Phytochemical Screening of Cinnamon Cassia and Its Protective Effects Against Hepatotoxicity Induced By Difenoconazole in Male Albino Rats.

Reda El-shaat Mohamed (✉ redaelshaatmohamed@gmail.com)

Central Agricultural Pesticides Laboratory <https://orcid.org/0000-0001-6065-3183>

Magdy M. El-Said

Central Agricultural Lab, Agricultural Research Center.

Mohamed M. H. Arief

faculty of science, Benha University.

Research Article

Keywords: Difenoconazole, Cinnamon, Hepatotoxicity, Oxidative stress

Posted Date: February 10th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-173661/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

The objective of the present study has focused on the phytochemical analysis of Cinnamon cassia bark for determination of bioactive components, which have been associated with antioxidative stress induced by difenoconazole treatment in hepatic tissue of male albino rats. Ninety rats were assigned randomly to 9 groups, each group comprised of 10 animals. The first group served as control animals were administrated distilled water and the rest served as the experimental groups. Groups II and III animals were orally administrated with difenoconazole at doses of 58.9 and 117.8 mg/kg BW (represent of 1/20 and 1/10 of oral LD 50 , respectively) while the groups IV and V animals were received aqueous extract of cinnamon (AEC) at doses of 200 and 400 mg/kg BW , respectively . In addition groups VI and VII, animals were received AEC prior to 2h of administration with difenoconazole at low dose as well as groups VIII and IX, rats were received with AEC before treatments with high dose of difenoconazole for 28 days. Results of the present study indicated the presence of total phenolic, flavinoids and tannins as the main bioactive components in the AEC. Furthermore, the final body weight and liver index were increased markedly in difenoconazole-treated rats and these parameters values were comparable to control group following co-administration with AEC. However, difenoconazole-treatment induced a significant elevation in the level of (LPO) associated with adepletion of GSH level and an elevation in the activities of serum liver enzyme markers (i.e., AST ,ALT,ALP and GGT) was observed. These results confirmed with histopathological findings . In contrast, treatment with AEC in difenoconazole-treated rats elevated the level of endogenous hepatic antioxidant system (SOD, CAT and GSH) along with reducing the activities of serum liver enzymes. However, the hepatic protective property of AEC was further confirmed by histopathological findings. These findings may be attributed to the presence of total phenolic , flavonioids and tannins , which have anti-oxidative effect against oxidative injury- induced by tested fungicide.

Introduction

Pesticide are known to induce oxidative stress by production of reactive oxygen species (POS) besides releasing of highly reactive metabolites (Rastogi et al ., 2009).

The triazole is chemical family of fungicides, introduced in the 1980s, and it is the most important classes of pesticides in agriculture. In addition, they have the excellent protective and curative properties against a wide rang spectrum of crop diseases, where they are commonly used as environmental fungicides for grain, vegetable, fruits and flowers production world wide (Wang et al., 2011).

These triazole fungicide consists of numerous members such as difenoconazole, propicanazole, tridimofen and fenbucanazole .and they are designed to inhibit the activity of lanosterol 14 α -demethylase (CYP51), a key enzyme for ergosterol biosynthesis in fungi, causing membrane dysfunction (Chaâbane et al., 2016).

Difenoconazole is abroad spectrum fungicide belong to the triazole group of fungicides, and it used to control a broad spectrum of foliar, seed and soil-borne diseases.

By the oral route of exposure, these triazole would be considered as having low to moderate mammalian toxicology (Costa, 1997).

Recently it is suspected that long –term exposure to difenoconazole via drinking water and food may induce hepatotoxicity and tumorigenesis(Wang et al .,2011).

Abd-Alrahman et al. (2014) studied the effect of oral administration of difenoconazole at a dose of 10mg/kg to rats for 28 days. They found a significant increase in serum alanine aminotransferase (ALT), gamma-glutamyl transferase

(GGT) activities and total protein concentration than the control group. In contrast, no significant differences were observed in the activity of aspartate aminotransferase (AST) and albumin concentration.

Mona and Gehan (2016) reported that treatment rats with penconazole at the doses of 0.5, 25 and 100mg/kg BW for 28 days, caused a significant elevation in the activities of AST, ALT, ALP and GGT in compared with control group in all doses. Meanwhile, a significant decrease in the liver antioxidant enzymes activities i.e., SOD and CAT was observed.

Due to the possibility of occurring diverse effects by the use of synthetic antioxidant, then many studies have been carried out to investigate the biological properties of natural substances and identify the photochemical bioactive components which are playing a vital role in increase antioxidant defense systems (Roussel et al., 2009).

One of the most widely used as medicinal plant is cinnamon (*Cinnamomum cassia*) from the Lauraceae family, which has long history of safety and it is rich in polyphenolic components that have been shown to improve the antioxidant activities, consequently reduced the oxidative stress state. It is implicated in hepatic disorders including acute and lethal injury due to the production of the reactive oxygen species (ROS) which attacks the hepatic tissue (Shobana and Naidu, 2000).

The extract of Cinnamon cassia has been reported to have a better hepatoprotective activity against alcohol and carbon tetrachloride induced hepatic injury. This may be due to its free radical scavenging activity (Moselhy and Ali, 2009).

Oral administration of 200 mg/kg B.W of water and ethanolic extracts of cinnamon once a day for 7 days resulted in the activity of alanine amino transferase (ALT) and aspartate amino transferase (AST) in carbon tetrachloride – treated rats were restored to the level of untreated rats (Moselhy and Ali, 2009).

Also, Treated mice with *C. cassia* extract at doses of 200 mg/kg./day for 12 weeks, significantly increased the level of reduced glutathione and activities of GR, GSH, GPX, CAT and SOD in hepatic tissues (Kim et al., 2006).

Therefore, the current study was conducted to investigate the adverse effects following exposure to difenoconazole and to evaluate whether these effects ameliorated or prevented by treatment with Cinnamon cassia in male rats.

Materials And Methods

Tested fungicide.

Difenoconazole is commercial formulation containing 250g/L. 1-[[2-[2-chloro-4-(chlorophenoxy) phenyl]-4-methyl-1,3-dioxolan-2-yl]methyl]-1,2,4- triazole.

Plant material.

Cinnamon cassia bark (family Lauraceae) was purchased from local market of medical plant and herbs, Cairo, Egypt.

Determination of Total phenolic, Total flavonoid and Tannins in Cinnamon Cassia .

Total phenolic in Cinnamon extracts was determined by using Folin-Ciocalteu's reagent (Singleton et al., 1999), and Total flavonoid content (TFC) was determined by the aluminum chloride method using quercetin as a standard (Saenkod et al., 2013). However, Total tannins were measured using the Folin-Ciocalteu reagent assay according to the method of Tambe and Bhambar (2014).

Preparation of aqueous extract of Cinnamon (AEC).

The aqueous extract of cinnamon (AEC) was prepared according to method of (Sheng et al ., 2008). Briefly, the dried bark of cinnamon was grained into a fine powder. The dried powder (10g) was added to 100 ml distilled water and boiled at 90 °C for 10 min then the solution was cooled and filtrated through whatman paper no 1 to obtain the cinnamon aqueous extract. This extract was freshly prepared during the experimental period and administered orally to rats at the doses of 200 mg/kg B.W and 400 mg/kg B.W for 28 day.

Experimental animal and ethical approved.

In this study 90 male albino rats (Wister stain) aged 6-8 weeks and weighed 150 ± 10 g were used .The animals were obtained from the Department of Mammalian and Aquatic toxicology, at Central Agricultural Pesticides Laboratory (CAPL), Dokki, Giza.

They were kept under controlled condition, temperature at 25 ± 2 °C relative humidity 50 ± 15 % and normal photoperiod (12 h dark, 12 h light) the animals were provided with standard pallet diet from agricultural-industrial integration company, Giza and water ad libitum. All rats were acclimatized for 14 days prior to the beginning of the experiments. The experiments on rats carried out according to the guide lines for care and laboratory animals.

Experimental design.

After acclimatization all rats were divided randomly into nine groups of 10 rats each group as follow:-

Group I: Rats were administrated 1ml of distilled water /kg body weigh and served as control group.

Group II: Rats were administrated difenoconazole formulation at a dose of 58.9 mg/kg B.W, which equivalent of 1/20 of oral LD50 and served as low dose of difenoconazole group (L.F).

Group III: Rats were administrated difenoconazole formulation at a dose of 117.8 mg/kg B.W which equivalent of 1/10 of oral LD50 and served as high dose of difenoconazole (H.F).

Group IV: The animals were received the aqueous extract of cinnamon (AEC) at dose of 200 mg /kg B.W and served as low dose of Cinnamon group (L.C):

Group V: The animals were received the aqueous extract of cinnamon (AEC) at dose of 400 mg /kg B.W and served as high dose of Cinnamon group (H.C):

Group VI: The animals were received 200 mg/kg BW AEC orally 2hrs prior to treatment with low dose of difenoconazole for 28 days and served as (LF+LC).

Group VII: The animals were received 400 mg/kg BW AEC prior to treatment with low dose of difenoconazole and served as (LF+HC).

Group VIII: The animals were received 200 mg/kg BW AEC prior to treatment with high dose of difenoconazole and served as (HF+LC).

Group IX: The animals were received 400 mg/kg BW AEC prior to treatment with high dose of difenoconazole and served as (HF +HC).

All animals were observed for clinical signs of toxicity and the mortality was recorded within the experimental period. However, individual body weight was measured at the initiation of experimental and weekly during the treatment period (28 days). The study was conducted in compliance with [OECD guidelines ,No, 407.2008].

Collection of blood and tissue samples.

At the end of the experimental, rats were fasted overnight and weighted and the blood samples from each rat was collected by orbital sinus vein (Stone, 1954) in sterilized dry tubes and kept to coagulation for 30 min at room temperature. The clear sera obtained after centrifuged at 3000 rpm for 15 min and then stored at – 20°C till biochemical analysis.

After that, animals were sacrificed by cervical dislocation on day 29, the liver of each rat was collected and divided into two portions. Small portion were fixed in neutral buffered formalin (10%). for histopathological examination (Banchroft et al., 1996). The other portion of liver was washed with ice-cold saline, blotted dry, then wrapped in foil and immediately placed in liquid nitrogen and stored at -80°C for subsequent measurements.

Liver weight and liver weight index.

Fresh liver from the treated rats will be blotted dry and subsequently weighted. Absolute liver weights will be recorded and the change in liver weight were recorded relative to body weight.

Sero –biochemical measurements :-

The serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured according to the method of Reitman and Frankel (1957), alkaline phosphatase was determined according to the method of Klein et al. (1960) and gamma glutamyl transferase (GGT) was determined according to the method of Szasz and Persijn (1974). While the total protein and albumin concentrations were determined according to the method of Henry (1974) and Young (1975), respectively. However, the globulin concentration was calculated mathematically according to Coles (1986). All kits obtained from sigma chemical Co.

Determination of hepatic Oxidative Stress markers:

One portion of liver was washed in physiological saline, and then homogenized and the homogenate used for estimation of MDA and GSH levels as well as the activities of GGT, SOD and CAT.

Lipid peroxidation assay.

Malondialdehyde (MDA) is an end-product of membrane lipid peroxidation (LPO) and the enhanced production of MDA in tissue is an index of oxidative stress.

Therefore, MDA concentration was estimated by the method of Ohkawa et al. (1979). The level of lipid peroxides is expressed as n mol MDA /g tissue.

Determination of hepatic reduced glutathione (GSH) content.

Reduced glutathione content was determined in the hepatic tissue according to the method of Ellman (1959) the level of GSH was expressed as mg/g tissue.

Determination of Superoxide Dismutase (SOD) activity.

Superoxide dismutase (SOD) activity was assayed in liver tissue homogenate by the method of Marklund & Marklund (1974). This method is based on the auto-oxidation of pyrogallol,. The pyrogallol auto oxidation is highly dependant on O₂ and is inhibited by the presence of SOD. The SOD activity was determined spectrometrically at 440nm and expressed as U /mg protein.

Determination of Catalase (CAT) activity.

Catalase activity was determined according to the method of Cohen et al. (1970). By measuring the decomposition of hydrogen peroxide (H₂O₂) at 240 nm and expressed as U /mg protein.

Determination of Gamma-Glutamyl Transferase (GGT) activity.

Gamm –glutamyl transferase (GGT) activity was determined in liver tissue homogenates according to the method of Szasz and Persijng (1974).

The rate of colored liberation of yellow indicator 5-amino-2- nitrobenzoat is directly proportional to GGT activity in the sample and is quantitated by measuring the increase in absorbance at 405 nm.

Determination of tissue protein.

The level of protein in liver tissue was determined according to the method of Bradford (1976) .values were expressed as mg/g of tissue.

Histopathological examination.

Liver tissue samples intended for the histopathological investigation were fixed in 10% neutral formalin, prepared and stained according to Bancroft et al.(1996).The degree of damage was graded as follow as mild (+),moderate (++)and severe (+++)damage .

Statistical analysis.

All results are expressed as (mean $\pm$ SE). Within groups comparisons were preformed by the analysis of variance using the ANOVA Test .Significant differences between the control and the experimental groups were assessed by the Student T Test .The result were considered significant at P < 0.05 .all statistical analysis were preformed using SPSS(statistical package for social sciences).

Results

Phytochemical screening of Cinnamon cassia.

The results presented in Table (1) showed that the aqueous extract of Cinnamon (AEC) had the highest content of total phenolic (31.17 mg GAE/g) followed by flavenoids content (18.58 mg GAE /g), and Tannis (10.14 mg GAE/g).

Table (1) Phytochemical analysis of Cinnamon cassia.

Content of Cinnamon cassia		
Total Tannines content (mg/g)	Total Flavonoid content (mg/g)	Total Phenolic content (mg/g)
10.14	18.58	31.17
±	±	±
0.35	0.49	1.28

Data are expressed as (mean ± SE.M).

Changes in body weights and liver index.

Table (2) summarized the effect of difenoconazole alone and co-administration with aqueous extract of cinnamon (AEC) on body weight and liver index throughout the experimental period (28day).

Difenoconazole treatments for 28 days, caused a slightly significant increase in the final body weight

(by 3.8 and 5.7%, respectively) compared to control group. As shown in Table (2) with regard to liver index, a significant increase in the absolute and relative weights of liver was observed in rats treated with difenoconazole in compared with control group.

Supplemented rats with AEC alone did not induce any significant change in the final of body weight and liver index when compared with control group as presented in Table (2).

However, administration of AEC pre-treatment with difenoconazole resulted in insignificant decrease in the final body weight and liver index was observed when compared with difenocanazole–treated rats. Interestingly, no signs of toxicity in the experimental, and no mortality was recorded.

Table (2) Effect of difenocanazole–treatment and aqueous extract of cinnamon alone or concomitant with difenocanazole on body weight and liver index of the male albino rats for 28 days.

Treated groups									
HF+HC	HF+LC	LF+HC	LF+LC	HC	LC	HF	LF	Cont	Parameters
155 ± 2.23 0.0	155 ± 2.23 0.0	155 ± 2.23 0.0	157.8 ± 1.01 1.80	158.2 ± 0.91 2.06	157 ± 2.00 1.29	155 ± 2.23 0.0	154 ± 1.87 -0.64	155 ± 2.23	Initial Body weight (g)
219 ± 3.37 4.2	221 ± 4.00 5.2	216 ± 1.87 2.8	215 ± 1.63 2.3	216.8 ± 4.35 3.2	209 ± 2.91 0.47	222 ^a ± 2.91 5.7	218 ^a ± 3.00 3.8	210 ± 1.58	Final Body weight (Day 28) (g)
66.4 ± 5.33	66 ± 1.87	63.00 ± 4.06	57.6 ± 1.66	58.6 ± 1.20	54.00 ± 2.91	67 ± 2.00	66 ± 3.31	57 ± 4.64	Body weight Gain (gm)
7.57 ± 0.32	6.95 ± 0.46	6.58 ± 0.34	6.76 ± 0.29	7.09 ± 0.25	7.24 ± 0.31	8.73 ^a ± 0.24	7.89 ^a ± 0.18	6.85 ± 0.29	Absolute liver weight (g)
3.44 ± 0.09	3.14 ± 0.10	3.05 ± 0.15	3.13 ± 0.14	3.26 ± 0.13	3.45 ± 0.09	3.93 ^a ± 0.12	3.62 ^a ± 0.14	3.24 ± 0.05	Liver index (g/100 g bw)

Data are expressed as (mean ± S.E.M.).

Data between treated groups were analyzed using (one way ANOVA). (a) significant compared to control.

(+) increase compared to control group. (-) decrease compared to control group.

Changes in serum hepato-specific markers.

The extent of hepatic injury assessment by determining the serum liver enzyme markers, i.e, ALT, AST, ALP and GGT in blood circulation as well as the concentrations of serum total protein, albumin and globulin were determined.

Administration of difenoconazole at doses of 58.9 mg and 117.8 mg/kg BW for 28 day, caused a significant elevation in the activity of ALT (by 36.11 and 29.69), AST (by 7.54 and 22.43 %), ALP (by 19.70 and 22.54%) and GGT (by 46.52 and 38.50%) when compared to the control group (Table 3).

As shown in Table (3), after 28 days of AEC supplementation to male albino rats, there was no significant differences between the control group and each treatment group in the activities of ALT, AST, ALP and GGT.

The results presents in Table (3) revealed that co-administration of AEC, ameliorated the activities of serum ALT, AST, ALP and GGT, which are significantly lower than values of difenoconazole –treated rats and become near to normal values of control group.

However, administration rats with difenoconazole at high dose produced a significant elevation in the level of total protein (by 17.39%) and insignificant increase (by 11.43%) in this parameter was observed in rats treated with low dose of difenoconazole in compared with control group.

On the other hand, the albumin concentration did not change markedly in rats treated with difenoconazole throughout the experimental period.

Table (3) Effect of difenocanazole–treatment and aqueous extract of cinnamon alone or concomitant with difenocanazole on serum liver biomarker function of the male albino rats for 28 days.

Treated groups									
HF+HC	HF+LC	LF+HC	LF+LC	HC	LC	HF	LF	Cont	Parameters
69.65 ^{b,c} ± 0.73 11.77	.52 ^{b,c} ± 1.60 3.54	63.28 ^{b,c} ± 1.47 1.55	61.58 ^{b,c} ± 1.01 -1.17	60.95 ± 1.09 -2.18	58.91 ± 0.49 -5.45	80.81 ^a ± 1.02 29.69	84.81 ^a ± 2.21 36.11	62.31 ± 0.93 	ALT (U/L) Change %
170.79 ^c ± 0.91 6.18	169.17 ^{b,c} ± 0.97 5.17	160.19 ^{b,c} ± 1.67 -0.40	159.46 ^{b,c} ± 0.66 -0.85	159.80 ± 1.63 -0.64	161.09 ± 0.67 0.15	179.23 ^a ± 1.04 11.43	172.97 ^a ± 1.45 7.54	160.84 ± 0.65 	AST (U/L) Change %
2.44 ^{b,c} ± 0.02 -5.42	2.62 ^{b,c} ± 0.07 1.55	2.53 ^{b,c} ± 0.07 1.93	2.58 ^{b,c} ± 0.04 0.0	2.62 ± 0.06 1.55	2.73 ± 0.01 5.42	2.21 ^a ± 0.03 -14.34	2.05 ^a ± 0.06 -20.54	2.58 ± 0.04 	AST/ALT Change %
165.53 ^c ± 2.56 16.38	169.65 ± 1.50 19.27	161.28 ^{b,c} ± 1.73 13.39	166.75 ^c ± 3.09 17.23	142.40 ± 3.00 0.11	141.79 ± 2.49 -0.30	174.36 ^a ± 2.1 22.54	170.25 ^a ± 2.02 19.70	142.23 ± 1.49 	ALP (U/L) Change %
2.11 ± 0.14 12.83	2.16 ± 0.27 15.50	2.03 ^b ± 0.13 8.55	2.02 ^b ± 0.15 8.00	1.62 ± 0.12 -12.36	1.88 ± 0.28 0.53	2.59 ^a ± 0.18 38.50	2.74 ^a ± 0.37 46.52	1.87 ± 0.32 	GGT (U/L) Change %

Data are expressed as (mean $\pm$ S.E.M.).

Data between treated groups were analyzed using (one way ANOVA). (a) significant compared to control, (b) significant compared to low dose difenocanazole (L.F), (c) significant compared to high dose difenocanazole (H.F), (d) significant compared to low dose cinnamon (L.C) , (e) significant compared to high dose cinnamon (H.C),

(+) increase compared to control group. (-) decrease compared to control group.

Meanwhile, there was a significant elevation (by 40.19 and 54.60%) in the globulin level in rats treated with both doses of difenoconazole when compared with control group Table (4).

In contrast, Cinnamon treatments did not induce any significant alteration in the levels of TP, albumin and globulin within the experimental period.

Co-administration of AEC led to a significant decrease in the levels of TP and globulin in rats treated with low dose of difenoconazole in compared with difenocanazole-treated rats.

Also, a significant decrease in the level of TP was noticed in rats supplemental with high dose of AEC prior to treatment with high dose of difenoconazole when compared with difenoconazole- treated rats.

Table (4) Effect of difenocanazole-treatment and aqueous extract of cinnamon alone or concomitant with difenocanazol on the serum protein pattern of the male albino rat for 28 days.

Treated groups									
HF+HC	HF+LC	LF+HC	LF+LC	HC	LC	HF	LF	Cont	Parameters
6.5 ^c	6.77	6.37 ^c	6.48 ^c	6.38	6.24	7.29 ^a	6.92	6.21	TP (g/dl)
±	±	±	±	±	±	±	±	±	
0.22	0.20	0.43	0.25	0.24	0.28	0.22	0.21	0.10	
4.43	9.01	2.57	4.34	2.73	0.48	17.39	11.43		Change %
3.95	3.89	3.98	3.92	4.09	4.14	4.06	3.91	4.12	Alb (g/dl)
±	±	±	±	±	±	±	±	±	
0.92	0.11	0.22	0.14	0.17	0.10	0.14	0.06	0.11	
-4.12	-5.58	-3.39	-4.48	0.72	0.48	-1.45	-5.09		Change %
2.49	2.88	2.39 ^c	2.55	2.29	1.96	3.22 ^a	2.93 ^a	2.09	Glob (g/dl)
±	±	±	±	±	±	±	±	±	
0.21	0.30	0.27	0.16	0.36	0.35	0.27	0.26	0.08	
19.13	37.79	14.35	22.00	9.56	-6.22	54.60	40.19		Change %
1.62	1.43	1.74	1.54	2.06	2.45	1.30	1.37	1.98	A/G Ratio
±	±	±	±	±	±	±	±	±	
0.12	0.21	0.20	0.08	0.47	0.50	0.14	0.13	0.12	
-18.18	-27.77	-12.12	-22.22	4.04	23.73	-34.34	-30.80		Change %

Data are expressed as (mean ± S.E.M.).

Data between treated groups were analyzed using (one way ANOVA). (a) significant compared to control, (b) significant compared to low dose difenocanazole (L.F), (c) significant compared to high dose difenocanazole (H.F), (d) significant compared to low dose cinnamon (L.C) , (e) significant compared to high dose cinnamon (H.C)

(+) increase compared to control group. (-) decrease compared to control group.

Changes in hepatic oxidative stress markers and antioxidant defense status.

The formation of malondaldehyde (MDA) is an indicator of lipid peroxidation (LPO) and thus an indicator of development of oxidative stress.

Concerning the effect of difenoconazole-treatment on the hepatic lipid peroxidation, the level of MDA in hepatic tissue of difenoconazole –treated rats was significantly elevated (by 29.13 and 34.47%) when compared to the level of MDA in control group (Table 5).

With regard to reduced glutathione level (GSH), there was a significant decline (by 32.17 and 35.08%, respectively) in the glutathione level in hepatic tissue of rats treated with both doses of difenoconazole, respectively in compared with control group as shown in Table 5 .

On the other hand, the enhancement in the activity of GGT was observed in rat treated with difenoconazole in compared with control group.

Furthermore, as seen in Table (5) difenoconazole–treatments induced a significant decreased in the activity of SOD (by 15.81 and 25.82 %) and CAT (by 37.58 and 41.13%) when compared with control group.

Supplemented rats with AEC did not cause any significant alteration in the levels of MDA and GSH as well as in the activities of GGT and CAT, whereas a significant elevation in the activity of SOD (by 39.7 and 43.47%) was detected in compared with control group (Table 5).

Administration of AEC before treatment with difenoconazole resulted in reversing difenoconazole induced increase in MDA and decrease in GSH level when compared with difenoconazole-treated rats.

Supplementation rats with AEC prior to treatment with difenoconazole at low dose caused a significant decrease in the level of MDA and increase in the level of GSH whereas, a significant reduced in the activity of GGT was noticed in rats treated with difenoconazole after supplementation with AEC in compared with difenoconazole- treated rats .In addition, a significant increase in the activities of SOD and CAT was detected in rats treated with high dose of difenoconazole following administration of AEC in comparison with difenoconazole-treated rats.

But Cinnamon treatments markedly improved GSH level in compared to the difenoconazole treated rats especially at low dose of tested fungicide.

The results presented in table (5) illustrated that rats received AEC at high dose, pre-treatment with low dose of difenoconazole had a significant decrease in the levels of MDA and GGT activity, whereas a significant elevation in the level of GSH and the activities of SOD and CAT was noticed in compared with difenoconazole- treatment rats.

Rats treated with high dose of difenoconazole after received the AEC had a significant decrease in the level of MDA and GGT activity ,whereas a significant enhancement in the activities of SOD and CAT was detected in compared with difenoconazole-treated rats(at high dose). In contrast, the GSH level did not alter markedly in compared with difenoconazole- treated rats.

Table (5) Effect of difenocanazole-treatment and aqueous extract of cinnamon alone or concomitant with difenocanazole on hepatic oxidative stress markers in male albino rats for 28 days.

Treated groups									
HF+HC	HF+LC	LF+HC	LF+LC	HC	LC	HF	LF	Cont	Parameters
161.02 ^{b,c}	166.62 ^c	157.15 ^{b,c}	152.94 ^{b,c}	132.17	131.16	181.31 ^a	170.31 ^a	131.89	MDA (n mol MDA/ g tissue) Change %
±	±	±	±	±	±	±	±	±	
3.26	1.62	1.93	2.78	1.04	1.70	0.88	3.70	2.17	
22.08	26.33	19.15	15.96	0.21	-0.55	37.47	29.13		
18.27	19.53	21.90 ^{b,c}	22.58 ^{b,c}	24.73	27.10	17.05 ^a	17.98 ^a	26.51	GSH (mg/g tissue) Change %
±	±	±	±	±	±	±	±	±	
0.86	1.01	1.59	1.99	1.73	0.70	1.58	0.83	1.03	
-31.08	-26.32	-17.38	-14.82	-6.71	2.22	-35.68	-32.17		
12.77 ^{b,c}	13.37 ^c	10.44 ^{b,c}	10.52 ^{b,c}	9.71	9.87	15.13 ^a	14.41 ^a	9.38	GGT (n mol /mg protein) Change %
±	±	±	±	±	±	±	±	±	
1.03	0.63	0.38	0.37	0.32	0.22	0.58	0.33	0.25	
36.14	42.53	11.30	12.15	3.51	5.22	61.30	53.62		
7.33 ^c	7.79 ^{b,c}	7.97 ^{b,c}	7.48 ^{b,c}	10.89 ^a	10.61 ^a	5.63 ^a	6.39 ^a	7.59	SOD (U/mg protein) Change %
±	±	±	±	±	±	±	±	±	
0.37	0.28	0.30	0.24	0.44	0.53	0.35	0.43	0.32	
-3.42	2.62	5.00	-1.44	43.47	39.78	-25.82	-15.81		
10.56 ^{b,c}	10.91 ^{b,c}	11.90 ^{b,c}	12.44 ^{b,c}	12.57	12.02	.64 ^a ,	7.04 ^a	11.28	CAT (U/mg protein)
±	±	±	±	±	±	±	±	±	
0.35	0.49	0.49	0.29	0.19	0.17	0.22	0.14	0.35	

-6.38	-3.28	5.49	10.28	11.43	6.56	-41.13	-37.58	Change %
-------	-------	------	-------	-------	------	--------	--------	----------

Data are expressed as (mean ± S.E.M.).

Data between treated groups were analyzed using (one way ANOVA). (a) significant compared to control, (b) significant compared to low dose difenocanazole (L.F), (c) significant compared to high dose difenocanazole (H.F), (d) significant compared to low dose cinnamon (L.C) , (e) significant compared to high dose cinnamon (H.C)

(+) increase compared to control group. (-) decrease compared to control group.

Histopathological findings.

The histological changes in treated rats are graded and summarized in Table (6). The histopathological examination of the liver sections of the control group, showed normal histological structure Fig 1(A).

The most prominent histopathological findings were observed in the liver of difenoconazole-treatment rats were degenerative changes in the hepatocytes surrounded and adjacent the dilated central vein of rats following treatment with high dose of difenoconazole for 28 days, in addition, the portal area showed oedema and periductal fibrosis surrounding the bile duct, as well as hyperplasia in other bile ducts was detected Fig 1(B,C,D).

As shown in Fig 1(E), treatment rats with low dose of difenoconazole caused vacuolar degeneration in the hepatocytes, in addition to dilation in the central vein associated with fatty change in the surrounding adjacent hepatocytes Fig1.(F , G).

Liver of AEC supplemented rats showed a normal histological structure Fig 1(H) with slight degenerative change especially at high dosage level and congestion in rats received the AEC during the experimental period.

When compared to difenoconazole-treated rats, rats received AEC pre-treatment with difenoconazole showed improvement in the histology of liver as shown in the Fig 1(I, J).

Table (6) The grading of histopathological findings in hepatic tissue of rats in different experimental groups.

Parameters	Cont	LF	HF	LC	HC	LF+LC	LF+HC	HF+LC	HF+HC
Congestion	-	+++	++	-	+	-	-	-	-
Degenerative change in hepatocytes	-	+++	++	-	+	-	-	-	+
Portal fibrosis with newly formed bile ducts	-	-	+++	-	-	-	-	-	-
Periductal fibrosis	-	-	++	-	-	-	-	-	-
Hyperplasia in bile duct	-	-	++	-	-	-	-	-	-
Portal oedema	-	++	++	-	-	-	-	-	+

(-) normal.

(+) mild.

(++) moderate.

(+++) sever

Discussion

Exposure to chemicals including agro-pesticides and various environmental toxicants, has been reported to cause damage of cellular macro-molecules such as lipids, proteins and DNA through induce of oxidative stress, which result from an imbalance between radical generating and radical scavenging system of cell, via enhancement production of reactive oxygen species (ROS) [Yang et al ., 2018 and Verma et al .,2007].

Conazoles are azole antifungal agents used in agricultural and pharmaceutical .products (Zarn et al ,2003).The fungicidal properties of conazole are due to their abilities to inhibit ergosterol biosynthesis which is an essential component of fungal membranes, consequently its inhibition lead to cell death [Vanden Bossche et al .,1989, Ronis et al., 1994 and Debeljak et al .,2003].

In mammalian systems, conazole modulate many CYP enzymes involved in the metabolism of xenobiotics sterol, steroids and other xenobiotics (Osman et al., 2011).

Recently, considerable attention has been focused on medicinal plants, which play a vital role for medication and to determine the active principles responsible for protecting or preventing the development of oxidative stress, which is an important cause of many chronic diseases.

Therefore, the present study carried to evaluate the adverse effects of difenoconazole fungicide and attempt of the possible anti-hepatotoxic of Cinnamon cassia against difenoconazole induced hepatotoxicity in male rats.

Phytochemical Screening of Cinnamon cassia.

The phytochemical investigation of Cinnamon cassia revealed the presence of total phenolic, flavonoids and tannins as shown in Table (1).

Cinnamon is known to have contain of phenolic compound and flavonoids which act as potent antioxidants due to their ability to scavenge free radical and form relatively inert phenoxy radical intermediates [Ferguson, 2001].

The phenolic compounds ,such as flavonoids ,phenolic acid and tannins posses divers biological activities , and they are responsible for antioxidant action as well as a potent scavenger of hydrogen peroxide(H₂O₂),nitric oxide (NO)and lipid peroxide free radicals [Aravid et al .,2012].

In addition ,the antioxidant activities of phenolic compounds is mainly due to their redox properties such as adsorbing and neutralizing free radicals as well as quenching single and triple oxygen or decomposing peroxides [Yashin et al .,2017].

With regard to flavonoids , they are typical phenolic compounds, which in turn ,act as potent metal-chelators and free radical scavengers.

Generally, they have higher antioxidant activity against peroxy radical than phenolic acid due to multiple hydroxyl group [Yashin et al .,2017].

Our results in agreement with the previous findings reported by [Pogngothan ,2011 and Benmehdi et al .,2012] who found that Cinnamon composed of many bioactive compounds such as polyphenols and flovonoids.

Changes in Body Weight and Liver Index.

In general, an increase or decrease in the body weight of an animal has been used as indicator of an adverse effect of drugs and chemical (Teo et al., 2002).

Our results demonstrated that oral administration of difenoconazole slightly but significant increased in the final body weight of rats.

Meanwhile, the body weight of rats received only the aqueous extract of cinnamon (AEC) did not show any significant change when compared with corresponding group of control. This is indicative of absence of general toxicity. The increase in body weight of difenoconazole-treated rats could be attributed to the ability of difenoconazole to stimulate the accumulation of triacylglycerol in adipocyte [Masuro et al .,2005 and Wada et al.,2007] and so .the organs involved in metabolic syhlrom [Met-S][Marmugi et al .,2012] or due to hypothyrodism [Moriyama et al .,2002] .

There was a significant decrease in the levels of T4 and T3 in rats treated with difenoconazole for 28 days (data not shown).

In addition, difenoconazole could be induce accelerated differentiation of immature adipocytes into mature cells and may disrupt the lipid metabolism of difenoconazole-treatment rats [EFSAb].

The results of our study agree with the results of work done by Heba Hamdi et al (2019) who found that treated rats with epoxiconazole at doses of 8, 24, 40 and 56 mg/kg B.W ,induced a significant increase in the body weight after 28

days of treatment.

On the other hand, Allen et al (2006) and Wolf et al (2006) reported that propiconazole and triademofen treatment produced a significant decrease in the body weight at the end of experiment (90 days).

In contrast, supplemented rats AEC alone within the experimental period, did not induce any significant change in the body weight.

However, pre-treatment of AEC induced insignificant decrease in the body weight of rats treated with low dose of difenoconazole, this may be due to the ability of AEC to decrease fat mass percent [Ziegenfuss et al., 2006].

Lopes et al (2015) who found that oral administration of Cinnamon extract (400mg/kg BW) to healthy rats led to a lower body mass.

On the other hand, Kim et al (2006) demonstrated that treatment rats with Cinnamon extract at 200mg /kg BW did not induce any significant change in the final body weight, whereas this extract caused a significant decrease in the liver index of diabetic mice after 12 weeks of treatment.

Concerning the liver weights when expressed as absolute or relative to body weight, there was a significant increase in liver weight (hepatomegaly) in rats following exposure to difenoconazole. This could be explained by the induction of several xenobiotics metabolizing enzyme (FFSAb, 2008).

A hepatomegaly often accompanies with increase in hepatic –derived enzymes (i.e., Transaminase, ALP and GGT), that may appear in the plasma following liver enlargement.

Soliman et al. (2012) noticed that AEC did not induce any anti-obesity effects although noticeable decrease in liver fat and glycogen deposition was recorded.

Our findings were consistent with previous studies that have evaluated the toxic effect of some triazole compounds like propiconazole, myclobutanil, triademofen, epoxiconazole and bromuconazole, which in turn, induced a significant increase in the liver weight in rodent animals. [Allen et al., 2006, Martin et al., 2007, Nesnow et al., 2011 and Heba Hamdi et al., 2019].

Meanwhile, the absolute and relative weight of liver rats supplemented only with AEC comparable to control and no significant differences were found in their values.

However, it was observed that co-administration of AEC with difenoconazole, attenuated the increase in weight of the liver following treatment with difenoconazole and consequently caused recovery towards normalization comparable to the control group. This indicated that AEC could exert an *in vivo* hepatoprotective effect.

Previous studies have shown that cinnamon *zeylanicum* treatment reduced the relative weight of the liver [Shan et al., 1998].

Changes in Serum hepato –specific markers.

Assessment of liver injury by difenoconazole is usually made by determination of serum enzymes level of ALT, AST, ALP and GGT, which are important markers to evaluate hepato-biliary injuries.

In the current study, an increase significantly in the activities of serum ALT and AST was observed following treatment with difenoconazole, which have been attributed to leakage of cellular enzymes in circulation as a result of loss of function.

integrity of cell membrane of hepatocytes [Navaro et al .,1993 and Ozer et al .,2008]

A number of chemical including agro-pesticides and various environmental pollutants can cause severe cellular damage in different organs in the body .This occurred through metabolic activation to highly reactive substances such as free radicals (Satpute et al., 2017).

In addition, there was also a significant elevation in the activities of ALP and GGT in difenoconazole treated rats, this induction of ALP, may be attributed to presence of increasing of biliary pressure rather than increased release from damaged cells .and this elevation may be occurred during liver regeneration [Moss and Butterworth , 1974]. However, enhancement of ALP activity may be due to elevation of bile acids concentration during cholestasis is apparently necessary for the release and transport of solubilized hepatic ALP to serum.

Also, an induction in the activity of GGT was detected in rats treated with difenoconazole within the experimental period.

ALP and GGT activities are important biomarkers to evaluate hepatobiliary injuries or bile duct lesion in the rat liver, where ALP is primary marker of hepato-biliary effects and cholestasis (moderate to marked elevation (Ramaiah , 2007)

GGT is a cell surface enzyme that cleaves Y-glutamyl bonds (Godwin et al 1992).It is localized to the luminal surface of ducts and glands throughout the body (Hanigan . 2014).

However, GGT is a biliary enzyme that is especially useful in the diagnosis of intra-hepatic cholestasis and obstructive Jaundice (Stein et al ., 1989).

In addition, GGT is more responsive to biliary obstruction than alkaline phosphatase , where GGT has no origin in bone or placenta ,and hence, GGT is not increased in bone disorders as is ALP .

In rats, GGT activity is considered a reliable marker for cholestasis compared to alkaline phosphatase activity (Ozer et al ., 2008). In addition, the rat GGT assay detects bile duct hyperplasia and necrosis [Leonard et al ., 1984].

The biochemical markers in the liver viz., ALT, AST and ALP were significantly elevated in rats treated with expoxicanazole at doses 8, 24, 40, 56 mg/kg BW for 28 days [Heba Hamdi et al .,2019].

Also, there are findings were reported previously by Abd-El Rhaman et al ., 2014 showed that treatment rats with difenoconazole at dose of 10mg /kg BW (representing of LOEL dose) caused a significant increase in ALT activity and total protein level, whereas a significant decrease in the activity of GGT and insignificant change in the AST activity were observed after 28 days of treatment.

In contrast ,rats supplemented with AEC at selected doses of 200 and 400 mg /kg B.W did not induce any significant change in serum liver markers (i.e .ALT,AST ,ALP and GGT),in addition to serum protein pattern and A/G ratio.

It is worth mentioning that C.cassia was able to ameliorate all the altered hepatic biomarkers post-treatment with difenoconazole ,this suggests that the vital role of Cinnamon extract in protecting the integrity and the function of cells and tissue .

Co-administration cinnamon with difenoconazole showed significant decrement in the activities of ALT ,AST,ALP and GGT compared to difenoconazole-treated rats , this indicates that the extract may reduce hepatocellular damage.

Co-administration of cinnamon extract in doses of 200 and 400mg/kg B.W, attenuated the increased levels of serum enzymes[i.e., ALT,AST and ALP] produced by difenoconazole and caused subsequent recovery towards normalization comparable to the control group .this in turn ,indicate that AEC could reduce liver injury –induced by difenoconazole . Hence, cinnamon extract its hepatoprotective effects as a result of presence of wide range of bioactive compounds including phenolics.

Cinnamon extract at doses of 200 and 400mg/kg B.W when orally given to obese diabetic rats ,significantly lowered the high serum levels of ALT,AST and ALP enzymes ,when compared to the positive control groups [Shalaby and Saifan ,2014].

The hepatoprotective effect of Cinnamon extract was evident from the significant decrease in serum activities of liver enzymes (ALT,AST&ALP)in obese diabetic rats[Shatwan et al .,2013].

Moselhy and Junbi (2010) observed that the elevated serum AST and ALT activities induced by CCL4, were restored towards normalization significantly by orally administration of 200mg/kg cinnamaldehyde (CNN) once a daily for 7 days when compared to control rats.

Our biochemical finding were confirmed with histopathological findings of rat liver sections in the current study.

An elevation in the serum total protein concentration and globulin levels was observed in rat treated with high dosage level of difenoconazole, whereas no significant differences in the concentration of albumin.

In addition, the albumin /globulin ratio (A/G ratio) was significantly decreased in the groups of rats treated with difenoconazole .This could be attributed to increase of serum globulin level as the result of the immune response of toxic effect.

Meanwhile, in the present study co-administration of AEC lowered the levels of TP and globulin and their values were found comparable to control values.

Changes in the markers of oxidative stress and antioxidant status .

It is well-established that the liver is a target organ for xenobiotics substances and it is play crucial role in the detoxification process. Therefore, any injury or impairment of its function produces hepatotoxicity on living organism.

Oxygen free radical (superoxide, O₂.) and hydroxyl radical (OH.)and hydrogen peroxide (H₂O₂) called reactive oxygen species, which play a significant role in oxidative stress and they are also capable presenting a toxic action on self tissues causing lipid peroxidation [Sharmanov et al .,1990 and Baxter et al .,1983].

The elevation of oxidative stress can be monitored by several markers .The monolondialdehy (MDA) measure is considered as an indicator of lipid peroxidation (LPO) [De-Zwart et al ., 1990].

Difinoconazole-treated induced lipid peroxidation in hepatic tissue, which observed by marked elevation in the level of MDA, this suggest that it mediated free radical induced lipid peroxidation in hepatocytes was strongly associated with tissue injuries.

In the present study, enhancement of MDA level in liver homogenate, suggest enhanced LPO leading to increase the preambility of the cell membrane of hepatocytes, consequently release of transaminases (ALT, AST) into circulation as shown in our result.

Therefore, the remarkable elevation of ALT and MDA in the current study supported that difenoconazole mediated lipid peroxidation in liver rat as a result of reducing the antioxidant potential and hence accelerating the oxidative damage of hepatocyte.

The MDA is by-product of lipid peroxidation, while the glutathione (GSH) content could be used to evaluate the antioxidant status of cellular system [Dall-Donn et al., 2007, Circu and Aw, 2008 and Pallarda et al., 2009].

The glutathione (GSH) is non-enzymatic antioxidant and it is one of the most abundant tri-peptide and it present in the liver and its essential function are mainly concerned with maintain structure and function integrity of cell via removal of free radical. Therefore, it plays a vital role in production against oxidative stress [Halliwell and Gutteridge 1990].

Our results revealed that difenoconazole treatment induce a significant depletion of GSH content in hepatic tissue and production of antioxidant enzymes viz, SOD and CAT.

In contrast, there was a significant elevation in the activity of hepatic GGT in difenoconazole –treated rats.

Induction of GGT expression it is considered as an adaptive response or as part of natural hepatocyte protective mechanism for GSH turnover (repletion) in hepatic tissue. The body's antioxidant defences undergo consumptive depletion following oxidative injury. Therefore, low antioxidant defense status are also correlated with elevated GGT, particularly reduced levels of GSH. However, GGT is needed to metabolize glutathionylated xenobiotics in liver and hence its elevation association with increased exposure to xenobiotics [Koenig and Seneff, 2015].

Previous studies have shown that usually the deleterious effects of oxidative stress are counteracted by endogenous antioxidative enzymes, which provide a major defensive mechanism against free radical damage. The most important antioxidative enzymes i.e., SOD and CAT, when ROS begin to accumulate in the cells, exhibits defensive mechanism using various antioxidant enzymes. The main detoxifying system for peroxides are CAT and GSH

However, superoxide dismutase (SOD), a common enzyme play an important protective role by catalyzing the removal of superoxide radical ($O_2^{\cdot-}$), and converted to hydrogen peroxide (H_2O_2) and hence (H_2O_2) is degraded by catalase (CAT) which catalyzes the reaction between two hydrogen peroxide molecules (H_2O_2) and this reaction results in water and O_2 production and prevent form a highly reactive OH in presence of Iron as catalyst [Turner and Lysiad, 2008].

Previous reports have shown that treatment animals with propiconazole or bromuconazole induced a significant elevation in the level of MDA and depletion of GSH level in liver rat. Meanwhile, there was a decline in the activities of SOD and CAT following treatment rats with bromuconazole for 3 months [Nesnow et al., 2011 and Osmen et al., 2011].

Also, Abd-Alrahem et al. (2014) reported that treatment rats with difenoconazole at dose of 10mg/kg BW caused a significant elevation in the level of serum MDA and SOD activity, whereas a significant decline in the level of SH protein was detected after 28 days of treatment.

Our findings revealed that SOD activity was decreased in the hepatic tissue. The reason of the decreased SOD could be attributed to excessive ROS generation [Scott et al., 2008].

Also this decrease could be due to a feedback inhibition or oxidative inactivation of enzyme protein due to excess ROS formation.

However, the reduction of the activity of CAT reflect inability of hepatic tissue to eliminate H₂O₂ produced the activity of CYP and inactivation of this enzyme as a result of excess of ROS production and /or suppressing of heme biosynthes [Pigeolet et al ., 1990].

Recently, considerable attention has been focused on some medicinal plants like cinnamon which are posses diverse biological activities.

The total phenolic might be the main bio-active compounds which, a potent scavenger of hydrogen peroxide(H₂O₂),nitric oxide (NO)and lipid peroxide [Aravind et al .,2012].The antioxidant activities of phenolic compounds is due to their redox properties.

In addition, the phenolic hydroxyl groups is shown to denote electron to oxygen radicals and also reduce ferrious ion to ferric ion, thereby suppressing the oxidation [Morel et al .,1993].

However ,it is found that cinnamon contain proteins ,carbohydrates .vitamins (A,C,K and B3) and minerals like Calcium ,Iron ,Manganes (Mn⁺²), Magnesim (Mg⁺²) Phophorus and Zink (Zn⁺²)[Vanalapati et al .,2012].

The polyphenolic polymers found in Cinnamon and they have antioxidants activity and reduce oxidative stress through inhibition of 5-lipoxygenase enzyme [Anderson et al .,2004].

The hepatoprotective potential of Cinnamon cassia against the oxidative stress induced by difenoconazole was evaluated in this study.

Apparently, the intracellular defenses based on glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) are insufficient to inhibit these pro-oxidant reaction, but the nature antioxidant supplementation impart protection against oxidative stress, via increased the intracellular antioxidant defenses,which can be overwhelmed .

In the current study, remarkable elevation of SOD and CAT activities was observed following co- administration of AEC with difenoconazole.

It is probable that initial increase in hepatic SOD and CAT level was occurred as adapative nature of the defense system against damage effect of superoxide radicals (O₂.) in liver brought about by difenoconazole treatment.

Since, polyphenol effectively removes oxygen radicals, and diminished degradation or inactivation of antioxidants enzymes, partly explains increased SOD activity during phenol–rich intervention [Casten-miller et al ., 1999].

Moreever, Cinnamon oil (Co) exhibited SOD like activity of pyrogallol auto-oxidation this catalyzed by the superoxide radical [Kim et al .,1995].

Also, Murcia et al . (2004) reported that cinnamon extract exhibited protection effect against irradiation–induced LPO in liposome and quenched hydroxyl radical (OH.) and H₂O₂.

Furthermore, the protective action of Cinnamon extract against radiation induced oxidative stress and inflammatory damages was attributed to inhibiting effect on No production through inhibition of NF-Kb activation as a result of presence of phenolic and flavonioids compounds.[Lu et al .,2005 , Azab et al ., 2011 and Morgen et al .,2014].

Bej et al . (2018) found that treatment rats with Cinnamon for 4 weeks significantly increased to near normal levels of SOD, CAT and GPX in the pancreatic tissue of diabetic rats.

Histopathological Findings.

The major histopathological findings were observed in the liver of rats treated with high dose of difenoconazole as shown in Table (6) and Fig (1).

These findings might further confirm that hepatic injury induced by difenoconazole is associated with generation of free-oxygen radicals and the biochemical results supported these findings. The cell injury causes the release of cytokines, especially Tumor Necrosis factor- α [TNF- α] which generate ROS from the tissues, and consequently produce lipid peroxidation [Lachleitner et al., 2000].

The degeneration condition observed in liver of difenoconazole-treated rats correlated with the detected biochemical alteration wherein, an increase in the level of ALT, AST, ALP, GGT and LPO was noticed.

However, histopathological observation in AEC-supplemented rats confirmed with biochemical results showing the normal cellular architecture, in particular low dose of AEC (200mg/kg B.W) this in turn substantiates the safety profile of the cinnamon extract at low dosage level.

In addition, our result revealed that rats received cinnamon extract prior to difenoconazole-treatment showed improvements in the histology of hepatocytes in comparison to difenoconazole-treated rats. This suggests that cinnamon has a role in counteracting the oxidative damage-induced by generation of free radicals via difenoconazole treatment.

Indeed, administration of rats with low dose of AEC did not induce any degenerative effect, although this extract at high dose (400mg/kg B.W) showed degenerative change.

In such cases, low dose of AEC could not damage mitochondria and antioxidant compounds were not oxidized and could scavenge free radicals.

In contrast, high dose level of AEC could damage and permeabilize and could react as pro-oxidant damaging rat hepatocytes. It seems that switch from anti-oxidant reactions occurs at low antioxidant concentration in a very narrow range.

Moreover, some phenolic components of essential oils are oxidized by contact with ROS, consequently producing very reactive phenoxyl radicals which add to ROS released by mitochondria and these types of radical reactions enhanced by the presence of cell transition metal ions such as Fe²⁺, Cu²⁺, Zn²⁺ and Mg²⁺ [Stadler et al., 1995 and Sakihama et al., 2002].

In addition, our results demonstrated that AEC has anti-fibrotic effect, as seen in Table (5) and Fig (1). This could be attributed to diminishing the oxidative stress, which occurred through difenoconazole-treatment. Thus, AEC supplementation leads to reduce inhibitors of metalloproteinase [which are a group of zinc-dependant endopeptidases] in tissues, consequently produce an increase in collagenase level which degrades fibrous tissue.

Lin et al. (2003) reported that Cinnamon cassia powder reduced significantly the expression of α -smooth muscle actin (α -SMA), which plays an important role in fibrogenesis and connective tissue growth factor (CTGF), Transforming growth factor β -1, and tissue inhibitor of metalloproteinase-1, which is elevated by oxidative stress in Sprague-Dawley rats with acute liver injury –induced by dimethyl-nitrosamine.

Conclusions

In conclusion, the present results suggest that the deleterious effects of difenoconazole in hepatic tissue could be due to the induction of oxidative stress as the results of generation of excessive oxy-radicals .Therefore, AEC to be useful in the attenuation of difenoconazole-induced lipid peroxidation (LPO) and showed more or maximum protective effects of the AEC when administrated once daily at 200mg/ kg BW for 28 days ,where it largely minimized the histopathological changes in hepatocytes associated with difenoconazole toxicity .

Declarations

Authors' contributions: All authors are contributed in this research work and work on it. M.M.El-S conceived the study and designed the experiment. R.M carried out the experiment and performed the data analysis and drafted the manuscript. M.M.H reviewed all the results. All author read, revised and approved the final munscrip .Also, the authors acknowledge the Central Agricultural Pesticides Lab (CAPL), Agriculture Research Center (ARC) Giza, Egypt, for providing necessary facilities for the accomplishment this research work.

Consent to publish: All the author consent to publish this research article.

Consent to participate: All the author have been participate in this research article.

Competing interest: The authors declare that they have no competing interest.

Availability of data and materials: All the data and materials are available upon request.

Funding: The author decline that they have no sources of funding .

References

1. Abdel- Alrahman, S. H., EL- halwagy, M. E.A., Kotb., G.A., Hoda, Farid, Farag A.G., Draz, H.M. and Isa, A.M. and Sabico, S. (2014) Exposure to diferonconzole, diclofop-methyl alone, and combination alters oxidative Stress and biochemical Parameters in albino rats. *Int. J clin Exp. Med.* 7 (10): 3637- 3646.
2. Allen ,J.W., Walf , D.C., George ,M.H., Hester , S.D., Sun, g., Thai ,S.F ., Dlker, D.A. (2006) Toxicity profiles in mice treated with hepatotumorigenic triazole fungicides propiconazole ,triadimefon and myclobutamil .*Toxicologic Pathology* 34: 853-862.
3. Anderson ,R.A., Broadhurst, C.L., Polansky, M.M., Schmidit, WF., Khan, A, Flanagan, V. (2004) Isolation and characterization of polyphenol type- A Polymers from cinnarmon with Insulin the biological activity. *J. Agri. Foodhem..* 52 (4): 65- 70.
4. Aravind, R., Aneesh, T., Bindu, A., Bindu, K. (2012) Estimation of phenolics and evaluation of antioxidant activity of cinnamomum malabattrum (Burn. F). *Blume Asian J Res. Chem .5:* 628- 632.
5. Azab, K.S., Moustafa, A.H.A., Ali, E.M.M., Abdel- Aziz, M.A.S. (2011) cinnamon extract ameliorates Ionizing radiation- indented cellular injury in rats. *Ecotoxicol. Environ. Saf.* 74, 2324- 2329.
6. Bancraft, JD., Stevens, A., Turner, DR. (1996) Theory and practice of histological techniques. 4th Ed., Churchill Livingstone, New York, London, San Francisco, Tokyo.
7. Baxter, M., Leslie, Rard Reeves W. (1983) The stimulation of superoxide anion production in guinea-pig peritoneal macro phages and neutriphils by phorbol myristate acetate, OP sonized zymosan and IgO2- containing soluble-complexes- *Immunology* 48: 657- 665.
8. Beji,R.S .,Khemir ,S .,Wannes, W.A., Ayari ,K .,Ksouri, R.(2018) Antidiabetic ,antihyperlidemic and antioxidant influences of the spice cinnamon (cinnamomoun Zeylanicumun)in experimental rats .*Braz .J.Pharm.Sci* 54(2)1-

8.

9. Benmahadi ,H .,Hasnaoni ,O ., Benali ,O., Salhi, F.(2012) Phytochemical investigation of leaves and fruits extracts of chamaero Ps himilish .J.Mat .Env. Sci 3(2):320-337.
10. Betro, M.G., Oon, R.C., Edwards, J.B. (1973) Y- glutamyl transpeptidase in diseases of the liver and bone. Am. J. clin. Pathol. 60: 672- 8.
11. Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry. 72: 248-254.
12. Casten-miller ,JJM ., Lauridsen ,ST ., Dragsted , L.O., Vanhet ,H ., Linssen JPH., West ,CE. (1999) B.Carotene does not change markers of enzymatic and non enzymatic antioxidant activity in human blood J.Nutr.129: 2162-2169.
13. Chaâbane, M., Tir ,M., Hamdi ,S., Boudawara ,O., Jamoussi ,K., Tahia Boudawara, T., Ghorbel ,RE., Zeghal ,N ., Soudani, N .(2016) Improvement of heart redox states contributes to the beneficial effects of selenium against penconazole-induced cardiotoxicity in adult rats. Biol Trace Elem Res 169:261-270.
14. Circu , M.L., Aw, T.Y.(2008) Glutathione and apoptosis Free Radic .Res .42:689-709.
15. Cohen, G., Dembiec, D., Marcus, J. (1970) Measurement of catalase activity in tissue extract. Analytical Biochemistry. 34: 30-38.
16. Coles, E. H. (1986) Veterinary clinical pathology. 4th Ed., W. B. Saunders CO., pp. 190-193.
17. Costa, L.G.(1997):Basic toxicity of pesticides .Occup.Med. State of the Art Rev.12:252-268.
18. Dalle –Donne ,I., Rossi ,R ., Giustarini ,D., Colomba ,R ., Milzani ,A.(2007) S-Glutathionylation in protein redox regulation .Free Radic .Biol .Med .43:883-898.
19. Debeljak, N., Fink,M.,and Rozman ,D.(2003):Many facets of mammalian lanosterol 14- alphi-demethylase from the evolutionary conserved cytochrome P450 family CYP51 .Arch .Biochem .Biophys .409,59-71.
20. De-Zwart, H.H., Meerman, J.H. N., Commandeur, J.N.M., and Vermeulen, N. P.E. (1990) Biomarkers free radical damage: Applications in experimental animals and in humans. Free radical Biology and Medicin. 26 (1- 2) 202-226.
21. EFSA Scientific Report (2008b) conclusion on the peer review of penconazole 175 :1-104 [http // ww. Efsa . europa. Eu .](http://www.efsa.europa.eu)
22. Ellman, G. L. (1959) Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics. 82: 70-77.
23. Fergeson , L.R .,(2001) Role of plant polyphenols in genomic stability .Mutat Res .475 :89-111.
24. Godwin ,AK ., Meister ,AO., Dwyer, PJ .,Huang CS., Hamilton ,TC., Anderson ,ME.(1992) High resistance to cisplatin in human ovarian cancer cell lines is associated with marked increase of glutathione synthesis .Proc Natl Acad Sci USA 89 :3070 -3074.
25. Halliwell B, Gutteridge JMC.(1990) The antioxidant of human extracellular fluids .Arch Biochem Biophys 280 :1-8.
26. Hanigan , M.H.(2014) Gamma-glutamyl transpeptidase redox regulation and drug resistance .Adv in Cancer Res 122 :103-141.
27. Heba Hamadi, Othmane, Y. B., Ammar, O., Kilifi ,A., Hallara., E. Ben. Ghali, F., Houas, Z., Najjar M.F. and Salwa Abid-Essaefi . (2019) oxidative stress, genotoxicity, biochemical and histopathological modifications induced by epoxiconazole in liver and Kidney of waster rats. Environ, Sci. and Pollut. Res. 26: 17535- 17547.
28. Henry, R. J. (1974) Clinical Chemistry: Principles and Techniques. 2nd ed., Harper & Row, Hagerstown, MD. Pp: 411-442.

29. Kim ,S,J .,Han .D .,Moon ,KD .,Rhee ,J.S. (1995) Measurement of superoxide dismutase like activity of natural antioxidant .Biosci .Biotechnol .Biochem .59 822-826
30. Kim, S.H., Hyun, S.H., Choung. S.Y. (2006) Antioxidative effects of cinnamon cassia, and Rhadiols rosea extracts in Liver of diabetic mica. Bio Factors 26: 209- 219.
31. Klein, B., Read, P. A., Babsona, L. A. (1960) Optical condition for the determination of serum alkaline phosphatase by a New kinetic method. Clin. Chem., 6:260-275.
32. Koenig, G., and Seneff, S. (2015) Gamma- Glutamyl transferdse,: A predictive biomarker of cellular antioxidant in adequacy and disease risk. Disease Markers. (Article ID 818570, 18 pages).
33. Lachleitner ,M., Koch, T., Herold ,M., Dzien ,A., Hoppichler ,F .(2000) Tumer necrosis factor –alpha- plasma level inpatients with type I diabetes mellitus and its association with glycaemic control and cardiovascular risk factor .J. Intern. Med.248:67-76.
34. Leonard, T.B., Neptun, D.A., and Popp. J.A. (1984) Serum gammaglutamyl transferase as a specific indicator of bile duct lesions in the rat liver- AmJ. Pathol. 116, 262- 296.
35. Lin, C. C., WU, S.J., Chang, C.H. and Ng. I.T. (2003) Antioxidant activity of cinnamomum cassia. Phytother. Res. 17 (7): 726- 730.
36. Lopes, B., Gaique, T.G., Souza, L.L., Panla, G.S.M., Kluck, G, E.G., Allella, G.C., Gome, C.C., Simay, N.K., Kuster, R.M., Ortega- Carvalho, T.M., Pazos- Moura, C.C., Oliveria, K.J. (2015) Cinnamon extract improves the body composition and attenuates lipogenic processes in the liver and adipose tissue of rats Food Funct .6:3257-3265.
37. Lu, S.H., Lee ,SY ., Son ,DJ .,Lee ,H., Yoo, HS., Song ,S. (2005) Inhibitory effect of 2-hydroxy cinnamaldehyde on nitric oxide production through inhabition NF-Kappa B-activation in RAW264.7cells .Biochem Phamacol.69 :791-9.
38. Marklund, S. and Marklund, G. (1974) Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem. 47: 469-474.
39. Marmugi, A., Ducheix, S., Lasserre, F., Polizzi, A., Paris, A., Priymenko, N. ,Berland- Michel, J., Pineau, T., Guillou, H., Martin, P.G., Mselli- Lakhal, L. (2012) Low doses of bisphenol A induce gene expression related to lipid synthesis and Trigger triglyceride accumulation in adult- mouse liver. Hepatology 55(2): 395- 407.
40. Martin, M. T., Brennan, R., Hu, W., Ayanoglu, Lau, C., Ren, H., Wood, C.R., Cortor, C., Kavlock, R.J. and Dix, D.J. (2007) Toxicogenomic study of triazole fungicides and perfluoroalky Acids in rat livers predicts toxicity and categorizes chemicals based on mechanisms of toxicity. Toxicol. Sci. 97 (2) 595- 613.
41. Masuro, H., Iwanani, J., Kidam, T., Sakayama, K., Honda, K. (2005) Bisphenol Accelerates remain of differentiation of 313- L1 cells into adipocytes through the phosphatidy linositol 13- kinase pathway toxic. Sci. 84, 319- 327.
42. Mona , A., Gehan ,M.(2016) Potential therapeutic effect of tumeric (Curcuma longa) against adverse effect of penconazole fungicide to white rats .Inter .J. of Phama and Toxi .4(2):178-186.
43. Morel ,I ., Lescoat ,G .,Cogrel ,P,Sergent ,O., Padeloup ,N., Bissot ,P, Cillard ,P, Cillard ,J. (1993) Antioxidant and iron-chelating activities of the flavonoids catechin quercetin and diosmetin on iron loaded rat hepatocytes culture .J.Bio. Chem.pharmacol 45:13-19.
44. Morgan ,A.M., El-Ballal ,S.S., EL-Bialy ,B.E ., EL-Borai ,N.B.(2014) Studies on the potential protective effect of Cinnamon against bisphenol-A and actyl phenol –induced oxidative stress in male albino rats .Toxicol .Reports .1:92-101.
45. Moriyama, K., Tagami, T., Akamizu, T., Usui, T., Soijo, M., Kanamoto, N., Hataya, Y., Shimatsu, A., Kuzuya, H., Nakao, K. (2002) Thyroid hormone action in disrupted by Bisphenol A as an antagonist. J.Clin , Endocrinol. Metab. 87 (11), 5185- 5190.

46. Moselhy ,S.S., Ali ,H.K. (2004) Hepatoprotective effect of cinnamon extract against carbontetrachloride induced oxidative stress and liver injury in rats .Boil .Res.42,93-98.
47. Moselhy ,S.S., Junbi, H.H.(2010) Antioxidant properties of ethanolic and aqueous Cinnamon extracts against liver injury in rats .Intar .J.of Advances in pharmacal science . 1(2):151-`55.
48. Moss D.W. and Butter Workh, P.J. (1974) Ezymology and medicine (Pitman Medical, London). 39.
49. Murcia, M.A., Egen, I., Romojaro, F., Parras, P., Jimenez, A.M., Martinez, Tome, M. (2004) Antioxidant evaluation in dessert spices compared with common food additives .In fluency of irradiation, procedure, J. Agric. Food. Chem 52: 1872- 1881.
50. Navarro , C.M., Mantilla, P.M., Martin ,A., J imenez, J .Utrilla ,P.M. (1993) free radicals scavenger and antihetotoxic activity of Rosmarinus. Plant .Medicin 59: 312-314 .
51. Nesnow, S., Grind staff, R. D., Lambert, G., Padgett, W., Bruno, M., Ge, Y., chen, P. J., Wood, C.E., Murphy, L. (2011) Penconazole increase reactive oxygen species levels in mouse hepatic cells in culture and in mouse liver by acyto chrome P 450 enzyme mediated process. Chemico- Biological interactions 194: 79- 89.
52. Nesnow, S., Padgentt, W.T. and Moore, T (2012) Propiconazole induces alterations in the hepatic metabolome of Mice: Relevance to Propiconazole- Induced mepatocarcinogeneses toxicological sciences 120 (2) 297- 309.
53. Ohkawa, H., Ohishi, N. and Yagi, K. (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95: 351-358.
54. Organization of Economic Co-operation and Development Guide line for testing of chemicals. (2008) Acute Oral Toxicity, No. 407. (OECD, 2008).
55. Osman, A.H., El- Shamas. S., Osman A. S., Abdel- Hammed, A. K. (2011) toxicological and pathological evaluations med. J. Cairo Univ. 79 (1): 555- 564.
56. Ozer, J., Ratner, M., Shaw, M., Bailey ,W., Schoma- Ker, S. (2008) The currents state of serum biomarkers of hepatotoxicity. Toxicology, 245: 194- 205.
57. Pallarda ,F.V.,Markovic ,J.,Garacia ,JL.,Vin ,J .(2009) Role of nuclear glutathione as a key regulator of cell proliferation .Mole Aspec of Medi 30 (1-2)77-85.
58. Pigeolet E,Corbisier P,Houbion A,Lambert D ,Michiels C,Raes M,Zachary M-D,Remacle J .(1990) Glutathione peroxidase ,superoxide dismutase ,and catalase inactivation by peroxidase and oxygen derived free radicals .Mech Ageing Dev 51 (3)283-297.
59. Pogngothai, A., Sreena, K.P., Sressith ,K., Uthiralingam M., Annapoorani ,S. (2011)Preliminary photochemicals screen in ficus racemosalinn. Bark Int .J. Pharm . Bio .Sci 2(2): 431-434.
60. Ramaiah, SK. (2007) A toxicologist guide to the diagnostic inter pretion of hepatic biochemical parameters. Food hem. Toxicol. 145, 1551- 1557.
61. Rastogi ,Sk .,Satyanarayan , P.V.V ., Ravishankar ,V., Sachin Tripathi (2009) A study on oxidative stress and antioxidant status of agricultural workers exposed to organophosphorus insecticides during spaying . Indian .J. of Occup and Envir medicine . 13(3) 131-134.
62. Reitman, S. and Frankel, S. (1957) A colourimetric method of the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol. 28: 52-56.
63. Ronis, M.J., Ingelman-sundberg , M., Badger, T.M.(1994) Induction suppression and inhibition of multiple hypatic cytochrome P450isozymes in the male rat and bobwhite quail (Colinus Virginianus)by ergosterol biosynthesis inhibiting fungicides (EBTFS). Biochem. Pharmacal .48,1953-65.
64. Roussal .A., Hinininger ,I ., Benaraba ,R.(2009) Antioxidant effects of cinnamon extract in people with impaired fasting glucose that are over weight or obese .J. of the American collage of nutrition . 28(1)16-21.

65. Saenkod ,C., Liu, Z., Huang ,J .,Gong ,Y. (2013) Anti-oxidative biochemical properties of extracts from some Chinese and Thai rice Varieties .Afr J Food Sci 7(9):300-305.
66. Sakihama ,Y., Cohen ,M.F., Grace,S.C., Yamesaki, H .(2002) Plant phenolic antioxidant and prooxidant activities phenolics –induced oxidative damage mediated by metals in plants .Toxicology .177:67-80.
67. Satpute ,R.M., Pawar, PP., Putterwar ,S., Sawale ,SD., Ambhore ,PP.(2017) Effect of resveratrol and tetracycline on the subacute paraquat toxicity in mice Human Exp.Toxicol .36:1303-1314.
68. Saxena ,J., Sahu ,R.(2012) Evaluation of phytochemical constituent in conventional and non-conventional species curcuma . Int .Res .J . Pharm 3(8):203 -204.
69. Scott, R.B.,Reddy,K.S., Husain ,K., Schlorff ,E.C.,Rybak, L.P., Somani ,S.M.(2008) Dose response of ethanol on antioxidant defense system of liver ,lung and kidney in rat .Path –Physiology . 7:25-32.
70. Shalaby ,M.A, and Saifan ,H.Y.(2014) Some pharmacological effects of cinnamon and ginger herbs in obese diabetic rats .J Intercult Ethnopharmacol .(3) 144-149.
71. Shan, A. H., Al- Shareef, A.H., Ageel, A.M., and Qureshi, S. (1998) Toxicity studies in mice of mice of common spices cinnamomum zeylanicum bark and piper longum fruits. Plant foods for Human Nutrition. (Formerly Qulaiates plahtarun) 52 (3): 231- 239.
72. Sharmanov, A.T., Aidarkhanov, BB., Kurmangelinov, SM. (1990) Effects of vitamin E deficiency on oxidative enzyme activity of macrophage. Annals of Nutrition and metabolism. 34: 143- 146.
73. Shatwan, I.A., Ahmed, L.A., Badkook, M.M. (2013) Effect of barley flour, crude cinnamon, and their combination on glycemiadys lipidemia and adipose tissue hormones in type 2 diabetic rats. J. Med. Food 16: 656- 662.
74. Sheng ,X., Zhang ,Y.,Gong ,Z .,Huang ,C. and Zang ,Y .(2008) Improved insulin resistance and lipid metabolism by cinnamon extract through activation of peroxisome proliferator –activates receptors .PPAR Res.
75. Shobana,S., Naidu,K.A. (2000) Antioxidant activity of selected spices .Prostaglandins Leukot Essent Fatty acids .62(2)107-110.
76. Singleton,V.L., Orthofer, R., Lamuela-Raventos, R.M. (1999) Analysis of total phenols and other oxidation substrates and antioxidant by means of Folin-ciocalteu reagent Methods Enzymol 299:152-178.
77. Soliman ,M.M., Attia ,H.F., EL-Shazly ,S.A., Saleh ,O.M.(2012) Biomedical effects of Cinnamon extract on obesity and Diabetes Relevance in wister rats .American .J.Biolchemistry and Mole .2(3):133-145.
78. Stadler ,R.H.,Markovic ,J. and Turesky, R.J.(1995) In vitro anti and prooxidative effects of natural polyphenols .Biol .Trace .Elem.Res .47:299-305.
79. Stein ,T.A.,Burns ,Gp and Wiseh(1989) Diagnostic value of liver Function Tastes in Bile Duct obstruction J. Surg.Res.46(3)226-9.
80. Stone, S. H. (1954) Method for obtaining venous blood from the orbital sinus of the rat or mouse. Science 119: 100-103.
81. Szasz, G., Persijng, J.P. (1974)New substrates for measuring gamma-glutamyle transpeptidase. Clin. Chem Clin. Biochem, 12:228
82. Tambe, V.D. and Bhamber, S.R (2014) Estimation of total phenol ,tannin, alkaloid and flavonoid in Hibiscus tiliaceus Linn.Wood extract.Res Rev J Pharm Phytochem 2(4):41-47.
83. Teo,S.,Stirling,D.,Thomas,S.,Hobermane,A.,Kiorpes,A.,Khetam,v.(2002) A90 day oral gavage toxicity study of D-methyl phenidate and D.L –methyl ph indicate in Spragne –Dawley rats . Toxicology .179; 183-196.
84. Turner, T.T. and Lysiak, J. (2008) Oxidative Stress: a common factor in testicular dysfunction J. Androl. 29: 488-498.

85. Vanden Bossche ,H., Marichal,p., Gorrens,J., Coene ,M.C .,Willemsens ,G .,Bellens ,D.,Roels,I .,Moereals ,H., and Janssen,P.A.(1989) Biochemical approaches to selective antifungal activity .focus on azole antifungals *Mycoses* 32 (suppl),35-52.
86. Vangalapati M, Sree Satya N, Surya Prakash DV, Avanigadda S.(2012) A review on pharmacological activities and clinical effects of cinnamon species . *J Pharma ,Biol and Chem* 3(1) :653-663.
87. Verma, R.S., Mehta, A., Srivastava, N. (2007)"In vitro Chlopyrifos Induced Oxidative Stress : Atten kated by antioxidant vitamins " *Pesticide Biochemistry and Physiology* 88:191-196.
88. Wada, K., Sakamoto, H., Nrishikawn, K., Sakuma, S., Nakajima, A., Fujimoto, Y., Kamisaki, Y.,(2007) Live- styl- related diseases of the digestive system: endocrine disruptors, stimulate lipid accumulation in target cells related metabolic syndrome. *J. pharmacol. Sci.* 105: 133- 137.
89. Wang.C., Wu ,Q, Wu, c. ,Wang Z .(2011) Application of dispersion –solidification liquid micro-extraction for water samples by high performance liquid chromatography. *J .Hazard Mater .*, 185 ,71-86.
90. Wolf ,D.C., Allen ,J.W., Geory ,M.M., Hesten ,S.D., Sun ,G., Moore ,T., Thai ,S.F., Delker ,D.A. (2006) Toxicity profiles in rats treated with tumorigenic and nontumorigenic triazole conazole fungicides :propiconazole ,triadimefon and myclobutanil .*Toxicologic Pathologic* 34 :895-902.
91. Yang ,JD., Liu, SH ,Liao, MH., Chen, RM, Liu PY ,Veng, TH (2018) Effects of tebuconazole on cytochrome P450 enzymes oxidative stress and endocrine disruption in male rats *Environ .Toxicol* .33 :899-907.
92. Yashin ,A., Yashin ,Y .,Xia ,X. and Newzer ,B.(2017) Antioxidant activity of spices and their impact on human health :A Review .*J Antioxidants* .6:1-18
93. Young, D. S. (1975) Effects of drugs on clinical laboratory tests. *Clin Chem.* 18: Id- 432D.
94. Zarm, J.A., bruschweiler ,B.J.,Schlatter ,J.R. (2003) Azole fungicides affect mammalian steroid genesis by inhibiting sterol 14- alpha –demethylase and aromatase *Environ .Health .PersPect* .111,255-261.
95. Ziegenfuss, T. N., Hofheins, J.E., Mendel, R.W., Landis, J., And Anderson, R.A. (2006) Effect of a water- soluble cinnamon extract on body composition and features of the metabolic syndrome in pre-diabetic men and women. *J. Int. Soc. Sports. Nutr.* 3, 45- 53.

Figures

Figure 1

Histopathology of liver tissue (A): liver section of normal control showing the normal histological structure of the central vein and portal area. (B, C, D): liver section of difenoconazole-treatment rats at high dose showing degenerative changes in the hepatocytes surrounded and adjacent the dilated central vein in addition, the portal area showed oedema and periductal fibrosis surrounding the bile duct and hyperplasia in bile ducts. (E, F, G): liver section of difenoconazole-treatment rats at low dose showed vacuolar degeneration in the hepatocytes, in addition to dilation in the central vein associated with fatty change in the surrounding adjacent hepatocytes. (H): liver section of cinnamon rats at high dose showed slight degenerative change. (I, J): liver section of rats received AEC pre-treatment with difenoconazole showed improvement in the histology of the liver section.