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The ameliorative effects of *cinnamum zeylanicum* extract on renal functions and oxidative stress against fluoxetine drug in male rats

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ABSTRACT

Objective: To determine the possible protective effects of cinnamon against fluoxetine impact on the renal function, lipid profiles and oxidative stress.
Design: Thirty-two males mature albino rats weighing about 250 ± 5 g were divided into four random experimental groups, control group, FLX group (administered fluoxetine drug), Cin group (administered cinnamon extract) and FLX+ Cin group for 60 days.
Samples: Blood samples were collected from retroorbital venous sinus for separation of serum for measurement of kidney function, liver function and lipid profiles. After dissection, the left kidney was taken for determination of antioxidants and oxidative stress parameters, histopathological evaluation of changes moreover detection for gene expression analysis of caspase, renin and NRF2 genes.
Procedures: **The control group** received distilled water only and standard ration. **FLX group** administered oral dose of fluoxetine 10 mg/kg Bwt by gavage tube. **Cin group** administered an aqueous cinnamon extract oral dose of 50 mg/kg body weight. **FLX+ Cin group** received fluoxetine at a concentration of 10 mg/kg body weight and after 30 minutes received an aqueous cinnamon extract at dose of 50 mg/kg body weight.

Results: Administration of fluoxetine exhibited a higher level of urea, uric acid and creatinine with a lower level of total protein compared to control group. Administration of Cin alone or with FLX improved kidney functions in addition to improvement in antioxidant parameters. Marked increase was detected in FLX group compared to the Cin and Cin+FLX groups. In addition, Administration of cinnamon with fluoxetine drug for two months leads to a significant improvement in the lipid profiles when compared to control group. Moreover, there was a significant upregulation ($p < 0.05$) in the mRNA expression level of renin and caspase 3 genes in the kidney of FLX and FLX + Cin treated groups when compared to the control group. Conversely, the expression level of the renal NRF2 was statistically downregulated ($p < 0.05$) in FLX group counterweight to the control group. Also, there was a marked elevation ($p < 0.05$) in the renal NRF2 transcriptional level in Cin treated group. Histopathological pictures of H&E-stained renal sections showing normal glomeruli and tubules in control group and Cin group. However, tubular dilation with renal epithelial cells degeneration, necrosis and fibrosis were detected in FLX group.
Conclusion and clinical relevance: Cinnamon is a medicinal plant, when used to counteract the negative effect of long administration of fluoxetine drug on renal tissue and protect the kidney to develop renal failure through antioxidant effects.
Keywords: Cinnamon, Fluoxetine, Renal function, oxidative stress, Histopathology and Renal genes.

1. INTRODUCTION

Depression is the most common mental illness. The depression could strike all ages, usually characterized by feeling of sadness, anxiety, insomnia, loss of interest in pleasure thing, and suicidal idea [1]. According to WHO there are more than 350 million people and one in ten men worldwide suffering from depression[2]. Therefore, it is considered one of the mental disorders which need immediate proper treatment as early as possible. Antidepressants and antipsychotics are widely used in the short- and long-term treatment. Fluoxetine (FLX) is a type of antidepressant known as an SSRI (selective serotonin reuptake inhibitor). It is often used in treatment of depression [3]**.** It is also used to treat neurological diseases such as depression and anxiety [4].

[5, 6] demonstrated that fluoxetine is well absorbed after oral administration, widely distributed, and metabolized within the liver, and added that, it has an elimination half-life (t ½) of 24 to 96 hours and its metabolite t ½, norfluoxetine, ranges between 168 and 360 hours. Therefore, this drug should be used with caution in patients with decreased liver function and metabolic activity and [7] declared that fluoxetine drug may cause the following side effects as sedation, constipation, blurred vision, and urinary retention due to its anticholinergic effects.

[8] showed that fluoxetine is effective in treatment of panic and other anxiety disorders. Clinical observations on patients with panic attacks have confirmed its effectiveness and reliability in decreasing panic attacks, depression, fear and anxiety symptoms. Fluoxetine is mainly excreted in urine and kidney plays a central role in maintaining homeostasis by conserving water and electrolytes and by disposing metabolic waste [5].

Cinnamon (*Cinnamomum zeylanicum*) is a traditional medicine native to India that is widely used. [9]**.** Cinnamon is a really essential plant in conventional medication with plenty of advantages including fats reduction, antibacterial and antifungal effect in addition to sexual enhancement ability[10].

Cinnamon is an antioxidant drug that contains aldehyde, terpenes, cinnamyl alcohol, and safrole compounds that play an important role in the modulation of stress in obese fasting people with low blood sugar and has a role in reducing infertility risk, cardiovascular, inflammatory and oxidative stress-related diseases [11].

[12] confirmed that cinnamon extract has been improved reproductive system function and increase spermatogenesis in rats. Cinnamon contains high concentrations of antioxidants [13] and so protect hepatic and renal tissue from toxicity as well as from cancer [14]**.**

[15] demonstrated that oral fluoxetine administration can cause tissue toxicity such as erectile dysfunction, hepatotoxicity, and nephrotoxicity, which may be caused by an excess of free radicals, an inability of the antioxidant response system, and accelerated lipid and protein peroxidation.[16] showed that A 14-day therapy with 10 mg/kg fluoxetine was shown to increase oxidative stress markers in rats. [17] showed that taking 20 mg/day of fluoxetine hydrochloride for 9 days for depression treatment resulted in hyponatremia, which causes serious neurological dysfunction and fluid accumulation inside the body's cells, especially in elderly people whose bodies are unable to maintain fluid levels.

To the best of our information, there are not investigations concerning the renal protective role of cinnamon against fluoxetine drug in rat. So, in the current study, it explored the possibility of using cinnamon in reducing fluoxetine side effects in rat model and detects if cinnamon can be used in the treatment of these side effects on kidney. It is also aimed to study the effects of fluoxetine and cinnamon on renal function through detection of some biochemical parameters and kidney related genes.

2.MATERIALS AND METHODS

2.1. Ration

A standard commercial ration meets the nutrient requirements of rats according to NRC was used in this study.

2.2. Chemicals

1. Fluoxetine was obtained as a capsule (20mg) from **Misr Company for Pharmaceuticals**, **Egypt**, then dissolved in 2ml distilled water to be at a concentration of 10 mg/kg Bwt. And was administrated dose of 0.2 ml orally for each rat daily by gavage tube according to [18, 19].

2. Cinnamon was obtained as a capsule (250mg) from **PURITAN PRIDE, INC, Ronkonkoma, NY 11779 U.S.A.**, then it was dissolved in 10ml distilled water at concentration of 250 mg and administrated at a dose of 50mg/kg body weight daily by gavage tube for 60 days.

2.3. Experimental Animals:

A total number of thirty-two male mature albino rats weighing about 250 ± 5 g were used in this study. Food and water were supplied ad libitum. The rats were acclimatized to the laboratory conditions (Physiology Lab in Faculty of Veterinary Medicine, Mansoura university) for 2 weeks. Moreover, a 12 h dark: 12 h light photoperiod was provided.

2.4. Experimental design:

Thirty-two mature male rats were divided into four random experimental groups (n = 8):

The control group received distilled water only and standard ration. **FLX group** received fluoxetine at a concentration of 10 mg/kg Bwt by gavage tube. **Cin group** received an aqueous cinnamon extract at a dose of 50 mg/kg body weight [20]. **FLX+ Cin group** received fluoxetine at a concentration of 10 mg/kg body weight and after 30 minutes received an aqueous cinnamon extract at dose of 250 mg/kg body weight. All rats were treated orally according to their group using a stomach tube for 60 days in all groups during the period of the experiment.

2.5. *Sample collections***:**

At the end of the experiment, Rats were anaesthetized by ether cotton soaked and the anesthesia was confirmed by loss of reflexes and loss of movement. Blood samples were collected in a plain test tube from the retro-orbital venous sinus of each rat.

Then, they were left for 30 min to clot and centrifuged at 3000 rpm after that serum were aspirated by Pasteur pipette. Serum was stored at –80◦C for further analysis. After dissection of rats, the left kidney was removed quickly, rinsed with cold normal saline and was divided into parts and dried with filter paper. First part was frozen in -80 °C for determination of antioxidants and oxidative stress parameters. The second part was preserved in 10% formalin for histopathological investigation for histopathological evaluation of changes in the structure of the renal tissues. The third part was put in eppendorf contained RNA later and kept at −80 °C for performing quantitative real time polymerase chain reaction (real-time PCR) test for genes expression analysis.

2.6. Biochemical Analysis

2.6.1. Kidney function tests (Serum urea, uric acid, creatinine and total protein)

Urea levels in serum were determined calorimetrically by using ready kits provided by Diamond, Egypt, according to [21]. As well as serum levels of Uric acid were determined by colorimetric method using ready kits provided by Spinreact, Spain, according to [22]. While, Creatinine levels in serum were assayed according to the method of [23] using kinetic kits provided by Human, Germany. Moreover, serum total

protein concentration were determined using ready kits provided by Spinreact, Spain according to [24].

2.6.2. Serum lipids profile

Cholesterol, triacylglycerol, HDL and LDL were measured in rats serum samples from each groups. Cholesterol was determined calorimetrically by using ready kits provided by Spinract, Spain according to [24]. Triacylglycerol was determined by enzymatic colorimetric test using assay kits provided by spinract, Spain according to [24]. HDL-cholesterol was determined by enzymatic colorimetric test by using ready kits provided by spinreact, Spain according to [25] LDL-cholesterol Determined by the following equation:

LDL-c = Cholesterol – (triacylglycerol/5 + HDL-c).

2.6.3. Oxidative and antioxidative stress biomarkers in renal tissues

MDA was determined by enzymatic colorimetric method by using ready diagnostic kits provided by Bio-Diagnostics, Dokki, Giza, Egypt according to [26]. Super oxide dismutase activity (SOD) was determined by enzymatic colorimetric method using ready diagnostic kits provided by Bio-diagnostic, Egypt according to [27]. GSH levels were measured calorimetrically using ready diagnostic kits provided by Bio-diagnostic, Egypt according to [28].

2.7. Histopathological examination

Renal tissues were kept in 10% formalin. Then, standard histological technique was applied including dipping in serial ascending dilution of ethanol. After that, the tissue specimens were embedded in paraffin. The obtained paraffin blocks were sectioned at 4 µm thickness and then the sections were stained with hematoxylin and eosin (H&E), as declared by [29]**.**

2.8. Real time PCR of some renal genes

2.8.1. RNA extraction and cDNA synthesis

RNA was extracted from renal tissue of rats using the QIAamp RNeasy Mini kit (Qiagen, Germany, GmbH) following the manufacturers' protocol. The integrity and concentration of the obtained RNA were determined with spectrophotometeric NanoDrop® ND-1000. The synthesis of first strand of cDNA from the obtained RNA was achieved through the use of QuantiTect Reverse Transcription kit (Qiagen, Heidelberg, Germany) and procedures of the manufacture were applied.

2.8.2. Quantitative real-time PCR

Quantitative real-time PCR was determined using Rotor-Gene Q cycler (Qiagen, Heidelberg, Germany) by SYBR Green QuantiTect PCR kits (Qiagen, Germany). Relative expression of mRNA level was carried for each gene. The sequence of the used primers was illustrated in **Table** 1. The reaction mixture consisted of 12.5 µl of 2x SYBR Green PCR Master mix, 0.25 µl of Revert Aid Reverse Transcriptase (200 U/µL) (Thermo Fisher), 0.5 µl of each primer of 20 p mol concentration (**Biobasic, Canada)**, 9.25 µl of water, and 2 µl of RNA template. The reaction was performed in a Strata gene MX3005P real time PCR machine. The thermal cycling conditions were: initial denaturation at 94 °C for 15 min for a number of 40 cycles followed by initial heat activation at 94 °C for 15 s; primers annealing at 55 °C for 30 sec for β actin gene, 60°C for 30 sec for renin and NRF2 genes, 53°C for 30 sec for caspase 3; finally, elongation at 72 °C for 30 sec. The relative fold changes in the mRNA expression of the studied genes were calculated by "ΔΔCt" method stated by [30] using the following ratio: ($2^{-\Delta\Delta ct}$).

2.9. Statistical analysis

The statistical analysis was carried out using the Analysis of variance (ANOVA) for detection of the effect of different treatments on the level of the studied variables under the study, followed by the Duncan Multiple Rang test (DMRT) for detection of the significances between the studied groups. Analysis of variance was made using **SPSSPC+** computer program (Version, 26) [31].

3.3. RESULTS AND DISCUSSION

3.1. Effect of different treatments on levels of Urea, Uric acid, Creatinine and total protein level:

The results of kidney functions were dissipated in table (2). Fluoxetine induced a significant increase in levels of uric acid, urea and creatinine in serum $p< 0.05$ as compared to other groups. Administration of Cin induced a significant decline $p< 0.05$ in serum uric acid, urea and creatinine levels in groups (Cin and Cin+Flx). In addition, Flx drug induced a significant decrease $p< 0.05$ in levels of serum total protein $p <0.05$ in Flx group compared to the other groups, rats treated with Cin alone or with Cin + Flx return to normal levels as control group.

3.2. Effect of different treatments on Level of MDA, SOD and GSH among different treatments:

To assess lipid peroxidation in renal homogenate, MDA was measured as shown in Table 3. Marked increment ($P < 0.05$) was detected in Flx group compared to the Cin and Cin+Flx groups, but there was no significant difference in comparison with control group. Similarly, no significant difference was detected between Cin, Cin+Flx as compared to control group.

Regarding GSH activity marked decline ($P < 0.05$) was observed in GSH activity in Flx exposed group as compared to Cin group, but no significant difference was observed when compared to control and Cin+Flx treated groups. Furthermore, no significant variations were detected between Cin group and Cin+Flx compared to control group. As well as the results revealed no significant alterations in SOD levels between control and other all treated groups ($p >$ 0.05).

Table 1. primer sequences of target gene used Real time PCR:

Target gene	Primers sequences (5'-3')	References
β. Actin	F: TCCTCCTGAGCGCAAGTACTCT R: GCTCAGTAACAGTCCGCCTAGAA	[68]
Renin	F: TGGATCAGGGAAGGTCAAAG R: CCCTCCTCACACAACAAGGT	[69]
Caspase 3	F: AGTTGGACCCACCTTGTGAG R: AGTCTGCAGCTCCTCCACAT	[70]
NRF2	F: CACATCCAGACAGACACCAGT R: CTACAAATGGGAATGTCTCTGC	[71]

Table 2. Levels of urea, uric acid, creatinine and total protein among different treatments:

Groups	Uric acid mg/dl	Urea mg/dl	Creatinine mg/dl	Total protein g/dl
Control	3.70±0.32[b]	47.00±1.53[b]	0.93±0.09[b]	5.60±0.06[a]
Cin	5.43±0.62[a]	55.33±5.84[a]	1.40±0.25[a]	5.19±0.15[a]
FLX	3.20±0.06[b]	46.33±0.88[b]	0.93±0.09[b]	5.69±0.10[a]
FLX+Cin	3.53±0.26[b]	48.67±0.67[b]	1.03±0.07[b]	5.52±0.25[a]

FLX: fluoxetine drug Cin: cinnamon.
Means within the same column of different litter are significantly different at (P < 0.05)
Means within the same column of same litter are not significant different at (P < 0.05).

3.3. Effect of different treatments on level of Cholesterol, TG, HDL and LDL level

The results of lipid profiles were presented in (Table, 4) cleared that, the level of cholesterol, triacylglycerol (TG) and low-density lipoprotein (LDL) differ significantly (P < 0.05) among different treatments groups as compared to control group. Fluoxetine was induced a significant decrease in level of cholesterol, triacylglycerol and LDL as compared to control group. As well as administration of Cin induced a significant decrease in level of cholesterol, Triglyceride and LDL as compared to control group.

Table 3. Represents Level of MDA, SOD and GSH among different treatments.

Groups	MDA U/mg	SOD U/mg	GSH U/mg
Control	33.67±1.86 [ab]	349±7.55 [a]	4.77±0.32 [ab]
FLX	37.67±1.45 [a]	321.67±16.17 [a]	4.3±0.2 [b]
Cin	28.67±1.2 [b]	357.33±12.44 [a]	5.47±0.2 [a]
FLX+Cin	32.33±1.45 [b]	350.33± 4.84 [a]	4.9±0.38 [ab]

FLX: Fluoxetine Cin: cinnamon

Means within the same column of different litter are significantly different at (P < 0.05).
Means within the same column of same litter are not significant different at (P < 0.05).

Table 4: Lipid profiles among different treatment groups.

Groups	Cholesterol mg/dl	TG mg/dl	HDL mg/dl	LDL mg/dl
Control	156.67± 10.37 [a]	227.67± 4.63 [a]	51.33± 1.86 [c]	52.67±8.19 [a]
FLX	147.33± 12.03 [b]	204.33± 20.87 [b]	61.67± 1.76 [b]	43.33±9.74 [b]
Cin	134.67± 3.53 [d]	152.33± 19.89 [d]	63.00± 1.15 [a]	34.33±4.06 [d]
FLX+Cin	139.67± 3.76 [c]	171.67± 8.41[c]	61.67± 4.91 [b]	39.33±4.70 [c]

FLX: fluoxetine Cin: cinnamon

Means within the same column of different litter are significantly different at (P < 0.05).
Means within the same column of same litter are not significant different at (P < 0.05).

While there was a significant increase in the level of high-density lipoprotein (HDL) in Cin group followed by flx and Cin + flx groups as compared to control group.

3.4. Effect of different treatments on the relative gene expression of renin, caspase 3 and NRF2 genes

The quantitative real-time PCR (qRT-PCR) findings displayed a significant upregulation (p < 0.05) in the mRNA expression level of renin and caspase 3 genes in the renal tissues of FLUX and FLX + Cin treated groups of the present study with respect to the control group (figure 1,2) treated group counterweight to the control group. Also, there was a marked elevation (p < 0.05) in the renal NRF2 transcription level was recorded in Cin treated group (figure 4).

3.5. Effect of different treatments on histopathological examination of renal tissue

Microscopic pictures of H&E-stained renal sections (figure 4) showing normal glomeruli and tubules with minimal interstitial tissue in control group (C) and Cin group (T). Renal sections from flx group (D) showing multifocal areas of tubular dilation (thin black arrow), renal epithelial cells degeneration (dashed black arrow), necrosis (*), interstitial lymphocytic cells infiltration (thick black arrow) and small focal area of fibrosis (red arrow). Renal sections from Cin+ Flx group (T+D)) showing mild tubular dilation (thin black arrow). Low magnification X: 100 bar 100 and high magnification X: 400 bar 50.

Fluoxetine is one of SSRIs used in treatment of depression, with fewer anti-cholinergic side effects, less toxicity, However, it may be associated with pharmacokinetic drug interactions, and inhibit the metabolism of other drugs and increased the risk of adverse effects [32-35]. Furthermore, the kidney is considered one of the most affected organs by the fluoxetine drug because it is mainly excreted through kidney in urine [5].

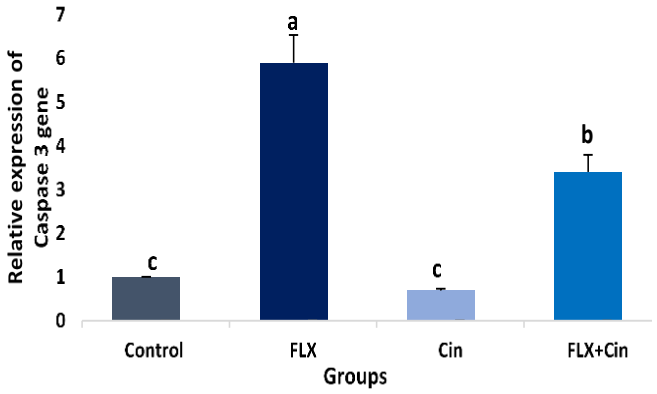

Figure (1): Relative gene expression of caspase 3 gene.

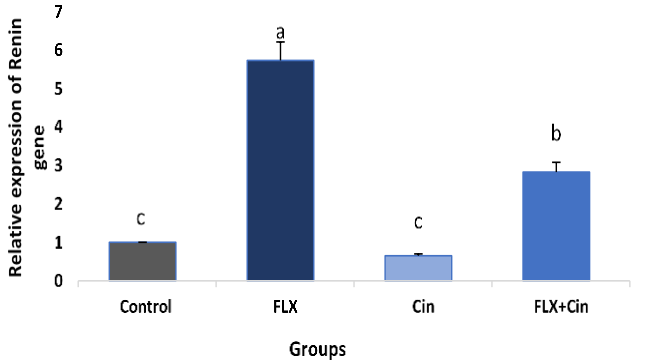

Figure (2): Relative gene expression of Renin gene.

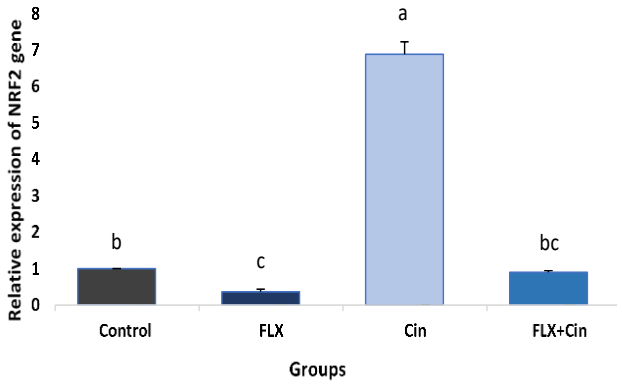

Figure (3): Relative gene expression of NRF2 gene.

The current study investigated the protective effect of Cinnamon on renal tissue of fluoxetine treated rats. However, FLX has inevitable side effects such as weight gain, dyslipidemia, type 2 diabetes and the risk of coronary vasoconstriction lead to heart attacks [36, 37]**.** Few studies have been investigated the FLX effect on renal tissue. For this purpose, this study payed an attention on the effects of FLX administration on rat kidney and the possible nephroprotective effect of Cinnamon against these effects.

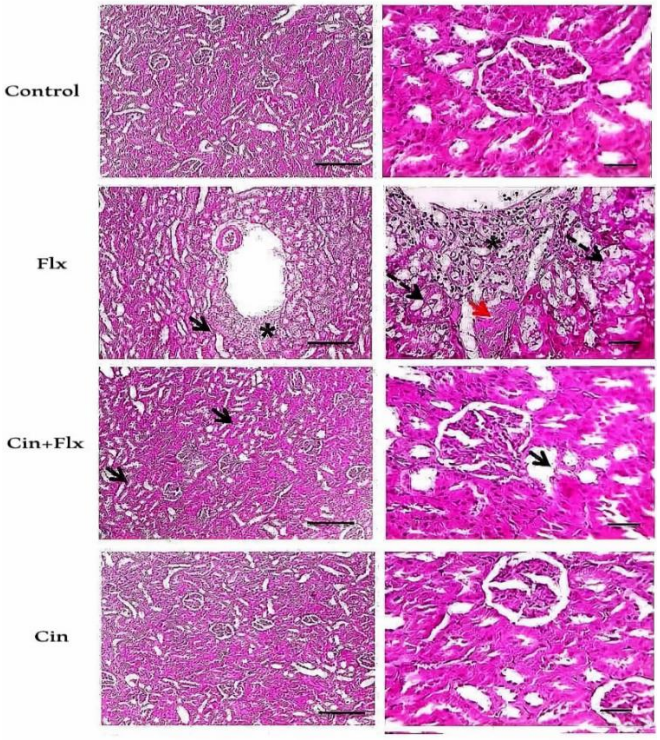

Figure 4. Microscopic pictures of H& E-stained renal sections of four groups showed multifocal areas of tubular dilation, renal epithelial cells degeneration with necrosis and small focal area of fibrosis in FLX group as compared to control and Cin group**.**

Serum creatinine is the most reliable marker of kidney function where it is produced from the muscle at constant rate and almost completely filtered at the glomerulus .As well as , high urea level can indicates kidney dysfunction [38]. In the current study, FLX treated rats exhibited a higher level of urea, uric acid and creatinine associated with a lower level of total protein (TP) as compared to control group. This was confirmed by upregulation in mRNA expression level of renin gene. This may be related to deleterious effects of FLX on renal tissue as histopathological examination of this group declared degeneration of renal epithelial cell and necrosis. The mechanism by which fluoxetine induces renal damage is due to changes in glomerulus and also in tubules which effect the normal filtration and reabsorption process which may lead to filtration of RBCs, proteins, electrolytes and casts in urine [39] . Parallel to these findings, [40] indicated that using fluoxetine in early pregnancy may be associated with cystic kidney or kidney agenesis. The results of the present investigation coincide with the study of [35] who declared that administration of FLX results in a significant elevation in urea levels, but creatinine level had not shown a significant changes. On the contrary the study of [16, 41, 42]**.** The later indicated that neither amitriptyline nor fluoxetine caused significant changes in the markers of renal function, including concentrations of total protein, urea, creatinine and β2-microglobulin.

In the present study, histopathological findings showed multifocal areas of tubular dilation, renal epithelial cells degeneration with necrosis and small focal area of fibrosis in FLX group as compared to control and Cin group**.** Moreover, fluoxetine caused alterations in kidney rats in treated group, the cortex region showed shrinking of glomeruli, hemorrhage and dead cells while in the medullary region, thickened wall of kidney tubules and degeneration of some kidney tubule epithelial cells were investigated by [43].

Reactive oxygen species formation is a normal consequence of a variety of essential biochemical reactions. It is also known that oxygen radicals could be formed in excess amount in chronic diseases [44]. Therefore, an adequate range of antioxidative defenses within and outside the cells, has also been considered to be very important to offer protection against oxidative damages of cell components including membrane phospholipids [45]. Considerable attention has recently been focused on some nutritional factors such as polyphenols that could counteract oxidative damages and therefore be beneficial through their antioxidant properties**,** among plants that contain polyphenols is Cinnamon that may be of special interest, since Cinnamon has been documented to be in vitro and in vivo antioxidant [46-48]. In our study, fluoxetine caused a significant increase in lipid peroxidation with reduced GSH activity suggesting oxidative status and deleterious effects of this reagent [49],while the effects of fluoxetine were evidently diminished by the combination of cinnamon and fluoxetine. Verifying the results of the current experiment, there was a significant downregulation of renal NRF2 gene. NF-E2–related factor 2 is an essential transcription factor that regulates an array of detoxifying and antioxidant defense gene expression. It plays a role in adaptation to oxidative stress. This remarkable down regulation points out their exhaustion in removing ROS. These results indicated that the drug could reduce the antioxidant capacity and cause oxidative stress. These results were confirmed by our histopathological findings. Additionally, fluoxetine induces oxidative stress possibly by the generation of reactive oxygen species (ROS) [50] which are capable of initiating oxidative damage to the cell and cause lipid peroxidation as the result of generation of large amount of free radicals induced by the drug [49]. This was confirmed by the significant decrease in antioxidant markers (SOD and GSH) and the significant increase in MDA as depletion of these markers may be due to impaired cellular membrane or reduction in some enzyme activity. It has been documented that the excessive release of free radicals and oxidative stress can trigger cell apoptosis. Based on our previous study, caspase 3 gene which is a marker for apoptosis [51, 52] was overexpressed in FLUX and CIN treated groups when compared to the control group. This was attributed to that fluoxetine significantly induced apoptosis, unregulated extrinsic (activation of first apoptosis signal protein and ligand (Fas/FasL), and caspase-8) and intrinsic (loss of mitochondrial membrane potential (ΔΨm) pathways and increased Bcl-2 homologous antagonist killer (BAK) apoptosis signaling [53]**.** In the same line, [54] demonstrated that the antidepressants including fluoxetine had a potent apoptotic activity in different cell lines. Due to rapid increase in p-c-Jun levels, Cyt c release from mitochondria, and increased expression level of caspase-3-mRNA. Previous literatures indicated that excess ROS can impair the mitochondrial membrane permeability, leading to the expulsion of cytochrome c (Cyt c) in to the cytosol, which, in turn, attaches to apoptotic activating factor 1 (APAF-1), resulting in caspase stimulation and, ultimately, apoptosis [55, 56].

Different underlying mechanisms have been proposed for fluoxetine side effects in various tissues and organs [57]. Our results affirmed the previous data of [15], who acclaimed that chronic high dose of fluoxetine raised the levels of MDA in the serum. It has been stated that the fluoxetine has a cytotoxic effect when cells expose to pharmacologic concentrations. Therefore, one could hypothesize that cinnamon different effects in a dose-dependent manner [58]**.** Also, the present study confirmed the previously recorded antioxidant effect of cinnamon in vitro [11] and in vivo [13] that was indicated by decreased MDA levels and increased antioxidant enzymes activities. [59] found that Cinnamon extract exhibited protective capacity against irradiation induced LPO in liposomes, and quenched hydroxyl radicals (OH) and H2O2.

In the present study, there was a significant decrease in cholesterol, TG, LDL and increase in HDL levels after FLX administration for two months. [60] in their study showed that fluoxetine can decrease total cholesterol and triglyceride level in short term. In contrast, [61] demonstrated that fluoxetine did not alter the lipid profile of the patients. Moreover, [37] reported that SSRIs as a group were associated with hypercholesterolemia. However, our study showed the opposite effects for fluoxetine. This may be due to the duration of this study may not be long enough to show the chronic effects of the studied antidepressants on lipid profile.

Since the ameliorative effect of cinnamon toward fluoxetine mediated oxidative stress and apoptosis and cellular damage in renal tissue has not been investigated, this article was planned to study the possible mechanisms of antioxidant and the anti-apoptotic activity of cinnamon against fluoxetine drug in renal tissue of rats. This ameliorative role of Cinnamom extract was reflected from the restoration of normal control level of antioxidant markers and mRNA levels of Renin, Caspase 3 and NRF2 in rats received Cinnamon Administration of Cin alone or with FLX in this investigation improved kidney functions parallel to hisopathological findings. Cin and FLX+ Cin groups were revealed significant declines in serum levels of urea, uric acid and creatinine, whereas led to a significant increase in TP compared to FLX group. Corresponding to our results, [62] declared that rats treated with cypermethrin and Cinamon (200 mg/kg b.w) for 6 weeks showed significant decreases in levels of creatinine and urea and an improvement in the

histological structure of the kidney. [38] declared the safety of Cinamon administration at dose for1.5 ml for 30 days in Type2 diabetes.

Cinnamon is extensively used in folk medicine due to its high content of natural antioxidants. [63] announced that the protective action of Cinnamon extract against renal oxidative injury is due to its positive role on the antioxidant enzymes and inhibitory action on ROS synthesis and revealed that the great content of polyphenolic substances in Cinnamon extract qualified it to serve as a nutritional supplement of natural antioxidants**.** Similarly, previous researchers have recorded the antioxidant action of Cinnamon in vitro and in vivo [11, 13]. The antioxidant activity of Cin may be attributed to its phenolic and flavonoids components, which act as free radicals scavengers, redox active transition metal chelators, and enzyme modulators [64]**.** Moreover, former investigators have declared that Cin exerted an anti-inflammatory action in different organs via inhibiting the expression of inducible nitric oxide synthase (iNOS) and COX-II [65, 66]. In addition, our findings indicated that administration of Cinnamon and fluoxetine drug for two months leads to a significant improvement in the lipid profile as compared with control group. [10] showed that Cinnamon, had a role in reducing cholesterol, LDL, and TG levels in subjects with type 2 diabetes by different mechanisms such as improved insulin sensitivity and increasing the activity of antioxidant enzymes. [67] demonstrated that Cinnamon had antioxidant activity by removal of free radicals and inhibition of 5-lipoxygenase enzyme in addition to decrease lipid peroxidation and increase total antioxidant power as mentioned by [58].

4. CONCLUSION

Cinnamon exhibited positive protective effect against negative effect of fluoxetine drug after 60 days of administration shown by its marked improvement on kidney and liver function. This improvement may be due to its ability to increase activity of antioxidant parameters. Fluoxetine drug shouldn't be used for long time to avoid its side effects on our health. Further research on fluoxetine is necessary to detect more and more about dose and duration of administration dependent effect.

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Conflict of interest statement

No conflict of interest.

Animal Ethics Committee permission

The current study was authorized to be carried out according to standards of the Research Ethics committee, Faculty of Veterinary Medicine, Mansoura University, Egypt with code No: R/91.

Authors contributions

Nada Hashem and Mona Elghareeb carried out the lab work and conceptualized the study and planned for the research activity. Huda El-Emam made the genetic part of the work and Eman El-Ashry helped us through the work. All of us wrote the manuscript and planned for the research activity and prof. dr. Nabil Abu Heakal helped us through the work and revised the final version. All authors have read and approved the final version of the manuscript for publication.

4.5. REFERENCES

[1] Soliman ME, Mahmoud BL, Kefafy MA, Yassien RI, El-Roghy ESJMMJ. Effect of antidepressant drug (fluoxetine) on the testes of adult male albino rats and the possible protective role of omega-3. 2017;30:1135. https://doi.org/10.4103/mmj.mmj_521_17

[2] Lim GY, Tam WW, Lu Y, Ho CS, Zhang MW, Ho RCJSr. Prevalence of depression in the community from 30 countries between 1994 and 2014. 2018;8:1-10. https://doi.org/10.1038/s41598-018-21243-x

[3] Sheehan DV, Mao CGJPb. Paroxetine treatment of generalized anxiety disorder. 2003;37:64-75.

[4] Guze BH, Gitlin MJJofp. New antidepressants and the treatment of depression. 1994;38:49-59.

[5] Pawlina W, Ross MH. Histology: a text and atlas: with correlated cell and molecular biology: Lippincott Williams & Wilkins; 2018.

[6] Altamura AC, Moro AR, Percudani MJCp. Clinical pharmacokinetics of fluoxetine. 1994;26:201-14.
https://doi.org/10.2165/00003088-199426030-00004

[7] Levin GM, DeVane CLJAoP. Prescribing attitudes of different physician groups regarding fluoxetine. 1993;27:1443-7.
https://doi.org/10.1177/106002809302701204

[8] Starcevic V, Linden M, Uhlenhuth E, Kolar D, Latas MJPr. Treatment of panic disorder with agoraphobia in an anxiety disorders clinic: factors influencing psychiatrists' treatment choices. 2004;125:41-52.
https://doi.org/10.1016/j.psychres.2003.11.001

[9] Anderson RA, Broadhurst CL, Polansky MM, Schmidt WF, Khan A, Flanagan VP, et al. Isolation and characterization of polyphenol type-A polymers from cinnamon with insulin-like biological activity. 2004;52:65-70.
https://doi.org/10.1021/jf034916b

[10] Khan A, Safdar M, Khan MMA, Khattak KN, Anderson RAJDc. Cinnamon improves glucose and lipids of people with type 2 diabetes. 2003;26:3215-8. https://doi.org/10.2337/diacare.26.12.3215

[11] Shobana S, Naidu KAJP, Leukotrienes, Acids EF. Antioxidant activity of selected Indian spices. 2000;62:107-10. https://doi.org/10.1054/plef.1999.0128

[12] Kamath JV, Rana A, Roy Chowdhury AJPRAIJDtP, Derivatives TEoNP. Pro-healing effect of Cinnamomum zeylanicum bark. 2003;17:970-2.
https://doi.org/10.1002/ptr.1293

[13] Eidi A, Mortazavi P, Bazargan M, Zaringhalam JJEJ. Hepatoprotective activity of cinnamon ethanolic extract against CCl4-induced liver injury in rats. 2012;11:495.

[14] Elkomy A, Aboubakr M, Soliman A, Abdeen A, Abdelkader A, Hekal HJIJPT. Paracetamol induced hepatic toxicity and amelioration by cinnamon in rats. 2016;4:187. https://doi.org/10.14419/ijpt.v4i2.6529

[15] Inkielewicz-Stępniak IJPR. Impact of fluoxetine on liver damage in rats. 2011;63:441-7. https://doi.org/10.1016/S1734-1140(11)70510-2

[16] Herbet M, Gawrońska-Grzywacz M, Jagiełło-Wójtowicz EJApp. Evaluation of selected biochemical parameters of oxidative stress in rats pretreated with rosuvasatin and fluoxetine. 2015;72:261-5.

[17] Kirpekar VC, Joshi PPJIjop. Syndrome of inappropriate ADH secretion (SIADH) associated with citalopram use. 2005;47:119.
https://doi.org/10.4103/0019-5545.55960

[18] Aggarwal A, Jethani S, Rohatgi R, Kalra JJG. Effect of Fluoxetine on epididymis of albino rats: A Histological study. 2013;5:0.0000053.

[19] Sakr HF, Abbas A, Elsamanoudy A, Ghoneim FJJoP, Pharmacology. Effect of fluoxetine and resveratrol on testicular functions and oxidative stress in a rat model of chronic mild stress-induced depression. 2015;66:515-27.

[20] Elgawish RAR, Abdelrazek HMJTR. Effects of lead acetate on testicular function and caspase-3 expression with respect to the protective effect

of cinnamon in albino rats. 2014;1:795-801. https://doi.org/10.1016/j.toxrep.2014.10.010
[21] Numann U, Ziegenborn JJCLI, 147: abstract. Determination of serum blood urea nitrogen. 1977;97.
[22] Fossati P, Prencipe L, Berti GJCc. Use of 3, 5-dichloro-2-hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. 1980;26:227-31. https://doi.org/10.1093/clinchem/26.2.0227
[23] Henry RJCcP, techniques. Hagerstown MH, Row. Cannon DC, Winkelman JW. 1974:842-53.
[24] Young DJP, Washington, DC. Effects of disease on clinical Lab. tests, AACC. 2001.
[25] Tietz NJWSCP, Toronto, London. Biuret method for the determination of total protein in serum In: Fundamental of clinical chemistry. 1976;503:602-9.
[26] Kei SJCca. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. 1978;90:37-43.
https://doi.org/10.1016/0009-8981(78)90081-5
[27] Nishikimi M, Rao NA, Yagi KJB, communications br. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. 1972;46:849-54. https://doi.org/10.1016/S0006-291X(72)80218-3
[28] Beutler EJJlcM. Improved method for the determination of blood glutathione. 1963;61:882-8.
[29] Bancroft JD, Layton CJBst, techniques poh. Connective and mesenchymal tissues with their stains. 2012:187-214. https://doi.org/10.1016/B978-0-7020-4226-3.00011-1
[30] Yuan JS, Reed A, Chen F, Stewart CNJBb. Statistical analysis of real-time PCR data. 2006;7:1-12. https://doi.org/10.1186/1471-2105-7-85
[31] George D, Mallery P. IBM SPSS statistics 26 step by step: A simple guide and reference: Routledge; 2019. https://doi.org/10.4324/9780429056765
[32] Schneier FR, Liebowitz MR, Davies SO, Fairbanks J, Hollander E, Campeas R, et al. Fluoxetine in panic disorder. 1990. https://doi.org/10.1097/00004714-199004000-00007
[33] Levine LJAGP. Fluoxetine in the treatment of bulimia nervosa. 1992;49:139-47. https://doi.org/10.1001/archpsyc.1992.01820020059008
[34] Su T-P, Schmidt PJ, Danaceau MA, Tobin MB, Rosenstein DL, Murphy DL, et al. Fluoxetine in the treatment of premenstrual dysphoria. 1997;16:346-56. https://doi.org/10.1016/S0893-133X(96)00245-X
[35] Karimi-Khouzani O, Heidarian E, Amini SAJPr. Anti-inflammatory and ameliorative effects of gallic acid on fluoxetine-induced oxidative stress and liver damage in rats. 2017;69:830-5. https://doi.org/10.1016/j.pharep.2017.03.011
[36] Amsterdam JD, Garcia-Espana F, Fawcett J, Quitkin FM, Reimherr FW, Rosenbaum JF, et al. Blood pressure changes during short-term fluoxetine treatment. 1999;19:9-14. https://doi.org/10.1097/00004714-199902000-00004
[37] Raeder MB, Bjelland I, Vollset SE, Steen VMJJoCP. Obesity, dyslipidemia, and diabetes with selective serotonin reuptake inhibitors: the Hordaland Health Study. 2006;67:1974-82. https://doi.org/10.4088/JCP.v67n1219
[38] Khan R, Khurshid A, Zakkia K, Safdar H, Nawab Z, Mohammad SJAPIMS. Cinnamon on the functions of liver and kidney in type 2 diabetic individuals. 2012;8:145-9.
[39] Aggarwal A, Jethani S, Rohatgi R, Kalra JJJoC, Research D. Effect of Fluoxetine on Kidney of Albino Rats-A Histological Study. 2019;13. https://doi.org/10.7860/JCDR/2019/42396.13232
[40] Reis M, Källén BJPm. Delivery outcome after maternal use of antidepressant drugs in pregnancy: an update using Swedish data. 2010;40:1723-33. https://doi.org/10.1017/S0033291709992194
[41] Levy NB, Blumenfield M, Beasley Jr CM, Dubey AK, Solomon RJ, Todd R, et al. Fluoxetine in depressed patients with renal failure and in depressed patients with normal kidney function. 1996;18:8-13. https://doi.org/10.1016/0163-8343(95)00073-9
[42] Blumenfield M, Levy NB, Spinowitz B, Charytan C, Beasley Jr CM, Dubey AK, et al. Fluoxetine in depressed patients on dialysis. 1997;27:71-80.
https://doi.org/10.2190/WQ33-M54T-XN7L-V8MX
[43] Hamad SHA, Hassan DH, Rasul KH, Mahmood NMSJZJoP, Sciences A. Histological Interfaces of Liver, Kidney and Cerebrum in Male Rats exposed to Fluoxetine. 2016;28:126-35. https://doi.org/10.21271/ZJPAS.28.5.14
[44] Beal MFJFRB, Medicine. Oxidatively modified proteins in aging and disease. 2002;32:797-803. https://doi.org/10.1016/S0891-5849(02)00780-3
[45] Skrzydlewska E, Sulkowski S, Koda M, Zalewski B, Kanczuga-Koda L, Sulkowska MJWjogW. Lipid peroxidation and antioxidant status in colorectal cancer. 2005;11:403. https://doi.org/10.3748/wjg.v11.i3.403
[46] Azab KS, Mostafa A-HA, Ali EM, Abdel-Aziz MAJE, safety e. Cinnamon extract ameliorates ionizing radiation-induced cellular injury in rats. 2011;74:2324-9. https://doi.org/10.1016/j.ecoenv.2011.06.016
[47] Lin CC, Wu SJ, Chang CH, Ng LTJPR. Antioxidant activity of Cinnamomum cassia. 2003;17:726-30. https://doi.org/10.1002/ptr.1190
[48] Qin B, Polansky M, Anderson RJH, Research M. Cinnamon extract regulates plasma levels of adipose-derived factors and expression of multiple genes related to carbohydrate metabolism and lipogenesis in adipose tissue of fructose-fed rats. 2010;42:187-93.https://doi.org/10.1055/s-0029-1242746
[49] Zlatković J, Todorović N, Tomanović N, Bošković M, Djordjević S, Lazarević-Pašti T, et al. Chronic administration of fluoxetine or clozapine induces oxidative stress in rat liver: a histopathological study. 2014;59:20-30. https://doi.org/10.1016/j.ejps.2014.04.010
[50] Hwang S, Kim J-KJCmj. Fluoxetine induces apoptotic and oxidative neuronal death associated with the influx of copper ions in cultured neuronal cells. 2020;56:20-6. https://doi.org/10.4068/cmj.2020.56.1.20
[51] Elmore SJTp. Apoptosis: a review of programmed cell death. 2007;35:495-516. https://doi.org/10.180/01926230701320337
[52] Sanz AB, Santamaría B, Ruiz-Ortega M, Egido J, Ortiz AJJotASoN. Mechanisms of renal apoptosis in health and disease. 2008;19:1634-42. https://doi.org/10.1681/ASN.2007121336
[53] Chen W-T, Hsu F-T, Liu Y-C, Chen C-H, Hsu L-C, Lin S-SJIjoms. Fluoxetine induces apoptosis through extrinsic/intrinsic pathways and inhibits ERK/NF-κB-modulated anti-apoptotic and invasive potential in hepatocellular carcinoma cells in vitro. 2019;20:757. https://doi.org/10.3390/ijms20030757
[54] Levkovitz Y, Gil-Ad I, Zeldich E, Dayag M, Weizman AJJomn. Differential induction of apoptosis by antidepressants in glioma and neuroblastoma cell lines. 2005;27:29-42.https://doi.org/10.1385/JMN:27:1:029
[55] Siddiqui WA, Ahad A, Ahsan HJAot. The mystery of BCL2 family: Bcl-2 proteins and apoptosis: an update. 2015;89:289-317. https://doi.org/10.1007/s00204-014-1448-7
[56] Kawakami M, Inagawa R, Hosokawa T, Saito T, Kurasaki MJF, toxicology c. Mechanism of apoptosis induced by copper in PC12 cells. 2008;46:2157-64. https://doi.org/10.1016/j.fct.2008.02.014
[57] De Long NE, Hyslop JR, Raha S, Hardy DB, Holloway ACJJoad. Fluoxetine-induced pancreatic beta cell dysfunction: New insight into the benefits of folic acid in the treatment of depression. 2014;166:6-13. https://doi.org/10.1016/j.jad.2014.04.063
[58] Ranjbar A, Ghasmeinezhad S, Zamani H, Malekirad AA, Baiaty A, Mohammadirad A, et al. Antioxidative stress potential of Cinnamomum zeylanicum in humans: a comparative cross-sectional clinical study. 2006;3:113. https://doi.org/10.1586/14750708.3.1.113
[59] Murcia MA, Egea I, Romojaro F, Parras P, Jiménez AM, Martínez-Tomé MJJoA, et al. Antioxidant evaluation in dessert spices compared with common food additives. Influence of irradiation procedure. 2004;52:1872-81. https://doi.org/10.1021/jf0303114
[60] Ananloo ES, Ghaeli P, Kamkar M-Z, Sadeghi MJDJoPS. Comparing the effects of fluoxetine and imipramine on total cholesterol, triglyceride, and weight in patients with major depression. 2013;21:1-7. https://doi.org/10.1186/2008-2231-21-4
[61] Sahraian A, Jelodar S, Javid Z, Mowla A, Ahmadzadeh LJAjop. Study the effects of saffron on depression and lipid profiles: A double blind comparative study. 2016;22:174-6. https://doi.org/10.1016/j.ajp.2015.10.012
[62] Sakr SA, Albarakai AYJIJAR. Effect of cinnamon on cypermethrin-induced nephrotoxicity in albino rats. 2014;2:578-86.
[63] Morgan AM, El-Ballal SS, El-Bialy BE, El-Borai NBJTr. Studies on the potential protective effect of cinnamon against bisphenol A-and octylphenol-induced oxidative stress in male albino rats. 2014;1:92-101. https://doi.org/10.1016/j.toxrep.2014.04.003

[64] Rice-Evans C, Miller N, Paganga GJTips. Antioxidant properties of phenolic compounds. 1997;2:152-9. https://doi.org/10.1016/S1360-1385(97)01018-2
[65] Elshopakey GE, Elazab STJVS. Cinnamon Aqueous Extract Attenuates Diclofenac Sodium and Oxytetracycline Mediated Hepato-Renal Toxicity and Modulates Oxidative Stress, Cell Apoptosis, and Inflammation in Male Albino Rats. 2021;8:9. https://doi.org/10.3390/vetsci8010009
[66] Rao PV, Gan SHJE-BC, Medicine A. Cinnamon: a multifaceted medicinal plant. 2014;2014. https://doi.org/10.1155/2014/642942
[67] Roussel A-M, Hininger I, Benaraba R, Ziegenfuss TN, Anderson RAJJotACoN. Antioxidant effects of a cinnamon extract in people with impaired fasting glucose that are overweight or obese. 2009;28:16-21. https://doi.org/10.1080/07315724.2009.10719756
[68] Banni M, Messaoudi I, Said L, El Heni J, Kerkeni A, Said KJAoec, et al. Metallothionein gene expression in liver of rats exposed to cadmium and supplemented with zinc and selenium. 2010;59:513-9. https://doi.org/10.1007/s00244-010-9494-5
[69] Ismail TA, Nassan MA, Alkhedaide AQ, Soliman MM, Mohamed DIJNJoP, Pharmacy, Pharmacology. Molecular, biochemical, and pathological impacts of energy drinks on renin-angiotensin-aldosterone pathway in Wistar rats. 2018;8:1140-6. https://doi.org/10.5455/njppp.2018.8.0411517042018
[70] Shi Y, Song Y, Wang Y, Liang X, Hu Y, Guan X, et al. p, p'-DDE induces apoptosis of rat Sertoli cells via a FasL-dependent pathway. 2009;2009. https://doi.org/10.1155/2009/181282
[71] Yamashita Y, Ueyama T, Nishi T, Yamamoto Y, Kawakoshi A, Sunami S, et al. Nrf2-inducing anti-oxidation stress response in the rat liver-new beneficial effect of lansoprazole. 2014;9:e97419. https://doi.org/10.1371/journal.pone.0097419