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Study the Potential Effects of Safflower Seeds on some Biological Parameters and Fertility Hormones in Male Rats

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Abstract:

This search aimed to study the effects of *Carthamus tinctorius* on rats inflicted with infertility. Thirty male Sprague Dawley rats weighing 160-170 g were used in this study. The animals were divided into six main groups. The first main group (n=5) was kept as a negative control group (-ve), and the second group was (n=5) injected with cadmium chloride to induce infertility in male rats. In contrast, the third infertile group was fed on a diet containing 2% safflower seeds powder, 4th group of infertile rats fed on a diet containing 5% safflower seeds powder, 5th group of infertile rats was fed on a diet containing 7% safflower seeds powder while 6th group infertile rats fed on a diet containing 10% safflower seeds powder for 28 days. At the end of the experiment, rats were fasted overnight before sacrificing, and blood was collected and then centrifuged to separate the serum. Serum glucose, liver functions, kidney functions, lipid profile, testosterone hormone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) were determined. The obtained results revealed that treated groups with safflower seeds led to a significant increase of two hormones (Testosterone, FSH) while LH reduced Serum glucose, cholesterol, triglycerides, LDL-c, VLDL-c, uric acid, urea, creatinine, ALP, ALT, AST decreased, and HDL-c increased.

***Keywords**: Sexual hormones, cadmium chloride, infertility, safflower seeds.*

Introduction

Normal male fertility defined as ability to engage in sexual intercourse and ejaculate fertile sperm cells. (1) David and Jessy (2) suggested about factors affecting male fertility which were: Obesity, Tobacco, Alcohol, Caffeine, Disorder Sexual hormones, Oxidative stress, and reactive oxygen species & Lack of antioxidants.

Oluyemi (3) reported that cadmium toxicity, possible cause of male infertility, results indicated that Cd exhibits a deleterious effect on the productive system, reported decrease

significant in seminal plasma levels, sperm density, sperm motility, counts and sperm concentration. Therefore, cadmium has a strong toxic effect on spermatogenesis.

Rekha (4) studied the exposure of rats to different doses (1mg/kg BW), (2mg/kg BW) of cadmium showed a decrease in the testicular weight and sperm count and increase in the testicular level of lipid peroxidation and in the incidence of abnormal sperms. Therefore, the exposure to high dose of cadmium showed a significant decrease in testicular weight and sperm count and increase in lipid peroxidation compared to low dose group, therefore, cadmium has deleterious effect on spermatogenesis and one of the possible mechanisms in cadmium induced oxidative damage on rat testis cell might be mediated through its effect on reducing ascorbic acid level.

The causes of infertility are wide ranging including diagnoses such as, ovulatory disorders, tubal disease, endometriosis, chromosomal abnormalities, sperm factors and unexplained infertility. Infertility can be caused by a huge number of factors such as hormone AST balance, polycystic ovarian syndrome, endometriosis, anovulatory cycles, physical blockage, inadequate hormone production, short luteal phase and lack of luteinizing hormone. (5)

Studies carried out to test the hypolipidemic effect of powdered safflower seed (SSP) and safflower seed extracts prepared with ethanol (SSE) or hot water (SSW) on high fat and high cholesterol-fed rats for 5 weeks showed that all the safflower seed preparations significantly lowered the plasma cholesterol concentration while the plasma triglyceride concentration was only lowered by the supplementation of SSE and SSW. The hepatic total cholesterol contents were significantly lowered in the SSW group compared with the control group, whereas the hepatic triglyceride contents were significantly lower in both the SSE and SSW groups compared with the control group. The results suggested that supplementation of SSE or SSW is more effective than SSP in improving the atherogenic risk factors in high cholesterol-fed rats. (6)

A study on the effects of safflower seed extract (SSE) supplementation on cardiovascular risk markers in healthy human beings revealed significant reductions in circulating oxidized LDL, autoantibody titers to malondialdehyde-modified LDL, the soluble form of vascular cell adhesion molecule-1 (sVCAM-1), and urinary 8-isoprostane. The study also indicated the index of arterial stiffness, brachial-ankle pulse wave velocity, to be lower than the baseline in 11 of 20 subjects. This was also accompanied by a reduction in blood pressure. The study concluded also that individuals with elevated oxidative stress, inflammation, and/or arterial stiffness may receive more benefits from SSE supplementation. (7)

Traditional herbal medicine is just one of the many different approaches using plants in the remedy of diseases. Carthamus tinctorius (CT) or safflower is a popular plant that is used for coloring and flavoring in food industries. The effect of CT on spermatogenesis

and sperm parameters has been reported in traditional medicine but has not yet been confirmed scientifically. The results indicated that the percentage of sperm with good morphology, motility, and count increased significantly in the group treated with 10 mg/kg CT (p=0.002, p=0.03, and p=0.00001, respectively). The effects on hormonal changes and genital organ weights were also positive. (8)

Safflower (Carthamus tinctorius L.) is an annual herbaceous plant, cultivated mainly for the seed which is used for edible oil extraction provide for its pigmented variety. (9)

This study aims to investigate the effect of safflower seeds (Carthinus tinctrorius L.) on FSH, LH, testosterone hormones and the other parameters of infertility male rats.

Materials And Methods

Materials

Safflower seeds (Carthamus tinctorius L.) was obtained from herbs market in Cairo, Egypt.

Cadmium Chloride (CdCl2) was purchased from Merk chemical company from Germany.

Thirty adult male Sprague Dawley rats, weighting (160-170g). From Medical Insects Research Institute, Doki, Cairo were used in this study. Rats were housed in wire cages under the normal laboratory condition and were fed on basal diet for a week as an adaptation period. Diet was offered to rats in special feed cups to avoid looser conditions of feed, water was provided to the rats by glass tubes supported to one side of the cage, feed and water provided ad-libium and checked daily.

Methods

Preparation of materials

All safflower seeds milled into powder by using sunrise to dry, then milled to give a powder and kept in dusky stoppered glass bottles in a cool and dry location until use according to Russo (10), who reported that all plants are kept in a cool, dry, and dark location to reduce oxidation of their content.

Induced infertility for rats

Rats were injected by cadmium chloride (CdCl2, 0.1% solution) at 1ml/kg body weight to induce male infertility for rats (4).

Experimental design

The experimental was done in the Faculty of Home Economics, Menoufia University, Shebin El-Kom. Rats were housed in wire cages in a room temperature 25°C and kept under normal healthy conditions. Rats were divided into the following groups:

Group (1) as Negative control group which fed on basal diet Infertility rats (25 rats) were divided into five groups, the first group was positive control group and the other groups were infertility rats were fed on 2, 5, 7 and 10% safflower seeds powder.

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), feed efficiency ratio (FER), organs weight were determined according to Chapman (11). Using the following equations:

$$BWG = \quad Final\ weight - Initial\ weight$$

$$FER = \frac{Gain\ in\ body\ weight\ (g/rat)/28}{Feed\ consumed\ (g/rat)/28}$$

$$Relative\ Organs\ weight = \frac{Organs\ weight\ (g/rat)/28}{Final\ weight\ \ (g/rat)\ /28}$$

Blood Sampling and Organs

Blood samples were collected after 12 hours fasting at the end of the experiment using the abdominal aorta in which the rats were scarified under ether anesthetized. Blood samples were received in to clean dry centrifuge tubes and left to clot at room temperature, then centrifuged for 10 minutes at 3000 rpm to separate the serum. Serum was carefully separated, transferred into clean centrifuge tubes, and stored frozen at -20°C for analysis. All serum samples were analyzed for total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL-C), low density lipoprotein (LDL-C), very low-density lipoprotein (VLDL-C), urea, creatinine, uric acid, glucose, aspartate amino transferase (AST), alanine amino transferase (ALT), and alkaline phosphatase (ALP), testosterone hormone, follicle stimulating hormone (FSH), luteinizing hormone (LH).

At the same time, the organs: Heart, kidney, liver, lungs, spleen and testes were removed, washed, and stored frozen in formalin solution 10% for histopathological testing according to method mentioned by Drury and Wallington (12).

Analytical Methods

The principal use of total cholesterol determination was according to Allen (13).

Phosphotungstic acid and magnesium ions selectively precipitating all lipoproteins except the HDL fraction-cholesterol present in the supernatant which can be determined by the same method used for total cholesterol, according to Lopez (14).

The determination of VLDL (very low-density lipoproteins) and LDL (low- density lipoprotein) were calculated according to the method of Lee and Nieman (15).

VLDL (mg/dl) = Triglycerides /5.

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

Urea was determined according to the enzymatic method of Patton and Crouch (16).

Creatinine was determined according to kinetic method of Henry (17).

Uric Acid was determined according to the enzymatic method of Patton and Crouch (16).

Enzymatic colorimetric determination of alkaline phosphatase was carried out according to Belfied and Goldberg (18).
AST and ALT activities were measured according to method of Yound (19).
Enzymatic determination of plasma glucose was carried out colorimetrically according to Trinder (20), Youung (21), and Young (22).
FSH hormone was determined colorimetrically according to the method of Fahim (23).
LH hormone was determined colorimetrically according to the method of Fahim (23).
Testosterone hormone was determined colorimetrically according to the method of Pardelles (24).

Histopathological examination

Small Specimens from liver and testes were collected from all experimental groups, fixed in 10% 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 Carleton (25).

Statistical analysis:

Statistical analysis was performed by using computer program (costat) when a significant main effect was detected. Differences between treatments at ($p \leq 0.05$) were considered significant Sendcor and Cochran (26).

Results and Discussion

Table 1 shows the mean value of body weight gain of infertility rats fed on various diets. It could be noticed that the mean value of BWG (g/day/rat) of negative control group (-) group was higher than positive control (+) group. The best BWG (g/day/rat) was recorded for group 6 fed on (10% safflower seeds) when compared to positive control (+) group. The results of this study agree with that of Soraya (9) who found that Safflower (Carthamus tinctorius L.) is an annual herbaceous plant, cultivated mainly for the seed which is used for edible oil extraction and bird feeding. The results showed that oral administration of safflower seeds significantly increased the body weight of male rats in a dose-dependent manner ($p<0.05$). Table (1) indicated the value of feed intake (g/day/rat) of infertility rats fed on variable diets. Data revealed that the mean value of (FI) of negative control group (-) group was lower than positive control group (+). Numerically the best FI was recorded for group 3 (infertility rats fed on 2%) safflower seeds when compared to positive control group (+) group. Data in the same table illustrated the mean value of FER of infertility rats fed on different diets. Data showed that the mean value of FER of negative control group (-) group was higher than positive control (+). The better FER was recorded for group6 (infertility rats fed on 10% safflower seeds) when compared to positive control group (+).

Table (1): Effect of Different levels of SSP on body weight gain (BWG), feed intake (FI) and feed efficiency ratio (FER) of infertility rats

Parameters / Groups	BWG /day (g/day/rat) Mean ± SD	FI (g/day/rat) Mean ± SD	FER (g) Mean ± SD
G1: Control –ve	1.214a±0. 0005	14.2e ±0.2	0.085a±0.53
G2: Control +ve	0.764f±0.0004	15.7a ±0.7	0.049b±0.006
G3: safflower seeds (2%)	1.00e±0. 005	14.2e ±0.5	0.070b±0.04
G4: safflower seeds (5%)	1.021d±0.0003	14.6c±0.6	0.0719b±0.0008
G5: safflower seeds (7%)	1.092c±0.0002	15.5b±0.5	0.0704b±0.0007
G6: safflower seeds (10%)	1.128b±0.0001	14.5d±0.5	0.077b±0.05
LSD	0.003	0.08	0.3

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

Results in table (2) revealed that show the mean value of liver weight (g) of infertility rats fed on various diets. It could be noticed that the mean value of liver weight (g) of negative control group (-) group was higher than positive control group (+). The best liver weight was recorded for group 6(10% safflower seeds) when compared to positive control group (+). The mean value of heart weight (g) of infertility rats fed on different diets. It could be indicated that the mean value of heart weight (g) of negative control group (-) group was higher than positive control group (+). The best heart weight was recorded for group 6 (infertility rats fed on 10% safflower seeds) when compared with positive control group (+). The mean value of kidneys weight (g) of infertility rats fed on different diets. It could be noticed that the mean value of kidneys weight (g) of negative control group (-) group was higher than positive control group (+). The best kidneys weight was revealed for group 6 infertility rats fed on 10% safflower seeds when compared to positive control group (+). The mean value of lungs weight (g) of infertility rats fed on various diets. It could be revealed that the mean value of lungs (g) of negative control group (-) was higher than positive control group (+). The better lungs weight was observed for group 6 (infertility rats fed on 10% safflower seeds) when compared to positive control group (+). The mean value of spleen weight (%) of infertility rats fed on various diets presented in table (2). It could be noticed that the mean value of spleen (%) of negative control group (-) group was higher than positive control group (+). The best spleen weight was recorded for group 6 (infertility rats fed on 10% safflower seeds) when compared to positive control group (+). The mean value of testes weight (g) of infertility rats fed on various diets shown in table (2). It could be observed that the mean value of testes (%) of negative control group (-) group was higher than positive control group (+). The best testes weight was recorded for group 6 (infertility rats fed on 10% safflower seeds) when compared to positive control group (+). The results of this study agree with Matboo and Modaresi (27)

who found that safflower seeds extract occurs increasing in organs weight like testes weight when compared to the control group.

Table (2): Effect of different levels of SSP on weight of kidney, liver, spleen, lungs, testes and heart of infertility rats

Parameters / Groups	Kidney (g)	Liver (g)	Spleen (g)	Lungs (g)	Testes (g)	Heart (g)
G1: Control-ve	2.50a±0.05	6.80a±0.06	1.90a±0.001	2.70a±0.06	4.00a±0.05	1.95a±0.005
G2: Control +ve	1.00f±0.06	3.60f±0.05	0.90e±0.002	0.80f±0.02	1.20f±0.003	0.65f±0.003
G3: safflower seeds (2%)	1.20e ±0.02	5.00e±0.02	0.10f±0.005	0.88e±0.04	1.30e±0.004	0.80e±0.006
G4: safflower seeds (5%)	1.35d±0.04	5.25d±0.04	1.20d±0.003	1.00d±0.01	1.40d±0.005	0.82d±0.001
G5: safflower seeds (7%)	1.50 c±0.07	5.50c±0.03	1.30c±0.008	1.40c ±0.03	1.50c± 0.006	0.88c±0.002
G6: safflower seeds (10%)	1.70 b±0.02	6.00b±0.07	1.50b±0.006	1.50b±0.05	1.60b±0.002	0.99b±0.004
LSD	0.08	0.08	0.008	0.06	0.007	0.006

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

Table (3) revealed that the mean value of serum glucose (mg/dl) of infertility rats fed on different diets. It could be noticed that the mean value of glucose for negative control group (-) group was lower than positive control group (+). The better serum glucose was observed for group 6 (infertility rats fed on 10% safflower seeds) when compared to positive control group (+). The results of this study agree with Hamza and Farrag (28) who revealed that safflower seeds significant decreased glucose in diabetic rats which fed 200 mg safflower seeds plus basal diet. Sedigheh (29) indicated that triglyceride, cholesterol, LDL-C and VLDL-C had a meaningful decreasing in diabetic rats treated with Carthamus tinctorius and diabetic rats treated with glipalamide as compared with diabetic rats with no treatment. Insulin level increased significantly in diabetic groups received treatment (glipalamide or Carthamus tinctorius L) in comparison with diabetic group with no treatment. Nasreen (30) showed that insulin levels were significantly increased in glipalamide treated as well as Carthamus tinctorius treated groups as compared to diabetic control, revealed that Carthamus tinctorius has significant hypoglycemic effect at 200 mg/kg and 300 mg/kg doses as compared to diabetic control group. Parivash (31) suggested that levels of blood glucose decreased in alloxan induced diabetic rats after treatment with 200 mg/kg safflower seed. Huijuan (32) found that safflower seeds significantly reduced the fasting blood glucose and increased insulin sensitivity of high fat diet. Safflower seeds significantly reduced fasting blood glucose and increased insulin sensitivity. Yanuo (33) found that safflower seeds decreased fasting blood glucose in diabetic rats treated with 100 mg/kg safflower seed.

Table (3): Effect of Different levels of SSP on serum glucose (mg/dl) of infertility rats

Groups	Glucose(mg/dl) Mean ± SD
G1: Control –ve	59.00f ± 0.5
G2: Control +ve	137.00a ± 0.4
G3: safflower seeds (2%)	115.40b ± 0.1
G4: safflower seeds (5%)	86.00c± 0.3
G5: safflower seeds (7%)	63.00d± 0.2
G6: safflower seeds (7%)	61.40e ± 0.6
LSD	0.69

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

Table (4) illustrated that the mean value of serum total cholesterol (TC) (mg/dl) of infertility rats fed on treated diets. It could be observed that the mean value of (TC) of negative control group (-) group was lower than positive control group (+). The better serum (TC) was obtained for group 3 (infertility rats fed on 2% safflower seeds) when compared to positive control group (+). Mean value of serum triglycerides (TG) (mg/dl) of infertility rats' diets is shown in table (4). It could be noticed that the mean value of (TG) of negative control group (-) group was lower than positive control group (+). The superior serum (TG) was recorded for group 5 (infertility rats fed on 7 % safflower seeds) when compared to control (+). The results of this study agree with Shimomura (34) found that serum triacylglycerol level was markedly lower in the rats fed high fat diet then treated by safflower seeds. Kwang (35) indicated that powdered safflower seed lowered the plasma cholesterol concentration in high-fat and high-cholesterol fed rats. These studies were designed to test the hypolipidemic activity of safflower seed extracts prepared with ethanol or hot water. Male rats were fed a high-cholesterol (1% wt./wt.) or high-cholesterol diet supplemented with safflower seed powder (5% wt./wt. SSP), safflower seed ethanol extract (0.15% wt./wt. SSE), or safflower seed water extract (0.5% wt./wt. SSW) for 5 weeks. All the safflower seed preparations significantly lowered the plasma cholesterol concentration, whereas the plasma triglyceride concentration was only lowered by the supplementation of SSE and SSW.

Results indicated in table (5) the mean value of serum (HDL-c) (mg/dl) of infertility rats fed on different diets. It could be observed that the mean value of HDL-c for negative control group (-) group was higher than positive control group (+). The best serum (HDL) was observed for group 5 (infertility rats fed on 7% safflower seeds) when compared to positive control group (+). The mean value of serum (LDL-c) (mg/dl) of infertility rats fed on different diets is shown in table (5). It could be observed that the mean value of (LDL-c) of negative control group (-) group was lower than positive control group (+).

Rats fed on (7% safflower seeds) (group 5) recorded the best serum (LDL). The mean value of serum (VLDL-c) (mg/dl) of infertility rats fed on different diets is shown in table (5). It could be observed that the mean value of (VLDL-c) of negative control group (-) group was lower than positive control group (+). The best treatment was recorded for group 5 (7% safflower seeds) when compared with control (+).

Table (4): Effect of Different levels of SSP on total cholesterol (TC) and triglycerides (TG) of infertility rats

Groups	TC (mg/dl) Mean ± SD	TG(mg/dl) Mean ± SD
G1: Control –ve	92.00f± 0.05	43.00e ± 0.5
G2: Control +ve	152.00a ± 0.06	169.00a ± 0.6
G3: safflower seeds (2%)	96.00e ± 0.07	56.00b ±0.2
G4: safflower seeds (5%)	98.00b±0.08	49.00c± 1.5
G5: safflower seeds (7%)	97.20 c± 0.02	43.00e± 0.4
G6: safflower seeds (10%)	97.00d± 0.01	47.00d±0.7
LSD	0.09	1.3

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

The results of this study agree with Teerakul (36) found that Carthamus tinctorius L. (safflower) is in Thailand traditionally used for a herbal tea for health to reduce cholesterol and prevent atherosclerosis. At dose of 250 mg/kg body wt. during the 4-week study, body weight, food intake, organ weight, and plasma cholesterol levels were evaluated. Animals treated with 2%-cholesterol diet and dichloromethane fraction for a week exhibited decreased body weight. After treatment for 14 and 30 days, a significant reduction in total cholesterol and total cholesterol/HDL-cholesterol and a significant induction in HDL-cholesterol were observed in the hypercholesterolemic rats treated with the dichloromethane extract. Sedigheh (29) found that triglyceride, cholesterol, LDL-c and VLDL-c had a meaningful decrease in diabetic rats treated with Carthamus tinctorius as compared with diabetic rats with no treatment. Parivash (31) found that TC, TG, LDL-c decreased, and HDL-c increased in alloxan induced diabetic rats after treatment with 200 mg/kg safflower seed for 28 days.

Data in table (6) illustrated the mean value of serum urea (mg/dl) of infertility rats fed on various diets. It could be noticed that the mean value of urea of negative control group (-) group was lower than positive control group (+). The best treatment compared to positive control group (+) group of serum urea was that of group 6 (10%safflower seeds). The mean value of serum creatinine (mg/dl) of infertility rats fed on various diets is shown in table (6). It could be observed that the mean value of creatinine of negative control group (-) group was lower than positive control group (+). The best treatment was

recorded for group 6 (10%safflower seeds) when compared to positive control group (+). The mean value of serum (UA) (mg/dl) of infertility rats fed on various diets is illustrated in table (6). It could be observed that the mean value of uric acid of negative control group (-) group was lower than positive control group (+). The best treatment was observed for group 6 (10% safflower seeds) when compared to positive control group (+). The results of this study agree with Yanuo(33) found that safflower seeds decreased creatinine and urea in rats treated by safflower seeds compared to control group. Soraya(9) showed that BUN (Blood urea nitrogen) and Cr (creatinine) were not significantly changed in A82 seed treated groups. Rofida(37) found that the effect of Carthamus tinctorius L. showed increasing in the levels of creatinine and urea compared to normal rats, being contrary to the results of table (6).

Table (5): Effect of safflower seeds on (VLDLc), (HDLc), and (LDLc) (mg/dl) of infertility rats

Parameters / Groups	VLDL-c(mg/dl) Mean ± SD	HDL-c(mg/dl) Mean ± SD	LDL-c(mg/dl) Mean ± SD
G1: Control –ve	8.6e± 0.005	64.00a± 0.8	19.4e± 0.1
G2: Control +ve	33.8a± 0.001	48.00d± 0.5	70.2a± 0.5
G3: safflower seeds (2%)	11.2b ± 0.002	52.00c± 0.6	32.8d± 0.3
G4: safflower seeds (5%)	9.8 c± 0.006	49.00d± 0.4	39.2c± 0.6
G5: safflower seeds (7%)	8.6e± 0.004	56.00b± 1.5	32.6d±0.2
G6: safflower seeds (10%)	9.4d± 0.003	45.00e± 1.2	42.6 b± 0.7
LSD	0.006	1.6	0.80

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

Table (6): Effect of Different levels of SSP on uric acid (UA), creatinine and nitrogen urea (mg/dl) of infertility rats

Parameters / Groups	Urea(mg/dl) Mean ± SD	UA (mg/dl) Mean ± SD	Creatinine (mg/dl) Mean ± SD
G1: Control –ve	27.00f± 0.5	1.63f± 0.006	0.79f± 0.001
G2: Control +ve	55.00a ± 0.6	3.9a± 0.002	1.09a± 0.002
G3: safflower seeds (2%)	41.00c± 0.7	2.00b±0.004	0.98b± 0.003
G4: safflower seeds (5%)	43.00b± 0.3	1.80c± 0.005	0.97c± 0.004
G5: safflower seeds (7%)	37.00d± 0.4	1.77d± 0.003	0.93d± 0.006
G6: safflower seeds (10%)	35.00e± 0.8	1.65 e± 0.001	0.91e± 0.008
LSD	1.02	0.006	0.008

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

The mean value of serum (ALP) (U/ L) for infertility rats fed on various diets (table 7). It could be noticed that the mean value of (ALP) of negative control group (-) group was lower than positive control group (+). Group 6 (10% safflower seeds) showed the better treatment of serum ALP. Data of table (7) revealed the mean value of serum (ALT) (U/L) of infertility rats fed on various diets. It could be observed that the mean value of (ALT) for negative control group (-) was lower than positive control group (+). The better serum (ALT) was recorded for group 6 (10% safflower seeds) as compared to positive control (+).

The mean value of serum (AST) (U/L) of infertility rats fed on various diets is revealed in table (7). It could be observed that the mean value of (AST) for negative control group (-) was lower than positive control group (+). The better serum (AST) was recorded for group (3) 2% safflower seeds as compared to positive control (+). The results of this study agree with Parivash (31) who revealed that levels of ALT, AST and ALP decreased in alloxan induced diabetic rats after treatment with 200 mg/kg safflower seed for 28 days. Zhi (38) found that plasma concentrations of alanine transaminase (ALT) and aspartate aminotransferase (AST) decreased significantly in comparison with the control positive group, the degree of liver fibrosis, cirrhosis and necrosis decreased in safflower seeds treated rats, safflower seeds significantly inhibited malondialdehyde (MDA) and superoxide dismutase (SOD) changes in diethyl nitrosamine-treated rat liver compared with control group. Yanuo (33) found that safflower seeds can improve the general condition of rats with hepatic fibrosis and relieve cellular swelling of the liver, fatty degeneration, necrosis, inflammatory cell infiltration and fibroblastic profile ration. Safflower seeds decreased the level of alanine aminotransferase, aspartate aminotransferase. Soraya (9) found that safflower (Carthamus tinctorius L.) is an annual herbaceous plant, cultivated mainly for the seed which is used for edible oil extraction which showed that oral administration of A82 seeds significantly increased the body weight of male rats in a dose-dependent manner ($p<0.05$). Biochemical tests showed that A82 seeds significantly increased the serum levels of AST (Aspartate amino transferase) ($p<0.05$), slightly reduced the serum levels of ALT (Alanine amino transferase) and significantly reduced ALP ($p<0.05$) levels in a dose dependent manner. Rofida (37) found that Carthamus tinctorius L. there was significant decreasing alanine aminotransferase (ALT), aspartate amino transferase (AST) and serum alkaline phosphatase (ALP).

Table (8) illustrated the mean value of testosterone hormone (ng/ml) of infertility rats fed on various diets. It could be noticed that the mean value of Testosterone of negative control group (-) group was higher than positive control group (+). The group 3 (2% safflower seeds) recorded the better treatment of testosterone hormone. The mean value of (FSH) hormone (MIU/ml) of infertility rats fed on various diets is shown in table (8). It could be noticed that the mean value of (FSH) for negative control group (-) group was

higher than positive control group (+). The group 6 (10% safflower seeds) recorded the better treatment of FSH hormone. Also, the mean value of (LH) hormone (MIU/ml) of infertility rats fed on various diets illustrated in table (8). It could be noticed that the mean value of (LH) of negative control group (-) group was lower than positive control group (+). Group 6 (10% safflower seeds) found to be the better treatment of LH hormone. The results of this study agree with Matboo (27) found that safflower seeds extract caused some changes like the number of sperm and the density of FSH, LH and testosterone hormones existing in the serum of blood when compared to the control group. The results of this research showed that safflower can be a modifier agent for the male reproduction potential and it is able to change the reproduction activity and can be effective on the testes and increased of the testosterone density. Mi (39) determined the effect of safflower seeds extract on the levels of serum sex hormone which caused significant increasing in serum testosterone levels. Soghara (8) found the effect of C. Tintorius on spermatogenesis. The semen was collected from the epididymis and the reproductive organs were assessed. Sperm count and motility were measured and smears were prepared for assessment of the other parameters. The results indicated that the percentage of sperm with good morphology, motility, and count-increased significantly in the group treated with 10 mg/kg CT. Ali(40) found that C. tinctorius extract reduced the number of ovarian follicles, the blood levels of the FSH were decreased in the experimental groups compared with those of the control animals. Smith (41) found that safflower decoctions have been used successfully for treatment of male sterility and dead sperm excess disease.

Table (7): Effect of Different levels of SSP on AST, ALT and ALP (U/L) of infertility rats

Parameters / Groups	AST(U/L) Mean ± SD	ALT(U/L) Mean ± SD	ALP(U/L) Mean ± SD
G1: Control –ve	50.00f ± 0.5	33.00f±0.7	190.00e± 0.5
G2: Control +ve	159.00a ± 0.7	59.00a±0.8	408.00a± 0.4
G3: safflower seeds (2%)	67.00b± 0.6	52.00b±0.5	270.00b± 0.3
G4: safflower seeds (5%)	64.00c± 0.4	50.00c± 0.6	263.00c± 0.2
G5: safflower seeds (7%)	61.00d± 0.1	47.00d± 0.4	236.00d± 1.5
G6: safflower seeds (10%)	54.00e± 0.2	35.00e± 1.5	190.00e± 1.2
LSD	0.83	1.4	1.4

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

Photos (1-7) showed the histological changes which occurred for infertility rats. Feeding on safflower seeds ameliorated these changes which were more pronounced for liver and tetes. Safflower seeds tended to show better results.

Table (8): Effect of Different levels of SSP on LH, FSH, testosterone (mIU/ mL) of infertility rats

Parameters / Groups	LH hormone (mIU/mL) (Mean±SD)	FSH hormone (mIU/mL) (Mean±SD)	Testosterone hormone (mIU/mL) (Mean±SD)
G1: Control -ve	0.11c±0.009	0. 27a±0.01	2.35a±0.01
G2: Control +ve	0.17a±0.002	0.10e±0.03	0.38f±0.05
G3: safflower seeds (2%)	0.14b±0.005	0.18b±0.05	0.86b±0.02
G4: safflower seeds (5%)	0.115c±0.0009	0.12c±0.01	0.39e±0.01
G5: safflower seeds (7%)	0.11c±0.003	0.15b±0.07	0.73c±0.01
G6: safflower seeds (10%)	0.09d±0.006	0.11d±0.01	0.44d±0.03
LSD	0.009	0.01	0.02

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

Testes of control positive rats that treated with (2% safflower seeds powder) showed marked protection of the seminiferous tubules with active spermatogenesis in most of them and active sperms in their lumen with nuclear pyknosis of some spermatogoneal cells (Photo 1). Livers of control positive rats that treated with (5% safflower seeds powder) showed marked protection of the hepatic parenchymal cells with only few scattered necrotic cells (Photo 2).

The testicular tissue of control positive rate that treated with (5% safflower seeds powder) showed marked protection of the testicular tissue and active spermatogenesis in most of the seminiferous tubules (Photo 3).

Liver of control positive rats that treated with (7% safflower seeds powder) showed good degree of protection of the hepatic parenchyma with mild degeneration with dilated and congested central vein (Photo 4).

Photo 5: Testis of control positive rat that treated with (7% safflower seeds powder) showing coagulative necrosis of the seminiferous tubules, some of them showing dystrophic calcification and few inflammatory cells infiltration and edema in the interstitial spaces.

Liver of control positive rats that treated with (10% safflower seeds powder) showing good protection of the hepatic cells, notice the congestion of the portal vessel and few proliferated bile ductules in the portal area (Photo 6) with very mild necrobiotic changes of the hepatic cells. Testis of control positive rats that treated with (10% safflower seeds powder) showed coagulative necrosis of the seminiferous tubules with calcium globules deposition in some of them and thickened blood vessels' wall (Photo 7).

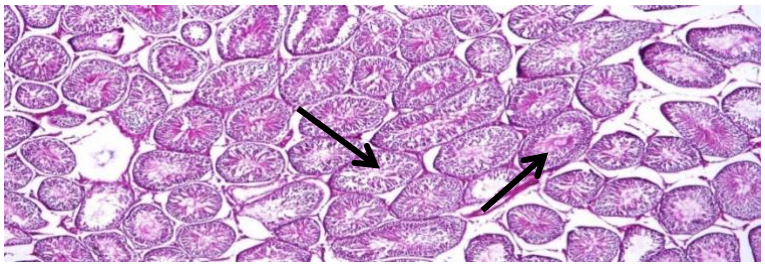


Photo (1): Testis of control positive rat that treated with 2% C. tinctorius showing marked protection of the seminiferous tubules with active spermatogenesis (arrow) in most of them and active sperms in their lumen. (H&E, X100).

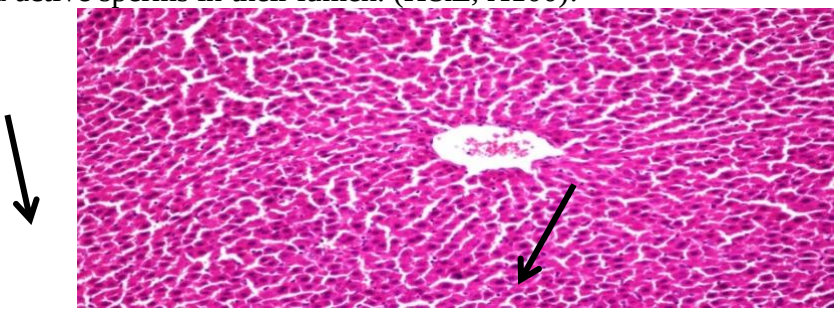


Photo 2: Liver of control positive rat that treated with 5% C.tinctorius showing marked protection of the hepatic parenchymal cells with only few scattered necrotic cells (arrow). (H&E, X200).

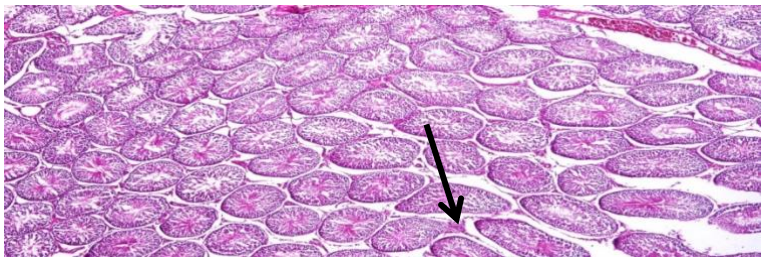
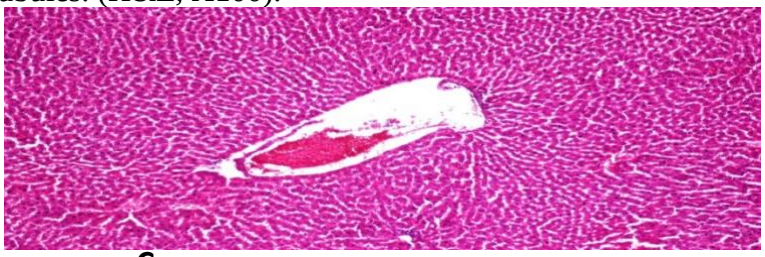


Photo (3): Testis of control positive rat that treated with 5% C. tinctorius showing marked protection of the testicular tissue and active spermatogenesis (arrow) in most of the seminiferous tubules. (H&E, X100).



Co

Photo 4: Liver of control positive rat that treated with 7% C. tinctorius showing good degree of protection of the hepatic parenchyma with mild degeneration with dilated and congested central vein (Co). (H&E, X200).

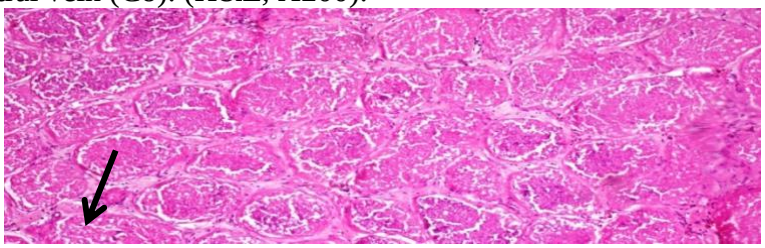


Photo (5): Testis of control positive rat that treated with 7% C. tintorius showing local coagulative necrosis of the seminiferous tubules (arrow). (H&E, X200).

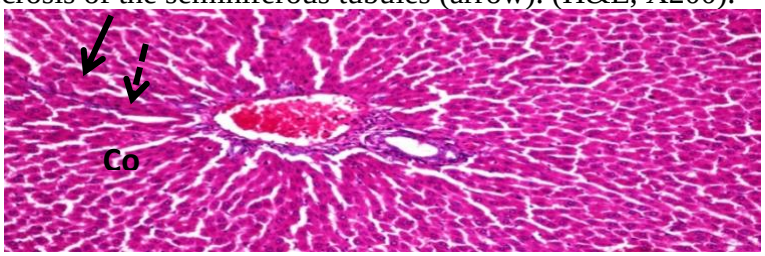


Photo (6): Liver of control positive rat that treated with 10% C. tinctorius showing good protection of the hepatic cells, notice the congestion of the portal vessel (Co) and few proliferated bile ductules (dotted arrow) in the portal area. (H&E, X200).

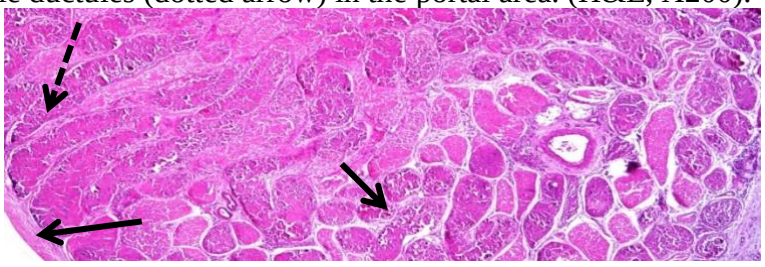


Photo (7): Testis of control positive rat that treated with 10% C. tinctorius showing local coagulative necrosis of the seminiferous tubules (arrow) with calcium globules deposition (dotted arrow) in some of them and thickened blood vessels' wall (short arrow). (H&E, X100).

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دراسة التأثير المحتمل لبذور القرطم على بعض المقاييس البيولوجية وهرمونات الخصوبة فى الفئران

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صممت هذه الدراسة لمعرفه التأثيرات المحتملة لبذور القرطم على مستوى الخصوبة في ذكور الفئران. أجريت هذه الدراسة على (30) فأر ذكر اسبراجو داولى تتراوح أوزانهم بين 160- 170 جم تم تقسيمهم إلى ست مجموعات رئيسية: المجموعة الأولى الرئيسية (الضابطة السالبة) (5 فئران). المجموعة الثانية (5 فئران) تم حقنهم بكلوريد الكادميوم بهدف إصابة ذكور الفئران بضعف الخصوبة. بينما المجموعة الثالثة المصابة بضعف الخصوبة تم تغذيتها ب2% من مسحوق بذور القرطم والمجموعة الثالثة المصابة بضعف الخصوبة تم تغذيتها ب5% من مسحوق بذور القرطم والمجموعة الرابعة المصابة بضعف الخصوبة تم تغذيتها ب7% من مسحوق بذور القرطم والمجموعة الخامسة المصابة بضعف الخصوبة تم تغذيتها ب7% من مسحوق بذور القرطم بينما المجموعة السادسة المصابة بضعف الخصوبة تم تغذيتها ب10% من مسحوق بذور القرطم وذلك لمده 28 يوم. فى نهاية التجربة تم وزن الفئران ثم ذبحها وتجميع عينات الدم بعد صيام 12 ساعة ثم فصل المصل لتقدير سيروم الجلوكوز، وظائف الكبد، وظائف الكلى، ودهون الدم، الهرمونات الجنسية (Testosterone-FSH-LH). وقد أوضحت النتائج ما يلي: المجموعات التي تم معالجتها ببذور القرطم لوحظ فيها زيادة مستوى اثنين من الهرمونات الجنسية (Testosterone-FSH) بينما لوحظ نقص فى هرمون LH ونقص فى (سيروم الجلوكوز والكوليسترول والجليسريدات الثلاثية والليبوبروتينات منخفضة الكثافة والليبوبروتينات المنخفضة جدا فى الكثافة وحمض اليوريك واليوريا والكرياتينين وناقلة أمين الألانين وناقلة أمين الأسبارتات والفوسفاتاز القلوى) وزيادة فى مستوى الليبو بروتينات عالية الكثافة.

الكلمات المفتاحية: هرمونات الخصوبة، كلوريد الكادميوم، مستوى الخصوبة، بذور القرطم