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The Impact of The Non-Polar Extract from *Cyperus Rotundus* Tubers on the Rats' Hepatotoxicity Induced by Alpha-Cypermethrin

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In Loving Memory of Late Professor Doctor ""Mohamed Refaat Hussein Mahran""

Abstract

Background: One of the most widely used insecticides, is alpha-cypermethrin (α-CYP). Excessive dependence on such insecticides leads to severe environmental pollution, negatively affecting human and animal health. Aim: The goal of the current investigation based on utilizing *Cyperus rotundus* tuber non-polar extract, to assist reducing the deleterious impact of α-CYP on liver tissues of male albino rats. Results: FT-IR analysis identified the most important active compounds present in the extract. While the active biochemical ones were confirmed by individualization and identification utilizing GC/MS. The results indicated that the most important active compounds in the hexane extract were oxygenated compounds,17-octadecynoic acid (60.51%) and 9-octadecenoic acid, methyl ester (6.54%) in addition to nitrogenated compounds, such as histidyl histidine (4.75 %). The results showed that the *C. rotundus* extract administration decreased liver enzymes; alanine aminotransferase (ALT), and aspartate aminotransferase (AST). Also, it increased antioxidant; glutathione (GSH), superoxide dismutase (SOD) enzyme, decreased oxidant; malondialdehyde (MDA), and nitric oxide (NO). Furthermore, it decreased liver inflammatory markers; nuclear factor-κB (NF-κB), adiponectin, and lipocalin. Conclusion: Results indicated that *C. rotundus* hexane extract exerts a potent protective effect against α-CYP-induced oxidative damage and inflammation in rats' liver tissues.

Keywords: Alpha-cypermethrin, *Cyperus rotundus*, Antioxidants, Fibrosis, Inflammation, Oxidative stress.

1. Introduction

Alpha-cypermethrin is a synthetic compound derived from the pyrethroids group insecticides, and are utilized for insects in gardens, industrial areas, and household due to their low toxicity to mammals. They are even used to treat ectoparasitic diseases in pets and sheep. Pyrethroids are considered one of the three major widely used pesticides in different countries, as the market of global pyrethroids production in 2016 was estimated to be $1633.03 million [1 ,2] .

However, overdependence and random utilization of these insecticides resulted in severe environmental pollution, consequently affecting human and animal health and finally causing the development of vector pest resistance and increasing the cost of pests control [3,4]. Generally, α-CYP exerts a mitochondrial dysfunction and induces oxidative stress, therefore the accumulation of these compounds impacts the human and animal tissues, causing hazardous involved in several diseases such as cardiovascular diseases and hepatotoxicity in addition to affecting the skin, nervous system, eyes, kidneys, gastrointestinal tract, blood, endocrine and reproductive system [5 ,2 ,6] .

Many studies have been conducted utilizing medicinal plants to treat a variety of disease situations, and the findings have shown that they offer effective and affordable therapeutic options. One such plant is *C. rotundus* ; *Cyperus* plant belongs to the botanical family *Cyperaceae*, which includes about 5,600 species and 100 genera. The family is spread all over the world and the genus *Cyperus* is considered one of the largest genera of the family. Although certain regions use *Cyperus* spp. for nutritional and therapeutic purposes, they are nonetheless considered weeds [7, 8] .

Generally, *Cyperus* spp. found worldwide predominantly in wetlands in tropical and subtropical regions, they serve as a source for the production of tubers, shoots, and fruits in large quantities as well as a food source for amphibians and aquatic animals [9] . The roots and tubers of *C. rotundus* have been mentioned in oriental traditional medicine for the treatment of fever, gastrointestinal disorders, menstrual disorders, epilepsy, ophthalmia, and inflammatory disorders in many countries including China, India, Iran, and Japan [10].It is commonly known as yellow nut, chufa, Tiger nut, Zulu nuts , and earth almond. In Egypt , Imam et al.,[11]indicated that

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the dry tubers of the *Cyperus* plant have been considered a food item since ancient times, especially in ancient Egypt in the Nile Valley region. They were also found in the tombs of the ancient Egyptians in the pre-dynastic era, about 6,000 years ago [12].

It has been stated that the plant contains flavonoids, epoxides, sesquiterpenes, alkaloids, glycosides, saponins, and essential oils [13, 14] .Clinical reports suggested 2 % aqueous extract of *C. rotundus* resulted in potent anti-inflammatory activity in conjunctivitis [15] . The liver is a substantial organ for the toxicity of drugs and xenobiotics due to its engagement with the gastrointestinal tract in addition to the singularity and complexity of its metabolic functions and anatomical structure [16] . Pesticides mainly cause liver damage, which can manifest in different forms, from hepatic steatosis to inflammation, or hepatobiliary dysfunction. On the other hand, chronic exposure is usually followed by cirrhotic or neoplastic damage 17] . The liver is the target organ because, among other functions, it takes up numerous toxic compounds and drugs from the portal circulation and detoxifies them. Therefore, the toxicity of substances is measured by the biochemical compounds and enzymes produced by the liver (liver makers) in plasma [18] .

Given the rarity of data on the effect of *Cyperus rotundus* tuber hexane extract on liver toxicity, this study aims to the characterization of *C. rotundus* hexane extract, and shed light on the effect of the extract to mitigate and eliminate the toxic effect of alpha-cypermethrin pesticide on some liver functions and inflammatory markers in rats.

2. Materials and methods

2.1. Plant Material

The dried tubers of *C. rotundus* were obtained from the local market in Giza Governorate. The dried tubers were ground by an electrical grinder (DING CANG DC-500A) at 25,000 rpm and then kept at – 4 °C.

2.2. Preparation of C. rotundus extract

The milled tubers powder of *C. rotundus* (100 g) was soaked with1000 ml *n*-hexane in a 2 L conical flask and shaken at 160 rpm in an orbital shaker (Stuart, England) for 24 h, at 25 °C, then re-extracted 3 times at the same conditions. Then, with Whatman No.1 filter paper, the extract was filtered to obtain the hexane filtrate. The filtrate was then concentrated by a rotary evaporator at 40 °C (HeidolphUnimax 2010, Germany) to obtain the crude *C. rotundus* nonpolar extract. After that, part of the crude extract was redissolved in n-hexane for chemical analysis, and the remaining portion was redissolved in water using tween 80 for animal treatment.

2.3. Chemical characterization of C. rotundus extract

2.3.1. Fourier Transform Infrared (FT-IR) spectra of hexane extract

Attenuated total reflectance (ATR)-FTIR evaluation was performed on a VERTEX 80 Bruker combined Platinum Diamond ATR (Germany), utilizing a diamond disc as an internal reflector from 4000–400 cm^{-1} range, with a resolution of 4cm^{-1} and a refractive index of 2.4.

2.3.2. Gas Chromatography mass spectrometry (GC-MS) analysis

The n-hexane extract of *C. rotundus* GC-MS analysis was performed by Thermo Scientific capillary gas chromatography (Trace GC ULTRA) equipped with TG-5MS nonpolar phenyl methylpolysiloxane (5%), with column dimensions of 30 m × 0.25 mm ID × 0.25 µm. The processing condition of GC oven temperature was 40 ºC for 3 min, to a final temperature of 280 ºC (rate 5 ºC/min). The GC–MS detection, was conducted with an electron ionization system (70 eV). The hexane extract (10 µl), was injected then detection was accomplished in full scan mode (40 to 500 m/z). The components identification was performed based on the retention time (Rt) compared with NIST (2010) library.

2.4. Animal experiment

Sixty-four male albino rats (150-170 g) were obtained from the breeding, faculty of Veterinary Medicine, Cairo University. Animals were housed in standard cages and fed with a standard laboratory diet. The experiment was implemented according to the guide of laboratory animals published by the National Institute of Health.

2.4.1. Toxicity test

Twenty-four rats were orally administered with α-CYP with different concentrations. Five rats were separated as a control group and the mortality was determined in different groups. The median lethal dose (LD50) was assessed according to Weill (1952) [19]. Rats were caged for 24 hours and observed for 14 days.

2.4.2. Animal treatment

Forty rats were randomly divided into 4 equal groups whereas, group1: represents the control , group 2: rats were orally administered a dose of *C. rotundus* extract (100 mg /kg BW) and 1/20 LD50 of α-CYP (0.533 mg/kg BW), group 3: rats were orally administered α-CYP (0.533 mg/kg BW) for 60 days, group 4: rats were orally administered α-CYP (0.533 mg/kg BW) in addition to 1 ml of *C. rotundus* extract via gastric intubation for 60 days.

2.4.3. Tissue sampling

Livers were removed after rats were sacrificed by decapitation and then quickly perfused with iced phosphate-buffered saline (PBS) at pH 7.4 for blood cells removal. The liver of each rat was isolated and frozen at -80 °C for biochemical analysis.

The frozen tissues were cut into small pieces and then homogenized in 5 ml/g tissue of cold buffer containing 0.5 g of Na_2HPO_4 and 0.7 g of NaH_2PO_4 per 500 ml in deionized water at pH 7.4. The mixture was then centrifuged at 4 °C for 15 min and 4000 rpm, the supernatant was then collected for different biochemical analyses[20] .

2.5. Biochemical analysis

2.5.1. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) analysis

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined using kit (BioMed Diagnostics) based on the method of Bergmeyer [21]. MDA, GSH, and NO were estimated in liver homogenates according to Ruiz-Larrea et al . [22] , Gabbianelli et al.[23] , and Moshage et al . [24] respectively.

2.5.2. Determination of liver superoxide dismutase (SOD)

Liver SOD was determined based on the method described by Nishikimi et al. [25] .

2.5.3. Determination of serum adiponectin

The adiponectin in serum was estimated by an enzyme-linked immunosorbent assay (ELISA) kit (Orgenium Laboratories, Finland) according to Watanabe et al.[26] .

2.5.4. Determination of serum nuclear factor-κB (NF-κB)

NF-κB was determined by an enzyme-amplified sensitivity immunoassay (EASIA) according to the manufacturing kit.

2.5.5. Determination of serum lipocalin-2

Lipocalin-2 levels in serum were assayed utilizing a lipocalin-2 Quantikine ELISA kit.

2.6. Statistical analysis

The SPSS statistics program 16 (Windows) was used to perform statistical analysis on the data. The data was presented as mean ± SE. The student T-test was used to compare the two groups. A one-way ANOVA test was used to perform multiple comparisons.

3. Results

3.1. Chemical characterization of C. rotundus tuber hexane extract

3.1.1. FT-IR spectrum of C. rotundus extract

The analysis using FT-IR spectroscopy at wave numbers between 400-4000 cm^{-1} of *C. rotundus* hexane extract showed the characteristic functional groups represented by carboxyl, esters, amine, alkene, and alkane groups as shown in Fig 1. The analysis indicated the presence of strong absorption peaks at 3286.77 cm^{-1} and 1710.16 cm^{-1} corresponding to O-H stretching of carboxylic acids. A strong peak (N-H stretching) also appeared at 2922.49 cm^{-1} related to amine salt. The strong absorption peaks (1280.26 and 1194.86 cm^{-1}) corresponded to the C-O stretching of esters. Alkene groups (C=C bending) were absorbed at 927.52, 869.09, and 721.40 cm^{-1}, while, alkane groups (C-H) appeared at 2853.01 and 1462.01 cm^{-1}. Finally, the absorption peak at 1377.51 cm^{-1} due to the –O–H stretching vibration of alcohol [27, 28 ,29].

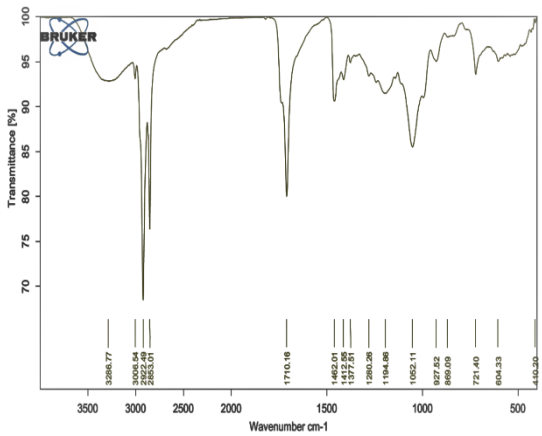

Fig 1. ATR-FT-IR spectrum of *C. rotundus* tuber hexane extract.

3.1.2. GC/MS analysis of the C. rotundus tuber hexane extract.

The separation and analysis by GC/MS of *C. rotundus* extract components are shown in Table 1. Twenty-five compounds were detected in the obtained extract and identified compared to the GC/MS library (NIST-11 and WILEY). The analysis revealed the exitance of oxygenated compounds, 17-octadecynoic acid (60.51%), 9-octadecenoic acid, methyl ester (6.54%), and hexadecanoic acid (5.33%). While the identified major hydrocarbon compounds were, dodecane (2.67 %) followed by undecane (2.62%). On the other hand, nitrogenated compounds were represented by histidyl histidine (4.75 %), 2-phenylglycine (1.03 %), and debrisoquine (0.86 %). The rest of the identified compounds were in a small amount.

Table (1). The main compounds identified in *C. rotundus* tuber hexane extract by GC/MS.

S.N.	R_t[a]	Compound name	Area %[b]	Molecular formula
1	5.51	(R)-(-)-2-Phenylglycine	1.03	$C_8H_9NO_2$
2	5.61	Octane, 4-methyl-(CAS)	0.52	C_9H_{20}
3	7.32	2-Allylcyclohexa-1,3-diene	0.54	C_9H_{12}
4	7.83	7,13,19,25-Tetra-tert-butyl-27,28,29,30-tetrahydroxy-2,3-bishomo-3-oxacalix[4]arene	0.32	$C_{45}H_{58}O_5$
5	8.20	(Z)-3-(3'-Hydroxyphenyl)-1,1-diphenylprop-2-en-1-ol	0.58	$C_{21}H_{18}O_2$
6	8.32	Docosane (CAS)	0.75	$C_{22}H_{46}$
7	10.52	*O*-Menthan-8-ol	0.38	$C_{10}H_{20}O$
8	11.21	Undecane (CAS)	2.62	$C_{11}H_{24}$
9	12.19	Cyclohexane, pentyl-(CAS)	0.21	$C_{11}H_{22}$
10	13.04	Pentadecane	0.52	$C_{15}H_{32}$
11	14.06	Dodecane (CAS)	2.67	$C_{12}H_{26}$
12	20.11	Dihydro-nor-dicyclo-pentadienyl acetate	0.19	$C_{12}H_{16}O_2$
13	20.62	1,2-Benzenedicarboxylicacid, diethyl ester (CAS)	0.25	$C_{12}H_{14}O_4$
14	21.70	Hexadecane,2,6,10,14-tetramethyl-(CAS)	0.13	$C_{20}H_{42}$
15	24.26	1,2-Benzenedicarboxylic acid, diethyl ester (CAS)	1.64	$C_{12}H_{14}O_4$
16	25.70	1-(4-Isopropylphenyl)-2-Methylpropyl Acetate	0.55	$C_{15}H_{22}O_2$
17	26.03	n-Hexyl salicylate	0.44	$C_{13}H_{18}O_3$
18	26.52	Heptadecane,2,6,10,15-tetramethyl-(CAS)	0.26	$C_{21}H_{44}$
19	27.37	Debrisoquine	0.86	$C_{10}H_{13}N_3$
20	27.55	Octanal,2-(phenylmethylene)-(CAS)	0.26	$C_{15}H_{20}O$
21	32.17	Hexadecanoic acid	5.33	$C_{16}H_{32}O_2$
22	34.11	9,12-Octadecadienoic acid(Z,Z), methyl ester(CAS)	1.16	$C_{19}H_{34}O_2$
23	34.18	Histidyl histidine	4.57	$C_{12}H_{16}N_6O_3$
24	34.21	9-Octadecenoic acid, methyl ester (CAS)	6.54	$C_{19}H_{36}O_2$
25	35.70	17-Octadecynoic acid	60.51	$C_{18}H_{32}O_2$

3.2. Biological analysis

Levels of liver enzymes ALT and AST were significantly low in the *C. rotundus* treated group compared to rats that received α-CYP only (Table 2). Results also have shown that treatment with *C. rotundus* significantly reduced liver NO and MDA levels and conversely elevated GSH contents and SOD activity in liver tissues (Table 3).

Regarding the levels of NF-kB, Lipocalin, and Adiponectin as shown in Table 4, results were significantly higher in rats that were orally administered 0.533 mg/kg BW of α-CYP only compared with rats that orally administered α-CYP in a dose level of 0.533 mg/kg BW beside 1 ml of *C. rotundus* extract.

Table 2: liver enzymes in different studied groups.

Groups	ALT (U/L)	AST (U/L)
Control	55.7±0.6	35.0±1.1
C.rotundus	53.2±.4	37.4±1.9
α-CYP	105.7±4.0[a]	95.0±9.2[a]
α-CYP +*C.rotundus*	69.6±7.5[b]	49.3±4.8[ab]

Data presented as mean ± SE, Number of rats per group n = 10.

[a] Significant difference at $P \leq 0.05$ compared to the control group. [b] Significant difference at $P \leq 0.05$ compared to the α-CYP group.

Table 3: oxidant and antioxidant parameters in different studied groups.

Groups	NO µmol/g tissue	SOD U/mg protein	GSH µg/g tissue	MDA µmol/g tissue
Control	6.8±0.4	8.5±0.8	27.6±0.8	85.5±2.3
C. rotundus	5.8±0.4	8.6±0.3	28.3±1.2	83.6±3.1
α-CYP	15.0±1.1[a]	4.3±0.6[a]	16.3±0.8[a]	135.3±4.5[a]
α-CYP +*C. rotundus*	10.0±0.5[ab]	6.5±0.4[b]	23.3±0.9[b]	102.2±3.6[ab]

Data presented as mean ± SE, Number of rats per group n = 10.

[a] Significant difference at $P \leq 0.05$ compared to the control group. [b] Significant difference at $P \leq 0.05$ compared to the α-CYP group.

Table 4. Liver inflammatory markers in different groups.

Groups	NF-kB µg/ ml tissue	NGAL µg/g tissue	Adiponectin µg/g tissue
Control	0.066±0.008	1.6±0.14	4.9±0.08
C.rotundus	0.07±0.003	1.7±0.1	5.2±0.2
α-CYP	1.08±0.08 [a]	2.8±0.05 [a]	9.8±0.5 [a]
α-CYP +*C.rotundus*	0.07±0.006[b]	1.9±0.5 [b]	6.5±0.7 [b]

Data presented as mean ± SE, Number of rats per group n = 10. [a] Significant difference at $P \leq 0.05$ compared to the control group. [b] Significant difference at $P \leq 0.05$ compared to the α-CYP group.

4. Discussion

Synthetic pyrethroids are a group of unique insecticides, they present over 30% of globally used insecticides [30] . α-CYP is a common pyrethroid insecticide utilized to control a wide range of insect pests in agricultural products [31] .

In this study, GC-MS analysis revealed that *C. rotundus* hexane non-polar extract has active constituents that may be responsible for pharmacological actions. The majority of these constituents are oxygenated, nitrogenated, and hydrocarbon compounds, in addition to the presence of several phytoconstituents such as alkaloids, sterols, resins, flavonoids, cyanogenic glycosides, saponins, vitamins, and tannins which are demonstrated by Ekeanyanwu et al. [32] .

17-octadecenoic acid, 9-octadecenoic acid, 12-octadecadienoic acid, and hexadecenoic acid were among the oxygenated compounds that have been detected in the extracting. The major component identified was 17-octadecanoic acid as known to possess antioxidant and anti-inflammatory effects[33] [34]. On the other side, nitrogenated compounds debrisoquine and histidyl histidine contribute to the biosynthesis of protein [35] .

To our knowledge, this was the first study showing that the constituents of *C. rotundus* debilitated α-CYP-induced liver toxicity by modulation of the damage exerted by oxidative, inflammation, and fibrotic alterations in liver tissues.

The obtained results indicated that oral administration of α-CYP has an obvious negative effect on the liver. This was demonstrated by increased levels of oxidative stress, proinflammatory cytokines, liver damage, and fibrosis, all of which were associated with a decrease in antioxidant defense. Liver enzymes, such as ALT and AST are specific indicators of liver damage. Their high activities in the serum may indicate cellular leakage and loss of functional integrity of cell membranes [36] . Results in the present study revealed that α-CYP exposure increased enzyme activities of ALT, and AST in rats. The obtained results were in agreement with Ali et al.[2] reported that the administration of α-CYP to rats increased ALT and AST activities indicating hepatic damage. Several studies have shown that α-CYP can damage the liver through the deterioration of biological membranes by causing oxidative stress [37] . One of the most common forms of oxidative damage caused by reactive oxygen species (ROS) is lipid peroxidation, which has been related to altered membrane structure and enzyme deactivation [38] . Peroxynitrite (ONOO-) is a highly reactive oxidant formed when superoxide anion and NO combine. Peroxynitrite has the ability to damage DNA and limit the function of endogenous superoxide dismutase (SOD) enzymes [39].

SOD is an antioxidant enzyme that converts the potentially harmful free radical superoxide ($O_{2^{\cdot}}^{-}$) to H_2O_2 and O_2, protecting cells from oxidative damage [40] .On the other hand, a significant thiol antioxidant, glutathione (GSH) is a multifunctional intracellular nonenzymatic antioxidant that can protect cell membranes from damage, scavenge free radicals, and prevent peroxidation [41, 42].Accordingly, oxidative stress was determined in this study by elevated MDA and NO and lowered levels of the two antioxidant defense molecules the SOD and GSH. These results were consistent with earlier research by Ali et al.[2] , who showed that α-CYP led to an increase in MDA and NO as well as a reduction in antioxidant defense.

However, ROS are produced in the liver during the metabolism of pyrethroids through cytochrome P450 oxidative pathways [43]. Pyrethroid-induced oxidative damage may be induced by their lipophilicity, which allows them to easily penetrate cell membranes and result in membrane lipid peroxidation. Therefore, this might be responsible for the liver's elevated MDA levels in this study. Furthermore, Wang et al., 2009 reported that cypermethrin in male rats increased the concentration of inducible NOS (iNOS), as well as total NOS (T-NOS), and thus raised NO concentrations. The observed decrease in SOD and GSH activity indicates their depletion in counteracting the ROS production generated by α-CYP [44] . This oxidative condition can cause organ damage as well as biochemical and physiological changes.

NF-kB is one of the most essential transcription factors proven to be highly sensitive to oxidative stress, and its activation is critical in the regulation of gene coding expression for pro-inflammatory cytokines and mediators [45]. When NF-kB is activated, pro-inflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor

necrosis factor-alpha (TNF-α) are released, resulting in an inflammatory response[46] .

In addition, adiponectin acts as a surrogate marker for inflammation by inducing the release of pro-inflammatory cytokines, with the participation of TNF-α [47] .

Recently Ranasinghe et al., 2023, stated that under noxious situations such as intoxication, infection, inflammation, and other forms of cellular stress, neutrophil gelatinase-associated lipocalin (NGAL) is expressed. NGAL synthesis was also induced rapidly and sustainably by injured hepatocytes during experimental liver injury [48].

Consistently, the present investigation demonstrated that rats exposure to α-CYP markedly increased NF-kB, adiponectin, and NGAL levels in the serum. These findings support previous work, which found a link between α-CYP and liver inflammation in adult rats [2] . Arafa et al. [44] attributed increased NF-kB mRNA expression in the lungs of α-CYP-treated rats to the excessive production of ROS by α-CYP.

NF-kB is present in an inactive state in the cytoplasm as a result of sequestration with the inhibitory protein $I_{\kappa}B$ [49] . Also, Arafa et al. [44] suggested that α-CYP causes phosphorylation of $I_{\kappa}B$ Ser32/36 and thus the translocation of NF-kBp65 into the nucleus where it is changed into an active state.

Highly accessible antioxidants or compounds with antioxidant enzyme activity may modify the inflammatory processes that are crucial to the pathogenesis of liver inflammation, in addition to providing protection against the direct harmful effects of oxidants [50] .

In the present study, treatment with *C. rotundus* non-polar extract significantly reduced liver NO and MDA levels and conversely elevated GSH contents and SOD activity in liver tissues. These observations clarified that *C. rotundus* extract could eliminate the oxidative stress induced by α-CYP through the quenching mechanism of the free radicals production, thereby inhibiting the chain reactions of the free radicals. The study attributed the antioxidant effect of *C. rotundus* hexane extract to the presence of antioxidant compounds such as 17-octadecynoic acid which act as inhibitors of cytochrome P450 ῲ-hydroxylase and epoxygenase systems.

Previously, *C. rotundus* tuber extract has been reported to contain a high amount of β-carotene and Vitamin A, in addition to the presence of α- tocopherol which prevents damage to polyunsaturated fatty acids by free radicals in membranes. The α- tocopherol particularly is converted to tocopheryl radical. This radical is converted back to α- tocopherol [51, 52] . Also, vitamin E exerts its antioxidant property by preventing chain propagation as a result of its ability to transfer phenolic hydrogen to a peroxyl free radical of a peroxidized polyunsaturated fatty acid, minimizing lipid peroxidation [53]. In this context, the presented vitamin E in the *C. rotundus* tuber extract may potentiate the effect through regeneration from vitamin E radical formed during the antioxidant action of vitamin E [53] . The present study demonstrated a significant increase in SOD activity and GSH levels with *C. rotundus* administration. This is in response to decreased free radical activity and lipid peroxidation. Moreover, *C. rotundus* is rich in tannins that may act as antioxidants to scavenge free radicals and protect against oxidative damage [54].

The present study confirms the significant reduction in the levels of NF-kB adiponectin and NGAL after the treatment with *C. rotundus* hexane extract. Results attributed these effects to the 9-octadecynoic acid, which has anti-inflammatory effects against inflammatory mediators. According to Xie et al. [55] , 9-octadecenoic Acid inhibited the production of pro-inflammatory cytokine TNF and reduced the protein expression and mRNA levels of iNOS and COX2, phosphorylated MAPK family members P38, ERK, and JNK, and controlled the nuclear translocation of NF-B in LPS-induced RAW264.7 macrophages.

Furthermore; phytochemical analysis of *C. rotundus* revealed the presence of a number of phytoconstituents such as sterols and saponin that inhibit the expression of pro-inflammatory cytokines through the NF-kB pathway[56] . In addition, sterol and saponin inhibited IκB phosphorylation and degradation also the translocation of the p65 subunit of NF-kB into the nucleus [57] .

The rats treated with *C. rotundus* hexane extract showed a significant decline in the AST and ALT ALP levels, compared to the treated rats group that received α-CYP alone. In accordance with the obtained results, Innih et al. [58] reported that the aqueous extract of *C. rotundus* treatment at doses of 400 and 600 mg/kg in triton-induced toxicities in rat liver significantly decreased serum activities of ALT and AST to normal levels. Furthermore, El-Naggar [59] reported that tiger nut oil may be enhancing the detoxicated properties of the liver and attributed this to its higher content of monounsaturated fatty acid and phytochemicals. These beneficial substances provide a protective effect against lipid oxidation in the liver and consequently improve liver functional enzyme activity.

5. Conclusion

The findings demonstrated that *C. rotundus* hexane nonpolar extract significantly protected rats' livers against oxidative damage and inflammation caused by α-CYP. This hepatoprotective activity of *C. rotundus* may be attributed to its active constituents that reduced the harmful effect of α-CYP through its antioxidant , and anti-inflammatory properties.

6. Declaration of Competing Interest

Authors declare no conflict of interest.

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