Ameliorative effect of wheat germ and/or seed oils on grape functions lipid profiles and rats hematological, kidnev in coadministreted chlorpyrifos

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SUMMARY

Organophosphorous pesticides such as chlorpyrifos (CPF) are substances worldwide for agricultural purposes. CPF induce oxidative stress leading to the generation of free radicals. The purpose of this study was to assess the biochemical role of wheat germ and grape seed oils on the kidney function tests and the oxidative stress alteration induced by chlorpyrifos in rats, moreover the heamatological, lipid profile. Throughout this study a total number of 70 rats were subjected to experimentation for 4 weeks and divided into 7 groups. The results demonstrated that there were significant decrease in the total counts of RBC's, WBC's. erythrocyte indices. hemoglobin concentration and hematocrit level experimental rats fed diets containing low and high levels of CPF. CPF caused a significant increase in serum creatinine and urea the

increase reached 211.39% and 104.29, % respectively for high dose of CPF received rats as compared to control rats. The treatments with, wheat germ oil, grape seed oil at the tested doses significantly reduced serum creatinine by 27.84% and 15.19% for G6 and G4 respectively. Concerning the values of lipid profile measurement, it was clear that low and high dose CPF received rats induced a significant increase in the level of serum total lipid, total cholesterol and triacylglycerols, also this reflects an elevation in the level LDL-C, VLDL-C and LDL/HDL ratio. Meanwhile, wheat germ oil supplementation reduced the serum LDL-C, VLDL-C and LDL/HDL ratio and increased serum HDL-C. It can be concluded that wheat germ oil and grape seed oils, used are valuable natural antioxidants for protecting against oxidative toxic effects of CPF and cell damage caused by toxic chemicals

Key words: chlorpyrifos, wheat germ oil, grape seed oil, biochemical analysis, rats

INTRODUCTION

Chlorpyrifos (CPF) is a broad-spectrum organophosphorous insecticide utilized extensively in agriculture and for residential pest control throughout the world under different registered trademarks (Saulsbury et al., 2009). CPF elicits a number of additional effects. including hepatic dysfunction. heamatological and immunological abnormalities, embryotoxicity, genotoxicity, neurotoxicity and neurobehavioral changes (Mehta et al., 2009).

Xenobiotics, including pesticides, are known to increase the production of reactive oxygen species (ROS), which in turn generate oxidative stress in different tissues (Rai and Sharma, 2007; Mehta *et al.*, 2009). Chlorpyrifos also induces oxidative stress, and in rat studies this results in the accumulation of lipid peroxidation products in different organs (Verma *et al.*, 2007; Mansour and Mossa, 2009).

Antioxidants have been shown to inhibit free radical formation (Durak *et al.*, 2010). Human diets also contain phytochemicals, such as flavonoids, that are metabolized by the same pathway as toxic man-made chemicals, such as

pesticides and other environmental pollutants (Panemangalore and Bebe, 2009). The antioxidant properties of flavonoids are due to their ability to directly scavenge some radical species. Flavonoids may also act as chain-breaking antioxidants and/or may recycle other chain-breaking antioxidants, such as atocopherol, by donating a hydrogen atom to the tocopheryl radical (Uzun et al., 2010).

Grape (Vitis vinifera) is one of the world's largest fruit crops and grape seed is a complex matrix containing approximately 40% fiber, 16% oil, 11% proteins, and 7% complex phenols including tannins, in addition to sugars, mineral salts, etc. grape seed extract (GSE), a well-known dietary supplement, contains important vitamins, minerals, and polyphenols including flavonoids, proanthocyanidins and procyanidins. It has recently become clear that grape seed oil (GSO) has shown various beneficial pharmacological effects such as its chemoprotective properties against reactive oxygen species and oxidative stress as well as being anti-inflammatory, anti-bacterial, and anti-cancer. Moreover, epicatechin is able to scavenge hydroxyl radicals, peroxyl radicals, superoxide radicals. Procyanidins are reported to have potent antioxidant activity both in vitro and in vivo. (Suwannaphet et al., 2010).

Wheat germ is a rich source of antioxidants that include carotenoids,

tocopherols, flavonoids and phenolic acids. (Vaher et. a l., 2010). Since the rapid increase of the global demand for protein consumption, wheat germ may represent one of the most attractive and alternative source of proteins from cheap vegetable sources (Ge et al., 2000) Most of the essential amino acids from wheat germ proteins are present at concentrations higher than in the reference egg protein pattern (Ge et al., 2001).

MATERIALS AND METHODS

Materials:

- Chlorpyrifos (CPF): It was obtained from Kafr EL-Zayat Pesticides & Chemicals Company, and used as a toxic organophosphorous pesticide. It was added to the experimental tested diets at two levels either low or high (25 and 50 mg/kg diet, respectively).
- Wheat germ oil (WGO) and grape seed oil (GSO) were obtained from El- Gomhoria company. Extracted natural oils and herbs were

added to the experimental diet at a level of 200 mg/kg diet.

Experimental animals and housing:

The health experimental animals used throughout the present work were 70 adult male albino Sprague-Dawely strain, body weight ranged between 98 to 117g. They were obtained from El-Salam-Farm, Giza, Egypt. The animals were divided into 7 homogenous groups and housed individually in plastic cages fitted with a wire mesh bottoms and fronts in a room maintained at 25-30 °C with about 50% relative humidity. The room was lightened on a daily photoperiod of 12hr light and dark. Then, they were allocated to the various experimental diets for 30 days.

The purified experimental diet used in the present study was the balanced diet prepared according to AIN-1993 adjusted by Reeves et al., (1993). The composition of the purified diet is presented in Table (1) during the accommodation period and through out the experiment, food and water were provided ad libitum.

Table (1): Composition of the purified diet * (g/100 g diet)

(g / 100 g diot)
g/100 g diet
62.07
14.00
10.00
3.50
1.00
4.00
5.00
0.18
0.25
0.0008

^{*}AIN-1993, adjusted by Revees et al., (1993)

Calculation the metabolizable energy /Kg

ME (K cal / Kg) =
$$10 [(3.5 \times CP) + (8.5 \times CF) + (3.5 \times NFE)]$$

Where ME = metabolizable energy K cal / Kg

CP = % crude protein

CF = % crude fat

NFE = % nitrogen – free extract (carbohydrate)

The metabolizable energy = 3335 K cal / kg (Jobling 1983)

Experimental design:

The experimental groups received different dietary treatments as follows:-

Group (1) received normal purified diet (control).

Group (2) received (CPF) at low dose (25 mg/kg diet).

Group (3) received (CPF) at high dose (50 mg/kg diet).

Group (4) received (CPF) at low dose with WGO (200 mg/kg diet).

Group (5) received (CPF) at high dose with WGO (200 mg/kg diet).

Group (6) received (CPF) at low dose with GSO (200 mg/kg diet). Group (7) received (CPF) at high dose with GSO (200 mg/kg diet).

Parameters:

1- Animals were weighed weekly, and feed efficiency ratio FER was calculated as described by Guo *et al.*, (2002).

Feed Efficiency Ratio (FER):

$$FER = \frac{Gain in body weight}{Food intake}$$

2- At the end of the experimental period, the animals were fasted for 12hrs, and then anesthetized under diethyl ether anesthesia and

whole blood samples were taken from hepatic portal vein in three centrifuge tubes. The first tube contained ethylene diamine tetra acetic acid (EDTA) and was used for haematological analysis. The second tube contained heparin then centrifuged for 10 minutes at 4000 rpm and the separated plasma was kept in plastic vials at -20 °C till used for the biochemical analysis. The third tube was left for 15 minutes at 37 °C then centrifuged at 4000 rpm for 20 minutes for

separating serum, and then serum was removed and kept in plastic vials at -20° C until analysis.

3. Haematological measurements:

Haematological measurements are one of the most important laboratory work from which enabled to make diagnosis of many obscure conditions. The haematological procedures included methods of absolute counting of red blood cells (RBC's), white blood cells (WBC's) and platelets as well as erythrocyte indices were processed with a blood counter model Kx-21 system (system coulter, electronic kobe, Japan) according to the method described by Flaherty (1991).

4. Kidney function tests:-

4.1 Determination of creatinine concentration:-

The concentration of creatinine in serum was determined by the colorimetric method described by Bartles *et al.* (1972).

4.2 Determination of urea concentration:-

The concentration of urea in serum was determined by the colorimetric procedure described by Fawcett and Soctt (1960).

5 -Determination of lipid profile:-

5.1 Determination of total lipid concentration:

The concentration of total lipid in serum was determined by the colorimetric procedure described by Zollner and Kirsch (1962).

5.2 Determination of total cholesterol concentration:-

The concentration of total cholesterol in serum was determined by the colorimetric method described by Richmond (1973).

5.3 Determination of high density lipoprotein cholesterol (HDL-C) concentration:-

The concentration of HDL-C in serum was determined by the colorimetric method described by Lopez (1977).

5.4 Determination of triacylglycerols concentration:-

The concentration of triacylglycerols in serum was determined by the following colorimetric procedure as described by Fassati and Prencipe (1982).

5.5 Calculation of low and very low density lipoprotein (VLDL and LDL-cholesterol):-

The calculation of LDL and VLD-cholesterol concentration in serum was performed according to the method of Arcol (1989) by using the following two equations: VLDL-C conc. (mg/dl) =

Triacylglycerols

5

LDL-C conc. (mg/dl) = Total cholesterol concentration - (VLDL-C+ HDL-C)

LDL-C conc. (mg/dl) = Total cholesterol concentration – (VLDL-C+ HDL-C)

6-Statistical analysis:-

Statistical analysis was done by using SPSS 11.5 statistical software completely randomization design in factorial arrangement (ANOVA; F-test) and one way classification to determine least significant difference (L.S.D) Levesque (2007)

RESULTS

The results of the effect different dietary treatments WGO and GSO oils on hematological parameters in CPF toxicated rats are presented in (table2)

The results revealed that normal RBC's, WBC's, platelets, Hb and Hct in the control group. There were significantly decreased when comparing the values of control group and values of untreated groups G2 and G3. The percent of reduction in RBC's, WBC's, platelets, Hb and Hct reached -36.69%,-27.61%, -49.71%, -37.33% and -34.91% for G2 and -56.69%, -51.24%, -59.18%, -55.11% and -52.12% for G3 respectively when compared to control. It is clear from the results that the treated groups by wheat germ oil and grape seed oil had achieved a ppositive the improvement in effect upon the deleterious impact of CPF. The increment RBC's levels in treated groups fed on low CPF were 35.27% and 26.38%, G6 and G4 respectively when

compared to G2. Moreover, the RBC's increase for were 57.84% and 56.95% for treated groups, G7 and G5 respectively when compared to G3

The results demonstrated that the effect of WGO oil and GSO oil on creatinine and urea levels in CPF toxicated rats are presented in table 3 low CPF dose causes a significantly increased in creatinine and urea levels but highly increased in case of high CPF dose which cause an increment in urea and creatinine levels by 101.26% and 67.38% for low CPF dose and 211.39%, 104.29% for high dose respectively as compared to health control group. The results demonstrated that induction, WGO (G4) and GSO (G6) diets plus low CPF dose caused a significant decrease in creatinine level. The decrement reached-42.76 and -36.47% when compared to G2 while, GSO (G7) and WGO (G5) diets plus high CPF dose cause a decrease in a creatinine level by -50% and -45.52% when compared to high dose only of CPF (G3) (table 3).

The treatment with wheat germ oil and grape seed oil at CPF tested doses had reduced the concentration of total lipids but the reduction was more evident in G6 (low CPF plus grape seed oil) and percent of change from control level reached 2.57%. While in total cholesterol and TG the improvement were found in G4, G5 received low and high CPF

plus wheat germ oil then G6 received CPF plus grape seed oil then G7 (table 4) From the results shown in table (5) it was clear that there were significant differences between G2, G3 and control by increasing the level of LDL-C, VLDL-C, and LDL/HDL ratio, the percentage of change reached 69.59%, 13.08% and 164.70% in case of groups treated with low

CPF dose respectively, while the percent was 129.33%, 34.22% and 343.5% in group that fed on high CPF dose as compared to control group respectively. Wheat germ oil supplementation reduced the serum of LDL-C, VLDL-C, and LDL/HDL ratio and increased serum of HDL-C.

Table (2): Effect of different dietary treatments (WGO), (GSO) on some hematological parameters in chlorpyrifos toxicated rats:

Rats group Parameters	*RBC's (10 ⁶ /μl)	**WBC's(10³/μl)	Platelets (10³/μl)	*** Hb (g/dl)	**** Hct (%)
Control (G1)	a	ab	a	a	a
	5.15±0.31	8.04±1.80	560.00±85.27	9.00±0.44	28.36±1.39
LCPF (G2)	ь 3.26±0.46	ad 5.82±2.78	bc - 281.60±40.41	ь 5.64±0.76	b 18.46±2.68
HCPF (G3)	c	cd	b	c	c
	2.23±0.64	3.92±1.56	228.60±10.44	4.04±0.86	13.58±3.1
LCPF+WGO (G4)	de	ae	ad	de	b
	4.12±0.51	7.04±1.59	478.00± 94.92	7.24±0.90	19.32±9.72
HCPF+WGO (G5)	be	ac	cef	be	bd
	3.50±0.31	5.34±0.71	372.80±16.75	6.22±0.47	20.20±1.74
LCPF+GSO (G6)	df	ae	de	df	a
	4.41±1.08	7.34±1.93	431.20±115.26	7.80±1.98	24.96±6.17
HCPF+GSO (G7)	be	af	bf	b	b
	3.52±0.49	6.64±1.45	305.20±44.94	5.94±0.58	19.50±1.77

^{*} Red blood cells count (RBC's), ** white blood cells count (WBC's,),), *** hemoglobin,(Hb), and **** hematocrit (Hct %)

[•] Values are expressed as means ± S.D, n=10

[•] There was no significant difference between means have the same alphabetical superscripts in the same column.

Significantly different at P≤ 0.05

Table (3): Effect of different dietary treatments (WGO), (GSO) on kidney functions in chlorpyrifos toxicated rats:

Rats group Parameters	Creatinine mg/dl	Urea mg/dl
Control (G1)	a 0.79±0.13	a 24.22±4.04
LCPF (G2)	b 1.59± 0.18	b 40.54±4.10
HCPF (G3)	c 2.46±0.34	c 49.48± 4.95
LCPF+WGO (G4)	a 0.91±0.22	de 29.34± 3.84
HCPF+WGO (G5)	d 1.34± 0.31	b 39.42± 3.08
LCPF+GSO (G6)	a 1.01± 0.19	df 31.82± 5.04
HCPF+GSO (G7)	d 1.23± 0.23	f35.01± 3.16

- Values are expressed as means ± S.D, n10
- There was no significant difference between means have the same alphabetica superscripts in the same column Significantly different at P≤ 0.05

Table (4): Effect of different dietary treatments on :Serum total lipid, total cholesterol and triacylglycerols in chlorpyrifos toxicated rats:

Rats group Parameters	Total lipids mg/dl	Total Cholesterol. mg/dl	Triacylglycerols mg/dl
Control (G1)	a 369.39± 20.69	a 99.10± 5.83	ab 143.32± 19.20
LCPF (G2)	b 635.01± 38.07	b 130.05± 17.01	a 162.06± 24.64
HCPF (G3)	c 791.53± 48.97	c 159.45± 17.26	c 192.35±18.36
LCPF+WGO (G4)	de 417.39± 60.65	a 101.27± 10.69	bd 132.70± 16.77
HCPF+WGO (G5)	f 485.16± 49.95	ad 105.59± 15.39	ad 152.43± 18.77
LCPF+GSO (G6)	ad 378.88± 35.39	a 95.69± 13.62	ad 146.82± 14.28
HCPF+GSO (G7)	eg 423.40± 46.46	bd 117.03± 11.46	ae 154.95± 16.89

- o Values are expressed as means \pm S.D, n=10.
- o There was no significant difference between means have the same alphabetical superscripts in the same column.
- Significantly different at P≤0.05

Table (5): Effect of different dietary treatments on Serum high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein (VLDL-C), and LDL/HDL ratio in chlorpyrifos toxicated rats

Rats group	HDL-C	LDL-C	VLDL-C	LDL/HDL
parameters	mg/dl	mg/dl	mg/dl	ratio
Control (G1)	a 62.01± 6.85	ab 52.65±12.44	ab 28.66± 3.84	ab 0.85± 0.17
LCPF (G2)	b 40.36± 4.46	c 89.29±19.47	a 32.41±4.93	c 2.25±0.62
HCPF (G3)	c 33.14± 5.14	d 120.74± 16.27	c 38.47± 3.67	d 3.77± 1.12
LCPF+WGO (G4)	d 58.28± 7.89	a 47.88± 9.35	bde 26.54± 3.35	ab 0.83± 0.17
HCPF+WGO (G5)	abcd 55.18± 5.87	be 66.76± 18.72	adf 30.49± 3.75	ae 1.25±0.47
LCPF+GSO (G6)	abcd 53.80± 7.77	abe 63.65± 12.66	aef 29.36± 2.86	ae 1.22±0.36
HCPF+GSO (G7)	abcd 50.60± 9.43	ce 73.36± 9.09	af 30.99± 3.38	e 1.49±0.32

- Values are expressed as means ± S.D, n=10.
- There was no significant difference between means have the same alphabetical superscripts in the same column.
- Significantly different tat P≤ 0.05

DISCUSSION

Chlorpyrifos is a lipophilic molecule which can easily pass through the cell membrane into the cytoplasm. Once inside the cell, CPF can generate a lot of damages. For these reasons, it is necessary to find solutions against this danger. Within this context, nature can provide us many substances that can attenuate this oxidative stress. Most of these substances are found in our alimentation, as a wheat germ oil, grape seed oil. (Jett and Navoa, 2000).

CPF caused decrease in RBC, Hb and Hct, which might be due to the effect of pesticide on blood forming organs suggesting the anaemic condition of the treated animals. The anemia may be due to the inhibition of erythropoiesis and hemosynthesis and to an

increase in the rate of erythrocytes destruction in hemopoietic organs. (Kanter et al. 2009) As well as the leucocytosis observed in present study indicates an immune system to protect the rats against infection that might have been caused by chemical and also secondary infections, which may be contracted after the weakening condition of the rats. Leucocytosis, which may be directly proportional to the severity of the causative stress condition, may be attributed to an increase in leukocyte mobilization, also the present study investigated the protective effects of wheat germ, grape seed oils supplementation in animals subjected to CPF intoxication. Here, Our results are in harmony with the results of Akhtar et al. (2009) who reported that, the Hb and Hct content were significantly decreased in animals exposed to CPF. Tripathi and Srivastav, (2010) concluded

that CPF is extremely effective in causing alterations in red blood cells and white blood cells. These changes may be potentially harmful for the survival of the organism if exposed for long-term to the toxicant, decreased red blood cell number/hemoglobin content may be accounted for by the destruction of red blood cells hemolysis and also degenerating white blood cells have been noticed after toxicant treatment to rats.

Findings of Simonetti et al., (2002) showed that when adminstrated the study group two capsules, each containing 110mg of procyanidins from grape seed extract for total of 30 days grape seed extract may have a significant sparing effect on a-tocopherol in RBC membranes, help reduce oxidative DNA damage, and also might increase the level of polyunsaturated fatty acid in RBC membranes. Also, Keevil et al., (2000) reported that polyphenols may participate in the regulation of vascular tone or in the inhibition of platelet aggregation. Shi et al. (2003) Grape seeds contain lipid, protein, carbohydrates, and 5-8% polyphenols depending on the variety. Polyphenols in grape seeds are mainly flavonoids. including gallic acid. the monomeric flavan-3-ols catechin, epicatechin, gallocatechin, epigallocatechin, and epicatechin 3-O-gallate, and procvanidin dimers, trimers, and more highly polymerized procyanidins.

Grape seed extract is known as a powerful antioxidant that protects the body from premature aging, disease, and decay. Grape seeds contain mainly phenols such as proanthocyanidins (oligomeric proanthocyanidins). Scientific studies have shown that the antioxidant power of proanthocyanidins is 20 times greater than vitamin E and 50 times greater than vitamin C.

Kidney is one of the target organs of experimental animals attacked by acute, sub-OP chronic and chronic exposure experimental compounds. Clinical and evidences of renal damage mediated by oxidative stress will be grouped in glomerular, tubulointerstitial, and endothelial alterations. Oxidative injury may alter the structure and function of the glomerulus mainly due to the effect of ROS on mesangial and endothelial cells (Klahr et al., 1997). These results have been previously reported in a number of researches (Tikoo et al., 2007 and Mansour et al.. 2002). Concomitant, grape seed proanthocyanidin extract (GSPE) treatment lowered the levels of plasma creatinine and urea than the CPF treated group, indicating an increase in the glomerular filtration rate. GSPE treatment provided a significant protection against increased urea and creatinine levels induced by CPF. Rodrigo and Rivera. (2002) demonstrated that together with scavenge free

radicals; polyphenols may avoid their formation through the Haber-Weiss/Fenton reactions, due to their chelating properties. Thus, quercetin chelates intracellular iron thereby avoiding its catalyzing effect on the formation of ROS. Also, quercetin is able to inhibit the activity of transcription factors involved in the production of inflammatory lesions of the kidney thus behaving as an anti-inflammatory agent. Resveratrol, another grape polyphenol, was shown to inhibit the expression of adhesion molecules of the endothelium.

Hence it can be expected that an results antioxidant therapy in relevant glomerular protective effects. Thus. α tocopherol administration was shown diminish glomerulosclerosis in a nephrectomy remnant kidney model in the rat (Hahn et al... 1999). Lipoprotein glomerulopathy has been characterized by a relatively rapid progression to renal impairment and the development of glomerulosclerosis.

Park et al. (2008) recorded that grape seed extract supplements in high fat diet might normalize lipid concentrations, decreased triacylglycerol and total cholesterol concentrations in serum and liver and increased serum HDL-cholesterol and HDL-cholesterol/triglyceride ratio.

The polyphenol-rich grape product was effective in reducing serum total cholesterol

and LDL-cholesterol and in improving the LDL/HDL ratio and the atherogenic index in hypercholesterolemic rats. Kim et al., (2010) demonstrated that GSO fed rats had a significant reduction in total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), and atherogenic index (AI), and the ratio of high density lipoprotein cholesterol (HDL-C) to TC was significantly higher than those of soybean oil (SO) and lard groups. These data suggest that SO supplementation has significant health benefits through favorable alterations in plasma lipid profiles in rats. GSO may be good dietary oil and may provide health benefits in hyperlipidemia and related complications.

Natella et al. (2002) found that grape seed may also have some benefit in the prevention of the progression of atherosclerosis. It has been suggested that an increase in plasma, LPO is associate with imbalance between oxidant/antioxidant leading to an increase in the susceptibility of LDL (low density lipoprotein) to oxidation. This leads to postprandial hyperlipemia that is a risk factor for atherosclerosis. Moreover he stated that oligomeric proanthocyanidins supplementation resulted in decreased lipid peroxidation, increased plasma antioxidant levels, and improved resistance of LDL to oxidation in volunteers consuming a lipid-rich test meal. Also Dugas et al., (2000) reported that

biological actions of polyphenols include the reduction of the susceptibility of low density lipoproteins (LDL) to oxidation both in vitro and in vivo an effect likely due to the property of these compounds to scavenge free radicals.

Johanna et al. (2003) demonstrated that octacosanol is the main component of a natural product wax extracted from plants found in a wheat-germ oil extract has many uses for treating various conditions. The most widely studied of these are its cholesterol lowering properties, and many studies have shown that octacosanol is very effective in lowering LDL and increasing HDL, and also the effect of octacosanol on the enzymes involved in lipid metabolism and found that the rate-limiting step in the esterification of fatty acid into triacylglycerol was decreased by octacosanol in rats fed a high-fat diet. This indicated that a step in the cholesterol biosynthetic pathway was inhibited by octacosanol, which was dependent on dietary fat content. Said et al. (2008) reported that wheat germ oil is a natural unrefined vegetable oil. It is an excellent source of vitamin E, octacosanol, linoleic and linolenic essential fatty acids, which may be beneficial in neutralizing the free oxygen radicals.

This study provides an easy and relatively cheap natural product (wheat germ oil and grape seed oil) as natural antioxidants for protecting against oxidative toxic effects of CPF and cell damage caused by toxic chemicals. The results confirmed that their administration improved the complete blood picture, reduced the lipid profile thus enhance kidney function.

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التأثير المحسن لزيت جنين القمح وزيت بذور العنب على الدم ووظانف الكلى، ومستوى الدهون في الجرذان المعطاة كلور بيرفوز

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اجريت التجربة على ٧٠ من ذكور الفنران البالغين Sprague Dawely وقسمت الى 7 مجموعات بالتساوى و استمرت التجربة لمدة ٤ اسابيع وكانت المجاميع المختبرة كالتالى:

المجموعة الاولى: فتران اصحاء تغذت على غذاء متوازن (مجموعة ضابطة).

المجموعة الثانية: فنران تغنت على تركيز منخفض من الكلوربيرفوز (٢٥مجم/كجم) من الوجبة الغذائية. المجموعة الثالثة: فنران تغنت على تركيز مرتفع من الكلوربيرفوز (٥٠مجم/كجم) من الوجبة الغذائية.

المجموعة الرابعة: فنران تناولت التركيز المنخفض من الكلوربيرفوز (٢٥مجم/كجم) + زيت جنين القمح ٢٠٠مجم/كجم من الوجبة الغذائية.

المجموعة المقامسة: فنران تناولت التركيز المرتفع من الكلوربير فوز (٥٠مجم/كجم) + زيت جنين القمح ٢٠٠ مجم/كجم من الوجبة الغذائية.

المجموعة السادسة: فنران تناولت التركيز المنخفض من الكلوربير فوز (٢٥مجم/كجم) + زيت بذور العنب

المجموعة السابعة: فنران تناولت التركيز المرتفع من الكلوربيرفوز (٥٠مجم/كجم) + زيت بنور العنب ١٠٠مجم/كجم من الوجبة الغذائية.

اظهرت نتائج هذه الدراسة ما يلى:

- أظهرت نتائج التقيم الهيماتولوجي للدم الى انخفاض كرات الدم الحمراء وكرات الدم البيضاء والصفائح
 الدموية وكلا من محتوى الهيموجلوبين والهيماتوكريت بصورة معنوية كبيرة فى المجموعتين المتناولتين
 لتركيزين الكلوروبيرفوز ،بينما تحسنت المجموعات المعالجة الاخرى بزيت جنين القمح وزيت بذور العنب
- أكدت نتائج وظائف الكلى أن هناك أرتفاع معنوى في قيم الكرياتينين واليوريا يصل الى ٢١١.٣٩%، ٢٠٤٢% على التوالى بالنسبة للمجموعة المتناولة التركيز العالى من الكلوروبيرفوز، بينما التحسن لوحظ

- فى المجموعات المتناولة،زيت جنين القمح ثم زيت بذور العنب فأنخفض الكرياتينين بنسبة ٢٧.٨٤%، ، ٩ ١ . ٥ ١ % لكل من المجموعة السادسة والرابعة على التوالي .
- أوضحت النتائج البيوكيميائية لمكونات ليبيدات الدم انه في المجموعة ذات التركيز المنخفض والمرتفع من تناول الكلوروبيرفوز كان ظهور الزيادة المعنوية لكلا من الليبيدات الكلية والكوليستيرول الكلى وثلاثي آسيل الجليسرولات في السيرم بالمقارنة بالمجموعة الضابطة وقد انعكس هذا على الزيادة في مستوى الكوليستيرول المرتبط بالليبوبروتين المنخفض الكثافة والمنخفض الكثافة جدا ونسبة ال LDL/HDL ، بينما أشارت النتائج الى ان تناول زيت جنين القمح أدى الى انخفاض في VLDL-C ، LDL-C ونسبة ال HDL-C ونسبة ال

يوصى البحث باضافة زيت جنين القمح ، زيت بذور العنب الى الوجبات حيث أنهمايحتويان على مضادات أكسدة ولهم فوائد متعددة لتحسين الصحة العامة و صورة الدم الكاملة وانخفاض مستوى الدهون فى الدم ووظائف الكلى ،كما أنهما يساعدان على تخلص الجسم من الشقوق الحرة والسموم.