peroxidase (HRP), the remaining H_2O_2 reacts with 3, 5-Dichloro-2-hydroxybenzene sulphonic acid (DHBS) and 4-aminophenazone (AAP) to form chromophore with color intensity inversely proportional to the amount of Catalase in the original sample as follows:

$$2H_2O_2 + DHBS + AAP \xrightarrow{HRP} \text{Quinoneimine Dye} + 4H_2O$$

Calculation:

$$\text{Tissue Catalase Activity U/g} = \frac{\text{A standard} - \text{Asample}}{\text{Astandard}} \text{X } 1/\text{ gm tissue used}$$

2) Determination of Reduced glutathione concentration (GSH):

GSH concentration was determined according to ***(Beutler, 1963)*.**

The kit was purchased from Biodiagnostic -Egypt.

Principle:

The method based on the reduction of 5, 5 dithiobis 2-nitrobenzene (DTNB) with glutathione to produce yellow compound. The reduced chromagen was directly proportional with GSH concentration.

Calculation:

Tissue GSH concentration = $\frac{\text{Asample x 66.66}}{\text{g tissue used}}$ mg/g tissue

3) Determination of Glutathione-S-Transferase (GST) activity:

GST activity was determined according to ***(Habig et al., 1974)*.** The kit was purchased from Biodiagnostic –Egypt.

Principle:

Total GST activity (cytosolic and microsomal) was determined by measuring the conjugation of 1- chloro- 2, 4-dinitrobenzene (CDNB) with reduced glutathione. The rate of increase was directly proportional to the GST activity in the sample.

Calculation:

GST activity:

Tissue (U/g tissue) = $\frac{\text{A(sample)X 2.812}}{\text{g tissue}}$

4) Determination of lipid peroxide (Malondialdehde) (MDA):

Thiobarbituric acid reacts with MDA in acidic medium at temperature of 95 C for 30 min. to form thiobarbituric acid reactive product the absorbance of the resultant pink product can be measured at 534 nm. It is determined according to ***Kei (1978)***

Calculation:

MDA in sample:

Tissue

$$(\text{nmol / g}) = \frac{\text{A(sample) x 10}}{\text{A(standard)x g tissue used}}$$

5) Determination of Nitric Oxide (NO):

NO was determined according to ***Montgomery and Dymock (1961)*.** The kit was purchased from Biodiagnostic-Egypt.

Principle:

Presence of both acidic medium and nitrite forms nitrous acid diazotize sulphanilamide. This product couples with N-(1-naphthyl) ethylene diamine. The resulting azo dye has a bright reddish – purple color, which can be measured at 540 nm.

Calculation:

Nitrite in sample:

$$\text{Tissue } (u\text{mol/L}) = \frac{\text{Asample x 50}}{\text{Astandard}}$$

III) Hormonal analysis:

It is determined according to ***Lashansky et al. (1991)***

III.i Determination of serum testosterone hormone:

Principle:

The Testosterone rat ELISA kit is a solid phase enzyme –linked immune sorbent assay (ELISA), based on the principle of competitive binding, purchased from (DRG Instruments GmbH, Germany).

An unknown amount of testosterone presents in the sample and a defined amount of testosterone antiserum coated to the wells of a micro plate. After one-hour incubation on a shaker the micro plate is washed four times. After addition of the substrate solution the concentration of testosterone is inversely proportional to the optical density measured.

Calculation:

1- Calculate the average absorbance values for each set of calibrators, controls and samples.

2- Using semi logarithmic graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3- Using the mean absorbance value for each sample, determine the corresponding concentration from the calibration curve. Or Log it-Log is recommended.

The concentration of the samples can be determined directly from this calibrator curve. Samples with concentrations higher than that of the highest

calibrator have to be further diluted. For the calculation of the concentrations, this dilution factor has to be taken into account. Conversion to SI units: Testosterone (pg. /ml) x 3.47 = p mol.

III.ii Determination of Serum FSH(Follicle Stimulating Hormone) : It is determined according to ***Uotila et al. (1981)*.**

Principle:

In rat FSH ELISA kit (Biovendor Research and Diagnostic Products, Germany), biotin –conjugated anti-FSH and standard or sample are incubated in monoclonal anti-FSH antibody –coated wells, After 15–18 hours incubation and washing, HRP (horse radish peroxidase)-conjugated advin is added, and incubated for 30 minutes. After washing, HRP – complex remaining in wells are reacted with a chromogenic substrate 20 minutes and reaction is stopped by addition of acidic solution and absorbance of yellow product is measured spectrophotometrically at 450 nm (sub –wavelength is 620 nm). The absorbance is nearly proportional to FSH concentration. The standard curve is prepared by plotting absorbance against standard FSH concentrations. FSH concentrations in unknown samples are determined using this standard curve.

Calculation:

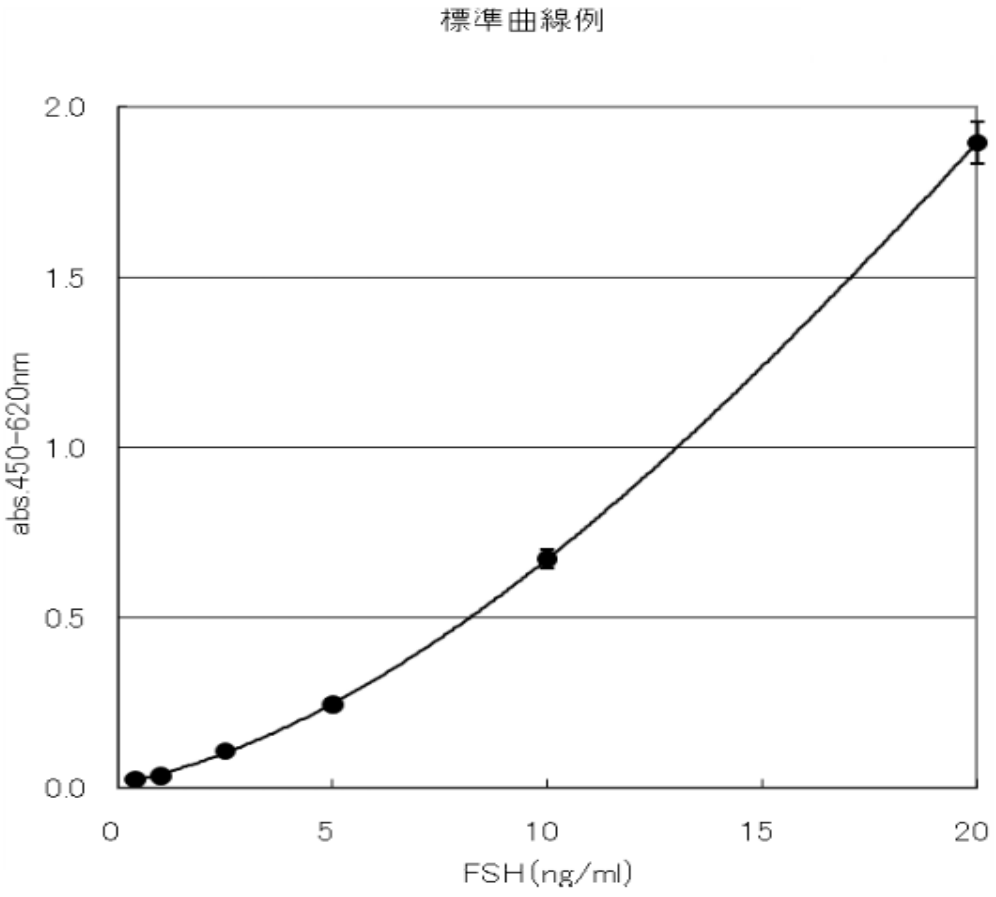
1- Prepare a standard curve in every assay by plotting absorbance (Y-axis) against FSH concentration (ng/ml) on X-axis. Absorbance at 450 nm minus absorbance at 620 nm.

2- Using the standard curve, read FSH concentration of samples at their absorbance, and multiply the assay value by dilution rate. Though the assay range is wide enough, in case the absorbance of some samples is higher than that of the highest standard, please

repeat the assay after proper dilution of samples with the buffer solution.

3- We recommend the use of 3rd order regression curve for log-log plot, or 4 parameters) calculation template for EXCEL on our home page.

Physiological or pathological situation of animals should be judged comprehensively taking other examination data into consideration.



FSH assay standard curve (an example above)
Absorbance may change due to assay environment.

III.iii Determination of serum LH:

It is determined according to ***Ross et al. (1981)***.

Principle:

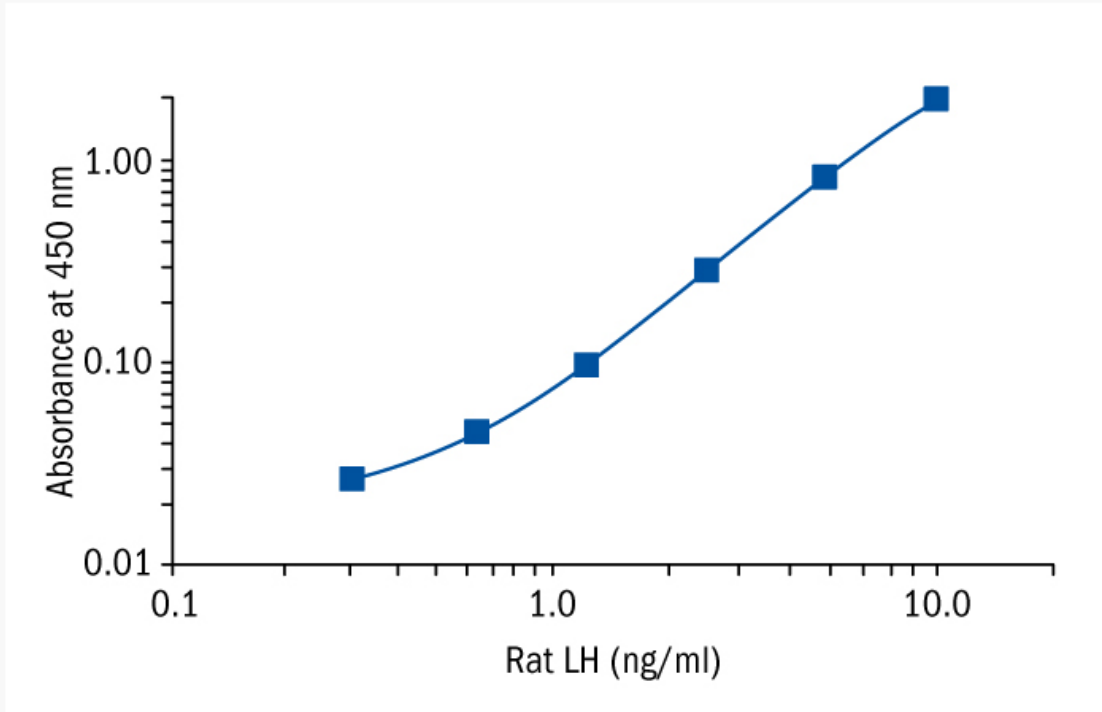
In Shibayagi's Rat LH ELISA Kit (BioVendor Research AND Diagnostic Products, Germany), standards or samples are incubated in monoclonal anti-LH β antibody-coated wells to capture LH. After 2 hours' incubation and washing, biotin-labeled anti-LH α antibody is added and incubated further for 1 hour to bind with captured LH. After washing, HRP (horse radish peroxidase)-labeled avidin is added, and incubated for 30 minutes. After washing, HRP-complex remaining in wells is reacted with a chromogenic substrate (TMB) for 20 minutes, and reaction is stopped by addition of acidic solution, and absorbance of yellow product is measured spectrophotometrically at 450 nm. The absorbance is proportional to LH concentration. The standard curve is prepared by plotting absorbance against standard LH concentrations. LH concentrations in unknown samples are determined using this standard curve.

Calculation:

1- Prepare a standard curve using semi-logarithmic or two-way logarithmic section paper by plotting absorbance (Y-axis) against LH concentration (ng/ml) on X-axis.
2- Using the standard curve, read the LH concentration of a sample at its absorbance, and multiply the assay value by dilution factor if the sample has been diluted. Though the assay range is wide enough, in case the absorbance of some samples is higher than that of the highest standard, repeat the assay after proper dilution of samples with assay buffer (c).

3- We recommend the use of 3rd order regression curve for log-log plot, or 4 parameters method for ion –normal plot in computer calculation.

Physiological or pathological situation of animals should be judged comprehensively taking other examination results into consideration.



IV. Histopathological examination:

After complete necropsy, testicular specimens were collected from all experimental groups and fixed in 10%neutral buffered formalin for the histopathological examination. After proper fixation ,the tissue was rinsed with water and dehydrated in ascending grades of alcohols, cleared in xylene and embedded in paraffin .Paraffin blocks were cut into 5 *u*m thick sections and stained with hematoxylin and eosin (H&E).About 50 sections of seminiferous tubules from each group were evaluated for their modified spermatogenesis index by Johnson's score ***(Johnsen, 1970).*** In Johnsons score a grade from 1 to 10 was given to each tubule cross section according to the range from no cells to complete spermatogenesis .spermatogenesis of these model rats was evaluated by H&E staining , and the maturity of seminiferous epithelium was marked by using a modified Johnson's score ***(Johnsen, 1970).***

Table (III): Modified Johnsen score

Score	Histological findings
10	Full spermatogenesis
9	Many late spermatids disorganized tubular epithelium.
8	Few late spermatids
7	No late spermatids, many early spermatids
6	No late spermatids, few early spermatids, arrest of spermatogenesis at the spermatid stage, disturbance of spermatid differentiation.
5	No spermatids, many spermatocytes.
4	No spermatids, few spermatocytes, arrest of spermatogenesis at the primary spermatocyte stage.
3	Spermatogonia only.
2	No germ cells, Sertoli cells only
1	No seminiferous epithelial cells, tubular sclerosis.

V. Statistical analysis:

Data were subjected to statistical analysis using statistical software Program (SPSS for Windows, version 16, USA).

Means and standard error for each variable were estimated. Differences between means of different groups were carried out using one way ANOVA with Duncan multiple comparison tests.

Statistical analysis was carried out after different variables were analyzed using Students (t) test ***(Snedecor and Cochran, 1984).***

RESULTS

In order to achieve the objectives of this investigation, fourty rats were divided into four groups **(**10 rats each**)** to obtain the required estimates to full fill the objectives stated earlier in the introduction of this thesis. The estimated values of all studied characters would be presented in the following orders:

I-Evaluation of weight of testis and sperm motility:

I.i-Effect of palm oil supplementation and oxidative stress induced by $cdcl_2$ on weight of testis:

Weight of rats testes supplemented with palm oil showed an increase(1.5 ± 0.11^{a}) than those obtained from control rats(1.3 ± 0.13^{a}), while testes of rats exposed to oxidative stress induced by $cdcl_2$ injection showed a decrease (0.41 ± 0.05^{b}), this decrease was protected in those supplemented with palm oil in co-operation with $cdcl_2$ (0.86 ± 0.03^{c}).

I.ii- Effect of palm oil supplementation and oxidative stress induced by $cdcl_2$ on sperm motility:

There is an increase in sperm motility in group supplemented with palm oil (85.83 ± 3.01^{a}) when compared to control group (80.83 ± 1.4^{a}), while there is no semen and so no sperm in both stress group and palm oil group under stress.

Table (1): Effect of palm oil supplementation and $cdcl_2$ injection on testicular weight and sperm motility (M±S.E):

Group	Testicular weight (gm)	Sperm motility
G1: Control	1.3 ± 0.13^{a}	80.83 ± 1.4^{a}
G2: Palm oil	1.5 ± 0.11^{a}	85.83 ± 3.01^{a}
G3:$cdcl_2$	0.41 ± 0.05^{b}	No semen, no sperm
G4: Palm oil + $cdcl_2$	0.86 ± 0.03^{c}	No semen, no sperm

Means with different letters are significantly differed ($p\leq0.05$)

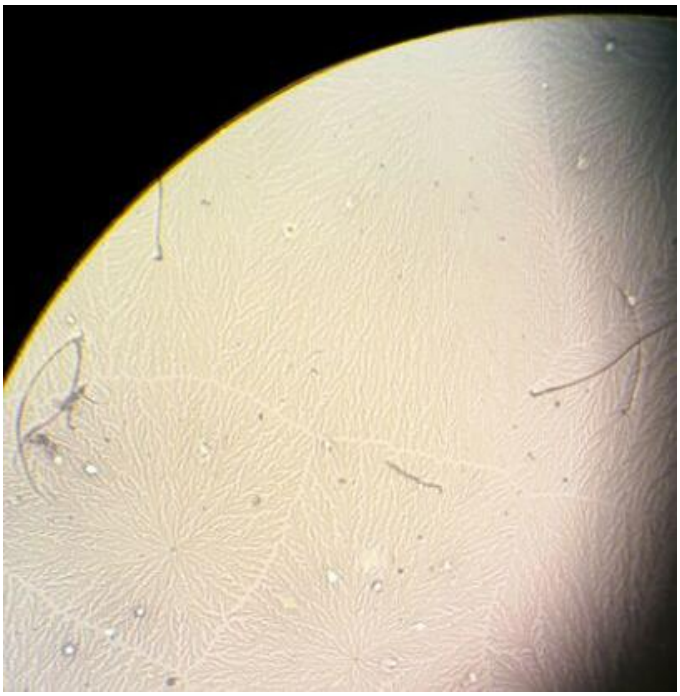


Photo (1): Control group, showing normal quality of sperms.

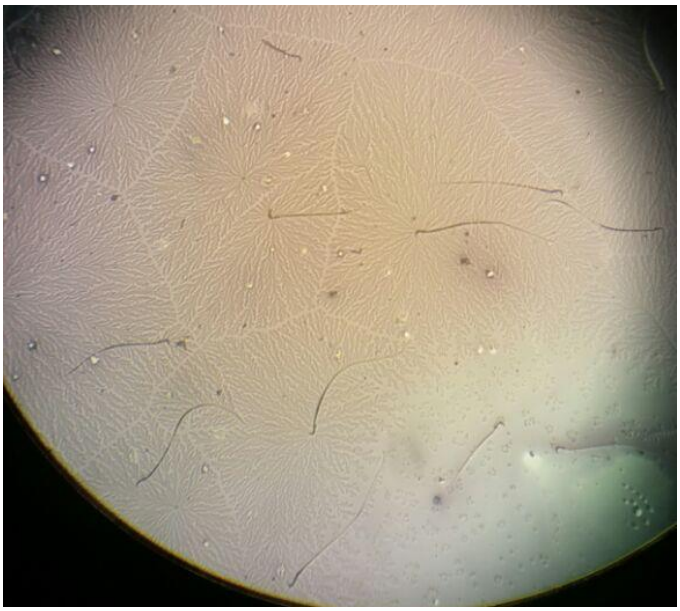


Photo (2): Palm oil supplemented group, showing an increase in quality of sperms.

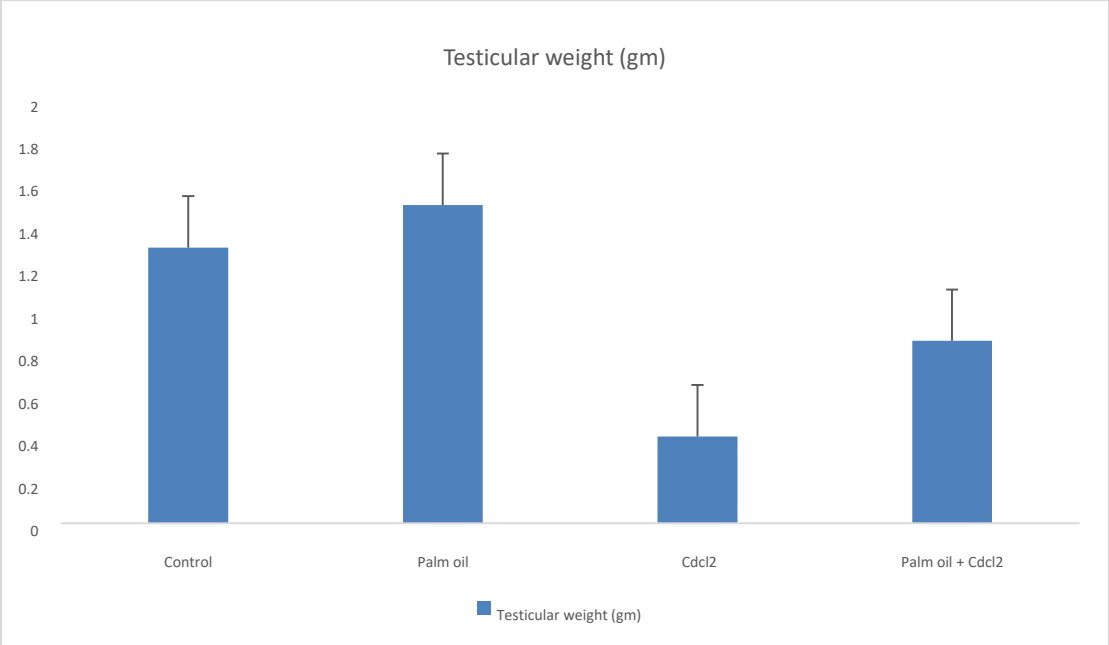


Figure (1): Effect of palm oil supplementation, $cdcl_2$ injection, co-operation of palm oil with $cdcl_2$ on weight of testis compared to control ones.

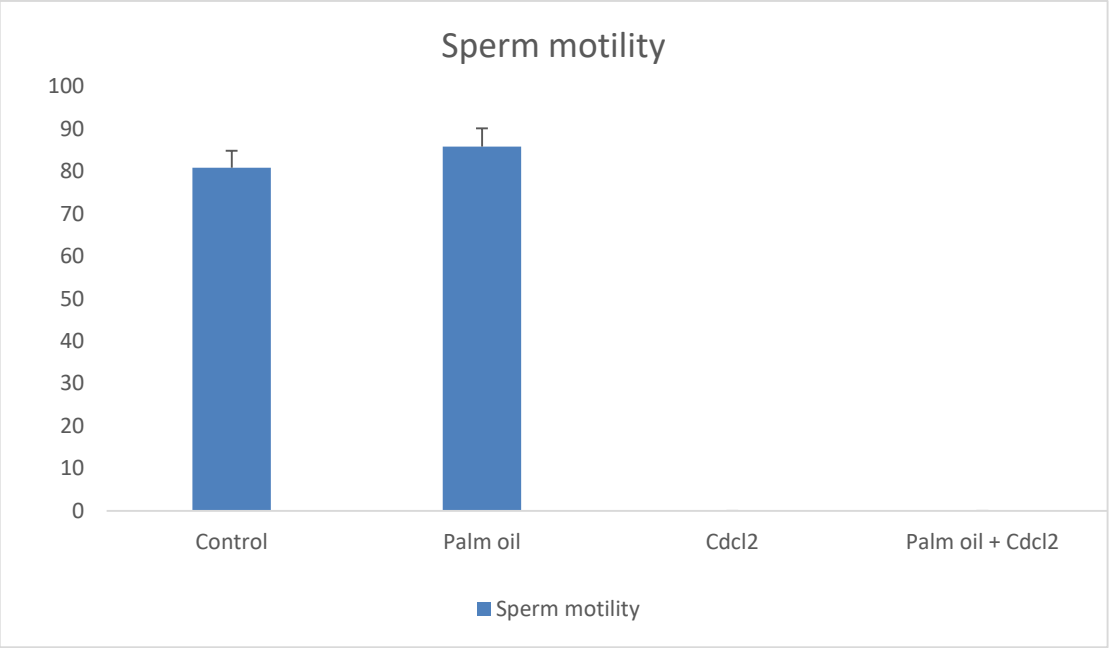


Figure (2): Effect of palm oil supplementation, $cdcl_2$ injection, co-operation of palm oil with $cdcl_2$ on Sperm motility compared with control ones.

II-Biochemical results:

- **Effect of Palm oil supplementation and $cdcl_2$ injection on oxidative and antioxidant activity in rat testis:**
    1- **On Catalase activity**
    2- **On Malondialdehde level**
    3- **On Reduced glutathione activity**
    4- **On Glutathione-S-Transferase activity**
    5- **On Nitric oxide level**

1-On Catalase activity:

In our study, there is an increase in the levels of Catalase in palm oil supplemented group (0.67±0.43), while in cadmium chloride injected group; the results evoked a decrease in the levels of serum catalase (0.67±0.43), but, in the group of palm oil and cadmium chloride, they cause a significant increase in catalase level (0.42±0.02) compared to that of control group (0.27±0.02).

2-**On Malondialdehde level:**

The level of Malondialdehde was non significantly decreased in the group supplemented with palm oil and it was (11.48±1.03), while in cadmium chloride injected group and the group of palm oil and cadmium chloride, the obtained data revealed an increase in Malondialdehde level (54.33±2.79) and (38.23±2.22) respectively compared to that of control group (13.22±0.64).

Table (2): Effect of Palm oil supplementation and $cdcl_2$ injection on Catalase activity and MDA level (M±S.E).

Group	Catalase activity (U/g)	MDA (mg/g)
G1: Control	0.27 ± 0.02^{c}	13.22 ± 0.64^{c}
G2: Palm oil	0.67 ± 0.43^{a}	11.48 ± 1.03^{c}
G3: $cdcl_2$	0.12 ± 0.01^{d}	54.33 ± 2.79^{a}
G4: Palm oil + $cdcl_2$	0.42 ± 0.02^{b}	38.23 ± 2.22^{b}

Means with different letters are significantly differed ($p\leq0.05$)

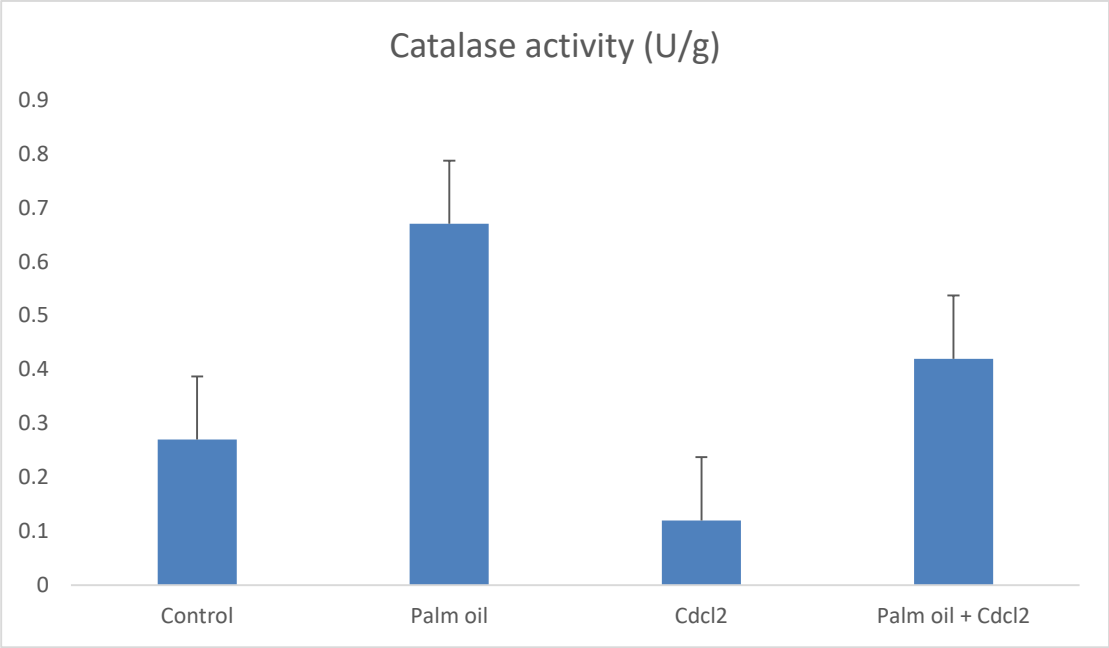


Figure (3): Effect of palm oil supplementation and $cdcl_2$ injection on Catalase activity.

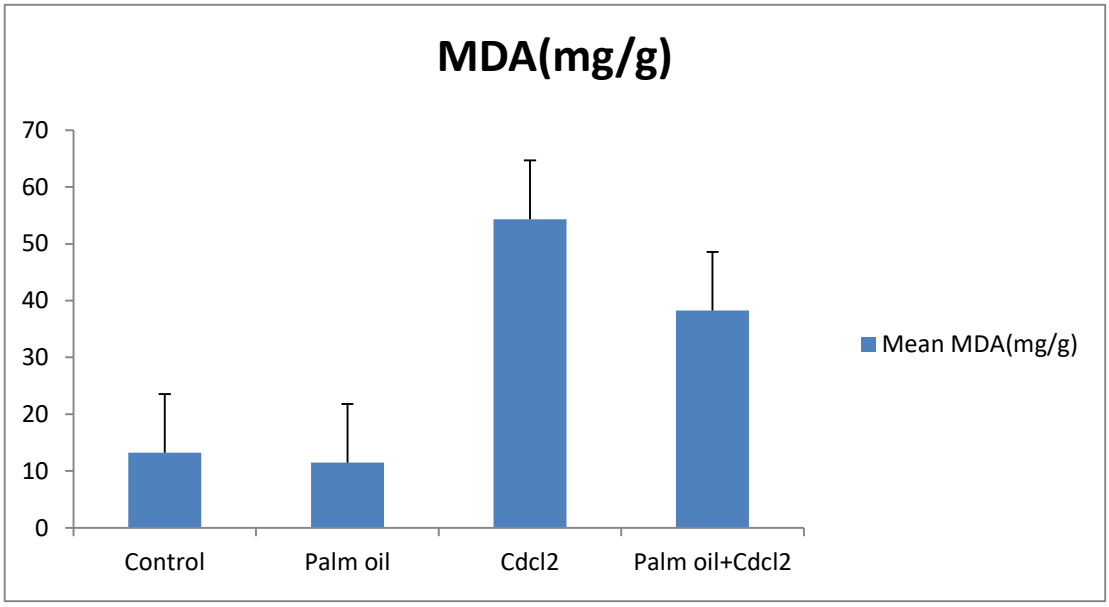


Figure (4): Effect of Palm oil supplementation and $cdcl_2$ injection on on MDA level.

3-On Reduced glutathione activity:

It was recorded that, there was an increase in the levels of serum GSH in palm oil supplemented group (17.03±0.78), while in cadmium chloride injected group and the group of palm oil and cadmium chloride, the obtained data revealed a decrease in reduced glutathione level (4.52±0.36 and 6.96±0.25respectively) compared to that of control group (12.82±0.74).

4- On Glutathione-S-Transferase activity:

Palm oil supplemented group induced an increase in the levels of GST (8.52±0.59) while in cadmium chloride injected group, the level of GST was significantly decreased (0.81±0.13). As in palm oil and cadmium chloride group evoked a significant increase in serum GST level (5.46±0.55) compared to that of control group (2.62±0.18).

5-On Nitric Oxide level:

The obtained result showed that there was a significant increase ($p \leq 0.05$) in NO level in all medicated groups, As (37.92±8.61)in Palm oil supplemented group, (89.23±4.53) in cadmium chloride injected group and (65.73±3.30) in palm oil and cadmium chloride group compared to that of control group (14.82±1.05).

Table (3): Effect of palm oil supplementation and oxidative stress induced by $cdcl_2$ injection on GSH, GST and NO activity (M±S.E).

Group	GSH (mg/g)	GST (U/gm)	NO (mg/g)
G1: Control	12.82 ± 0.74^{b}	2.62 ± 0.18^{c}	14.82 ± 1.05^{d}
G2: Palm oil	17.03 ± 0.78^{a}	8.52 ± 0.59^{a}	37.92 ± 8.61^{c}
G3: $cdcl_2$	4.52 ± 0.36^{d}	0.81 ± 0.13^{d}	89.23 ± 4.53^{a}
G4: Palm oil + $cdcl_2$	6.96 ± 0.25^{c}	5.46 ± 0.55^{b}	65.73 ± 3.30^{b}

Means with different letters are significantly differed ($p\leq0.05$)

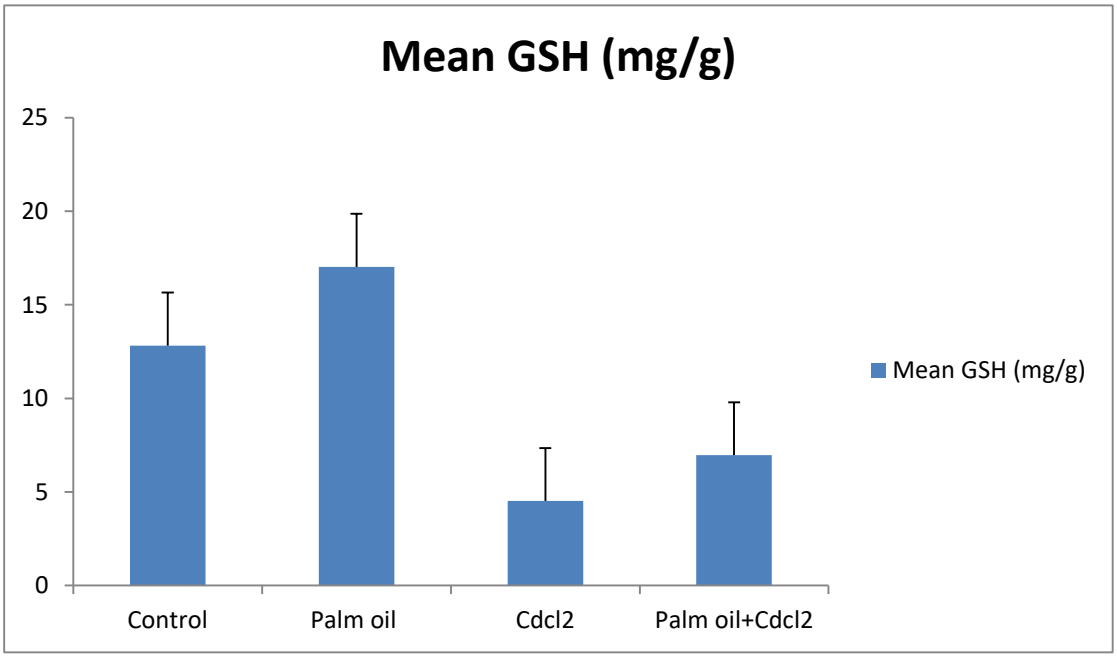


Figure (5): Effect of Palm oil supplementation and $cdcl_2$ injection on GSH activity

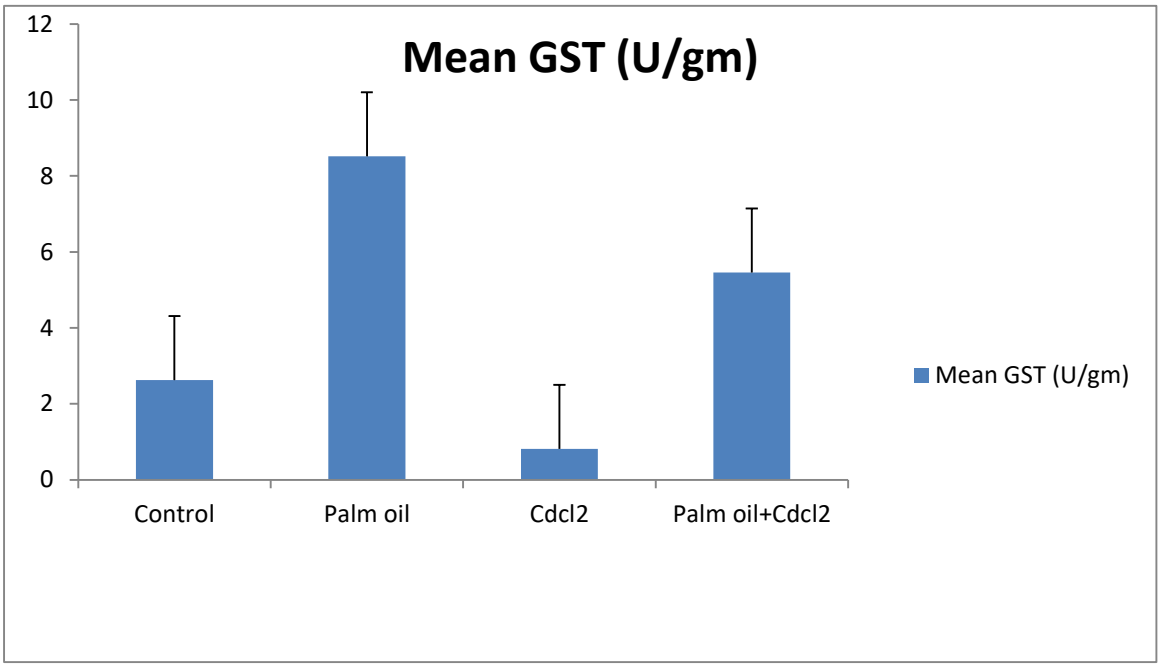


Figure (6): Effect of Palm oil supplementation and $cdcl_2$ injection on GST activity.

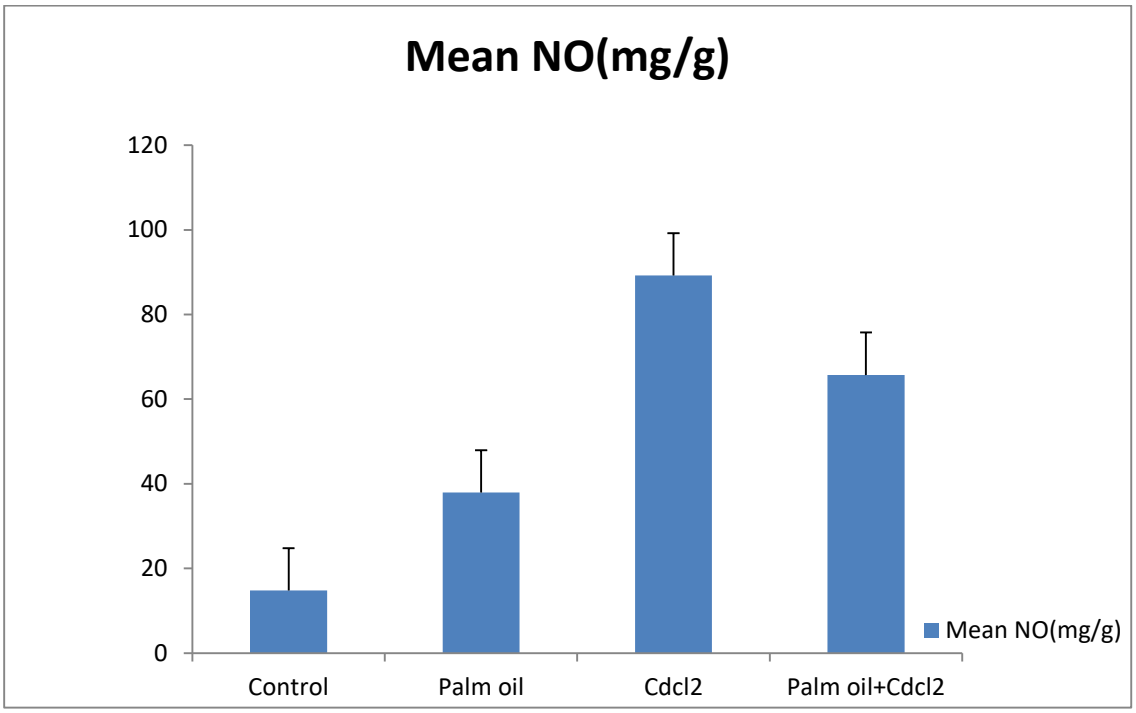


Figure (7): Effect of Palm oil supplementation and $cdcl_2$ injection on NO level

III. Hormonal analysis:

- **Effect of Palm oil supplementation on sexual hormones:**

  1-On Testosterone Hormone

  2-On Follicle Stimulating Hormone

  3-On Luteinizing Hormone

1-On Testosterone Hormone:

The total serum Testosterone level of palm oil medicated group was significantly increased ($p \leq 0.05$) (6.80 ± 0.44) while cadmium chloride medicated group, there was significant decrease in testosterone level (0.53 ± 0.038), In addition in palm oil and cadmium chloride group, serum testosterone level was a non-significantly increased (2.32 ± 0.046) compared to that of control one (1.79 ± 0.26).

2-On Follicle Stimulating Hormone:

The results reflect no change in FSH level in palm oil supplemented group, while in cadmium chloride injected group as well as palm oil and cadmium chloride group, the reflect a continuous significant increase ($p \leq 0.05$) in FSH level (19.07 ± 2.58 and 28.63 ± 2.14 respectively) compared to that of control one (13.57 ± 1.07).

3-On Luteinizing Hormone:

The obtained results evoked non-significant decrease ($p \leq 0.05$) in serum LH level in palm oil supplemented group (8.32 ± 1.37) while in cadmium chloride injected group, there was a significant decrease in serum level of LH (3.85 ± 1.27). While in group of palm oil and cadmium chloride, serum LH level was significantly increased (20.96 ± 1.69) compared to that of control one (11.29 ± 1.05).

Table (4): Effect of Palm oil supplementation and oxidative stress induced by $cdcl_2$ on Testosterone hormone, FSH and LH (M±S.E).

Group	Testosterone (ng/ml)	FSH (ng/ml)	LH (ng/ml)
G1: Control	1.79 ± 0.26^{b}	13.57 ± 1.07^{b}	11.29 ± 1.05^{c}
G2: Palm oil	6.80 ± 0.44^{a}	13.32 ± 1.90^{b}	8.32 ± 1.37^{b}
G3: $cdcl_2$	0.53 ± 0.038^{c}	19.07 ± 2.58^{b}	3.85 ± 1.27^{c}
G4: Palm oil + $cdcl_2$	2.32 ± 0.046^{b}	28.63 ± 2.14^{a}	20.96 ± 1.69^{a}

Means with different letters are significantly differed ($p\leq0.05$)

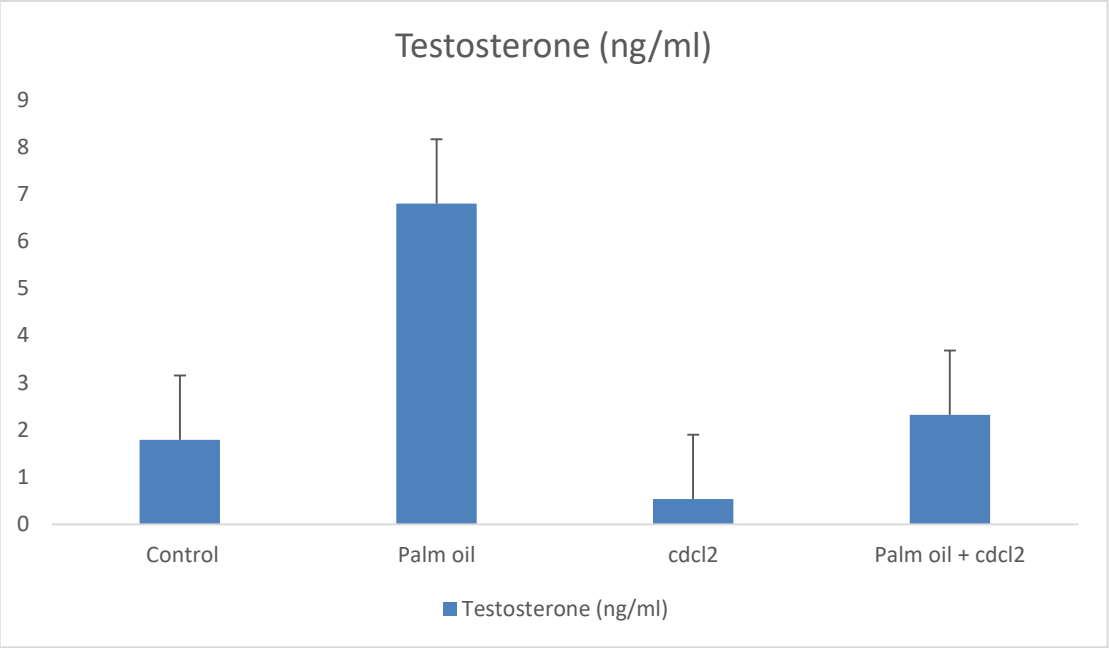


Figure (8): Effect of palm oil supplementation and oxidative stress induced by $cdcl_2$ on Testosterone hormone level

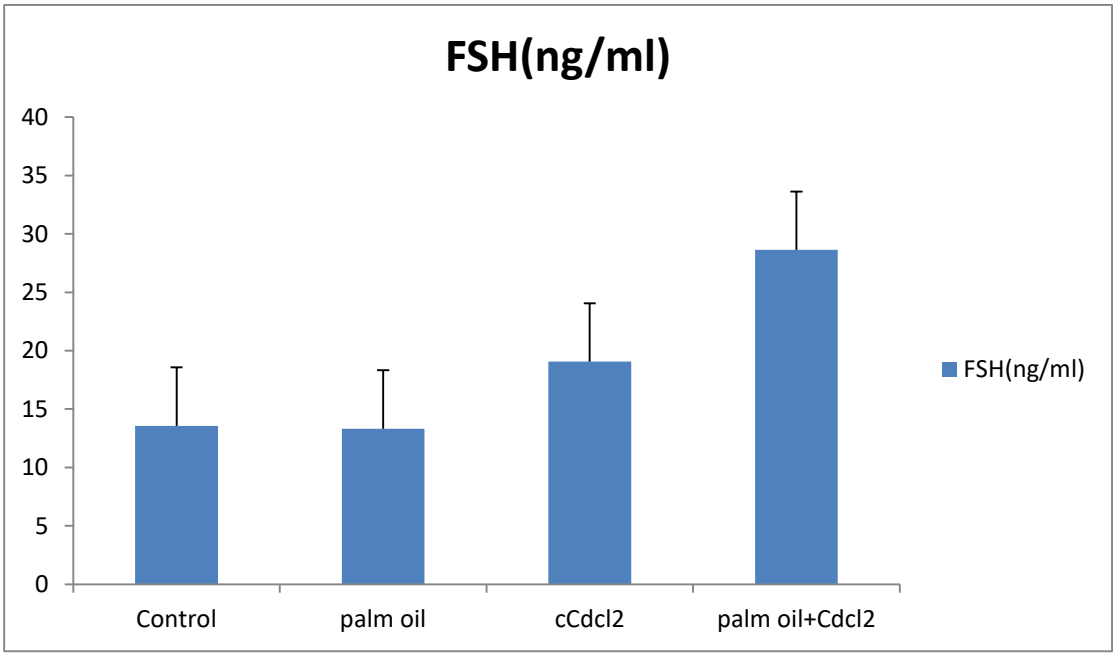


Figure (9): Effect of palm oil supplementation and oxidative stress induced by $cdcl_2$on FSH level.

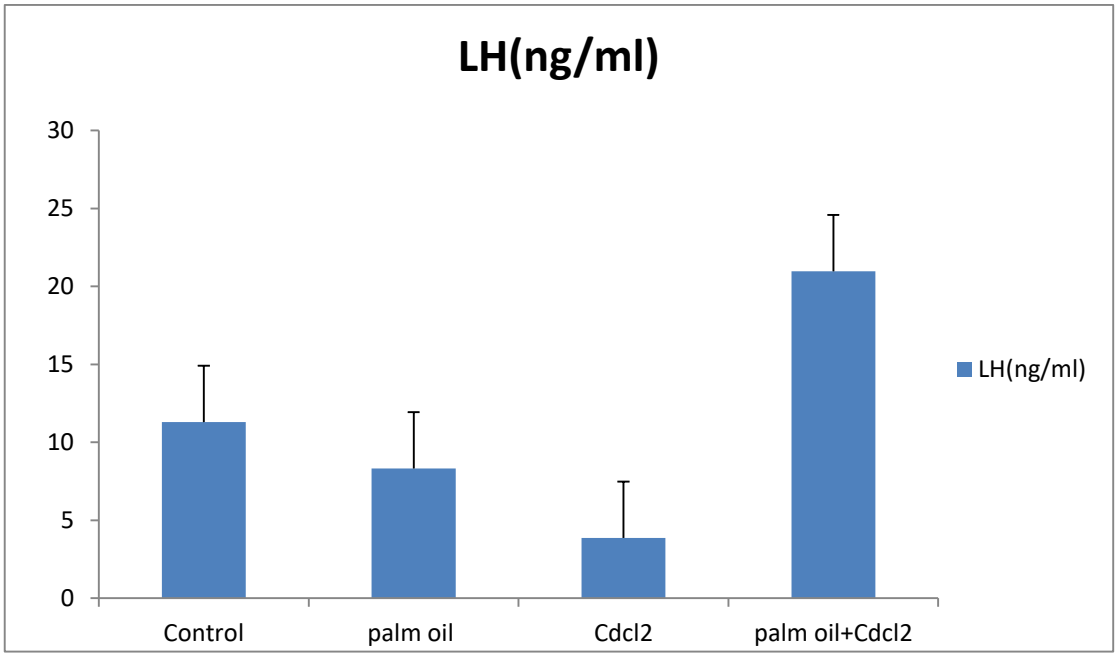


Figure (10): Effect of palm oil supplementation and oxidative stress induced by $cdcl_2$ on LH level.

IV. Histopathological Findings:

- **Effect of palm oil supplementation on spermatogenic efficiency on Testis using histopathological evaluation:**

The obtained data revealed that, Plam oil supplemented group, Testis showing seminiferous tubules with normal primary spermatocytes and normal spermatid. While cadmium chloride administered group, Testis showing mild necrosis of spermatogenic epithelium in seminiferous tubules with mild congestion. Moreover Palm oil and cadmium chloride injected group, Testis showing most seminiferous tubules having nearly regular contour and are lined by stratified germinal epithelium. Their lumina contain aggregations of sperms; the interstitial spaces are relatively narrow and contain clusters of cells.

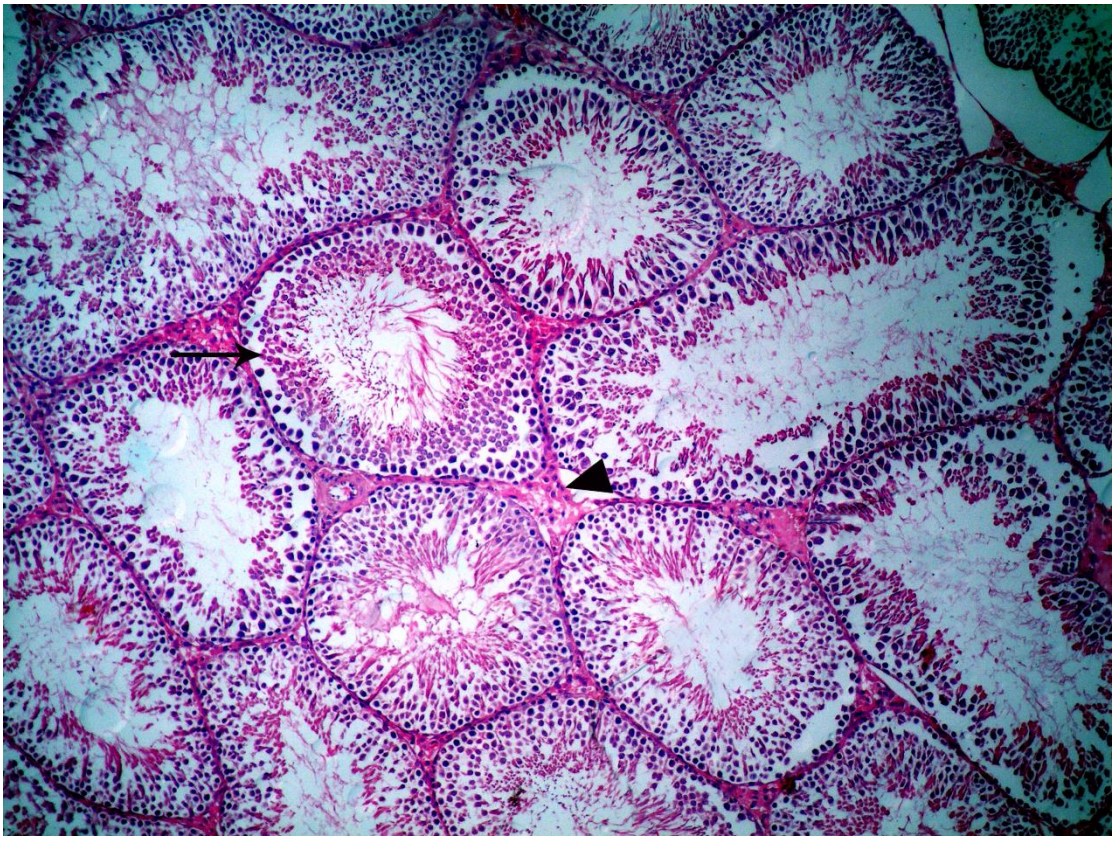


Photo (3): Control group, Testis is showing normal seminiferous tubules with normal spermatogenesis "arrow ", with normal leydig cells "arrowhead"(H&E, 100X).

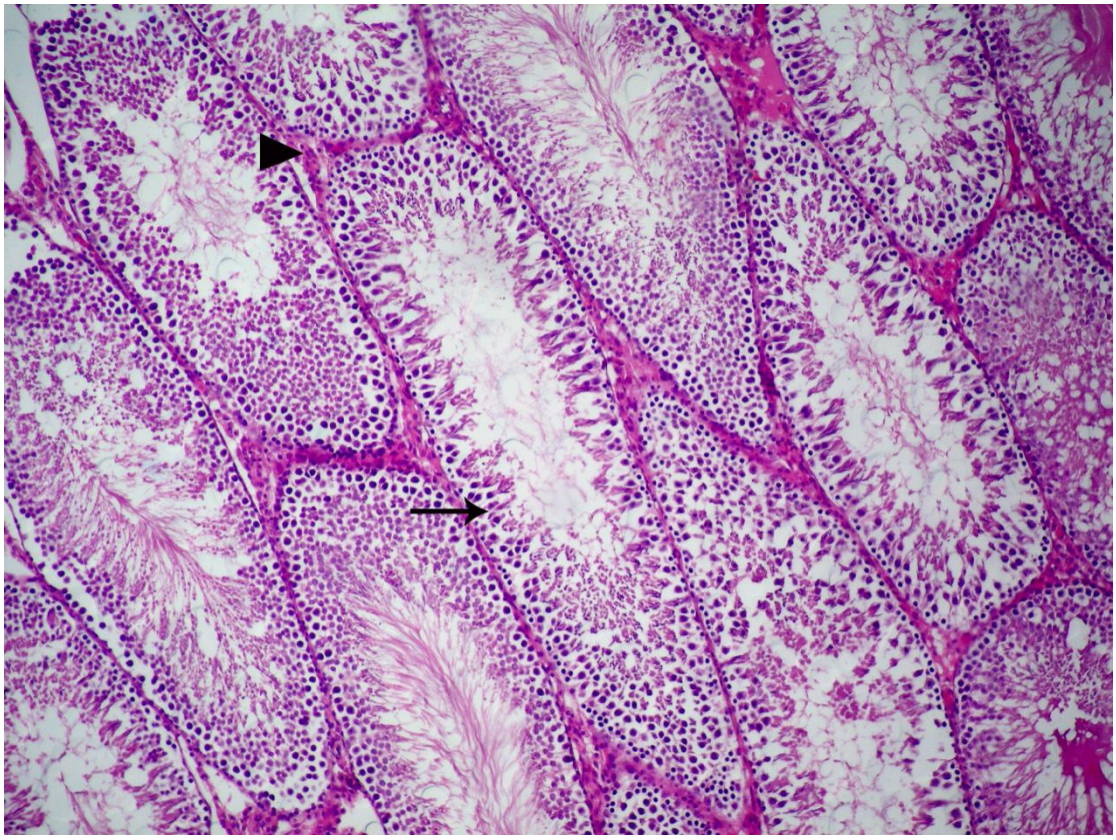


Photo (4): Palm oil administered group, Testis is showing seminiferous tubules with normal primary spermatocytes "arrow" and normal spermatid "arrowhead"(H&E, 100X).

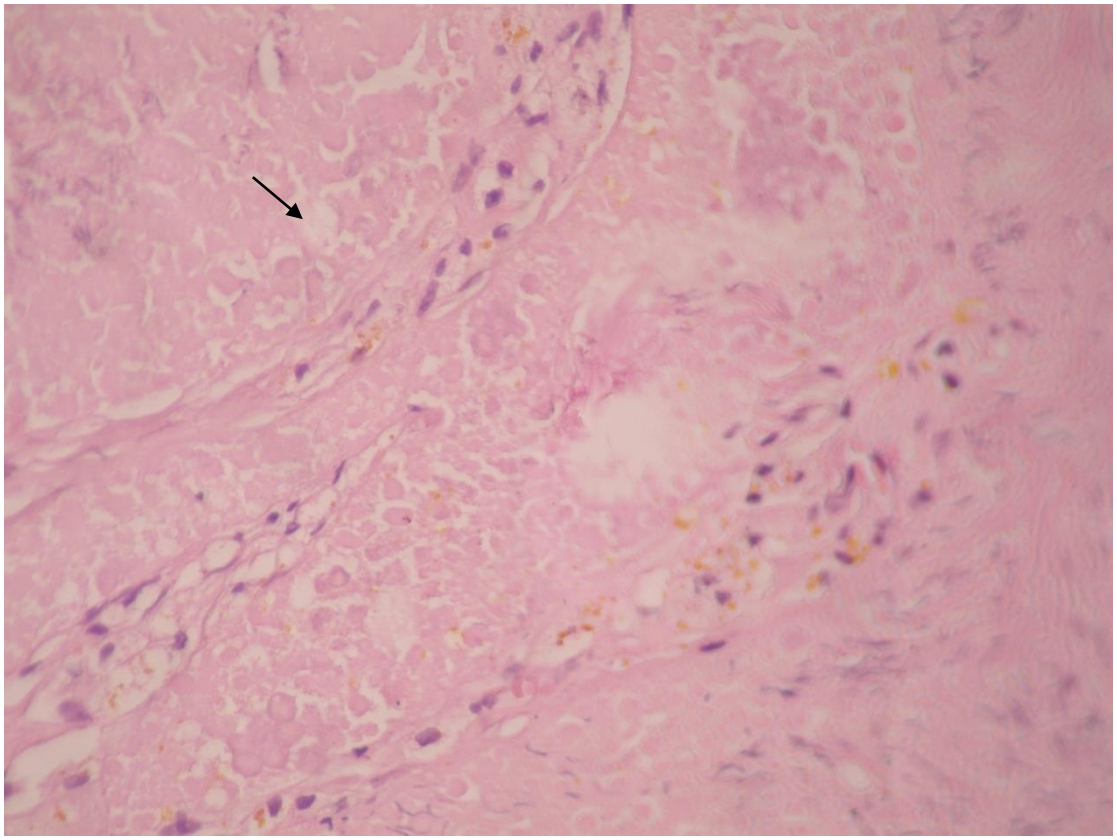


Photo (5): Stress group "cdcl$_2$ administered group", Testis is showing severe necrosis of spermatogenic epithelium in seminiferous tubules "arrow", with severe congestion "arrow head ' (H&E, 100X).

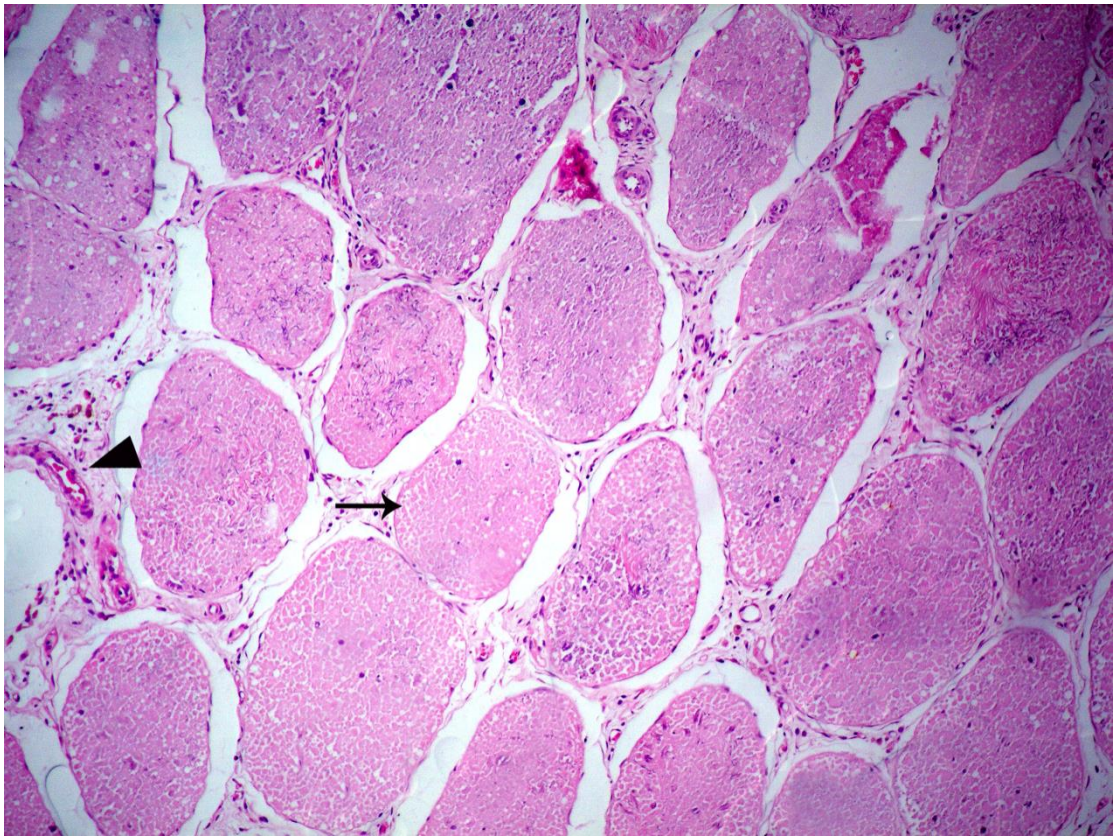


Photo (6): Palm oil + Stress group, Testis is showing mild necrosis of spermatogenic epithelium in seminiferous tubules "arrow", with mild congestion "arrow head ' (H&E, 100X).

DISCUSSION

Nowadays, Indigenous or traditional medicines are used mainly by 80% of people all over the world for their primary health needs according to World Health Organization ***(Pantsi et al., 2011)*.**

I-Evaluation of Semen:

I.iWeight of testis and fertility:

Weight of testis is affected by palm oil supplementation and stress induced by $cdcl_2$ as in table (1) fig. (1).

Palm oil is a potential supplement to increased fertility ***(Nicholson, 2000),*** in current study, palm oil supplementation results in increasing weight of testis (1.50±0.11) compared to control group (1.30±0.13) and this result is confirmed by ***Umeda et al. (1982),*** while there is a decrease in weight of testis in both stress group (0.41±0.05) and group of palm oil under stress (0.86±0.03) when compared to control group (1.30±0.13). This comes in agreement with ***Salama and El-Bahr (2007)*** who found a significant decrease in testicular weight after cadmium exposure.

The weight of the testes is correlated positively with the status of spermatogenesis process. Therefore, a decrease in seminiferous tubules size and spermatogenic arrest are main causes of decreased testicular weight ***(Chapin, 1997)*.**

In the current study, The exposure to cadmium chloride causes oxidative stress on spermatozoa parameters, so leads to harmful effects on germ cell ***(Zemjanis et al., 1970)*.**

I.ii Sperm motility:

In Stress group, sperm parameters were negatively influenced as sperm concentration correlated negatively to intracellular ROS levels. These results are in support of findings previously reported from our group ***(Aboua et al., 2009)***, the severity of cadmium-induced damage at the testicular tissue and the seminal vesicles ***(Laskey et al., 1984)***, which lead to decrease in reproductive organ weight and cause changes in the diameter of seminal vesicle and interstitial space ***(Coni et al., 1992)***. The results of this study were confirmed by reports from other studies, Some heavy metals like lead and cadmium could adversely affect the male reproductive system; either by causing hypothalamic-pituitary axis disruption or by directly affecting spermatogenesis, resulting in impaired semen quality ***(Jurasović et al., 2004)*** , Several metals, especially, lead and cadmium are considered reproductive toxicants and/or suspected endocrine disruptor compounds. ***Jurasović et al. (2004)*** reported that there is a positive associations between blood cadmium (B-Cd) concentrations and follicle stimulating hormone (FSH) and testosterone levels among men with no occupational exposure. Furthermore, it had been reported that there was a decline in semen quality associated with both lead ***(Telišman et al., 2007)*** and cadmium concentrations in blood. In another study performed by ***Benoff et al. (2009)***, they claimed that the presence of lead and Cadmium in the reproductive tract of men attending infertility clinics may be related to a moderate alteration of their seminal parameters. In Nigeriean population, ***Mendiola et al. (2011) and, Akinloye et al. (2006)*** reported that high plasma cadmium level can be a cause of oligo-astheno-teratozoospermia syndrome. Preliminary results of an ongoing study in Nigeria showed that 83.97% of the patients with history of herbal intake had abnormal seminal fluid analysis, whereas only 16.03% of subjects with no history of herbal intake had abnormal results. This gives a clue of the

possibility of male infertility from Nigerian herbal remedies, which has been reported to contain heavy metals ***(Enuh et al., 2012)***. The reproductive health damage from consumption of Nigerian herbal remedies should be identified with in depth risk assessment ***(Obi et al., 2006).*** There is an arrest of the sperm development as no fully matured cell can be seen which implies that the process of spermatogenesis is affected by lead. This result agreed with the report of ***Al-Azemi et al. (2010)*** where administration of cd, another heavy metal, was shown to be spermatogenic state specific. However, a slight increase was noticed in the groups treated with RPO as in Photo (1), this agree with previous report that palm oil is a potential supplement to increase fertility ***(Jegede et al., 2015)***, there were no semen in group supplemented with palm oil under stress, All these are shown in table (1) fig. (2).

II-Antioxidant status:

II.i-Testicular Catalase activity:

Catalase(CAT) has two different kinetic activities which are catalytic and peroxidatic activities ***(Wong and Whitaker, 2002)***

$$2H_2O_2 \xrightarrow{CAT} 2H_2O + O_2 \text{ (Catalytic activity)}$$

$$Rooh + AH_2 \xrightarrow{CAT} ROH+A+H_2O \text{ (Peroxidatic activity)}$$

The means of CAT activity in testes were investigated among different treated groups as shown in table (2) Fig. (3).

The mean catalase activity was decreased in stress group (0.10±0.009) when compared to control group (0.26±0.01), during oxidative stress, CAT activity levels progressively decreased due to high accumulation of H_2O_2 in tissues.

The significant decrease in CAT activity may be due to its inactivation by superoxide radical or due to decrease in the rate of reaction as a result of excess transform of H_2O_2 to water and oxygen, It may also be due to decreased absorption of essential trace elements , Fe, required for the activity of this enzyme ***(Jurczuk et al., 2004).*** There is an increase in mean level of catalase in palm oil group (0.66±0.04) and group of palm oil under stress (0.41±0.02) when compared to control group (0.26±0.01). It is evident from earlier work that different concentrations RPO have differential effects on the activities of antioxidant enzymes ***(Zamora Rodríguez et al., 2007)***.

II.ii-Testicular Reduced Glutathione (GSH) concentration:

GSH is an intercellular antioxidant which is usually in high concentration within cell. there were significant decrease in GSH levels in tissues as kidney and testis in mice exposed to heavy metals ***(Al-Attar, 2011)***. The current study results indicated that there was a significant reduction in GSH in stress group(4.51±0.36) compared to control group (12.82±0.74) as in table (3) Fig. (5), This marked low level of GSH is due to the excessive utilization of antioxidant in scavenging for the high amount of free radicals produced by cadmium chloride, but the combination of $cdcl_2$ and palm oil achieved a marked improvement in testicular GSH where Palm oil was seen to alleviate the generation of free radicals as it protect the testicular tissue and stimulate increase of GSH levels and also increase levels of GSH in palm oil group (17.03+0.77) compared to control group (12.82±0.74). The supplementation of RPO diet increased the level of GSH in sperm. This increased intracellular transport of GSH is essential for maintaining the redox state during oxidative stress. Due to its content in carotene tocopherol and phenolic-flavonoid-rich antioxidant complex, RPO was used in this study, to investigate its scavenging and antioxidant capacity. The antioxidant properties of carotenoids have been suggested to reflect, not only the rates of free radical scavenging, but also the reactivity of the resultant carotenoid-derived radicals. Carotenoid radical-cation or adduct radicals have been shown to be highly resonance stabilised and predicted to be relatively unreactive. They may further undergo bimolecular decay to generate non-radical products or in the case of carotene radical-adducts, may terminate radical reactions by binding to the attacking free radical ***(Everett et al., 1996).*** It can be mentioned that the role of palm oil is similar in to the role of GSH which is the most important protective mechanism for scavenging of free radicals and inhibition of electrophilic xenobiotics attack on cellular

macromolecules ***(Cnubben et al., 2001)***. RPO was seen to alleviate the generation of free radicals by induction of a significant increase in the level of GSH. It can therefore be postulated that the protective action of RPO is similar to the role of intracellular GSH which is the most important protective mechanism for free radical scavenging and inhibitor electrophilic xenobiotics attach on cellular macromolecules ***(Cnubben et al., 2001)*.**

II.iii-Testicular Glutathione-S-Transferase (GST) activity:

GST is a family of multifunctional proteins , it has an important role in catalyzing the conjugation of reduced glutathione (GSH) to xenobiotic substrates which are less toxic, easily soluble and easily eliminated),which is essential for cellular detoxification , Also, GST has the properties of isomerases, peroxidases and thiol transferases ***(Hayes et al., 2005)*.**

In our results as shown in table (3) Fig. (6), The mean GST was decreased in testicular tissue obtained from Stress group (0.80$\pm$0.128) than those obtained from control group (2.62$\pm$0.17) , This come in agreement with ***Kim et al. (2011)*** in rats and carp , respectively, The cause of reduced GST activity in the tissues exposed to $cdcl_2$ may be the over-consumption of GST enzyme to escape from the toxicity of peroxides under Cd insult , the reaction of thiol (-SH) group of GSH with electrophilic reagents is catalyzed by GST such as those generated by xenobiotic microsomal metabolism , this lead to neutralizing their electrophilic sites and rendering the product more water soluble ***(Han et al., 2006)***

The decrease in activity of GST in testicular tissues come in the agreement with ***El-Missiry and Shalaby (2000)*** where there was a significant decrease in GST activity in brain and testis in rats exposed to $cdcl_2$ as a result of generation of free radicals in injured tissues.

There is significant increase in GST levels in both palm oil group (8.51±0.59), and palm oil group under stress (5.46±0.55) compared to control group (2.62±0.17), Due to nucleophilic thiol group, It can detoxify substances in one of three ways: (1) Conjugation catalyzed by glutathione-*S*-transferases (GST), (2) Chemical reaction with a reactive metabolite to form a conjugate and (3) Donation of proton or hydrogen atom to reactive metabolites or free radicals. Reactive intermediates can react with GSH either by a direct chemical reaction or by a GST-mediated reaction preventing possible cell death. Regarding the role of glutathione in the protection against oxidative stress and detoxification of xenobiotics, its availability in the reduced form (GSH) may be a key factor in maintenance of health. It has been established in several different animal models, as well as in humans, that a decrease in GSH concentration may be associated with aging and pathogenesis of many diseases

II.iv-Testicular Malondialdehyde (MDA) concentration:

MDA is a stable product used to evaluate the degree of lipid per oxidation, It is formed from the breakdown of poly un saturated fatty acids ***(Bell et al., 1991)*.**

The effect of palm oil supplementation on testicular lipid peroxidation was compared among treatment groups**,** As shown in table (2) Fig. (4), The mean MDA was significantly lower in palm oil group (11.47±1.02), on the other hand, there was a significant increase of MDA level in stress group taking cadmium chloride (54.33±2.79) when compared to control group (13.22±0.63).

Importantly, Combination between palm oil and Cadmium Chloride as stress factor decrease the level of MDA (38.22±2.22) when compared to the stress group taking Cadmium chloride (54.33±2.79), there will be marked

increase in lipid per oxidation in testis due to the increase MDA levels in stress group taking cadmium chloride, Exposure of rats to cadmium lead to increased levels of MDA ***(Eybl et al., 2004)*.**

Cadmium and thermal stress lead to oxidative stress through binding with -SH groups of cellular glutathione with per oxidation of intracellular lipids and this is the cause of increase levels of MDA ***(Salama and El-Bahr, 2007)*.**

Supplementation of rats with palm oil had the ability to reduce MDA significantly ***(Dauqan et al., 2013)***.

This observation further confirms that palm oil has the ability to increase fertility, ***Packer (1992)*** **and** ***Atroshi et al. (1992)*** who confirmed that this property is conferred to palm oil by its possession of high level of alpha-tocopherol and other antioxidant vitamin .

Red Palm Olein contains (542 ppm) *Beta* carotene , other suggests that generation of singlet oxygen during lipid peroxidation might be attributed to breakdown reactions of lipid hydro per-oxy free radicals, therefore, the *B*-carotene and alpha- tocopherol, act as a potent antioxidants in protecting biological membranes or lipids against free radical damage ***(Zhang and Omaye, 2001)*.**

II.v-Testicular Nitric Oxide (NO) level:

Nitric Oxide (NO) requires nitric oxide synthase enzyme (NOS) to be formed in which it is increased in testicular tissue which exposed to oxidative stress condition as cryptorchidism ***(Ishikawa et al., 2005)*** and Varicocele ***(Shiraishi and Naito, 2007),*** It is called endothelium derived relaxing factor which is synthesized endogenously from L-arginine, Oxygen and NADPH by various NOS ***(Nunoshiba et al., 1993)*.**

As shown in table (3) Fig. (7), the mean NO was increased in testicular tissue obtained from palm oil group (37.91±8.60 mg/g), group taking cadmium chloride (89.22±4.52) and palm oil under stress group (65.73±3.30) than those obtained from control group (14.81±1.04).

Zhou et al. (2002) said that there is a positive correlation between vitamin E level and NO level in vitamin E supplementation.

RPO , vitamin E and vitamin C are non-enzymatic antioxidants that may prevent or reduce production of reactive Oxygen species as this leads to increase NOS levels ***(d'Uscio et al., 2003).***

III. Hormonal analysis:

Testosterone, FSH and LH evaluation is useful in the management of male infertility ***(Zabul et al., 1994).***

Testosterone, FSH and LH are primer regulators of germ cell development, the quantitative production of spermatozoa generally requires the presence of FSH, LH and testosterone. FSH acts directly on the seminiferous tubules where as leutenizing hormone stimulates spermatogenesis indirectly via testosterone. FSH plays a key role in stimulating mitotic and meiotic DNA synthesis in spermatogonia ***(Anderson et al., 1997).***

III.i Testosterone hormone:

Testosterone is a steroid hormone and a member of androgen group which is found in mammals, reptiles and other vertebrates ***(Cox and John-Alder, 2005).***

Mooradian et al. (1987) stated that mammalian testosterone is primarily secreted in testicles of males, although small amounts are also secreted by the adrenal glands. It is the principal male sex hormone and an anabolic steroid. In men, testosterone plays a key role in the development of male reproductive tissues, as well as, promoting secondary sexual characteristics.

As shown in table (4) fig. (8), Serum testosterone mean was increased in palm oil group (6.80$\pm$0.44) and group taking palm oil under stress obtained by cadmium chloride exposure (2.32$\pm$0.04) when compared with control group (1.79$\pm$0.24) which agree with ***Umeda et al. (1982)*** who said that a high uptake of exogenously administered vitamin E was also shown in pituitary gland and tocotrienols in palm oil help support testosterone

production by maintaining optimum and testicular function and testosterone level increased in quails fed 3% palm oil ***(Fitriyah et al., 2008)***, This suggests that vitamin E may play an important role in lipoperoxide formation and hormone production in these endocrine glands, While cadmium chloride group show significant decrease (0.53±0.03) when compared to control group (1.79±0.24) comes in agreement with ***(Valko et al., 2005)*** who have quoted the cadmium induced oxidative stress in the testicular tissue as a prime cause for hampered Steroidogenesis**,** Testosterone, estradiol and inhibin control the secretion of gonadotropins ***(Weinbauer and Nieschlag, 1995),*** For many years, vitamin E has been considered as an anti-sterility factor ***(Evans and Bishop, 1922)***.

III.ii Follicular stimulating hormone (FSH):

The successful and complete male germ cell development is dependent on the balanced endocrine interplay of hypothalamus, pituitary and testis, Gonadotropine releasing hormone (Gnrh) secreted by the hypothalamus elicits the release of gonadotrophins i.e. Follicle stimulating hormone (FSH) and Leutenizing hormone (LH) from the pituitary gland ***(De Kretser, 1979)*.**

FSH binds with receptors in the sertoli cells and stimulatesthe production of testosterone in leydig cells, which in turn may act on the sertoli and peritubular cells of the semineferous tubules and stimulates spermatogenesis ***(O'Donnell, 1994)***.

As shown in table (4) fig. (9), the mean FSH level is increased in stress group (19.06±2.58) compared to control group (13.56±1.07) which are in accordance with ***(Bergmann et al., 1994)*** who said that FSH levels increase in men with azoospermia or severe oligospermia indicate damaged seminiferous tubules and also agree with the studies of ***Sultan et al. (1984)***

and ***De Kretser (1979)*** reported elevated levels of serum FSH with increasing severity of seminiferous epithelial destruction, Our result show increased in serum levels of FSH after cadmium administration. This finding could be due to the accumulation of cadmium in the testis. In this context, it was shown that cadmium affects Sertoli cell activity by decreasing inhibin synthesis and release. Thus, the increased serum levels of FSH could be explained, as this peptideis the main inhibitory signal for FSH secretion ***(de Souza Predes et al., 2010)*** also, there is no significant difference in mean level in group supplemented with palm oil (13.32±1.90) compared to control group (13.56±1.07) , while There is an increase in mean FSH level in group taking palm oil under stress (28.63±2.14) compared to control group (13.56±1.07) which come in agreement with ***Gnessi (1997)*** who stated that the production of inhibin and testosterone , dihydrotestosterone and estradiol is affected causing disturbance in negative feedback mechanism resulting in elevated levels of FSH .

III.iii Leutinizing hormone (LH):

LH, a member of the glycoprotein hormone family, is a heterodimer composed of a common alpha subunit and a specific beta subunit that confers biological specificity for the hormone receptor in the target organ ***(Gharib et al., 1990).***

In our study, as shown in table (4) fig. (10), there is a decrease in mean level in both group supplemented with palm oil (8.31±1.37) and stress group (3.85±1.27) compared to control group (11.29±1.05),this agree with ***(Weinbauer and Nieschlag, 1995)*** who reported that the failure of pituitary to secrete FSH and LH will results in disruption of testicular function leading to infertility, cd has been considered as an important environmental endocrine disruptor ***(Li and Wu, 2002),*** this may lead to variations in

plasma LH levels, thus indicating that the metal may act at the hypothalamic level, modifying the activity of the endogenous clock, changing the mean concentration of LH secreted daily by the pituitary gland ***(Shirama et al., 1982),*** while mean level of group supplemented with palm oil under stress is increased compared to control group (11.29 ± 1.05^{c}), vitamin E administration could activate the production of pituitary gonadotropins. In our ultrastructural study of vitamin E supplemented rats, an increase in the number of secretory granules was observed in the anterior pituitary gonadotrophs ***(Umeda, 1978)*.**

Cadmium induced toxicity to the testis is probably the result of inter-digitating complex interactions which involve the disruption of the blood-testis barrier via specific signal transduction pathways and signaling molecules ***(Siu et al., 2009)*.**

IV. Histopathological examination:

The effects of palm oil administration under oxidative stress condition on histological parameters of testicular tissue were investigated, The testes obtained from either control or palm oil group showed normal diameter of seminiferous tubules with several spermatogenic layers in Photo (3) and (4) respectively, ***Nicholson (2000)*** said that palm oil is a potential supplement to increased fertility.

On the other hand, exposure of rats to oxidative stress perturbs the microscopical picture of the testes, Testis is showing severe necrosis of spermatogenic epithelium in seminiferous tubules, with severe congestion and spermatogenic arrest in Photo (5), ***Zavos and Zarmakoupis-Zavos (1999)*** said that Nicotine as a cause of oxidative stress has been implicated in the detrimental effects of smoking on sperm parameters, In the present results several showed that the sperm parameters were severely affected

following $cdcl_2$ injection. The oxidative stress induced by $cdcl_2$ effects included deleterious consequences, such as arrest of sperms, that could affect the fertility as described in other toxicants ***(Bustos-Obregón and González-Hormazabal, 2003; Sarkar et al., 2003)***.

There is an evidence that the disturbance of Sertoli structure resulted the damage of spermatogenesis ***(Born et al., 1988)***. These morphological changes reflect the decrease of sperm quality in oxidative stress group in which, testes showed dose dependent seminiferous epithelial necrosis, degeneration and loss of spermatozoa in albino rats exposed to cd ***(Devy, 2006).***

Importantly administration of palm oil helps to maintain some of normal histological picture of testicular tissue when compared to that of stress group in photo (6), RPO was seen to alleviate the generation of free radicals by protecting the testicular tissue and hence a significant increase in the level of GSH. It can therefore be postulated that the protective action of RPO is similar to the role of intracellular GSH which is the most important protective mechanism for free radical scavenging and inhibitor of electrophilic xenobiotics attack on cellular macromolecules ***(Cnubben et al., 2001).***

Preventative antioxidants, such as metal chelators and metal binding proteins, block the formation of new ROS, whereas scavenger antioxidants such as vitamins E and C, beta-carotene, and other antioxidants dietary supplements, glutathione and enzymes, remove ROS already generated by cellular oxidation. Dietary products such as vitamins C, E, and A are some of the excellent sources of antioxidant ***(Sandur et al., 2007)*** and RPO is a virtual powerhouse of nutrition, It contains, by far, more nutrients than any other dietary oil. A new dimension to the view above from our study is to

consider ranking RPO in the group of preventive antioxidants, since it appears to block the formation of ROS. Vitamin E is an effective antioxidant against the oxidative damage ***(Helen et al., 2000).***

The protective effect of vitamin E on lipid peroxidation has also been reported in rat liver cells ***(Shen et al., 1995)*** and in *in vitro* studies of aflatoxin effect ***(Cassand et al., 1993).*** Thus vitamin E is a useful nutritional antioxidants and their supplementation nullifies the tissue damage or oxidative stress caused by nicotine.

Increasing intake of dietary antioxidants may help maintain an adequate antioxidant status and, therefore, the normal physiological function of a living system ***(Record et al., 2001).***

SUMMARY

Exposure of testicular tissue to cadmium chloride ($cdcl_2$) results in severe testicular damage. Therefore, understanding the underlying molecular mechanism of cadmium-induced testicular injury is an indispensable goal and prerequisite to find the suitable tool that can abrogate its harmful effect. Palm oil has been shown to have a potent antioxidant activity. However, whether Palm oil has a protective effect on cadmium-induced testicular damage remains unknown.

This study was aimed to 1) Investigate the effect of cadmium chloride exposure on testicular function in rats, and 2) Evaluate the protective role of palm oil on oxidative stress-induced testicular damage. To achieve these aims, the effect of palm oil was investigated under oxidative stress condition induced by cadmium chloride in relation to 1) The fertilizing capacity of rat spermatozoa, 2) Biochemical antioxidant enzyme system, 3) Hormonal evaluation via hormonal analysis of testosterone hormone, FSH and LH and 4) The status of spermatogenesis via histopathological examination.

Exposure of rats to cadmium chloride caused a significant decrease in testicular weight, reduced motility of spermatozoa and decreased testosterone hormone level. Importantly, in contrast to the effect of palm oil under normal condition, administration of palm oil to rats under oxidative stress condition significantly improved the weight of testes and increased testosterone hormone level.

Exposure of rats to cadmium chloride resulted in pronounced increase in testicular lipid peroxidation as evidenced by the significant increase of MDA and the decrease of GSH. Importantly, administration of palm oil under oxidative stress condition could attenuate the oxidative stress and

testicular lipid peroxidation as evidenced by the significant decrease of MDA and increase of GSH.

Histopathological examination revealed that exposure of rats to oxidative stress induced by $cdcl_2$ induces degeneration of seminiferous tubules with complete arrest of spermatogenesis. On the other hand, in contrast to the effect of palm oil under normal condition, administration of palm oil to rats under oxidative stress condition significantly improved the spermatogenic status of seminiferous tubules.

From this study we can conclude that 1) Exposure of rat testes to cadmium resulted in oxidative stress-induced testicular damage and decreased sperm quality and 2) Palm oil has an ameliorating effect on testicular toxicity induced by oxidative stress in rats.

CONCLUSION

1) Palm oil is very effective on testicular function under normal function in rats through increasing their fertility.
2) Exposure of rat testes to cadmium results in oxidative stress-induced testicular damage.
3) Palm oil has an ameliorating effect on testicular toxicity induced by oxidative stress in rats.

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الملخص العربي

يعتبر زيت النخيل من الأكثر أهمية بين الزيوت النباتية حيث أن له استخدامات عديدة فهو يدخل في كثير من الصناعات الغذائية كما وجد أن له أهمية كبيرة في زيادة الخصوبة وكذلك له تأثير قوى كمضاد للأكسدة مما يجعل له دور وقائي لعلاج كثير من الأمراض.

وقد استهدفت هذه الدراسة إلقاء الضوء على تأثير زيت النخيل على الخصوبة في ذكور الفئران وكذلك تأثيره على ضغوط الأكسدة المحدثة باستخدام كلوريد الكادميوم كمعدن ثقيل والذي يؤدى إلى ضرر شديد في الخصية (تلف الخصية التاكسدى) عن طريق: معرفة جودة الحيوانات المنوية للفئران وقياس مستوى كل من هرمون التستوستيرون والهرمون المنشط للحويصلة والهرمون المحفز للجسم الأصفرفي الدم و قياس إنزيمات مضادات الاكسدة وكذلك فحص أنسجة الخصية.

أجريت هذه الدراسة على عدد ٤٠ من ذكور الفئران السليمة اكلينيكيا وتم تقسيمها إلى أربع مجموعات متساوية (بواقع ١٠ فئران لكل مجموعة) على النحو التالى::المجموعة الاولى : استخدمت كمجموعة ضابطة و المجموعة الثانية : اعطيت زيت نخيل فقط (٤ مللي) عن طريق التجريع بالفم يوميا لمدة شهر و المجموعة الثالثة : اعطيت كلوريد كادميوم فقط (٢,٥مجم كجرعة واحده اسبوعيا عن طريق الحقن لمدة شهر و المجموعة الرابعة: اعطيت كل من زيت النخيل (٤مللى) يوميا بالتجريع بالفم مع كلوريد كادميوم (٢,٥ مجم) كجرعة واحدة أسبوعيا بالحقن لمدة شهر.

وبعد انهاء التجربة تم اخذ العينات بعد ٢٤ ساعة؛ عينات دم من كل مجموعه للحصول على مصل لقياس مستوى الهرمونات ثم عينات الخصية بعد الذبح لقياس إنزيمات مضادات الأكسدة وكذلك لفحص أنسجتها.

<u>وقد أظهرت النتائج ما يلى :</u>

أن إعطاء زيت النخيل تحت الظروف الطبيعية يؤدى الى زيادة في وزن الخصية و تحسين في جودة الحيوانات المنوية وزيادة في هرمون التستوستيرون وقد قام بتخفيف ضغوط الاكسدة الناتجة عن كلوريد الكادميوم والذي اتضح من انخفاض معدل MDA وزيادة نشاط GSH وGST و CAT كما أن إعطاء كلوريد الكادميوم للفئران نجم عنه انخفاض في وزن الخصية و تلف في انسجتها مما أدى إلى تكسير الانابيب المنوية مع توقف كامل لعملية تخليق الحيوانات المنوية وانخفاض كبير في مستوى هرمون التستوستيرون و زيادة في كل من MDA و NO بينما في حالة إعطاء زيت

النخيل تحت ظروف ضغوط الأكسدة قد أدى إلى زيادة في هرمون التستوستيرون وقد خفف من تأثير ضغوط الأكسدة حيث أدى إلى تقليل مستوى كل من MDA وNO عن مستواهم في حالة إعطاء الفئران لكلوريد الكادميوم فقط.

ومما سبق يتضح أن تناول زيت النخيل له قدرة على زيادة الخصوبة وكذلك تحسين في أنسجة الخصية الناتجة عن ضغوط الأكسدة في الفئران .

السادة المشرفين ومساعديهم

عنوان الرسالة: تأثير تناول زيت االنخيل على الخصوبة وضغوط الأكسدة فى ذكور الفئران

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عنوان الرسالة: تأثير تناول زيت النخيل على ضغوط الأكسدة والخصوبة فى ذكور الفئران

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قسم الكيمياء الحيوية وكيمياء التغذية

تأثير تناول زيت النخيل على ضغوط الأكسدة والخصوبة فى ذكور الفئران

رسالة مقدمة من

ط.ب/ رنا رمضان أبوزيد العفيفى

بكالوريوس العلوم الطبية البيطرية (٢٠١٢)

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للحصول على

درجة الماجستير في العلوم الطبية البيطرية

(الكيمياء الحيوية وكيمياء التغذية)

(٢٠١٧)