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Correspondence:
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365 Ramses St- Ain Shams University, Faculty of Specific Education
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يسر **معامل التأثير والاستشهادات المرجعية للمجلات العلمية العربية (ارسيف – ARCIF)**، أحد مبادرات قاعدة بيانات **"معرفة"** للإنتاج والمحتوى العلمي، إعلامكم بأنه قد **أطلق التقرير السنوي السابع للمجلات للعام 2022**.

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أ.د. سامي الخزندار

رئيس مبادرة معامل التأثير" ارسيف Arcif"

+962 6 5548228 -9
+ 962 6 55 19 10 7
info@e-marefa.net
www.e-marefa.net
Amman - Jordan
2351 Amman, 11953 Jordan

محتويات العدد

تابع محتويات العدد

Effect of intake supplementation choline powder and food rich of it on immune response and oxidative stress induced by diazinon toxicity in rats

Dr. Hala R. A. Sopeah [1]

[1] Home Economics Department, Nutrition and Food Science, Faculty of Specific Education, Ain Shams University

Effect of intake supplementation choline powder and food rich of it on immune response and oxidative stress induced by diazinon toxicity in rats

Hala R. A. Sopeah

Abstract

Diazinon is an organic compound of organophosphate insecticide formerly used to control cockroaches, silverfish, ants, and fleas in residential. Choline is one of the essential nutrients for the body, which has the characteristic of solubility in water, and it is usually composed of B-complex vitamins. Generally choline refers to different types of quaternary ammonium salts, it is important for maintaining good health in the body and strengthening the immune system. This study aimed to verify the protective effects of food rich in choline against daizinon intoxication in rats infected with oxidative stress and on immune response. Forty male wistar rats weighing 170 ±20g were divided into four equal groups; 10 animals rats (negative group) and 30 rats were take Diazinon in dissolved (70mg/Kg bw orally daily for four weeks), which were classified into the control positive group and two treated rats groups that treated by choline powder (20 mg/kg orally daily for four weeks), peanuts (5, 5 g daily for four weeks, this average taken per rat) respectively. Rats were orally administered their relevant doses daily for four weeks. Blood samples were collected and picture (CBC) and serum was separated to evaluate the different immunological parameters and biochemical. The results revealed that, all tread groups and the negative group showed a significant increase in blood levels of IFN ɤ, RBCs, Hb, Mcv, WBCs, Lymphocytes, Monocytes count, GPx ,GSH , SOD and CTA, but a decrease in IL-1β, TNF-α, MCV, AST, ALT, creatinine, urea and uric acid compared with a positive group. While the fourth group (choline in nutrition) showed improvement than third group (choline powder) in IL-1β, TNF-α, Hb, Mcv, Lymphocytes, Monocytes, GPx, SOD,CTA, AST , ALT and Uric acid. Group 4 which intake food rich in choline revealed no histopathological changes in the liver and kidney. In conclusion, taking foods rich in choline significantly reduces the acute immune response and oxidative stress so it can be replaced with nutritional supplements.

Keywords: Choline, Immune response, Oxidative stress, Diazinon

Introduction

Diazinon (O, O-Diethyl O-[4-methyl-6-(propan-2-yl) pyrimidin-2-yl] is an organophosphate insecticide that is most widely used to combat cockroaches, silverfish, ants, and fleas **(Grafft et al., 2002 and Assaraj et al., 2018)**. In agriculture and horticulture, it has been used extensively to control insects in crops all over the world **(Goodman et al., 1979**). The mechanism of the toxic effect of diazinon is the same as that of other organic substances by inhibiting a whole series of enzymes, in particular acetylcholinesterase, which is an enzyme essential for the proper functioning of the nervous system. Toxic, non-essential toxic metal, a pollutant that endangers human and animal health, both environmental and occupational **(Sastry and Sharma, 1980 and Ansari et al., 1987)**.

Choline is a quaternary amine that can be formed in the liver, it is similar to the B vitamins, Foods such as liver, muscle meats, fish, nuts, peas, beans, spinach, wheat germ, and eggs also contain it. Peanuts contain choline at 61 mg per 100g **(Shaw et al., 2004 and Saliner et al., 2019)**. This is obtained from the diet and several organs, particularly the liver. For liver disorders, including chronic hepatitis and cirrhosis, choline is used. It is often used for depression, memory loss, Alzheimer's disease, a brain disorder called cerebellar ataxia, some forms of seizures, and a psychiatric illness called schizophrenia since it is necessary for the development of acetylcholine. In addition dietary choline decreased the oxidant damage and regulated the antioxidant in the immune system (**Pei et al., 2014)**. According to the United States Department of Agriculture, an estimated adequate intake (AI) of 150 mg of choline per day for children, while AI for males aged 20-59 was 450 mg of choline per day on average, and females need around 400 mg per day in the same age group (**USDA., 2022)**.

An imbalance in your body between free radicals and antioxidants is oxidative stress. Free radicals are molecules with an uneven number of electrons that contain oxygen. The uneven

number enables them to react with other molecules easily (**Halliwell, 2007)**. Free radicals in your body can cause large-chain chemical reactions because they react with other molecules so easily. Such responses are called oxidation. They can be helpful or damaging (**Hwang, 2013 and Roma et al., 2015)**.The damage can lead over time to a large number of illnesses (**Joseph et al., 2015)**. These include atherosclerosis, or the hardening of the blood vessels, cardiac disease, inflammatory conditions, high blood pressure, cancer and immune response such as immunodeficiency (**Haider et al., 2011)**.

By recognizing and responding to antigens, the immune system protects the body from potentially harmful substances. Substances (usually proteins) on the surface of cells, viruses, fungi, or bacteria, are antigens. Substances that are non-living, such as toxins, chemicals, narcotics, and foreign particles (**Azcutia et al., 2017)**. The immune system identifies and eliminates compounds that contain antigens, or attempt to destroy them. The immune response is how the body identifies and protects itself against foreign and harmful bacteria, viruses, and substances (**Bonilla and Oettgen, 2010 and Mohamed et al., 2014)**. At the site of infection, oxidative stress can affect the immune response, Immune activation releases highly reactive species that destroy pathogens but can also cause oxidative damage to host tissues (**Ercal et al ., 2001 and Viviana and Heinz., 2014)**.

Therefore, this study aims to confirm the effect of consuming foods rich in choline and choline powder on the toxicity of diazinon which causes an immune response oxidative stress in rats.

Materials and methods

Experimental animals and design:

Forty male wistar rats weighing about 170±20 g were obtained from the Animal House, Agricultural Research Center, Cairo. All animals were caged and maintained on a standard diet

and had free access to water. In a temperature-controlled room (25±2 ° C) with a 12 h light and 12 h dark exposure, the rats were housed in stainless steel cages, with free access to tap water and were acclimatized for one week before the experiment was completed. In both classes, the body weights of experimental rats were estimated weekly in order to adjust the dose of chemicals administered using an electronic balance.

The animals were randomly divided into four groups 10 animals rats served as the normal control group (negative group) and 30 rats were taken Diazinonin was bought from Al-Gomhoria Pharmaceutical Company Cairo) dissolved in distilled water to obtain the required dose concentration (70 mg/Kg bw orally daily for four weeks) **(Assaraj et al., 2018),** which classified into control positive group and two treated rats groups, group three and four that treated with choline powder (20mg/kg orally daily for four weeks) it was bought from Al-Gomhoria Pharmaceutical Company Cairo, and peanuts (5.5 g daily for four weeks this average taken per rat) respectively that bought from the local market, and this quantity of peanuts contains a similar amount of choline, where the average intake of choline per rat was about 3.4 mg /day from powder choline or peanuts that contain the same amount of choline.

Samples collection and parameters measured:

Blood samples were collected in test tubes containing ethylene diamine tetra acetic acid (EDTA) anticoagulant disodium salt and used to assess red blood cell counts (RBCs), haemoglobin (Hb) concentrations, hematocrit (HCT) and erythrocyte indices, mean corpuscular volume (MCV) and white blood cell counts (WBCs). With Giemsa stain, blood films were stained and differential leukocyte count and Lymphocytes Monocytes were performed by normal haematological techniques (**Hariri et al., 2018)**. The second blood samples were obtained without anticoagulant in the test tubes, the samples were centrifuged for 10 min at 3000 rpm and the clear serum was extracted carefully from all the samples. Enzyme Amplified

Susceptibility Immunoassay using microplates determined selective humoral immunological parameters Tumor Necrosis Factor-Alpha (TNF-α), Interleukin 1-Beta (IL-1β) and Interferon Gamma (IFN ɤ). For IL-1β-3.1 pg/mL, for TNF-α and IFN ɤ 6.2 pg/mL, the lower detection limits are 1.5 pg/mL and data is provided as cytokine pg /mL serum (**Donnelly et al., 2005)**.

From undiluted serum samples, antioxidant markers, reduced glutathione (GSH), superoxide dismutase (SOD) (**Satoh, 1978)**, catalase (CAT) and oxidized glutathione (GPx) were calculated using commercially available ELISA Kits.The plates were read on the computerized automated microplate reader ELISA and ALT, AST at 450 nm and a correction wavelength of 550 nm, while total protein, albumin, uric acid, urea, and creatinine were spectrophotometrically estimated (**Nishikimi et al., 1972)**.

The activity of serum alanine and aspartate amino transferees (ALT and AST) enzymes was carried out using the methods describe by **(Bergmeyer and Horder, 1980)**, and (Kind and Serum creatinine, urea and uric acid were enzymatically determined according to **(Bonsens and Taussky, 1984)**

Histopathological Examination:

The liver of the sacrificed rats was washed in slain solution, dried by filter paper, weighed, and stored frozen in formalin solution 10% for histopathological testing according to the method mentioned by, **Drury and Wallington, (1980).**

Statistical analysis:

Data were analyzed using SPSS 11.0 for windows. The significance was calculated using a one-way analysis of variance (ANOVA) and followed by Tukey multiple comparison procedures to calculate significance. $P< 0.05$ value was taken as statistically significant.

Results

Data presented in table (1), it could be showed that the mean value of (BWG) of the control (-) group was higher than the control (+) group. The best (BWG) level in the treatment group was in group 4. It could be noticed that the mean value of FI and FER of the control (-) group was higher than the control (+) group, besides group 4 was higher than group 3 in FI and FER.

Table 1: Effect of choline powder and food rich in it (peanuts) on body weight gain (BWG), feed intake (FI) and feed efficiency ratio (FER) on rats infected with immunodeficiency and oxidative stress

Parameters	Experiment groups			
	Group1 (Negative control)	Group 2 (Control positive)	Group 3 (choline powder)	Group 4 (peanuts)
BWG (g) Mean ± SD	0.63 ±0.006 [a]	0.11±0.004 [c]	0.52 ±0.004 [b]	0.54±0.005 [b]
FI (g) Mean ± SD	20.24 ±0.005 [a]	14.50 ±0.004 [b]	19.59±0.003 [a]	19.99 ±0.002 [a]
FER (%) Mean ± SD	0.031±0.0001 [a]	0.012±0.0008 [b]	0.031±0.0005 [a]	0.032±0.0005 [a]

Each value is the mean ±SD

The different letters means that there is a significant difference between groups at P <0.05 and vice versa.

The serum cytokines results from the present study indicate that (Interleukin-1 Beta) IL-1β and (Tumor Necrosis Factor-Alpha) TNF-α were significantly higher in the control positive group, while the serum level of (Interferon Gamma) IFN ɤ, significantly decreased in the control positive group as compared with the negative the control groups. Besides, groups 3 and 4 showed a significant increase in IFN ɤ and TNF-α while IL-1β significantly decreased in group 3 and increase in group 4 when compared with negative control rats as shown in Table 2.

Table 2: Effects of choline powder and food rich of it (peanuts) on cytokines markers (mean ± SD), 4 weeks post treatment.

Parameters	Unit	Experiment groups			
		Group1 (Negative control)	Group 2 (Control positive)	Group 3 (choline powder)	Group 4 (peanuts)
IFN ɤ	pg/ml	40.1 ± 1.1b	33.3± 0.6c	43.2± 1.2a	42.6± 1.3a
IL-1β	pg/ml	36.6 ± 0.9c	41.7± 2.2a	35.1± 0.6c	37.4± 1.6b
TNF-α	pg/ml	25.9 ± 1.2c	32.5± 1.3a	27.3± 0.9c	29.2± 0.7b

Each value is the mean ±SD
The different letters means that there is a significant difference between groups at P <0.05 and vice versa.

Diazinonin in the group two showed a significant decrease (P < 0.05) in RBCs , Hb, WBCs, Lymphocytes and Monocytes count, while Mcv only count was significantly increased when compared with the control negative group. There was a significantly increased in Mcv count in group3 (choline powder), but there was a significantly decreased in RBCs ,Hb, WBCs, Lymphocytes and Monocytes count as compared to the control negative group. In addition, there was a significantly increased in Hb and Monocytes count in group 4 (peanuts), but there were a significantly decreased in RBCs, Mcv, WBCs and Lymphocytes count as compared to the control negative group (Table 3).

Table 3: Hematological picture (mean ± SD), 4 weeks post treatment with choline powder and food rich of it (peanuts).

Parameters	Unit	Experiment groups			
		Group1 (negative control)	Group 2 (control positive)	Group 3 (choline powder)	Group 4 (peanuts)
RBCs	106/L	7.2 ± 0.9a	5.1± 0.8c	6.8± 0.2b	6.6± 0.3b
Hb	g/dL	12.7 ± 1.9a	9.5± 1.2c	11.5± 1.1b	13.4± 0.5a
Mcv	Fl	70.3 ± 2.2b	75.2± 0.9a	71.2± 0.7b	68.1± 1.1c
WBCs	103/L	14.5 ± 0.8a	10.7± 1.4c	12.6± 1.1b	11.9± 0.9b
Lymphocytes	103/L	7.7 ± 0.5a	6.1± 0.7c	7.1± 0.6b	7.4± 0.3a
Monocytes	103/L	0.51 ± 0.05a	0.33± 0.02c	0.45± 0.08b	0.52± 0.03a

Each value is the mean ±SD
The different letters means that there is a significant difference between groups at P <0.05 and vice versa.

Results in table 4 showed that in the positive group a significant decrease (P < 0. 05) in antioxidant enzymes, GPx, GSH, SOD and CTA when compared to the control negative group. On the other hand, group3 (choline powder) and group 4 (peanuts) were a significantly decrease in GPx, GSH, SOD and CTA as compared to the control negative group. While group4 (peanuts) showed an increase significantly in GPx, SOD and CTA as compared to group3 (choline powder).

Table 4: Effects of diazinonin and choline powder and food rich of it (peanuts) on antioxidant and oxidative stress markers (mean ± SD), 4 weeks days post treatment.

Parameters	Unit	Experiment groups			
		Group1 (negative control)	Group 2 (control positive)	Group 3 (choline powder)	Group 4 (peanuts)
GPx	µmol/mL_	19.2± 0.5a	14.5± 0.4c	16.1± 0.8b	17.8± 0.7b
GSH	µmol/mL_	21.2± 0.3a	13.9± 0.3c	18.8± 0.4b	18.1± 0.5b
SOD	U/mL	2.5± 0.2a	1.9±0.6c	2.1± 0.3b	2.4±0.5 a
CAT	U/mL	30.2± 0.7a	19.7± 0.7c	26.8± 0.8b	28.7± 0.4a

Each value is the mean ±SD

The different letters means that there is a significant difference between groups at P <0.05 and vice versa.

Table 5; include results pertaining to the hepatic markers, results showed that AST and ALT levels were higher in group 2 in comparison with the control negative group. All hepatic markers were none significantly changed in group 3 and 4, when compared with the control negative group. While there was a statically significant decrease in AST and ALT levels in group 4 compared with group 3.

In addition to the renal markers results showed that creatinine, urea and uric acid levels were higher in group 2 in comparison with the control negative group.

There were significant decreases in urea, creatinine and uric acid levels in group3, as compared to the control positive group. On another hand, there was a significant increase in, creatinine and urea levels and a decrease in uric acid in group4 as compared to the control negative group.

Table 5: Liver and kidney function biomarkers (mean ± SE), 4weeks days post treatment with choline powder and food rich of it (peanuts).

Parameters	Unit	Experiment groups			
		Group1 (negative control)	Group 2 (control positive)	Group 3 (choline powder)	Group 4 (peanuts)
AST	IU/L	55.2± 0.4c	69.1± 0.6a	57.1± 0.9b	53.2± 1.2c
ALT	IU/L	49.5± 0.8b	70.3± 0.4a	48.7± 0.9b	45.1± 0.4c
Creatinine	mg/dL	0.51± 0.05b	0.79± 0.06a	0.42± 0.08c	0.53± 0.03b
Urea	mg/dL	39.5± 0.4c	58.2± 0.3a	44.5± 1.2b	46.3± 0.9b
Uric acid)	mg/dL	0.89± 0.06b	1.2± 0.03a	0.97 ± 0.05b	0.83 ± 0.08c

Each value is the mean ±SD

The different letters means that there is a significant difference between groups at P <0.05 and vice versa.

Histopathological changes of liver:

Liver of rats from group 1 showed the normal histological structure of the hepatic lobule (Fig. 1). Besides, the liver of rats from group 2 revealed thickening of glissonian's capsule and cytoplasmic vacuolation of hepatocytes Liver of rats for control positive and focal area of hepatic necrosis completely replaced by leucocytic cells infiltration (Figs. 2). On the other hand, some sections from group 3 showed cytoplasmic vacuolation of centrilobular hepatocytes in some examined sections but in a slight way (Fig. 3). Moreover, liver of rats from group 4 revealed no histopathological changes (Fig. 4)

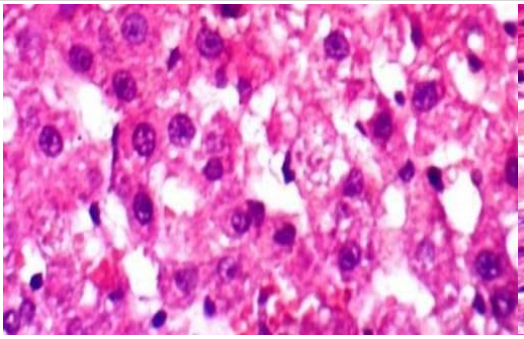

Fig. 1: Liver of rats for the control negative group showed the normal histological structure of hepatic lobule (Hand E X 200).

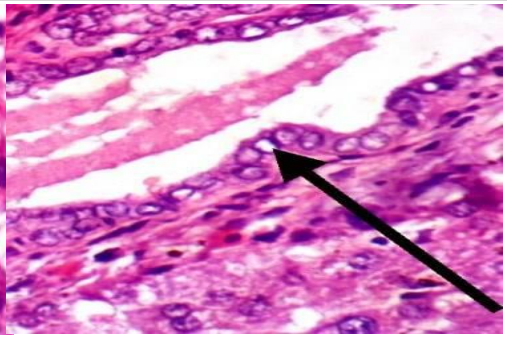

Fig. 2: liver of rats for the control positive group showed a focal area of hepatic necrosis completely replaced by leucocytic cells infiltration (Hand E X 200).

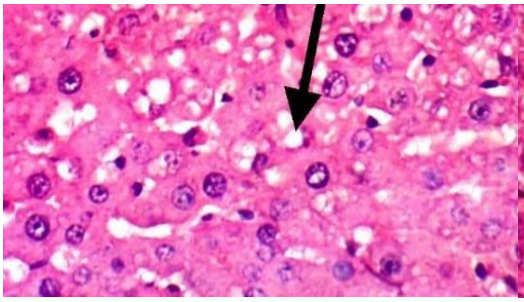

Fig. 3: liver of a rat from group 4 showed cytoplasmic vacuolation of centrilobular hepatocytes (Hand E X 200).

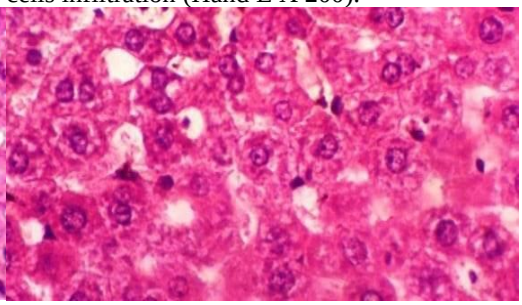

Fig. 4: Liver of rats from group 4 that intake choline in food showed no histopathological changes (Hand E X 200).

Histopathological examination of kidneys:

Kidneys microscopically, of rats from group 1 revealed the normal histological structure of renal parenchyma (Figs. 1). However, kidneys of rats from group 2 showed that proteinaceous materials in the lumen of renal tubules, cytoplasmic vacuolation of epithelial lining renal tubules,and congestion of glomerular tuft and focal inflammatory cells infiltration (Fig. 2). Beside group 3 and 4 showed no histopathological changes (Fig. 3) and (Fig. 4) respectively.

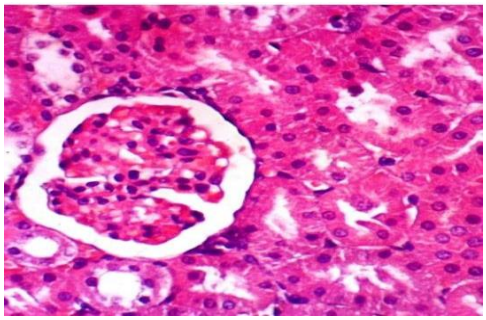

Fig. (1): Kidney of rat from group 1 showed the normal histological structure of renal parenchyma (Hand E X 200).

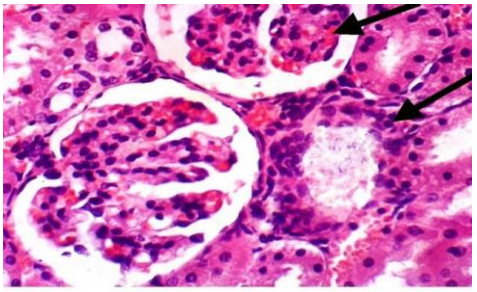

Fig. (2): Kidney of a rat from group 2 showed proteinaceous materials in the lumen of renal tubules and congestion of glomerular tuft and
focal inflammatory cells infiltration (Hand E X 200).

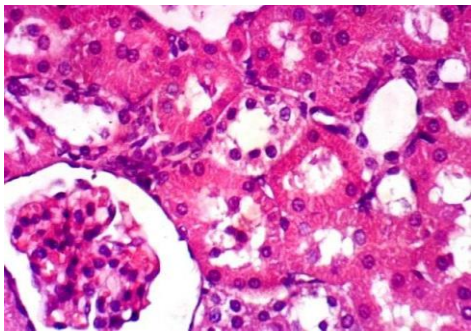

Fig. (3): Kidney of rat from group 3 showed the normal histological structure of renal parenchyma (Hand E X 200).

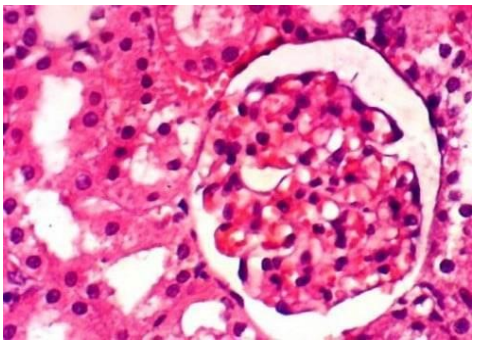

Fig. (4): Kidney of rat from group 4 showed the normal histological structure of renal parenchyma (Hand E X 200).

Discussion

According to the current study in table 1, showed that diazinon exposure to rats resulted a reduction in BWG, FI, FER and dose-related histopathologic damage in the liver and kidneys in particular. This is consistent with previous studies that also showed diazinon exposure to mice induced of reducing in BWG,FI, FER, beside the exposed mice's livers displayed vacuolization in hepatocytes, mononuclear cell infiltration, congestion, expansion of the veins, and an increase in Kupffer cells. Diazinon-treated groups showed mononuclear cell infiltration, glomerular degeneration, glomerular loss, and congestion in the kidney tissue. While diazinon may have negative impacts on organisms like humans, **Temitayo et al.,**

(2016) and Saeed et al., (2020) . While treatment with choline, whether natural from food or powder, showed an improvement in the cells and tissues of the liver and kidneys, and this result agrees with **Shaw et al., (2004) and (Viviana and Heinz. 2014).**

In the present study in table 2, showed a significant increase in IFN ɤ and TNF-α while IL-1β significantly decreased in group 3 and increase in group 4 when compared with negative control rats, This finding is in accordance with a study that examined the impact of lactoferrin on immune response in rats exposed to diazinon for seven weeks and DZN at a dose of 70 mg/kg body weight for four weeks. In this study, WBCs were found to be significantly lower in the (LF DZN) group, and IL-1 β, TP, albumin, and globulin were found to be significantly higher (P 0.05) **(Assaraj et al., 2018)**. Beside Management of mice with colitis was documented in the study as additional regulation of IL-1β and IL-10 anti-inflammatories was later discovered **(Guillen et al., 2002)**. Additionally, LF decreases colitis in rats and increases the release of the anti-inflammatory cytokines IL-1β **Togawa et al., (2002)**.

The immunomodulatory response of choline was assessed in the current study by a significant increase in IFN- and a decrease in IL-1β and TNF- in peanuts and the choline powder groups compared with the control positive group. It has been reported that IFN- activates macrophages, whereas IL-1β prevents macrophage activation) **(Saeed et al., 2020)**.

Although dietary choline has the potential to affect systemic immunity, it is yet unknown whether this is predominantly due to direct effects on immune cells or incidental consequences of changed metabolic function. Monocytes from cows in the middle of lactation that had been given choline linearly increased the abundance of numerous genes that code for choline metabolic enzymes. These findings show that choline controls immune cells' inflammatory response and imply that one or more of its metabolic products may be involved in the mechanism **Temitayo et al., (2016)**. Choline, a crucial nutrient, is

a selective nicotinic acetylcholine (ACh) receptor agonist at the 7-nm position. It is crucial for several metabolic and non-metabolic pathways, such as inflammatory pathways **Ahmed et al., (2019)**. A particular amount of dietary choline has been demonstrated to reduce TNF-, IL-1, and the relative expression of TGF- 2 mRNA in immunological organs, which attenuates inflammation brought on by the challenge of hydrophilia (intrapulmonary injection of Aeromonas as a semi-lethal dosage of endotoxin) **Wu et al., (2013)**, and agreement with liver TNF-expression (**Kawaratani et al., 2008)**.

In the present study in table 3, hematological picture results showed, Diazinonin group showed a significant decrease in RBCs ,Hb, WBCs, Lymphocytes and Monocytes count , while Mcv only count was significantly increased when compared with the control positive group and treatment groups, This result is consistent with Muhammad and Abdul whose exposed mice to two sub-lethal concentrations of diazinon for 30 days (0.815 mg/L and 1.63 mg/L) and found a significant decline in erythrocyte count, haemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular haemoglobin, plasma levels of total protein, albumin, globulin, and enzyme activity **(Muhammad and Abdul, 2014).** These concur with research conducted by **Saeed et al., (2020)** on rats given DZN (15 mg/kg, orally) for 4 weeks, then in comparison to age-matched control rats, the acquired data showed that DZN dramatically reduced RBCs, Hb, and MCV in adult and elderly rats . The considerable decrease in PCV, Hb, and RBC counts seen in the group of rats given DZN alone when compared to the control group suggests that anemia has developed in this group of rats **(El-Shenawy et al., 2009)**. The significant decreases in RBC count, haemoglobin concentration, and hematocrit percentage supported the conclusion that DZN caused anemia. Several earlier studies have supported the hematotoxic effects of DZN **Biochem, (1981) and El-Demerdash and Nasr (2013).**

There was a significantly increased in Mcv count in group3 (choline powder), but there were a significantly decreased in RBCs ,Hb, WBCs, Lymphocytes and Monocytes count as compared to the control group. In addition to there was a significantly increased in Hb and Monocytes count in group4 (peanuts), but there were a significantly decreased in RBCs, Mcv, WBCs and Lymphocytes count as compared to the control group. This outcome is consistent with **Biochem, (1981)** that showed choline raises RBC, Hb, and WBC levels in rats. And concur with **El-Shenawy et al., (2009)**, who claimed that feeding weanling rats a synthetic meal to which no choline was added caused choline deficiency, leading to a drop in monocytes, lymphocytes, RBCs, WBCs, and haemoglobin.

On the other hand SOD, CAT, GPx, and GSH are the primary components of the enzymatic antioxidant defense system, which guards cells against the toxicity of reactive oxygen species (ROS) and lipid peroxidation. Superoxide anion radical is changed by SOD into hydrogen peroxide, which is then broken down by CAT into water and oxygen **(Altuntas et al., 2006)**. A selenoenzyme called GPx catalyses the conversion of GSH to GSSG, scavenging H2O2 in the process, additionally, the most prevalent selenoprotein in mammals is GPx (**Husain et al., 1987 and Tinggi., 2008)**.

According to the current findings, therapy in table 4 for groups 3 and 4 (peanuts and choline powder, respectively) resulted in a considerably higher level of GPx, GSH, SOD, and CTA than the control positive group. As opposed to group 3, group 4 (peanuts) shown a considerable rise in GPx, SOD, and CTA. However, GPx, GSH, SOD, and CTA levels in group 3 (choline powder) were considerably higher than in group 2 positive control. This finding, which is consistent with **(pei et al., 2014)** whose demonstrated that choline administration led to a significantly higher level of GSH, SOD, CAT, and GPx activity in fish serum. And concur with **(Suhaniza et al., 2013)**, who demonstrated that a diet low in choline causes a drop in the

antioxidant enzymes superoxide dismutase (SOD), catalase (CTA), and glutathione peroxidase (GPx).

While the present study showed the positive group a significant decrease in antioxidant enzymes, GPx ,GSH , SOD and CTA when compared to the control negative group. On the other hand, **(Altuntas et al., 2002)** of the participants confirmed that adiazinon reduced erythrocyte SOD activity. Due to the impacts of pesticides on cells, SOD and CAT activity is reduced. The extent of this compensatory mechanism activation depends on the level of oxidative stress and, consequently, the stressor dose. The difficulties responding to the produced free radicals is the reason of the decreased CAT activity, showing that the entire antioxidant defense mechanism failed to protect the tissues from mechanical damage brought on by pesticides, as shown by lipid peroxidation **(Altuntas et al., 2006 and Ahmed, 2006)**. On the other hand agree with **(Pei et al., 2014)** who evaluates the effects of various levels of dietary choline on antioxidants in the spleen and head kidney of juvenile Jian carp, while increasing levels of SOD, CAT, GPx and GR gene in the spleen for a group that intakes high choline, dietary choline decreased the oxidant damage and regulated the antioxidant system in immune organs of juvenile Jian carp.

According to the current study in table 5, showed in group 2 AST, ALT, creatinine, and urea levels were greater than those in the control negative group. This might be because group 2 was exposed to a diazinon, which raises the levels of AST, ALT, creatinine, and urea. This outcome is consistent with Ahmed's findings from 1996, which showed that rats given diazinon for three weeks had transaminase (AST, ALT) elevations. Additionally, **Kalender et al., (2005)** found that rats given diazinon had higher levels of ALT, AST, ALP, total cholesterol, and triglycerides. Transaminases were thought to be a more accurate indicator of both liver damage and function. According to **Hatoff and Hardison (1980)**, acute hepatocellular injury, extrahepatic blockage, or both, were the main causes of increases

in these enzymes' serum levels. **(Sarhan et al., 2011)** demonstrated that diazinon caused renal tubule degeneration, glomerular enlargement, and leucocytic infiltrations.

These findings showed that diazinon metabolites were harmful to the kidneys and that the immune system is an effective defence against foreign invaders. Male albino rats given diazinon orally for two months displayed renal tubule deterioration **(Sherlock, 1981).** According to **El-Shenawy et al., (2009),** diazinon exposure resulted in interstitial inflammatory cell infiltrations, shrinkage of the glomeruli, and degeneration of the renal tubules in mice. In addition, a considerable rise in serum urea and creatinine in reaction to diazinon poisoning was also seen in **(Sarhan et al., 2011)**. **El- Shenawy et al., (2009)** obtained comparable outcomes in mice. In the present study in group 3 and 4 there were decreases in ALT, AST, creatinine, urea and uric acid levels compared with the control positive group. This outcome is consistent with **Rui et al (2008)** who findings that enough choline intake is sufficient to prevent increases in serum indicators of liver function. Foods with less choline raise blood levels of ALT, AST, creatinine, urea, and uric acid **(Alaa et al., 2018)** and concur with **Slow and Timothy (2006)** who investigated the impact of dietary choline on rat liver and kidney betaine and found a decrease in the levels of AST, ALT, creatinine, and urea. Additionally, a degree of fatty liver was seen in all rats fed a choline-deficient diet, demonstrating that too much methionine cannot make up for insufficient dietary choline.

Researchers discovered that a choline-deficient diet caused changes in renal gene expression in male weanling wistar rats, suggesting that changes in the lipid composition of cellular membranes may alter the production of second messengers and cell signal transduction pathways, which makes the cells vulnerable to damage due to choline deficiency **(Elmer et al., 2014 and Courreges, 2000).** Choline consumption alters the epigenetic control of gene expression via affecting the methylation of DNA and histones, when humans consume diets

low in choline, fatty liver is one of the earliest adverse events, and in some people significant hepatic damage occurs (as measured by release of hepatic enzymes into blood). The liver is the main organ in which methylation reactions occur, and many of the hepatic genes involved in pathways for the development of fatty liver, hepatic fibrosis, and hepatocarcinomas are epigenetically regulated **Mihai et al., (2013) and (Fischer and Costa., 2007).** The primary mechanism of haemorrhagic kidney degeneration in choline shortage has been proposed to be an imbalance between vasoconstrictor catecholamines and a vasodilator like acetylcholine, produced by a decrease in acetylcholine **(Costa et al., 1979).** And agreement with **(Wu et al., 2013)** who asserts that choline-rich foods improve kidney and liver function in rats

Conclusion:

In conclusion, results from this study showed that diazinon is capable of causing reduced immune response and occurrence of oxidative stress. Treatment with food rich in chorine could significantly attenuate the diazinon and improve immune response and oxidative stress besides renal damage and hepatotoxicity

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egyjournal@sedu.asu.edu.eg

الترقيم الدولي الموحد للطباعة : 6164 - 1687

الترقيم الدولي الموحد الإلكتروني : 2682 - 4353

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