



## Evaluation of the ameliorative effect of Cinnamon Cassia against metabolic disorder and thyroid hormonal disruption following treatment with difenoconazole fungicide in the male albino rats.

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### Abstract

The present study aimed to investigate the deleterious effects of difenoconazole exposure involving the blood glucose level and lipid profile indices [i.e. TC, TAG, LDL-C, VLDL-C, and HDL-C] as well as renal function markers and thyroid hormonal level (T4&T3) in male albino rats after 28 days of treatments. Therefore, we also evaluate the ameliorative effect of aqueous extract of cinnamon (AEC) as a prophylactic agent against metabolic disorders including hyperglycemia and dyslipidemia as well as the alterations in the renal function markers (creatinine and urea) and the thyroid hormone levels in rats flowering treatment with difenoconazole. Results revealed that treating rats with difenoconazole induced a significant increase in the level of blood glucose and so the lipid profile indices i.e. TC, TAG, LDL-C, and VLDL-C, and a marked decrease in the level of HDL-C, in addition, a significant elevation in the levels of creatinine and urea was observed. In contrast, there is a significant decrease in the levels of T4 and T3 in difenoconazole-treated rats. Pre-treatments with AEC alleviate the harmful effects of difenoconazole treatment revealed by reducing the blood glucose level (hypoglycemia) and lipid profile parameters as well as the biomarkers of renal function and improvements the hypothyroidism state via elevating the levels of T4 and T3 in comparison with difenoconazole treatment. Therefore, these findings indicated the safety and useful effects of cinnamon extract for preventing the development of lipid metabolic disorders and hypothyroidism state, consequently attenuated its complications involved diabetes and cardiovascular disease (CHD).

Keywords: Cinnamon; Difenoconazole; Lipid profile; Blood glucose; Thyroid hormone.

### 1. Introduction

Pesticides are a broad group of heterogenous chemicals and they became an essential component used in the agriculture system for increasing crop yield and food production all over the world [1]. Nowadays, fungicides are widely used for the keeping of consumed vegetables and fruits fresh for a long time. Triazole fungicides are important agricultural

pesticides, where they are used on crops and fruits [2]. Pesticide exposure may disturb the oxidative stress homeostasis, consequently induces many biological or biochemical disorders and hence the development of many diseases, such as diabetes mellitus and coronary heart diseases (CHD) in humans.

Treatment of cypermethrin to rats induced nephrotoxicity revealed by elevated BUN and creatinine levels in albino rats [3]. Moreover, oral administration of flusilazole fungicide exhibited a significant elevation in the levels of glucose, total cholesterol (TC), triacylglycerol (TAG), low-density lipoprotein-cholesterol (LDL-C) along with reducing the level of high density-lipoprotein-cholesterol (HDL-C) at the end of the experimental period (90 days) [4]. A study carried out by [5] revealed that penconazole treatment induced a significant reduction in the levels of thyroid (T4) and triiodothyronine (T3) after 60 days of treatments.

Medicinal herbal plants as an enriched source of biologically active components such as polyphenolic compounds and essential oils constituents that may be

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responsible for the health of humans through modulating the antioxidants status [6]. Cinnamon has received many alterations in the past several years as a natural product and it is the most popular spice used daily by people all over the world because it is rich in polyphenolic compounds that have been shown to improve the action of insulin in vitro [7].

Recently, many studies revealed that cinnamon has beneficial properties which play a key role in the human via controlling glucose levels and lowering lipid concentration in the dyslipidemia state [8,9]. also, cinnamon extracts are reported to have beneficial effects on people with normal and impaired glucose tolerance, metabolic syndrome, type 2 diabetes, insulin sensitivity, and insulin resistance [10].

The blood glucose level decline markedly in a dose-dependent manner with the most efficient at the dose level of 200 mg/kg B.W of cinnamon extract and also there is a decrease in the levels of TG and VLDL detected after received 400 mg/kg B.W of cinnamon for 6 weeks [11].

Furthermore, supplementation rats with 200 mg/kg BW of cinnamon bark extract (CBE) significantly decreased serum levels of total cholesterol (TC), low density-lipoprotein-cholesterol(LDL-C),and increased level of high-density lipoprotein- cholesterol (HDL-C), in addition to the ratio of HDL-C /LDL-C) as compared to the control group after 8weeks of treatment [12].

Therefore, this study was conducted to determine the harmful effects of difenoconazole which are related to metabolic disorders involving regularly the blood glucose level and dyslipidemia as well as impaired the function of kidney and thyroid gland and then evaluate the efficacy of cinnamon aqueous extract (CAE) as prophylactic against given before administration of difenoconazole in improving such these effect in the current study.

## 2. Materials and Methods

### 2.1. Tested fungicide.

Difenoconazole (Score 25% EC) is a commercial formulation containing 250g/L. It is manufactured by Syngenta crop protection Inc. Basel-Switzerland. And it is obtained from Central Agricultural Pesticides Lab (CAPL), Agriculture Research Center(ARC), Dokki, Giza, Egypt.

### 2.2. Plant material

Cinnamon cassia bark (family Lauraceae) was purchased from the local market of medicinal plants and herbs, Cairo, Egypt.

### 2.3. Preparation of aqueous extract of Cinnamon (AEC)

The aqueous extract of cinnamon (AEC) was prepared according to the method of [13]. 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 days.

### 2.4. Experimental animal.

In this study 90 male albino rats (Wister stain) aged 6-8 weeks and weighed  $150 \pm 10$  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 conditions, the temperature at  $25 \pm 2$  °C relative humidity  $50 \pm 15$  % and normal photoperiod (12 h dark, 12 h light) the animals were provided with a standard pellet diet from agricultural-industrial integration company, Giza, and water ad libitum. All rats were acclimatized for 14 days before the beginning of the experiments.

**Ethical approval:** The study was conducted in accordance with the national guidelines for the care and use of laboratory animals.

### 2.5. Experimental design.

The selected doses of difenoconazole (Score 25%EC) in the current study are based on the value of oral LD<sub>50</sub> in male rats, which was determined in our laboratory, and it was found to be 1178 mg/kg BW. With regard to selected doses of aqueous extract of cinnamon (AEC) the doses were chosen based on the earlier studies of [13,14].

After acclimatization, all rats were divided randomly into nine groups (10of rats) in each group as follows. The first group represented the rats were administrated distilled water (10ml/kg BW) and served as a control group, and the rest of the groups as follow.

The group LD and HD represented the rats were administrated the difenoconazole formulation at a dose of 58.94 and 117.88 mg/kg BW [which equivalent to 1/20 and 1/10 of oral LD<sub>50</sub>] and served as a low and high dose of the difenoconazole group.

The group LC and HC represented the animals have received the aqueous extract of cinnamon (AEC) at a

dose of 200 and 400 mg/kg BW and served as the low and high dose of the cinnamon.

The group LF+LC represented the rats were received AEC at a dose of 200 mg/kg BW before treatment with a low dose of difenoconazole. The group LF+HC represented the rats were received AEC at a dose of 400 mg/kg BW before treatment with a low dose of difenoconazole.

The group HF+LC represented the rats were received AEC at a dose of 200 mg/kg BW AEC before treatment with a high dose of difenoconazole. In the group HF+HC, the animals were received AEC at a dose of 400 mg/kg BW before treatment with a high dose of difenoconazole

All animals were observed for clinical signs of toxicity and the mortality was recorded within the experimental period.

### 2.6. Blood sample preparation.

On the last day of experimentation (day 28) overnight fasted animals and the blood samples were collected in clean Eppendorf tubes from the orbital sinus vein [15]. The blood was kept under refrigeration (4°C) allowed to clot and serum was separated by centrifugation at 3000 rpm for 15 minutes (by using centrifuge MSE, super – minor, England), the anthor clean Eppendorf tubes, containing heparin as an anticoagulant to obtain plasma for determination of glucose level. The serum and plasma samples were kept at – 40°C for further analysis.

### 2.7. Glucose and lipid profile measurements.

Glucose concentration in blood was estimated according to the method of [16]. Serum triacylglycerol concentration and total cholesterol concentration were measured according to the method of [17,18] by using the Gama trade kit. The very-low-density lipoprotein cholesterol (VLDL) concentration was estimated by employing the friedwald formula. Serum high-density lipoprotein-cholesterol (HDL-C) level was determined according to the method of and Serum low-density lipoprotein-cholesterol (LDL-C) level was calculated by using the [20] equation.

$$\text{LDL-C (mg/100 ml)} = \text{TC} - (\text{HDL-C} + \text{VLDL-C})$$

### 2.8. Kidney function measurements.

Serum urea nitrogen concentration and creatinine were determined according to the method of [21,22], respectively by using the Gama trade kit.

### 2.9. Determination of Serum Tri-iodothyronine (T<sub>3</sub>) and Thyroxine (T<sub>4</sub>) Level.

Serum triiodothyronine (T<sub>3</sub>) and thyroxine (T<sub>4</sub>) level were analyzed by the competitive enzyme-linked

immunosorbent assay (ELISA) according to the method of [23] by using a rat kit from USCN. Company (USA).

## 3. Results

### 3.1. Influence of difenoconazole treatment alone or in concomitant with AEC on the metabolic disorders in male albino rats.

The result presented in Table (1&2) showed that repeated oral administration of difenoconazole induces a significant elevation in the level of glucose by 8.59 and 12.83%, respectively, in comparison with the control group. In contrast, a significant reduction in the glucose level by -12.55 and -12.24 % was observed in rats supplemented only with AEC for 28 days.

Also, a significant reduction in the level of glucose by -12.45 and -11.09% was noticed in rats received AEC pre-treatment with a low dose of difenoconazole-treated rats (at low dose). Also, supplement rats with AEC before treatment with a high dose of difenoconazole exhibit a significant reduction in the level of glucose by -5.57 and -7.52 % in compared with difenoconazole-treated rats (at high dose).

Moreover, treatment rats with a high dose of difenoconazole caused a significant increase in the level of Triacylglycerol (TAG) by 9.47% in comparison with control rats. Also, insignificant elevation in the level of TAG was detected in rats treated with a low dose of difenoconazole in compared with the control group. However, supplemented rats only with AEC showed an insignificant increase in the TAG level in comparison with the control group.

In addition, rats treated with a high dose of difenoconazole for 28 days exhibited a significant elevation in the level of very-low-density lipoprotein (VLDL-C) by 10.51% in comparison with the control group. While there was an insignificant increase in the level of VLDL-C in rats treated with a low dose of difenoconazole in comparison with control rats. Also, this trend was observed in rats received only AEC, where there was an insignificant elevation in the level of VLDL-C.

Meanwhile, insignificant decrease in the level of VLDL-C by -2.80 and -7.36% in rats supplemented with AEC pre-treatment with a low dose of difenoconazole in compared with difenoconazole treated rats (at low dose). Also, treated rats with a high dose of difenoconazole after received AEC resulted in a significantly reduced in the level of VLDL-C by -4.48 and -4.59% in comparison with difenoconazole-treated rats (at high dose).

Table (1) Effect of difenoconazole and aqueous cinnamon alone or concomitant with difenoconazole at different doses on plasma glucose and serum lipid profile of the male albino rat for 28 days.

Parameters	Cont	LD	HD	LC	HC	LD+LC	LD+HC	HD+LC	HD+HC
Glu (mg/dl)	106.36 ±0.74	115.50 <sup>a,c</sup> ±0.68	120.01 <sup>a,b</sup> ±0.80	92.59 <sup>a,b,c</sup> ±0.89	95.46 <sup>a,b,c</sup> ±0.60	101.11 <sup>a,b,c</sup> ±0.50	102.69 <sup>a,b,c</sup> ±0.54	113.32 <sup>a,c</sup> ±1.50	110.98 <sup>a,b,c</sup> ±0.59
Change		8.59	12.83	-12.94	-10.24	-12.45	-11.09	-5.57	-7.52
TAG (mg/dl)	79.88 ±3.02	85.58 ±2.83	87.45 <sup>a</sup> ±0.86	83.44 <sup>c</sup> ±0.57	80.28 <sup>b,c</sup> ±0.25	84.19 ±2.15	79.3 <sup>b,c</sup> ±1.89	83.28 ±1.56	82.40 ±0.87
Change %		7.13	9.47	4.45	0.50	-1.62	-7.27	-4.76	-5.77
VLDL-C (mg/dl)	15.97 ±0.60	17.11 ±0.56	17.40 <sup>a</sup> ±0.11	16.61 ±0.07	16.09 <sup>b,c</sup> ±0.06	16.63 ±0.33	15.85 <sup>b,c</sup> ±0.39	16.62 ±0.31	16.60 ±0.07
Change %		8.35	10.19	5.19	1.89	-2.80	-7.36	-4.48	-4.59

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 difenoconazole (L.D), (c) significant compared to high dose difenoconazole (H.D).

Concerning total cholesterol (TC) treatment rats with both doses of difenoconazole for 28 days produced a significant increase in the level of TC by 9.82 and 13.18 %, respectively in comparison with the control group as shown in Table (2). Meanwhile, supplemented rats with AEC showed non-significant differences in the level of TC in comparison with the control group.

On the other hand, a significant decrease in the level of TC by -8.69 and -7.45 was detected in rats received AEC before treatment with a low dose of difenoconazole in compared with difenoconazole-treated rats (at low dose). Also, rats treated with a high dose of difenoconazole after received AEC has a significant reduction in the level of TC by -9.01 and -8.11%, in comparison with difenoconazole-treated rats (at high dose).

In addition, treated rats with both doses of difenoconazole produced a significant elevation in the level of low-density lipoprotein cholesterol (LDL-C) by 52.10 and 61.36 % respectively in comparison with the control group. On the other hand, there was a significant decrease in the level of LDL-C by -24.40 and -25.45% in rats supplemented only AEC in compared with the control group.

Administration difenoconazole rats following supplemented with CAE, resulted in a significant decline in the level of LDL-C by -41.00 and -33.23% as compared with difenoconazole-treated rats (at low dose). Also, this trend was observed in rats received the AEC pier to treatment with a high dose of difenoconazole, where the LDL-C level decreased markedly by -35.06 and -24.56 %, respectively in comparison with difenoconazole-treated rats (at high dose).

Moreover, the present study showed that treating rats with difenoconazole induced a significant decline in the high-density lipoprotein cholesterol (HDL-C) by -25.08 and -26.80% in comparison with the control group.

However, there was a significant increase in the level of HDL-C by 15.5% and 10.51% in rats treated with a high and low dose of AEC when compared with the control group.

Furthermore, a significant elevation enhancement in the level of HDL-C by 37.91 and 34.52% was seen in rats treated with a low dose of difenoconazole after received the AEC in compared with difenoconazole-treated rats (at low dose). Also, an elevation markedly in the level of HDL-C by 29.61 and 31.51% was observed in rats treated with a high dose of difenoconazole after received the AEC in compared with difenoconazole treated rats (at high dose).

With respect to the ratio of TC/HDL-C, the results presented in Table (2) exhibited that difenoconazole treatment induced a significant elevation in the ratio of TC/HDL-C by 53.72% and 50.58% respectively, in comparison with the control group.

While rats supplemented only with AEC had a significant decrease in this ratio particularly rats treated with a high dose of difenoconazole by -17.25% in comparison with the control group.

However, pre-treatment with AEC showed a significant decrease in the ratio of TC/HDL-C by -39.79 and -33.92 % respectively, in rats received CAE before treatment with a low dose of difenoconazole-treated rats (at low dose).

Also, supplemented rats with AEC before treatment rats with a high dose of difenoconazole showed a significant reduction in the ratio of TC/HDL-C by -30.46 and -27.34% in comparison with difenoconazole - treated rats (at high dose).

Table (2) Effect of difenoconazole and aqueous cinnamon alone or concomitant with difenoconazole at different doses on serum lipid profile of the male albino rat for 28 days.

Parameters	Cont	LD	HD	LC	HC	LD+LC	LD+HC	HD+LC	HD+HC
TC (mg/dl)	61.96 ±1.40	68.05 <sup>a</sup> ±1.00	70.13 <sup>a</sup> ±1.12	59.56 <sup>b,c</sup> ±1.66	60.76 <sup>b,c</sup> ±2.08	62.13 <sup>b,c</sup> ±2.32	64.58 <sup>c</sup> ±1.47	63.81 <sup>c</sup> ±1.97	64.44 <sup>c</sup> ±1.66
Change %		9.82	13.18	-3.87	-1.93	-8.69	-5.09	-9.01	-8.11
LDL-C (mg/dl)	20.94 ±0.73	31.85 <sup>a</sup> ±0.78	33.79 <sup>a</sup> ±0.77	15.83 <sup>a,b,c</sup> ±1.95	15.61 <sup>a,b,c</sup> ±1.11	18.79 <sup>b,c</sup> ±1.07	22.56 <sup>b,c</sup> ±2.02	21.94 <sup>b,c</sup> ±1.74	25.49 <sup>b,c</sup> ±2.95
Change %		52.10	61.36	24.40	-25.45	-41.00	-29.16	-35.06	-24.56
HDL-C (mg/dl)	24.36 ±1.11	18.25 <sup>a</sup> ±1.08	17.83 <sup>a</sup> ±1.06	26.92 <sup>b,c</sup> ±1.41	28.14 <sup>b,c</sup> ±0.98	25.17 <sup>b,c</sup> ±1.59	24.55 <sup>b,c</sup> ±1.54	23.11 <sup>b,c</sup> ±1.51	23.45 <sup>b,c</sup> ±1.72
Change %		-25.08	-26.80	10.50	15.51	37.91	34.52	-29.61	-31.51
TC/ HDL-C	2.55 ±0.10	3.92 <sup>a</sup> ±0.15	3.84 <sup>a</sup> ±0.16	2.30 ±0.06	2.11 ±0.04	2.36 ±0.05	2.59 ±0.13	2.67 ±0.16	2.79 ±0.27
Change %		53.72	50.58	-9.80	-17.25	-39.79	-33.92	-30.46	-27.34

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 difenoconazole (L.D), (c) significant compared to high dose difenoconazole (H.D).

### 3.2. Influence of difenoconazole treatment alone or in concomitant with AEC on renal function parameters in male albino rats.

Concerning biochemical parameters of renal function, there was a significant increase in the creatinine level by 12.50% and 7.81% in rats following treatment with a low and high dose of difenoconazole in compared with control rats. Meanwhile, non-significant differences in the levels of creatinine in rats received only AEC when compared with the control group.

However, treatment rats with a low dose of difenoconazole along with AEC induced an in significant decrease in the level of creatinine by -9.85 and -8.45% in compared with difenoconazole-treated rats.

Also, this trend was observed in rats treated with a high dose of difenoconazole post-treatment with AEC where there was an insignificant decrease in the

creatinine level by -4.34 and -8.69 % in compared with difenoconazole treated rats [at high dose].

In concern of blood urea nitrogen (BUN), there was a significant increase in the level of BUN by 41.97 and 48.39% in rats treated with difenoconazole in comparison with the control group.

Also, an insignificant decrease in the BUN level by 14.20 and 8.81% was detected in rats treated with AEC in compared with the control group.

Rats received the AEC at a low and high dose pre-treatment with a low dose of difenoconazole had a significant decrease in the level of BUN by -15.85 and -12.96%. and administration of a high dose of difenoconazole after supplementation with AEC induced in significant decline in the level of BUN by -7.85 and -6.01 % in comparison with difenoconazole treated rats.

Table (3) Effect of difenoconazole and aqueous cinnamon alone or concomitant with difenoconazole at different doses on kidney function of the male albino rats for 28 days.

Parameters	Cont	LD	HD	LC	HC	LD+LC	LD+HC	HD+LC	HD+HC
Creatinine (mg/dl)	0.64 ± 0.01	0.72 <sup>c</sup> ± 0.01	0.69 ± 0.055	0.65 ± 0.01	0.62 <sup>b</sup> ± 0.023	0.64 ± 0.01	0.65 ± 0.015	0.66 ± 0.032	0.63 <sup>b</sup> ± 0.016
Change %		12.50	7.81	1.65	-3.12	-9.85	-8.45	-4.34	-8.69
BUN (mg/dl)	18.37 ± 0.50	26.08 <sup>a</sup> ± 1.07	27.26 <sup>a</sup> ± 1.17	15.76 <sup>b,c</sup> ± 0.89	16.75 <sup>b,c</sup> ± 1.02	21.95 <sup>a,b,c</sup> ± 1.46	22.70 <sup>a,b,c</sup> ± 1.72	25.12 <sup>a</sup> ± 1.97	25.62 <sup>a</sup> ± 1.64
Change %		41.97	48.39	14.20	8.81	-15.85	-12.96	-7.85	-6.01

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 difenoconazole (L.D), (c) significant compared to high dose difenoconazole (H.D).

### 3.3. Influence of difenoconazole-treatment alone or in concomitant with AEC on the thyroid hormone levels in male albino rats.

Results presented in Table (4) revealed that difenoconazole-treatment induced a significant decrease in the level of thyroxine (T4) by -13.58 and -41.65% in compared with the control group.

Meanwhile, non-significant differences in the level of T4 in rats received only the AEC in comparison with the control group.

However, supplemented rats with AEC prior to administration of a low dose of difenoconazole caused a significant elevation in the level of T<sub>4</sub> by 13.07 and 12.37% in comparison with difenoconazole-treated rats [at low dose]. Also, a significant elevation in the T<sub>4</sub> level by 28.10 and 14.84% in rats received the AEC pre-treatment with a high dose of difenoconazole in compared with difenoconazole-treated rats [at high dose].

In concern of T<sub>3</sub> level, there was also a significant decrease in the level of T<sub>3</sub> by -27.39 and -33.56% in rats treated with difenoconazole compared with the control group.

Rats received a high dose of AEC exhibited a significant decrease in the level of T<sub>3</sub> by -8.90% in compared with control rats. Whereas non-significant differences in the level of T<sub>3</sub> in rats received the low dose of AEC in compared with the control group.

In contrast, supplemented rats with AEC before treatment with a low dose of difenoconazole produced a significant elevation in the level of T<sub>3</sub> by 20.75 and 24.52% in compared with difenoconazole-treated rats. Also, this tend detected in rats treated with a high dose of difenoconazole after received the AEC where a

significant increase in the level of T<sub>3</sub> by 24.74 and 12.37 % was observed in compared with difenoconazole-treated animals [at high dose].

## 4. Discussion

### 4.1. Effect of difenoconazole treatment alone or in concomitant with AEC on glucose and lipid profile in male albino rats.

The rise in blood glucose (hyperglycemia) by pesticide exposure may indicate disruption of carbohydrate metabolism as a result of an enhanced breakdown of liver glycogen, possibly mediated by increasing stress hormones such as adrenocorticotrophic hormones and reduced insulin activity [24]. Also, it has been reported that pesticides caused hyperglycemic as a consequence of glycogenolysis in muscle and liver [25].

Our results in the present study showed that difenoconazole treatment induced a significant elevation in the level of glucose [hyperglycemia], this could be attributed to an increase of catecholamines release, which causes glycogenolysis, consequently leading to elevation in the level of glucose in the bloodstream.

Administration of flusilazole fungicide to male rats produced a significant elevation in the level of glucose in comparison with the control group [4]. Also, [26] reported that treating rats with cypermethrin caused a significant increase in plasma glucose concentration as compared with control rats. In addition, rabbits were treated with talstar as a synthetic pyrethroid exhibited a marked increase in the glucose level [27].

Table (4) Effect of difenoconazole and aqueous cinnamon alone or concomitant with difenoconazole on the level of serum thyroxine (T4) and triiodothyronine (T3) of the male albino rats for 28 days.

Parameters	Cont	LD	HD	LC	HC	LD+LC	LD+HC	HD+LC	HD+HC
T4 (pg/ml)	34.88 ± 0.23	30.14 <sup>a,c</sup> ± 0.40	20.35 <sup>a,b</sup> ± 0.29	35.66 <sup>a,b,c</sup> ± 0.43	34.43 <sup>b,c</sup> ± 0.17	34.07 <sup>a,b,c</sup> ± 0.04	33.87 <sup>a,b,c</sup> ± 0.21	26.07 <sup>a,b,c</sup> ± 0.11	23.37 <sup>a,b,c</sup> ± 0.22
Change%		-13.58	-41.65	2.23	-1.29	13.03	12.37	28.10	14.84
T3 (ng/ml)	1.46 ± 0.03	1.06 <sup>a,c</sup> ± 0.04	0.97 <sup>a,b</sup> ± 0.01	1.48 <sup>b,c</sup> ± 0.04	1.33 <sup>a,b,c</sup> ± 0.02	1.28 <sup>a,b,c</sup> ± 0.02	1.32 <sup>a,b,c</sup> ± 0.03	1.21 <sup>a,b,c</sup> ± 0.01	1.09 <sup>a,c</sup> ± 0.05
Change %		-	-33.56	1.36	-8.90	20.75	24.52	24.74	12.37

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 difenoconazole (L.D), (c) significant compared to high dose difenoconazole (H.D).

Meanwhile, co-treatment with AEC significantly reduced the glucose level [hypoglycemia] as compared to the difenoconazole-treated group. Also, this trend was observed in rats received only AEC in compared with the control group.

These observations exhibited the positive effect in stressful conditions and refer to the potential of cinnamon extract as a hypoglycemic agent, this may be a result of the presence of antioxidant compounds such as water-soluble polyphenol Type-A Polymer which is effective and reducing insulin resistance [28,29].

There are several studies reported that cinnamon has been one of the most effective regulating blood glucose concentration in diabetic patients and animals. This may be attributed to the presence of active compounds of cinnamon as soluble polyphenol Type-A polymers as well as the cinnamaldehyde as an essential oil and cinnamic acid [30,31].

Regarding the mechanism of action of the cinnamon extract [32] reported that cinnamon extract promotes enhanced glucose transportation or uptake by glucose transporter particularly, GLU-4 in brown adipose tissue and muscles.

Also, other studies have shown that cinnamon extract promotes glucose uptake by activating insulin receptor kinase activity and autophosphorylation of insulin receptors and increasing glycogen synthesis along with inhibiting of gluconeogenesis via effects on key regulatory enzymes as well as stimulating insulin release and potentiating insulin receptor activity [33].

Also, polyphenols of cinnamon have been identified as up-regulator of mouse adipocyte insulin receptors [10]. However, the major active components in cinnamon, which are responsible for hypoglycemic effects in the aqueous extract are water-soluble polyphenol double-linked procyanidin Type-A Polymers, which are can up-regulation of glucose uptake and increase glycogen synthesis by activating glycogen synthesis and inhibiting glycogen synthase kinase 3B and reducing glucose absorption in the small intestine via increasing the glycosidase enzyme activity. Also, the aqueous extract of cinnamon markedly reduced the alanine in the rat intestine, which plays a vital role in gluconeogenesis in the liver [34].

A recent study by [35] who found that the cinnamon doses of 5,10 and 20 mg/kg BW, caused glycemic control in diabetic rats, due to enhanced insulin secretion. Cinnamaldehyde is an aromatic essential oil and it improves glucose metabolism modulation. This may be ascribed to increase the expression of the

levels of peroxisome proliferators which induces insulin receptors [36]. Moreover, hydro-alcoholic extract of cinnamon exhibited inhibition of the activity of pancreatic- $\alpha$ -amylase in Vivo and in Vitro [37].

Oral administration of cinnamon, essential oils markedly reduced the glucose level in diabetic rats [38].

Furthermore, flavonoids were also found to improve hepatic hexokinase and glucose-6-phosphates enzymes activities, two major enzymes involved in glucose homeostasis in the body [39]. Additionally, flavonoids are also known to remedy the damaged pancreatic B-cells and act as insulin secretagogues.

Flavonoids, steroids, terpenoids, and phenolic acid are known to be bioactive substances that act as anti-diabetic compounds [40].

It has been reported that the anti-hyperglycemic potency of the flavonoids could be attributed to the potentiation of the insulin effect by increasing the pancreatic secretion of insulin from pancreatic B-cells, and also its release from bound insulin (Pro-insulin) to insulin conversion by pancreatic cathepsin [41].

However, [28] reported that cinnamon extract (CE) supplementation to mice for 12 weeks (200 mg/kg B.W) led to reducing the level of glucose in comparison to the control group.

Rats were given cinnamon and then administration Glucose Tolerance test had decreased blood glucose levels. Hence, cinnamon has a direct anti-hyperglycemic effect by increasing insulin concentration [42]. Cinnamon can activate insulin-like growth factor [IGF-1] signaling that tends to lower insulin resistance and improvement of glycemic control [43].

Evaluation of rat lipid profile induces post-treatment with difenoconazole alone or in combination with AEC exhibited that difenoconazole treatment induced a significant elevation in the levels of TC, TAG, LDL-C, VLDL, whereas a marked reduction in the level of HDL-C was observed in comparison with the control group.

Hyperlipidemia is characterized by increased serum Triacylglycerol (TAG), Total cholesterol (TC), Low-density lipoprotein cholesterol (LDL-C) particles, and decreased high-density lipoprotein cholesterol (HDL-C) [45,46].

However, hyperlipidemia status is associated with altered physical properties of cellular membranes, this, in turn, may facilitate the escape of free radicals from the mitochondrial electron transport chain or the activation of NADPH-oxidase [47].

Hypertriglyceridemia is a common and important

component of the so-called metabolic syndrome (MS), which includes obesity and decreased high-density lipoprotein (HDL-C)[48].

Our findings are similar to those previously reported by [4] who found that flusilazole caused a significant increase in the level of TAG, TC, and LDL-C at a high dose of flusilazole, whereas a significant decrease in the level of HDL-C was detected in compared with the control group. Also, the administration rats with Carbofuran caused a significant alteration in the serum lipid profile including TAG, TC, LDL-C, and HDL-C [5].

Supplemented rats with a cinnamon aqueous extract (CAE) alone showed in significant differences in the serum lipid profile indices including TAG, TC, LDL-C, VLDL-C, and HDL-C in compared with the control group at the endpoint of experimental.

However, supplemented rats with AEC before treatment with difenoconazole results in significant reduction in the levels of TAG, TC, LDL-C, and VLDL-C. whereas a significant elevation in the level of HDL-C was observed as compared with difenoconazole-treatments.

This could be attributed to a decrease in de-novo hepatic lipogenesis as a consequence of reducing the activity of lipogenic enzymes such as acetyl Co-A carboxylase or ATP-Citrate lipase [94].

Also, it is likely that AEC treatment reduced the rate of lipogenesis in hepatic tissue this, in turn, led to a decrease of triglyceride in the bloodstream.

In addition, supplementation of cinnamon to rats induced a significant increase in the peroxisome proliferator-activated receptors -alpha (PPAR- $\alpha$ ) level which improvement of the serum lipid profile parameters and also reduced the level of sterol regulatory element-binding protein-1c (SREBP-1c), which, is a master of transcriptional regulator of fatty acids and de-novo triglycerides synthesis and it is responding to insulin in white adipose tissue (WAT) and in the liver [50].

Also, it has been shown that the SREBP-1C act to regulate the synthesis and secretion of VLDL-C, which is the predominant carrier of triglycerides

Our results demonstrated that supplemented rats with AEC before treated with difenoconazole reduced the level of the TAG correlated with under sedation of VLDL-C in serum, which contains less TAG and thus reflects a reduction of TAG in the bloodstream, and this is probably due to TAG utilization.

Also, our results exhibited that administration of difenoconazole to rats led to a significant elevation in the TC and LDL-C concentrations

[hypercholesterolemia] and a significant reduction in the HDL-C level in comparison with control rats.

High serum cholesterol levels have been associated with cardiovascular disease (CD), leading to the cause of death and disability in the western world [51]. also, cholesterol metabolism has been implicated in the development of Alzheimer's disease.

There was inhibition of hepatic cytochrome P450 in vivo by ketoconazole led to down-regulation of HMG-CoA reductase and steroidogenic carbon flux, possibly by preventing the inactivation of natural endogenous oxysterol regulators.

Cholesterol-7-alpha hydroxylase is a cytochrome P450 hem enzyme that oxidizes cholesterol in position 7, it is an oxidoreductase. CYP7A1 is located in the endoplasmic reticulum (ER) and it is important for the synthesis of bile acid and the regulation of cholesterol levels. There was inhibition of hepatic cytochrome P450 in vivo by ketoconazole led to downregulation of HMG-CoA-reductase and sterol genic carbon flux, possibly by preventing the inactivation of natural endogenous oxysterol regulators.

However, it has been reported that ketoconazole induced cholesterol-7-alpha hydroxylase (CYP7A1), which is responsible for the synthesis of bile acid and the regulator of cholesterol level.

In these studies, it was reported that peroxisomal proliferator (PP) increased hepatic Acyl-Co enzyme A cholesterol transferase (ACAT) is a key enzyme in controlling cholesterol metabolism, and thus ACAT inhibition leads to reduce cholesterol absorption, therefore, it is a key element in the treatment of hypercholesterolemia and atherosclerosis

Many studies have shown that elevated concentrations of total cholesterol (TC) and low-density lipoprotein (LDL-C) in the blood are powerful risk factors for coronary heart disease (CHD). Therefore, lowering LDL-C is associated with a reduction in the risk of cardiovascular disease (CVD)[52].

The results in the present study showed that supplement rats with AEC before treatment with difenoconazole induced a significant reduction in the level of serum TC and LDL-C levels when compared with difenoconazole treated rats.

Also, supplement rats with AEC alone caused before a marked reduction in the level of LDL-C in compared with the control group.

It has been found that received rabbits with iso-flavones, pre-treatment with cypermethrin had a significant decline in the level of cholesterol, could be attributed to an increase in the activity of cholesterol

7-B hydroxylase which regulate the bile acids synthesis from cholesterol and extraction [53], consequently, the removal of LDL-C and bile acid synthesis and excretion are increased. However, there is a significant increase in plasma HDL-C, which may hasten the removal of cholesterol from peripheral tissue to the liver for catabolism and removal [54].

Moreover, a cinnamate, a phenolic compound improved hyperlipidemia possibly by playing a vital role in lipid metabolism by inhibiting hepatic hydroxyl methyl glutamyl-CoA-reductase (HMG-CoA-reductase) activity. In addition, hepatic HMG-CoA-reductase expression correlated with sterol regulator element-binding protein-2 (SREBP-2), which is more specific for cholesterol metabolism[55]. However, bile acids, cholesterol and mevalonate are known to inhibit HMG-CoA-reductase expression.

Furthermore, hypertriglyceridemia and hypercholesterolemia were associated with oxidative modification of LDL, protein glycation, glucose-oxidation, and eventually leading to the excess formulation of lipid peroxidation products.

However, low HDL-C levels are also associated with other atherogenic factors, termed Metabolism Syndrome (MS), and correlated with the development of metabolic diseases, such as obesity, insulin resistance, and atherosclerosis.

The results in the current study demonstrated that prophylactic treatments of rats with AEC, ameliorated hyperlipidemia induced by difenoconazole as evidenced by a marked reduced the elevated levels serum TC, LDL-C, TAG, VLDL-C, and enhancement markedly of HDL-C level.

Many factors have been reported to control the plasma level of HDL-C. One of these factors, HDL-receptors, that expressed in the liver, and their role is reverse of cholesterol transport and hence effect on the plasma HDL-C levels and development of atherosclerosis.

The scavenger receptor-class B-Type 1 [SR-BI] is a major protein of the HDL-C receptor in the liver and control HDL-metabolism by mediating the cellular selective uptake of cholesterol esters from plasma HDL [56].

It was strongly suggested that the elevation of HDL-C was primarily due to delayed clearance of HDL-C rather than the whole HDL-particles [57] who found that the clearance of HDL-C from the blood was greatly diminished in scavenger receptor class B, Type 1 [SR-B1] deficient mice.

Also, [58] reported that there were markedly increased plasma HDL levels in SR-1 deficient mice was detected.

There are several studies reported that both rats and humans when intake polyphenol exhibited suppressed LDL-cholesterol concentration and susceptibility of LDL-C to oxidation and increased HDL-C.

One of the potential properties of AEC is the ability of one or more of its constituents to improve the dyslipidemia state.

Essential oils rich in phenolic constituents, such as cinnamaldehyde, have the highest antioxidant activity against LDL-oxidation and consequently protect against atherogenesis or sclerosis [59].

This observation referred to cinnamon contains an antioxidant compound, such as water-soluble polyphenol type-A polymers, which has a positive in enhancing insulin sensitivity and reducing its resistance, therefore, it regulates the levels of serum lipids and lipid fraction [28,29].

The previous studies demonstrated that the cholesterol-lowering property of cinnamon extract(CE) could be attributed to many factors like the presence of hypocholesterolemic compounds, such as hydroxymethyl glutamyl-CoA reductase (HMG-CoA reductase), which participate in the cholesterol synthesis or reduces the absorption of cholesterol from the intestine, the CE might in turn, inhibit lipoprotein lipase activity or reduces lipid peroxidation(LPO) [60].

In addition, in the current study, the ratio of TC/HDL-C increased often linked with overweight and hyper-insulinemia and correlated with high TG and low HDL-C.

Dislipidemia not only resulted from the decreased HDL-C level, but also from the slight increase in the TC level as more, TC may be associated with the calculated VLDL fraction, in hypertriglyceridemia individuals than in normal lipidemic people.

Also, the variations in the TC/HDL-C ratio seem to better reflect underlying metabolic alterations in the features of the insulin resistance syndrome (IRS) than the LDL-C/HDL-C ratio.

#### **4.2. Influence of difenoconazole treatment alone or in concomitant with AEC on renal function biomarkers in male rats.**

The results present in this study exhibited that an elevation markedly in the level of creatinine in rats treated with a low dose of difenoconazole (for 28days) in comparison with those in the control group.

In contrast, no significant differences in the levels of concentration of creatinine were noticed in rats supplemented only with AEC in comparison with the control group.

In respect of creatinine, it is breaking down product of creatine phosphate in muscle and is usually a product at a fairly constant rate by the body and thus depend on muscle mass. Therefore, the creatinine is filtrated only but is not reabsorbed, thus, the serum creatinine concentration is usually a relatively good marker of glomerular filtration rate (GFR).

In addition, a significant elevation in the level of urea in rats treated with both doses of difenoconazole for 28 days. Also, this trend was observed in the level of blood urea nitrogen (BUN) in difenoconazole-treated rats.

The previous report has shown that a reduced GFR lead to retention of nitrogenous was to products such as urea and creatinine (Azotomia) [61]. With regard to blood urea nitrogen (DUN), is referred to the amount of nitrogen in the blood, that comes from the waste product urea and their nitrogenous waste.

The renal dysfunction is proven by enlargement of the relative weight of the kidney. Interestingly, there is a significant increase in the relative weight of the kidney in the difenoconazole-treated group [Our Unpublished data].

Treatment rats with thioacetamide (TAA) fungicide induced a significant increase in the BUN and creatinine levels in compared with the control group [62]. In addition, treatment animals either rats or rabbits with cypermethrin exhibited an increase in the concentration of urea and creatinine in treated animals [3].

However, prophylactic treatment of rats with AEC, improvement the levels of renal function markers evidenced by a significant reduction in the level of urea, particularly in rats treated with a low dose of difenoconazole after supplementation rats with AEC in comparison with difenoconazole treated rats.

On the other hand, no significant differences in the levels of creatinine and BUN were noticed in rats supplemented with AEC pretreatment with a high dose of difenoconazole.

Pre-treatment rats with cinnamon aqueous extract ameliorated the nephrotic effects of bisphenol (BPA) and octyl phenol (OP), as evidenced by s reducing the serum urea and creatinine levels, compared with the BPA group and OP-treated group [63]. Furthermore, thyroid-deficient rats exhibited many renal function defects, such as decreased renal plasma flow and glomerular filtration rate.

The findings in the present study pertain to a reduced markedly in the levels of T4 and T3 in difenoconazole treated rats and this, in turn, is associated with increased of urea and BUN levels. Therefore, these findings confirmed that hyperthyroidism states showed a decrease in the GFR and vice versa. And also, our results as concern with renal and thyroid function markers supported the results of the study which, done by [64], in addition to interpreting the insufficiency of renal function in hypothyroid in the current study.

#### **4.3. Influence of difenoconazole treatment alone or concomitant with AEC on thyroid hormones Status in male albino rats.**

The results presented in this study demonstrated that treatment rats with difenoconazole caused a significant decrease in the levels of thyroxine (T4) and tri-iodothyronine (T3) (hypothyroidism) in rats after 28 days of treatments.

The thyroxin hormone (T4), produced entirely by the thyroid gland, while the majority of circulating T3 is derived from the deiodination T4 in non-thyroidal tissue such as liver and kidney via Type I and Type II deiodinase enzymes, and thus converts T4 to T3 in peripheral tissue.

The hypothyroidism state may be attributed to occur of immunoglobulin G (IgG) can either stimulate or interfere with thyroid function by stimulating membrane-bound adenylate cyclase (CA) or blocking the high-affinity TSH binding sites and thus inhibiting TSH action [65].

The results of the previous studies indicated that serum (TH), primary T4 are decreased in response to peroxisome proliferators (PPS) in rats, this may be a result of PPdisplace TH from serum carrier protein. And this decrease in serum TH could be attributed to the induction of hepatic uridine-diphosphate glucuronyl transferase may also be the result of increased TH in serum [66].

Treatment of difenoconazole to rats induced a significant decrease in the level of T4 and T3 in difenoconazole-treated rats [67].

It has been reported that penconazole treatment at both doses induced a significant decrease in the T4 and T3 levels in compared with the control group. whereas supplemented rats with sesame oil before administration of penconazole elevated the reduction in the T4 and T3 level, in compared with penconazole treated rats [5]. In contrast, treatment rats with a low dose of flusilazole fungicide induced a significant elevation of the serum thyroxin T4 and T3, whereas,

non-significant change in the level of T4 and T3 was observed in rats treated with a high dose of flusilazole after 90 days of treatments [4].

On the other hand, no significant alterations in the level of T4 was observed in rats supplemented only with AEC, whereas a significant decrease in the level of T3 was detected in rats supplemented with a high dose of AEC during the experimental period.

However, supplemented rats with CAE prior treatment with difenoconazole exhibited improvement in the thyroid, hormone concentrations, where cinnamon extract elevated the reduction in the levels of T4 and T3 in compared with difenoconazole-treated rats. Therefore these findings referred to the ability of AEC to ameliorate the adverse effects of difenoconazole on the thyroid hormones levels and this could be attributed to the presence of many bioactive components in the cinnamon extract which are responsible for improving and minimized the toxic effects of tested fungicide in the current study.

## 5. Conclusion

In conclusion, the results revealed that treatment rats with difenoconazole induced a significant increase in the level of blood glucose and the lipid profile indices (i.e. TC, TAG, LDL-C, and VLDL-C), also, a significant elevation in the levels of creatinine and urea. In contrast, there is a significant decrease in the levels of T4 and T3 in difenoconazole-treated rats.

Pre-treatments with AEC decrease the harmful effects of difenoconazole treatment. So, cinnamon extract can reduce or prevent the development of lipid metabolic disorders and hypothyroidism state, consequently attenuated its complications involved diabetes and cardiovascular disease (CHD).

## 6. Conflicts of interest

No potential conflict of interest was reported by the authors.

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