Biological and biochemical effect of maca root powder (Lepidium meyenii) for infertile rats injected by Chlorpyrifos (CPF).

التأثير البيولوجي والبيوكميائي لمسحوق جذور الماكا (*Lepidium meyenii*) في الفئران المصابة بضعف الخصوبة المحقونة بالكلوربيريفوس (CPF).

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Abstract

Organophosphorus pesticides such as chlorpyrifos (CPF), play a vital role in current agriculture. Pesticide residues have been found to be a notable cause of male reproductive injury (infertility). The aim of this study is to investigate the biological and biochemical effects of maca roots (*Lepidium meyenii*) levels in infertile male rats treated with CPF. Thirty adult male albino rats, weighing (170±10g), were divided into five groups .The first group (6 rats) fed on basal diet as negative control and the other twenty four rats were injected with 38ml Chlorpyrifos /1kg body weight to induce infertility, one of them kept as a positive control group. The other three infertility groups were given the basal diet and *Lepidium meyenii* (LM) at the levels 5, 10, and 15%. The biological parameters (body weight, feed intake and feed efficiency ratio) and biochemical variables (some sexual hormones, liver enzymes, lipid profile and some oxidative enzymes) were determined. The obtained results indicated that the mean values of body weight, feed efficiency ratio, tested sexual hormones, SOD, catalase and HDL_{-c} of the positive control group were significantly lower than negative control group while the feed intake, organs weight, MDA, lipid profile and liver enzymes of the positive control group were significantly higher than those of the negative control group. All infertility rats fed on different diets revealed significant improvement in mean values as compared to the positive control group. The level of 15% LM significantly increased TH, FSH and LH by the percentage of 293, 350 and 377% respectively as compared to the positive control group. In conclusion, this study found that using LM especially at the level 15% could improve male rat fertility and provide theoretical information for human infertility.

Keywords: Maca (*Lepidium meyenii*) - Chlorpyrifos - infertility - sexual hormones - Lipid Profile.

1- Introduction

Pesticides such as Organophosphate (OPs) are frequently used in agriculture, they are principal environmental pollutants and have a severe effect on the environment that promotes sudden hazards **(Khalaf *et al.*, 2017).** It causes many harmful influences on various organs and blood parameters including delayed polyneuropathy, immunotoxicity, carcinogenesis, endocrine development, and causes a varying degree of testicular dysfunction in man and also in experimental animals (**Zhou *et al.*, 2017).**

Chlorpyrifos (CPF), (*o*, *o*-diethyl-*o* (3, 5, 6-trichloro-2-pyridyl) phosphorothioate) is a broad-spectrum organophosphate insecticide that is used in both domestic and industrial purposes. The CPF toxicity induced inhibits acetylcholinesterase and oxygen species (ROS) production, alters the reproductive functions by changing the activity of both the pituitary thyroid and pituitary -adrenal axes-, reduction in epididymal and testicular sperm counts and decreases serum testosterone concentration in exposed males rats (**Colovic and Lazarevic, 2015 and Nasr *et al.*, 2016) .**

Fertility is the quality of a human's ability to produce offspring, which is dependent on age, health, and other factors. Human reproduction requires three elements: mature sperm, mature eggs and a person with a uterus to carry the pregnancy and give birth (**Del Prete *et al.*, 2018a).** Infertility is defined as failure to achieve clinical pregnancy after ≥12 months of regular unprotected sexual intercourse, and its prevalence is ~9%. It is known that the cause of infertility exists in the male partner in approximately half of all infertile couples. There are several causes of male infertility, including the production of defective spermatozoa, obstruction of the reproductive tract, inflammation, and sexual disorders such as erectile dysfunction and retrograde ejaculation **(Del Prete *et al.*, 2018b).** A recent review revealed that the prevalence of male infertility, defined as men reporting an experience of infertility (generally >12 months in duration), varied from 9.0% to 15.8% in surveys of general populations **(Tafuri *et al.*, 2019).**

Infertility may be caused by male or female factors. For female, the main causes of female infertility are uterine factors, menstrual and ovulation disorders, and uterine abnormalities. Whereas in case of male infertility, the Factors affecting male infertility are low sperm production, abnormal sperm function or blockages in genital tract. Illnesses, genital infections and testicles injury **(Obeagu *et al.*, 2023).**

LM, an annual or biennial plant of the Brassicaceae family, has grown in the Andes of South America at an altitude of 3,500–4,000 m for a long time. Maca root is one of the most medicinal plants which is rich in antioxidants that protect cells from mutations and damage caused by free radicals. It contains large amounts of vitamins and very rich in flavonoids , amino acids, essential fatty acids,

polysaccharides, and mineral elements, such as iron, calcium, zinc, copper, and potassium **(Wang and Zhu, 2019).** Moreover, it contains compounds N-benzyl-palmitamide, glucosinolates, phenolics, and benzyl isothiocyanate **(Turgud and Narinç , 2022).** For that Maca root has a major role in stimulating the immune system, affects sexual potency and increases fertility through its impact on sex hormones, and their receptors, and it plays a role in improving productive performance **(Korkmaz *et al.*, 2016 and Ali *et al.*, 2021).**

The current study was planned to determine the effect of different levels of maca roots on the biological and biochemical parameters of infertile rats treated with CPF.

2- Materials and Methods

2-1 Materials

plants

Maca root powder (*Lepidium meyenii)* were purchased from the Haraz Herbal Company in Cairo, Egypt.

Rats

Thirty healthy adult male albino rats, weighing (170±10g) were obtained from Medical Insects Research Institute, Doki, Cairo.

Chemicals

CPF was purchased from Egypt Chemical International Company for Agricultural Chemicals while kits and basal diet components were obtained from El-Gomhoriya Company for Trading Drugs, Chemicals and Medical instruments, Cairo, Egypt.

2-2 Methods

Preparation of maca roots

The dried roots were ground using a food mixer, sieved with a 45-mesh screen and stored in dusky stoppered glass bottles in a cool and dry location till use **(Russo, 2001).**

Induction of infertility

Rats were injected with CPF (38ml/1kg body weight) to induce infertility in male rats **(Babazadeh and Najafi, 2017).**

Experimental design

The study was once carried out at the Menoufia University, Shebin El-Kom, Faculty of Home Economics. The research protocol #14-SREC-04-2022 used to be accepted with-using Science Research Ethics Committee of the Faculty of Home Economics**.** The experiment was done in the Faculty of Home Economics, Menoufia University, Shebin El-kom. All rats were fed on basal diet for one week (the adaptation period). The basal diet consisted of protein (10%), cellulose (5%), salt mixture (4%), vitamin mixture (1%), corn oil (10%) and corn starch (70%) according to **Reeves *et al.* (1993)** .Rats were housed in wire cages at a room temperature of 25 C^{o} and kept under normal healthy condition. The first group fed on a basal diet as negative control and the second group was

infertility rats which treated with CPF and fed on basal diet as a positive control group. The other three infertility groups were given a basal diet and LM at levels 5, 10, and 15 %.

Biological evaluation

During the experimental period (28 days), the diet consumed was recorded every day and body weight was recorded every week. The body weight gain (BWG) and feed efficiency ratio (FER) were determined according to **(Chapman *et al.*, 1959).**

$$BWG = \text{Final weight} - \text{Initial weight}$$

$$F.E.R. = \frac{\text{Grams gain in body weight}}{\text{Grams feed consumed}}$$

Organs

The heart, kidney, liver, lungs, spleen and testis were removed, cleaned and weighted. Testis were fixed and stored in a 10% formalin solution for histopathological examination according to the method mentioned by **(Drury and Wallington, 1980)**.

Blood sampling

At the ending of experiment, blood samples of overnight fasted rats were collected from orbital plexus venous into centrifuge tubes at 3000 rpm for 10 min and the serum was separated and stored at -20 °C for biochemical analysis as described by **(Schermer, 1967)**.

Biochemical analysis

Serum testosterone (TH), Follicle Stimulating Hormone (FSH) and Luteinising Hormone (LH) levels were evaluated, according to the methods of **Pradelles *et al*., (1985) and Akram *et al*.(2012)** respectively. Superoxide Dismutase (SOD), Malondialdehyde (MDA) and Catalase (CAT) were measured by **Zhao (2001); Satoh (1998) and Luck (1974**) methods respectively. Serum Aspartate Aminotransaminase (AST) and Alanine Aminotransferase (ALT) were measured by the method of **Henry(1974) and Yound(1975)** respectively. Serum Total Cholesterol, Triglyceride, and HDL-c were determined according to the method described by **Allain(1974); Fassati and Prencipe** (**1982) and Lopez(1997**) respectively. LDL_{-c} and $VLDL_{-c}$ were calculated in mg/dl according to **Lee and Nieman, (1996)** using the following equations:

VLDL-c (mg/dl) = Triglycerides / 5

LDL-c (mg/dl) = Total cholesterol – HDL-c – VLDL-c

Histopathological examination of testis

Small specimens from testis were collected from all experimental groups, fixed in neutral buffered formalin, dehydrated in ascending concentration of ethanol (70, 80, and 90 %), cleared in xylene and embedded in paraffin. Sections of (4-6) µm thickness were prepared and stained with Hematoxylin and Eosin according to **Bancroft *et al.*, (1996).**

Statistical analysis

The data were statistically analyzed using a computerized Costat Program by one way ANOVA using a Completely Randomized Factorial Design **(SAS, 1985)** when a significant mean effect was detected, the means were separated with the Duncan's Multiple Range Test. Differences between treatments at $P \leq 0.05$ were considered significant. The results are presented as mean ± SD.

3- Results and discussion

Data in table (1) illustrate the mean value of body weight gain (g/d), feed intake (g/d/rat) and feed efficiency ratio of infertility rats injected by CPF and treated with different levels of maca roots. It could be noticed that the mean value of body weight and feed efficiency ratio of positive control group was lower than negative control group, with significant difference while the feed intake of positive control group was significantly higher than the negative control group .All infertility rats injected with CPF fed on different diets revealed significant decreases in mean values as compared to positive control group but still significantly were higher than negative control group. All treated groups with maca roots at different levels recorded changes by increasing the roots level with nonsignificant differences except the body weight of 15% maca roots group which recorded nonsignificant changes with negative control group.

The obtained results were matched with **Groh *et al.* (2017)** who found that CPF, as pesticides was one of these environmental chemicals, led to weight loss by affecting gut health through interfering with both the gut microbiota and the gut barrier. Also, **Fang *et al.* (2018)** showed that CPF caused weight loss by gut microbiota dysbiosis, increasing intestinal permeability and disrupting metabolism**.**

When adding LM in treated groups led to support weight gain and weight maintenance of the groups weight. It occasion balance hormones, increase appetite and help muscle building **(Gonzales-Arimborgo *et al.*, 2016).**

Also, **Groh *et al.* (2017)** reported that maca root contains nearly all of the essential amino acids and an abundance of others, along with crucial muscle-building nutrients like iron, potassium, and calcium which are effective for muscle growth and healthy weight gain.

LM contains many proteins, amino acids, fats and vitamins , as well as a variety of secondary metabolites such as maca alkaloids (including maca amide), glucosinolates, and other components. It is also a rich source of micro-

and macroelements, including iron, calcium, copper, zinc and potassium. So, Maca has been used by local people as a food ingredient and in traditional folk medicine to enhance energy, alertness, mental concentration, mood and physical immunity for that, Maca root added to drinks as energy source and aromatic sweetener **(Peres *et al.*, 2020).**

Zhu *et al.* (2020) reported that Maca roots enhance insulin secretion; however, it enhances the peripheral tissue insulin sensitivity or response. It additionally suppresses hepatic gluconeogenesis, causing decreases in blood glucose. Maca roots showed hypoglycemic, hypolipidemic, antioxidant, cardioprotective, and anti-inflammatory effects, aiding in alleviating metabolic syndrome in rats **(Choi *et al.*, 2021).** Also, **(Fei *et al.*, 2022)** reported that maca roots prevented weight loss, enhanced mitochondrial energy metabolism enzyme activity when taking toxic material, and improved spleen deficiency syndrome.

Table (1): Effect of LM (*Lepidium meyenii*) on body weight gain, feed intake and feed efficiency ratio of infertility rats.

Parameters / Groups	BWG(g/day)	FI(g/day/rat)	FER (%)
Control- Ve	$0.69^{a} \pm 0.04$	$15.2^{c} \pm 0.75$	$0.78^{a} \pm 0.04$
Control+Ve	$0.55^{b} \pm 0.03$	$19.56^{a} \pm 0.73$	$0.56^{c} \pm 0.02$
LM (5%)	$0.57^{b} \pm 0.01$	$17.26^{b} \pm 0.25$	$0.64^{b} \pm 0.02$
LM (10%)	$0.61^{b} \pm 0.02$	$17.73^{b} \pm 0.20$	$0.69^{b} \pm 0.03$
LM (15%)	$0.67^{a} \pm 0.04$	$16.8^{b} \pm 0.26$	$0.70^{b} \pm 0.02$
LSD ($P \leq 0.05$)	0.058	0.78	0.063

Values denote arithmetic means ± standard deviation .Means with different letters (a, b, c, d, etc,) in the same column differ significantly at $p \leq 0.05$ using ANOVA test, while those with similar letters are non-significantly different. LM = *Lepidium meyenii* – BWG = Body weight gain - FI = Feed Intake - FER = Feed Efficiency Ratio.

Data in table (2) revealed that the mean value of some organs weight (g) of infertility rats injected by Chlorpyrifos CPF and treated with 5, 10 and 15% LM. It could be noticed that the mean values of all organs weights for negative control were the lowest weights whereas the mean values in positive control group of LM were the highest with significant differences. Treating the infertility rats with different levels caused significant decreasing in organs weight and the best result was recorded in the group fed on 15% of maca roots. From the obtained results, it could be observed that the increasing of organs weight led to the toxic effect of insecticides which caused enlargement of internal organs especially testis weight whereas feeding toxic rats on different levels of maca roots led to decrease the chlorpyrifos effect and the increasing of maca roots level caused high effect.

The results carried by **Uzun *et al.* (2010)** explain the damage of internal organs in our results**.** They found that CPF gets inside the cytoplasm from cells as a lipophilic molecule and harms cellular molecules inside the cell**.** Also**, Ambali *et al.* (2011)** found that chlorpyrifos causes oxidative damage with the production of reactive oxygen types and causes damage in macromolecules such as lipid, DNA , protein and cell phospholipids which have an ability to react with a great number of organic and inorganic compounds, mainly multiple unsaturated fat acids.

These results are in agreement with **Ogirima *et al.* (2006)** who reported that, CRF in male rats had significantly increased the kidney weight. These results may partially due to reduced feed intake accompanied by excess albuminuria leading to malnutrition. The kidneys enlargement may be due to the marked elevation in the levels of monocytes, inflammatory infiltrates and crystalline tubulointerstitial deposits in renal tissues. **Tanvir *et al.*, (2015)** found significantly ($P \leq 0.05$) necrotic and degenerative changes in histopathological investigations of spleen in CPF exposed birds. Also, **Mansour *et al.* (2009)** assessed the effects of CPF in rat's liver structure, it caused histopathological changes such as hepatocyte degeneration, focal inflammatory cell infiltrations and diffuse Kupffer cell proliferation. Also, they showed that chlorpyrifos increased lipid peroxidation; decreased plasma superoxide dismutase (SOD), glutathione-S-transferase and serum acetyl choline esterase activities**.**

In case the effect of CPF on the lungs, **Carey *et al.* (2013**) found that its damage effect is due to overstimulation of the muscarinic receptors in the central nervous system. For the heart tissue, **Bayır *et al.* (2013)** showed that OPs produce high quantity of free radicals, become sensitive to oxidative stress, and damage, these effects cause cardiovascular changes include an increase in cardiac marker enzymes levels.

From the obtained results, CPF led to enlargement of testis of rats and this result was supported by **Uchendu *et al. (*2013)** who demonstrated significant associations between CPF and testicular damages by causing reduction in epididymal, testicular sperm counts and decreased in serum testosterone concentration in exposed males rats.

In this study adding maca root significantly decrease the liver weight and this result matched with **Rahman *et al. (*2021)** who found that maca roots decreased liver cell necrosis, inflammatory cell infiltration, and liver cell apoptosis **and also Fei *et al. (*2022)** showed that Maca pretreatment prevent liver injury though inhibition of oxidative stress damage such as lipid peroxidation , malondialdehyde , thiobarbituric acid reactive substance , alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH)**.**

The improvement effect of maca roots reported by **Yousef *et al*.(2006) and Kalender *et al.* (2010)** who stated that maca polysaccharides (MP), which are composed of galactose, rhamnose, arabinose, glucose, xylose, fucose, and mannose, could inhibit lipopolysaccharide-induced NO production in RAW cells and enhanced anti-inflammatory activities especially in spleen tissues**.** Finally**, Peres *et al.* (2020)** reported that maca roots enhance immunity, improve memory, resist fatigue, relieve depression, improve fertility and sexual function.

Table (2): Effect of LM on some organs of infertility rats.

Parameters / Groups	Liver (g)	Kidneys(g)	Spleen(g)	Lungs(g)	Heart(g)	Testis(g)
Control- Ve	$5.5^{e}\pm0.45$	$1.32^{e}\pm0.08$	$0.76^{d}\pm0.11$	$1.25^{e}\pm0.12$	$0.84^{d}\pm0.04$	$1.89^{e}\pm0.11$
Control+Ve	$6.79^{a}\pm0.51$	$1.54^{a}\pm0.05$	$1.13^{a}\pm0.14$	$1.54^{a}\pm0.11$	$1.05^{a}\pm0.02$	$2.31^{a}\pm0.18$
LM (5%)	$6.38^{b}\pm0.18$	$1.49^{b}\pm0.02$	$1.05^{b}\pm0.1$	$1.47^{b}\pm0.03$	$0.97^{b}\pm0.04$	$2.22^{b}\pm0.01$
LM (10%)	$6.04^{c}\pm0.43$	$1.43^{c}\pm0.07$	$0.96^{c}\pm0.08$	$1.41^{c}\pm0.07$	$0.91^{c}\pm0.05$	$2.09^{c}\pm0.03$
LM (15%)	$5.81^{d}\pm0.15$	$1.38^{d}\pm0.06$	$0.84^{d}\pm0.04$	$1.32^{d}\pm0.11$	$0.85^{d}\pm0.02$	$1.95^{d}\pm0.05$
LSD (P≤ 0.05)	**0.19**	**0.04**	**0.08**	**0.05**	**0.05**	**0.07**

Values denote arithmetic means ± standard deviation .Means with different letters (a, b, c, d, etc,) in the same column differ significantly at $p \leq 0.05$ using ANOVA test, while those with similar letters are non-significantly different.

Data presented in table (3) the mean values of some sexual hormones for infertility rats injected by CPF and fed on various diets. It could be noticed that the testosterone, Follicle Stimulating hormone and Luteinising hormone mean values of negative control group were higher than positive control group with significant differences. After treating with different levels of maca roots, the tested sexual hormones values increased as compared to positive control group and decreased when compared to negative control group. The fifth group was significantly higher than the other treated groups and recorded the best mean value. From the obtained results, it was found that chlorpyrifos treatment led to decrease the level of testosterone hormone and this result matched with the study carried by **(Alaa-Eldin *et al*., 2017),** they reported that chronic exposure to chlorpyrifos significantly decreased the concentrations of luteinizing hormone, follicule stimulating hormone and testosterone.

In this regard, **Zirkin and Papadopoulos, (2018)** showed that chlorpyrifos also led to a significant decrease in testosterone in rats fed the normal-fat diet when exposed from newly weaning. This decrease was partly due to the decrease in testosterone synthesis in the testis. Also, **Lahimer *et al*., (2023)** found that chlorpyrifos has significantly effect on testicular function though steroid hormone synthesis and the testis-pituitary axis.

LM has traditionally been used by Peruvian inhabitants living at high altitudes as a nutrient, an energizer and for aphrodisiac and/or fertility-enhancing properties. It has been proved to be effective in improving sexual desire in men and sexual behavior in male rats and mice. Certainly, sexual desire may be affected directly by increasing serum testosterone levels or by having a testosterone-like effect, or indirectly by affecting behavioral depression, stress (**Gonzales-Arimborgo *et al*., 2016).** While, **Peres *et al*., (2020)** showed that the administration of Maca root powder was significantly increased testosterone concentration and significantly decreased of serum FSH and LH concentration through its curative effects by its antioxidant potential, scavenging free radical ability and may have important role in overcoming centraldysfunction by direct stimulating central nervous system and hypothalamus pituitary-gonadal axis due to its phytochemicals. So, increased level of testosterone stimulates pituitary gland to decrease release LH and FSH. In the other hand**, Choi *et al*., (2018)** reported that Adding maca root powder led to an improvement in testosterone level and this to the high concentration of antioxidants, contains several secondary metabolites, which help in keeping ROS levels low. Maca root powder effect may be due to enhance of spermatogenesis and increased of testosterone level due to antioxidant components which contain alkaloid like flavonoids and anthocyanins **(Fei *et al*., 2022).**

Table (3): Effect of LM on Testosterone hormone (ng/ml), Follicle Stimulating Hormone and luteinising hormone of infertility rats.

Parameters / Groups	TH (ng/ml)	FSH (MLU/ml)	LH (MLU/ml)
Control- Ve	$3.54^{a}\pm0.64$	$1.9^{a}\pm0.87$	$18.36^{a}\pm2.95$
Control+Ve	$0.46^{c}\pm0.09$	$0.30^{e}\pm0.05$	$2.16^{d}\pm0.37$
LM (5%)	$0.75^{c}\pm0.13$	$0.54^{d}\pm0.07$	$6^{c}\pm1.25$
LM (10%)	$0.95^{c}\pm0.08$	$0.82^{c}\pm0.04$	$8.66^{bc}\pm0.47$
LM (15%)	$1.81^{b}\pm0.25$	$1.35^{b}\pm0.15$	$10.29^{b}\pm1.02$
LSD (P≤ 0.05)	0.552	0.06	3.213

Values denote arithmetic means ± standard deviation .Means with different letters (a, b, c, d, etc,) in the same column differ significantly at $p \leq 0.05$ using ANOVA test, while those with similar letters are non-significantly different. TH = Testosterone Hormone – FSH = Follicle Stimulating hormone – LH = Luteinising hormone.

Table (4) revealed the mean value of some serum antioxidant enzymes for infertility rats injected by CPF and treated with maca roots at different levels. It was observed that the mean values of serum superoxide dismutase and catalase of negative control group significantly were higher than positive control group whereas the mean value of malonaldehyde significantly was lower in negative control group as compared to positive control group. Treated with different levels of maca roots led to increase the serum superoxide dismutase and

catalase, while in the same time caused decreasing in malonaldehyde level .The best level was detected in the fourth group which fed on 15% maca roots.

Gan *et al.* (2017) support the obtained results**.** They revealed that maca roots help the body's cells from harmful free radicals to contain natural antioxidants. Also, they found that Polysaccharides in maca roots have nutritive value and some pharmacological activities such as antifatigue, antioxidant, immunomodulator and antimicrobial. Also, **Lee and Chang, (2019)** found that maca roots contained glucosinolates (Gls) as glucoalyssin, glucosinalbin, glucolimnanthin, 4-hydroxyglucobrassicin, 4-methoxyglucobrassicin and glucoraphanin which are the secondary metabolites with nitrogen and sulfur chains which have antioxidant and anticarcinogenic effects by acting on the ferric reducing antioxidant potential, hydroxyl radical scavenging ability, 2,2-diphenyl-1-picrylhydrazyl free-radical scavenging capacity , increase the levels of superoxide dismutase, catalase , glutathione and decrease MDA **.**

Table (4): Effect of LM on antioxidant enzymes of infertility rats.

Parameters / Groups	SOD (u/ml)	MDA (nmol/ml)	CAT (ng/ml)
Control- Ve	**$42.30^{a}\pm1.9$**	**$5.46^{d}\pm0.73$**	**$4.65^{a}\pm0.98$**
Control+Ve	**$17.30^{d}\pm2.5$**	**$11.10^{a}\pm0.2$**	**$0.61^{c}\pm0.09$**
LM (5%)	**$23.60^{c}\pm1.5$**	**$8.90^{b}\pm0.52$**	**$1.34^{bc}\pm0.23$**
LM (10%)	**$31.02^{b}\pm1$**	**$7.50^{c}\pm0.2$**	**$2.07^{b}\pm0.22$**
LM (15%)	**$33.60^{b}\pm2.08$**	**$6.46^{c}\pm0.25$**	**$3.95^{a}\pm0.43$**
LSD (P≤ 0.05)	**3.514**	**0.856**	**0.99**

Values denote arithmetic means ± standard deviation .Means with different letters (a, b, c, d, etc,) in the same column differ significantly at $p \leq 0.05$ using ANOVA test, while those with similar letters are non-significantly different.
SOD= superoxidase - MDA= Malonaldehyde - CAT= Catalase.

The mean values of serum lipid profile for infertility rats injected by CPF as affecting by tested diets were presented in table (5). It could be noticed that the mean values of serum lipid profile of positive control group significantly were higher than negative control group except HDL_{-c} which significantly recorded lower value in positive control group. All infertility rats injected with CPF and treated with levels of maca roots showed significant decreases in mean values of lipid profile with increasing the level of HDL_{-c} as compared to positive control group. The best results of lipid profile was recorded in group 5 that fed on 15% maca roots powder. The showing data revealed that the toxic material increased the level of total cholesterol and cholesterol fraction except HDL which was decreased to their effect of the organ's functions as liver while adding maca roots in different levels led to decrease the bad cholesterol and increase the good one and their effect increased by increasing the roots level to contain high fiber and the activity compounds.

Subchronic exposure to CPF significantly altered the lipid profile of rats. Plasma TC, TG and LDL_{-c} levels were significantly elevated in CPF treated rats when compared with the control group. CPF induced changes in liver cell membrane permeability and/or increased cholesterol synthesis in the liver are plausible mechanisms behind the increase in plasma TC levels. In addition, the blockage of the liver bile ducts and a consequent reduction in cholesterol secretion into the duodenum can result in elevated plasma cholesterol levels and indicates liver damage **(Zaahkouk *et al.*, 2000; Yousef *et al.*, 2006 and Kalender *et al.*, 2010). Goel *et al.*, (2005) and Ambali *et al.*, (2011**) showed a marked decrease in HDL_{-C} in CPF treated rats. Low HDL_{-C} may be attributed to CPF induced hepatic damage, resulting in the impairment of lipoprotein synthesis. These observations suggest that prolonged exposure to CPF may be a major risk factor for the development of degenerative heart diseases.

Xia *et al.* (2021) results undergird the obtained which revealed that maca root contains Macaenes , are derived from long-chain unsaturated fatty acids, which behave as peroxisome proliferator-activated receptor (PPAR) agonists, which are ligand-activated transcription factors that can influence lipid metabolism that may increase fatty acid oxidation, reduce circulating and cellular lipid levels in obese diabetic individuals.

Lee *et al.*(2023) and Gencoglu (2023) displayed that Maca root have antioxidant activity (1,1-diphenyl-2-picrylhydrazyl radical scavenging activity and ferric-reducing ability of plasma) and ameliorated hypercholesterolemia by attenuating mRNA levels of acetyl-coenzyme A (CoA) acetyltransferase 2, sterol regulatory element-binding protein 2, and 3-hydroxy-3-methyl-glutaryl-CoA reductase. This antioxidant activity led to contain fiber, many essential amino acids, fatty acids, vitamin C, copper, iron, calcium , saponins, phenols, and flavonoids which associated with protection against the excessive production of reactive oxygen species by decreasing TC, TG, LDL_{-c} and increasing HDL_{-C}.

Table (5): Effect of LM on lipid profile of infertility rats.

Parameters / Groups	TC (mg/dl)	TG (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)
Control- Ve	$88.33^{e}\pm2.08$	$59.33^{e}\pm5.50$	$51.66^{a}\pm0.57$	$26.33^{e}\pm1.41$	$11.86^{e}\pm1.10$
Control+Ve	$156.66^{a}\pm6.20$	$131.66^{a}\pm0.57$	$45.33^{d}\pm1.15$	$102.13^{a}\pm5.50$	$26.33^{a}\pm0.11$
LM (5%)	$144.09^{b}\pm6.11$	$121.01^{b}\pm5.76$	$47.04^{cd}\pm1.02$	$87.60^{b}\pm4.68$	$240.2^{b}\pm1.33$
LM (10%)	$129.33^{c}\pm2.08$	$87.66^{c}\pm3.51$	$48.03^{bc}\pm10.42$	$71.73^{c}\pm2.64$	$17.53^{c}\pm0.70$
LM (15%)	$117.01^{d}\pm5.56$	$69.02^{d}\pm5.29$	$49.66^{b}\pm0.57$	$75.4^{d}\pm3.12$	$13.8^{d}\pm1.05$
LSD (P≤ 0.05)	6.25	7.58	1.76	5.54	1.51

Values denote arithmetic means ± standard deviation .Means with different letters (a, b, c, d, etc,) in the same column differ significantly at $p \leq 0.05$ using ANOVA test, while those with similar letters are non-significantly different. TC = total cholesterol –

TG = triglycerides – HDLc = High density lipoprotein cholesterol – LDLc = low density lipoprotein cholesterol – VLDLc = very low density lipoprotein cholesterol.

Data in table (6) presented the mean value of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (U\L) for infertility rats injected by CPF. From the results, it was found that liver enzymes in the positive control group were significantly higher than their levels in the negative control group. Maca roots at different levels improved liver functions by decreasing the liver's enzymes as compared to positive control group and the group fed high maca roots level recorded the highest reduction in the liver enzymes as a result of injecting with CPF.

The study of **Celik and Suzek, (2009**) revealed that chlorpyrifos-treatment caused an increase in the activities of AST and ALT in serum of male rats. The increase in these enzymes may be due to liver dysfunction and disturbance in the biosynthesis of these enzymes with alteration in the permeability of liver membrane takes place. Moreover, chlorpyrifos is mainly due to the leakage of these enzymes from the liver cytosol into the blood stream. Researchers reported that enzymatic activities of AST and ALT were elevated in CPF treated group led to fibrosis and a reduction in the number of functional hepatocytes, plasma activities of liver enzymes may be within normal limits despite the presence of severe liver fibrosis **(El-Demerdash , 2004)** . In other study, CPF showed mild necrosis of glandular cells and accumulation of exfoliated cells in the lumen of proventricular glands. There was mild degeneration of the tips of the mucosal papillae, moderate necrosis of glandular cells and accumulation of desquamated cells in the lumen of the glands on day (**Tanvir *et al.*, 2015).**

After feeding on maca roots significantly decreased liver cell necrosis, inflammatory cell infiltration, and liver cell apoptosis. These findings showed that Maca pretreatment alleviated pathological changes, suggesting potential for the use of Maca in clinical application to prevent liver injury though inhibition of inflammation damage. Furthermore, studies revealed protective effects of ginseng in hepatic damages. In these studies, it was shown that extract of maca reduced treatment period of acute and chronic hepatitis. Maca treatments inhibited oxidative stress damage such as lipid peroxidation, malondialdehyde, thiobarbituric acid reactive substance, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH). Also, polysaccharides from maca regulate immunomodulatory mechanisms by enhancing the secretion of nitric oxide (NO), tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6) in macrophages (**Rahman *et al.*, 2021 and Fei *et al.*, 2022).**

Table (6): Effect of LM on AST and ALT (U\L) of infertility rats.

Parameters / Groups	AST (U\L)	ALT (U\L)
Control- Ve	44.5^{e}±8.23	31.1^{e}±2.16
Control+Ve	156.16^{a}±1.89	99.76^{a}±3.35
LM (5%)	133.83^{b}±2.36	86.83^{b}±5.34
LM (10%)	126.26^{c}±1.41	79.2^{c}±0.75
LM (15%)	117.5^{d}±1.67	63.36^{d}±2.04
LSD (P≤ 0.05)	7.374	4.36

Values denote arithmetic means ± standard deviation .Means with different letters (a, b, c, d, etc,) in the same column differ significantly at $p \leq 0.05$ using ANOVA test, while those with similar letters are non-significantly different. AST= aspartate aminotransferase – ALT= alanine aminotransferase.

- Histopathological examination of testis

Microscopically, testes of rats from group 1(negative control) showed the normal histological structure of seminiferous tubule with normal spermatogoneal cells and complete spermatogenesis (Photo 1). On the other hand, examined sections of testis of rats from group 2 (positive control) revealed degeneration of spermatogoneal cells lining some seminiferous tubules (black arrow) and interstitial edema (red arrow) (photo. 2) degeneration of spermatogoneal cells lining some seminiferous tubules (black arrow) and congestion of interstitial blood vessel (red arrow) (photo. 3). Meanwhile, testis of rats from groups 3 (LM 5%) and 4 (LM 10%) showed that interstitial edema and congestion of interstitial blood vessel (Photos 4 and 5) while, group 5 (LM 15%) exhibited no histopathological alterations (Photo 6). From the previous studies, it was found that CPF had significant associations with testicular damages. This caused reduction in epididymal and testicular sperm counts and decreased in serum testosterone concentration in exposed males rats **(Uchendu *et al.*, 2013).** Whereas adding LM especially at the level of 15% caused non histological changes and this obtained result matched with **Peres *et al.* (2020)** who reported that maca roots enhance immunity, improve memory, resist fatigue, relieve depression, improve fertility and sexual function to contain many proteins, amino acids, fats and vitamins, as well as a variety of secondary metabolites such as maca alkaloids (including maca amide), glucosinolates, and other components. It is also a rich source of micro- and macroelements, including iron, calcium, copper, zinc and potassium (**Fei *et al.*, 2022).**

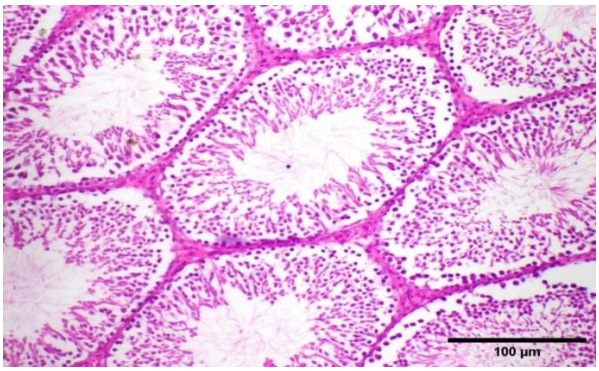

Photo. (1): Photomicrograph of testis of rat from group 1 (Control- Ve) (H & E X 100, scale bar, 100µm).

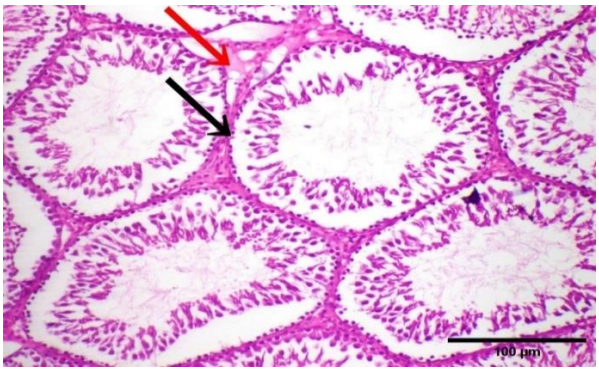

Photo. (2): Photomicrograph of testis of rat from group 2(Control +Ve) (H & E X 100, scale bar, 100µm).

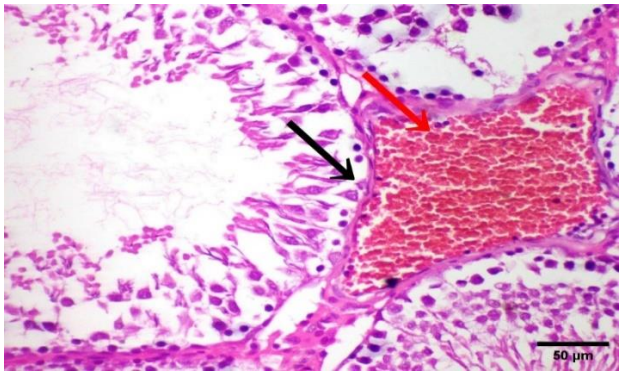

Photo. (3): Photomicrograph of testis of rat from group 2(Control +Ve) (H & E X 200, scale bar, 50µm).

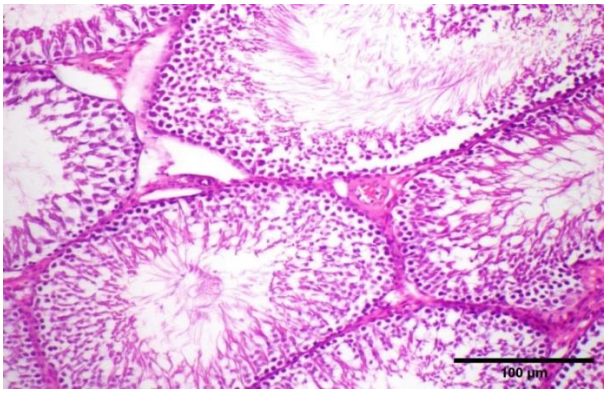

Photo. (4): Photomicrograph of testis of rat from group 3(Maca roots powder 5%) (H & E X 100, scale bar, 100µm).

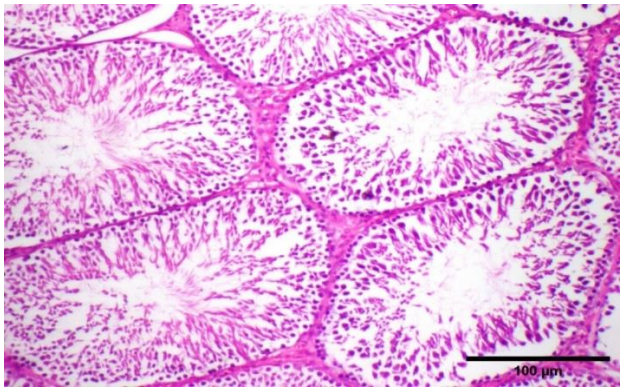

Photo.(5): Photomicrograph of testis of rat from group 4 (Maca roots powder 10%) (H & E X 100, scale bar, 100µm).

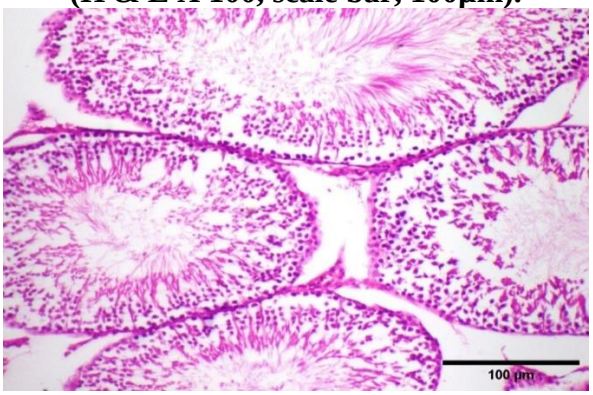

Photo.(6): Photomicrograph of testis of rat from group 5 (Maca roots powder 15%) (H & E X 100, scale bar, 100µm).

Conclusion

In this study, it became clear that injected rats with CPF caused injury and damage in internal organs and their function especially testis while the rats treated with different levels of LM led to decrease the adverse effect of CPF and improve body weight , sexual hormones, oxidative enzymes, liver enzymes and lipid profile and the best level was 15%.

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التأثير البيولوجي والبيوكميائي لمسحوق جذور الماكا *(Lepidium meyenii)* في الفئران المصابة بضعف الخصوبة المحقونة بالكلوربيريفوس (CPF).

الملخص العربى

تلعب المبيدات الفسفورية العضوية مثل الكلوربيريفوس دور حيوي في الزراعة الحالية. وقد لوحظ ان هذا النوع يسبب خطورة على خصوبة الرجال (ضعف الخصوبة). لذا تهدف الدراسة الحالية إلى التحقق من تأثير مسحوق جذور الماكا على الفئران المصابة بضعف الخصوبة لدى ذكور الفئران المستحثة بالكلوربيريفوس. تم تقسيم ثلاثون فأر من الذكور البالغين وزن (١٧٠جم ±١٠) إلى خمس مجموعات: المجموعة الاولى (٦ فئران) تغذت على الوجبة الأساسية فقط كمجموعة ضابطة سالبة، اربعة وعشرون فار تم حقنهم ب ٣٨مل بالكلوربيريفوس لكل كجم من وزن الجسم وتم تقسيمهم الى ٥ مجاميع واحدة منهم تمثل المجموعة الضابطة الموجبة . بينما المجموعات الثلاثة الاخرى تغذت على الوجبة الاساسية المضاف اليها (٥٪، ١٠٪، ١٥٪) من مسحوق جذور الماكا . تم تقدير القياسات البيولوجية (وزن الجسم-الماخوذ الغذائي- كفاءة الوجبة) والتحاليل البيوكميائية (بعض الهرمونات الجنسية- انزيمات الكبد- دهون الدم- وبعض مضادات الاكسدة). وقد توصلت الدراسة ان المجموعة الضابطة الموجبة اعطت نتائج معنوية منخفضة لكل من وزن الجسم – كفاءة الوجبة – الهرمونات الجنسية- الكتاليز- السوبر أوكسيديز- الليبوبروتينات عالية الكثافة عند المقارنة بالمجموعة الضابطة السالبة بينما سجل الماخوذ الغذائي واوزان الاعضاء – المالوندهايد – دهون الدم و انزيمات الكبد زيادة معنوية. وقد وجد ان المجموعة (٥) التى تغذت على ١٥% من مسحوق الماكا سجلت أفضل النتائج على TH, FSH ,LH بنسبة ٢٩٣و٣٥٠و ٣٧٧% بالمقارنة بالمجموعة الضابطة الموجبة على التوالى. الخلاصة: وجدت الدراسة أن إستخدام جذور الماكا وخاصة النسبة الأعلى أدى إلى تحسن خصوبة ذكور الفئران وهذا يعطى فوائد علاجية في حالة ضعف الخصوبة لدى الانسان .

الكلمات المفتاحية: مسحوق جذور الماكا، الكلوربيريفوس، ضعف الخصوبة، الهرمونات الجنسية، دهون الدم.