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ANIMAL SCIENCE

Nutritional Significance, Antimicrobial, Antioxidants, Anticancer, and Antiviral Activities of Lemongrass Leaves Extract and Its Application as Hepatoprotective Agent against CCl_4-Induced Hepatic Injury in Rats

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Abstract: This work investigated the antioxidant and hepatoprotective activities of lemongrass extract and its effects on rat hepatotoxicity. The lemongrass extract (LGE) contains bioactive components such as phenolic acids, flavonoid components, vitamin C, fibers, and tannins. The LGE had high phenolic content (397 mg/100g) and flavonoids (164 mg/100g), influencing its antioxidant activity of 91.25%. Additionally, it inhibited 81% of breast cancer, also, inhibited the growth of pathogenic bacteria and *Candida* at a concentration of 20-40 µg/mL. Additionally, it inhibited SARS-Cov-2 by 75%; however, increasing the activity of Cas-3. Quercetin-3-rhamnoside was the main phenolic compound in the HPLC profile; the phenolic compounds may be attributable to the beneficial effects of LGE. In this study, the CCl_4-challenged rats delivered two levels of LGE (100 and 300 mg/kg BW). LGE reduced ALT, AST, creatinine and urea by 50 and 37%, respectively. Generally, LGE mitigated the oxidative stress induced by CCl_4, which is evident in the histology of liver and kidney tissues, where significant improvement, with no cytoplasmic degradation in undamaged liver hepatocytes, improved kidney performance and shape. It can be concluded that polyphenolic-rich LGE can mitigate the oxidative stress induced by CCl_4 and other parameters while enhancing kidney and liver performance.

Key words: Antioxidant, antimicrobial, oxidative stress, blood lipid profile, hepatotoxicity.

INTRODUCTION

Free radicals or oxidative damage are the core cause of various neurologic and other human illnesses (Akbari et al. 2022). Numerous human disorders, such as cancer, Alzheimer, cataract, stroke, liver and kidney cancer, coronary heart disease, arthritis, and aging, are associated with high exposure to chemicals that generate free radicals or high accumulation of synthetic free radicals in human cells (Akbari et al. 2022). Unsaturated fatty acids contributing to membrane permeability, receptor orientation, and cell lysis are strong contenders for free radical reactions (Yammine et al. 2020). Free radicals cause damage to enzymes containing sulfur and other proteins, resulting in the denaturation, inactivation, and cross-linking of enzymes and proteins. DNA damage can result in mutations that may be cancerous (Tvrdá et al. 2022). Oxidative damage to carbohydrates can change any receptor function, including hormone and neurotransmitter responses

(Jakubczyk et al. 2020). Reactive oxygen species (ROS), such as superoxide anion (O2–), hydrogen peroxide (H_2O_2), and hydroxyl radical (HO•), cause lipid oxidation and peroxidation after attacking polyunsaturated fatty acids in cell membranes of living systems, which is directly associated with aging and carcinogenesis (Liu et al. 2022). Under standard physiological settings, tissues and cells contain minimal lipid peroxidation products. However, the damaged cells produced more lipid peroxidation products under oxidative stress into the serum, causing more damage to other cells (Shahidi & Ambigaipalan 2015).

The main field of application of natural products is in the prevention of oxidation of animals and their derivatives (Ebrahim et al. 2020, Bilal et al. 2021, Alagwany et al. 2021). Extra antioxidants from natural sources, such as plants, effectively guard against oxidative stress, leading to more food makers substituting natural antioxidants for synthetic antioxidants. As a result, natural additives have received considerable interest as they pose no threat to consumer health (Butnariu & Grozea 2012). The antioxidant defense system against free radical reactions contains enzymatic and nonenzymic compounds, some of which may be produced only in plants and could be received through food (Adebooye et al. 2008, El-Ashry et al. 2022). Natural antioxidants in leafy greens play an essential role in preventing free radical damage (Ashour et al. 2020, Swelum et al. 2021, Abd El-Hack et al. 2022 a, b). Various epidemiological studies have linked meals high in phenolics with considerable antioxidant potential to a reduced risk of coronary heart disease., diabetes, cancer, and neurological illnesses (Adebooye et al. 2008, Saad et al. 2021b, El-Saadony et al. 2022).

Lemongrass is high in carbohydrates, protein, vitamins, minerals, and a small amount of fat (Muala et al. 2021). Alcohols, terpenes, aldehydes, ketones, esters, flavonoids, and phenolic substances, such as iso-orientin, quercetin, luteolin, kaempferol, apigenin, and tannins, have been found in lemongrass *Cymbopogon citratus* leaves (Muala et al. 2021). Antihypertensive, antioxidant, hypoglycemia, anti-diabetic, anti-inflammatory, anti-amoebic, antibacterial, anti-filarial, antifungal, anticancer, and anti-mutagenicity characteristics are some of the pharmacological effects of *Cymbopogon citratus* leaf (Zulfa et al. 2016, Muala et al. 2021, Pan et al. 2022).

Diuretics such as lemongrass decoction and infusion treat gastrointestinal spasms, food poisoning, rheumatism, anorexia, and digestive issues (Mohamed et al. 2017). According to Koh et al. (2012), the ethanol extract of *C. citratus* may be employed as a medicinal treatment to protect the hepatic tissue from oxidative stress destruction. Also, fortification of lemongrass toast at levels 5 and 10%, then feeding mice, resulted in significant decreases in markers of kidney (creatinine and urea), liver functions (Alanine aminotransferase-ALT, Alkaline phosphatase-ALP, and Aspartate aminotransferase-AST), and lipid profile (total cholesterol-TC and triglyceride-TG) in serum, which proves that lemongrass improves kidney and liver health in sick rats (Radwan & Elmaadawy 2022). No findings included *in vitro* and *in vivo* studies of lemongrass application; therefore, this study investigates lemongrass extract's antioxidant, anticancer, antiviral, and antimicrobial activity and estimates the phenolic compounds by HPLC. The ability of lemongrass (*Cymbopogon citratus*) to change oxidative stress indicators and preserve liver and kidney function in CCl_4- rats harmed by hepatotoxicity was studied *in vivo*.

MATERIALS AND METHODS

Ethical Approval

The animal study has been reviewed and approved by ZU-IACUC committee. was performed in accordance with the guidelines of the Egyptian Research Ethics Committee and the guidelines specified in the Guide for the Care and Use of Laboratory Animals (2022). Ethical code number ZU-IACUC/2/F/394/2022.

Distinguishing materials

Serum total protein, cholesterol, liver enzymes, creatinine, urea, LDL, HDL, and triglyceride kits were obtained from ELIPSE, United Diagnostic Industry, Dammam, Saudi Arabia. 2,2-diphenyl-1-picrylhydrazil, carbon tetrachloride, Folin-Ciocalteu reagent, gallic acid, quercetin standards, aluminum chloride hexahydrate, thiobarbituric acid, and propane 1,1,3,3-tetra methoxy were acquired from Sigma company (USA). Figure (1) shows a graphical experimental design.

Preparation of lemongrass ethanolic extracts (LGE)

Cymbopogon citratus leaves were taken from a plantation in the Hail region of Saudi Arabia in October 2020. The leaves were rinsed under running water, cut into 2 cm pieces, dehydrated at 60 °C

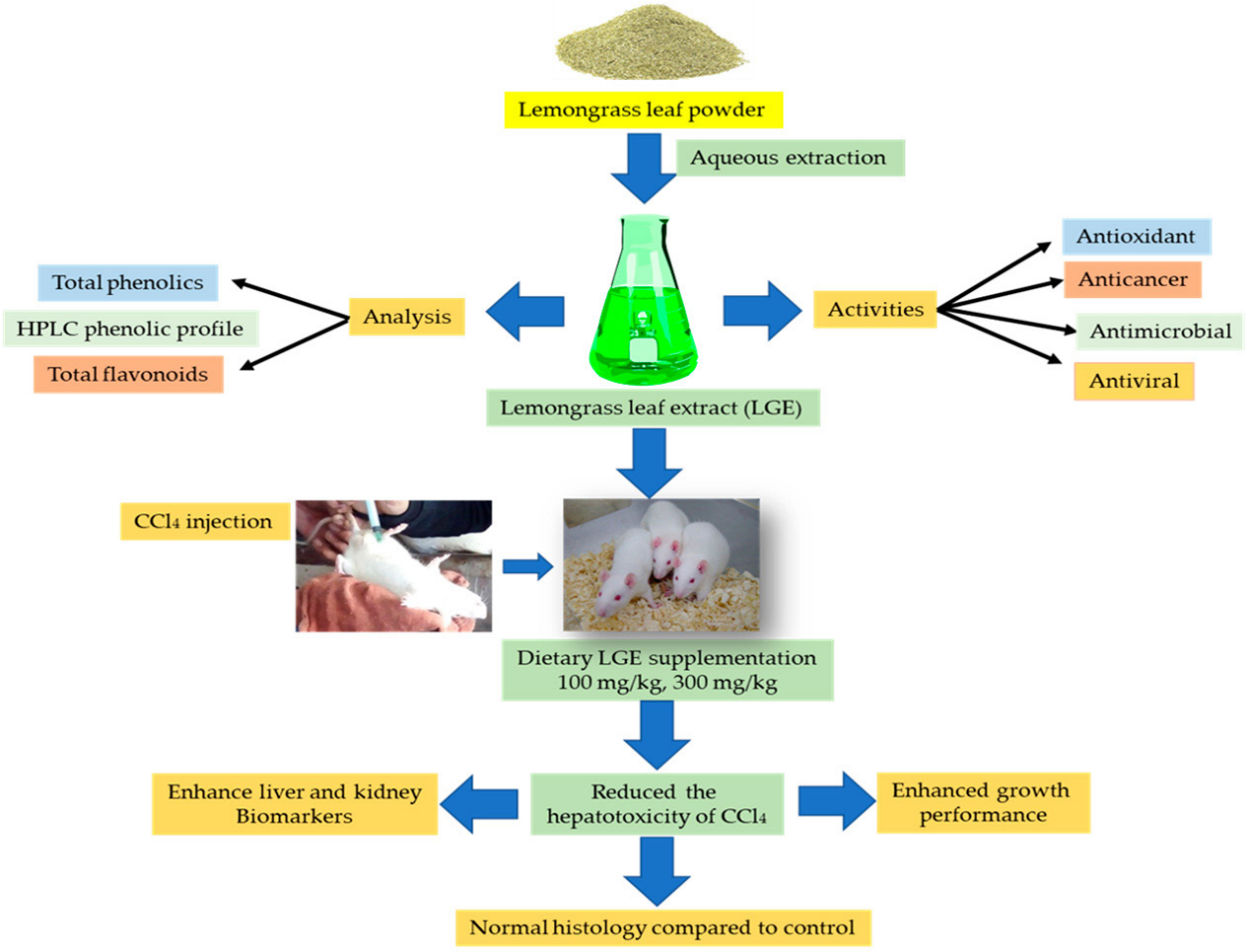

Figure 1. Graphical experimental design.

for three hours, ground, and sieved using a 1 mm sieve, packaged, and stored at 40°C. Lemongrass has been certified and verified as non-toxic by the Plant Production and Protection Department at Qassim University, Saudi Arabia. The lemongrass powder was stirred in 70% ethanol for three days at 150 rpm. The mixture was then filtered and evaporated the solvent using a rotary evaporator. The concentrated filtrate was then lyophilized to remove any remaining solvent. The extract was dark until the polyphenols, tannins, and flavonoid content were analyzed (Falah et al. 2015).

Polyphenolic content in LGE

Total phenolic and flavonoids content

The Folin-Ciocalteu reagent was used to measure the total polyphenols (TPs) in lemongrass extract (LGE) (Wolfe et al. 2003). The OD of yellow or purple color was estimated at 750 nanometers. The TPC was given in mg of gallic acid (GA)/g of LGE and was calculated using the standard curve of gallic acid.

$$y = 0.0201x + 0.0439, R^2 = 0.9968 \quad (1)$$

The $AlCl_3$ technique calculates TFs as quercetin equivalents (QE) (Saad et al. 2021a), whereas At 450 nm, the OD was detected using the QE standard calibration equation

$$y = 0.0144x - 0.0092, R^2 = 0.9985 \quad (2)$$

Phenolic compounds profile by HPLC

The HPLC Agilent 1200 series includes an auto-sampler, a quaternary pump, and a separation column (Zorbax-OD, 4.6×250 nm, 35°C) using a 1 mL/min mobile phase flow rate and a multiwavelength detector tuned at 330 and 280 to detect flavonoids and phenolic compounds, respectively and degasser. The phenolic and flavonoid compounds of *Cymbopogon citratus* ethanolic extracts were identified by HPLC, according to Saad et al. (2021a).

In vitro biological activities of LGE

Antioxidant

The LGE (50, 100, 150, 200, 250, and 300 µg/mL) The ability of lemongrass extract (LGE) to scavenge the DPPH free radicals was tested. First, 100 microliters (µL) of LGE were added to 100 µL of DPPH in a microtiter plate. The mixture was left in a dark place for 30 minutes. The absorbance of the solution at 515 nanometers (nm) was then measured using a BioTek microtiter plate reader (USA). The following equation applied the data to calculate antioxidant activity and IC50.

$$\% \text{ Antioxidant activity} = \frac{\text{Control absorbance} - \text{sample absorbance}}{\text{Control absorbance}} \times 100 \quad (3)$$

Cytotoxicity Effects

The sulforhodamine B assessed the viability of MCF-7 breast cancer cells. Cancer cells were cultivated in 100 µL of culture medium without the LGE for a day. Another 100 µL of culture medium was

supplemented with various doses of lemongrass extract (LGE) (50, 100, 150, 200, 250, and 300 µg/mL) under the same conditions.

The samples were fixed in 150 µL of 10% TCA for 1 h at 4°C, then washed several times with deionized water. Each well received a 0.4 percent SRB solution (70 µL), and the plate was kept in a dark place for 5 min, followed by washing with 1% acetic acid and air-dried overnight. The protein-bound SRB dye was dissolved in 150 µL of 10 mM Tris-HCl, and the OD of the resultant color was measured using a microtiter plate reader at 540 nanometers (nm) (BioTek Elx808, USA). The lowest LGE concentration that caused a 50 percent decrease in OD refers to (LC50) which was also calculated (Bahuguna et al. 2017).

Antimicrobial

The antimicrobial activity of LGE concentrations was performed against various bacterial and fungal strains. The tested strains were cultivated overnight at 37 °C in a shaking incubator with MHB to obtain 1×10^8 colony-forming units (CFUs) per mL. The disc diffusion method was then used to assess the antibacterial activity of the lemongrass extract (LGE). The spread plate approach infused the Petri plates with 100 µL active strains. Paper discs (6 mm) that had previously been moistened with LGE (50, 100, 150, 200, 250, and 300 µg/mL) were placed on the plates' surface. The plates were incubated at 37 degrees Celsius for 24 hours. The diameter of the inhibition zones surrounding the disks was then measured using a ruler in millimeters (mm). The results were compared to those obtained with levofloxacin, used as a positive control (El-Saadony et al. 2021)

Antiviral activity

The antiviral activity of LGE concentrations was performed against the binding between SARS-Cov-2 and Angiotensin-converting enzyme 2 (ACE-2). The antiviral activity was performed according to Alsubhi et al. (2022).

In vivo *Experimental Layout*

Thirty-two rats (160–180g) were obtained from King Saud University, KSA, and allocated randomly into four groups of 8 animals. The groups were

The rats were fed a basal diet without additions as a negative control.

As a positive control, the rats were intraperitoneally administered a single dose of CCl_4 (2 ml/kg-1 b-wt.).

Rats were given CCl_4 and *Cymbopogon citratus* extract (100 mg/kg-1 b-wt.).

Rats were given CCl_4 and *Cymbopogon citratus* (300 mg/kg-1 b wt.).

The treatments were administered for four weeks. At the end of the trial, rats were humanely euthanized, and liver and kidney specimens were surgically removed. The blood was drawn from the retro-orbital vein. Biochemical tests were done on liver function, total protein, total lipids, cholesterol, TG, malondialdehyde (MDA), and renal function.

Biochemical Examination

The levels of liver enzymes ALT, AST total protein, and albumin were evaluated using the (Schumann & Klauke 2003) method. Reduced glutathione (GSH) in serum was quantified using (Beutler 1994).

The Malondialdehyde (MDA) was measured as the thiobarbituric acid (TBA) reactive according to Beutler (1994). The kidney markers, urea, and creatinine were measured (Bulut et al. 2013). Total cholesterol, low-density lipoprotein (LDL) cholesterol, and high-density lipoprotein (HDL) cholesterol were determined using the enzymatic colorimeter method (Armbruster & Lambert 1996). TG were determined using the method of (Devi & Sharma 2004).

Histological examination

Histopathologic samples were taken from the slaughtered rats' livers and kidneys that were preserved in a 10% formalin saline solution for at least 10 h before being rinsed for 12 h in tap water. Before the tissue samples were kept in paraffin, they were rinsed with xylene. Under an optical microscope, hematoxylin and eosin were used to stain tissue slices on slides for histologic analysis (Bancroft & Gamble 2008).

Statistical evaluation

ANOVA was used to explore the variation between the data means with a confidence level of 95% on the triplicate findings. LSD was employed as a post-hoc test to compare the significant differences between findings mean. The statistical evaluation was conducted by SPSS 23.0 software.

RESULTS AND DISCUSSION

Proximate chemical structure of *Cymbopogon citratus* leaf

The herbal extract standard for certifying crude powdered plant materials is closely examined. The chemical compositions of *Cymbopogon citratus* powder leaf were determined in this study, including moisture content, ash, water-soluble ash, crude fat, crude fiber, and crude protein, as results are presented in Table I, moisture (13.00±2.92 %), ash (7.63± 0.23 %), crude protein (4.45± 0.13 %), crude fat (2.44± 0.58 %), and crude fiber (29.40 ±1.03 %) respectively. The findings agree with Nimenibo-Uadia & Nwosu (2020), but on a fresh basis, the leaves contained protein, ash, lipids, fiber, carbohydrates, and moisture at 15.86, 9.40, 6.90, 1.00, 66.54, and 72.95%, respectively.

Polyphenol components in lemongrass leaf extract

Total phenolics and flavonoids

Polyphenol molecules are important plant components with antioxidant properties due to their redox properties. The hydroxyl groups found in plant extracts facilitate the scavenging of free radicals. The antioxidant effect of a crude ethanol extract of *Cymbopogon citratus* leaf was determined using free radical scavenging antioxidant power tests. The findings were represented in gallic acid equivalents/g DW (Table II). Flavonoids are secondary metabolites with antioxidant activity that varies with the amount and position of OH groups. In ethanol extracts, total phenolic components, flavonoids, and radical scavenging activities were 397 mg GAE/100g, 164 mg QE/100g, and 91.25%, respectively.

Many factors affect Polyphenol content in raw plants, including genotype, ambient conditions, altitude, light, temperature, and the quantity of nutritional material in the soil (Kostić et al. 2012).

Table I. Chemical composition of *Cymbopogon citratus* leaf.

Parameters	Concentration g/100g
Moisture	13.03±1.23
Water soluble ash	6.24±0.13
Total ash	7.52±0.21
Crude fiber	29.63±1.23
Crude protein	4.56±0.11
Crude fat	2.46±0.44

Table II. Polyphenols, flavonoid compounds in LGE.

Items	Concentrate
Total phenolic compounds (mg GAE/100g)	397±6.8
Total flavonoids (mg QE/100g)	164.10±3.3

Identification of polyphenolic compounds by HPLC

Extraction techniques and solvents are responsible for dissolving the plant's endogenous components. Furthermore, plant components might be either polar or nonpolar. Because phenolic compounds include a hydroxyl group, they are more soluble in polar organic solvents; hence, ethanol was chosen as the extraction solvent (Kouassi et al. 2017). Compared to the literature, the phenolic concentrations in this investigation differed marginally. Because of the presence of various levels of sugars, carotenoids, or ascorbic acid, the length of time, regional variance, or extraction methods could affect the amount of phenolic (Althwab et al. 2019, Burri et al. 2017). The phenolic components found in lemongrass leaf extract ranged from 1.1 to 157.80 mg/100g, as shown in Table III and Figure 2. Swertiajaponin (157.0 mg/100g), ellagic acid (51.81 mg/100g), caffeic (31.06 mg/100g), and syringic acid (20.24 mg/100g) are the most abundant components in the lemongrass leaf extract. Flavonoid contents in the plant extracts were measured quantitatively using aluminum chloride in a colorimetric technique. According to the literature El-Zahar et al. (2021a,b), genetic variety and biological, ecological, seasonal, and year-to-year variations substantially influence vegetable flavonoid concentration. According to our findings, the most abundant components in lemongrass leaf extract are swertiajaponin, ellagic acid, caffeic, and syringic acids (Muala et al. 2021) caffeic (20.81 mg/100ml) and syringic acids (18.63, 7.390 mg/100ml) were the predominant phenolic components in *Cymbopogon citratus* extract at optimal extraction conditions.

Biological activity of LGE

Antioxidant

In our study, lemongrass leaf extract has high phenolic and flavonoid compound inclusion and free radical scavenging. The total phenolic content of *Cymbopogon citratus* methanol extract was 16.6 mg

Table III. Classification of polyphenol and flavonoid constituents of lemongrass leaf by HPLC.

Rt*(min)	Total phenolic compounds	Conc.(mg/100g)
5.56	Gallic acid	88.0±4
5.77	2-Carene	57±3
5.8	Quercetin	72±4
6.01	Allagic acid	51.81±4
6.25	Kaembferol	60±3
8.06	Phellandrene	10±1
8.88	Limonene	3.7±0.4
9.67	Resorcinol	50.8±4
10.1	Caffeic acid	31.8±2
11.7	2.5-Dihydroxybenzoic acid	20.1±2
12.12	a-Terpinene	44.4±3
13.46	Quercetin-3-rhamnoside	325.6±14
13.7	Swertiajaponin	157±7
13.9	Ferulic acid	98.3±6
19.12	Trans-cinnamic acid	97.2±6
28.3	Neochlorogenic acid	21.6±2
29.08	Chlorogenic acid	44±3
31.06	Caffeic acid	31.06±2
33.8	Agarospirol	33.8±2
35.60	Syringic acid	20.24±2
40.2	Catechin	12±1
46.14	*p*-coumeric acid	1.10±0.1
57.74	Rutin	12±2

Data are presented mean±SD, Rt, retention time.

GAE/g DW. DPPH and FRAP activity were reported at 1261.3 mg TE/100g DW and 1920.926 mg TE/100g DW, respectively. Total phenolic and flavonoid compounds and antioxidants were found to have 118.14 mg GAE/g and 178.069 mM TE/ml DW (Kouassi et al. 2017). Also, Ranjah et al. (2022) found that TPs in lemongrass leaves were 14.7 mg GAE/100 g, and antioxidant activity against DPPH was 86.3%, which may be an excellent source of bioactive compounds with significant antioxidant potential.

Anticancer

Figure 3 shows that lemongrass extract (LGE) possesses potent antitumor properties on breast cancer cells, compared to the commonly used chemotherapy drug doxorubicin (DOX). Cancer cell viability is enhanced in a concentration-dependent way. LGE (300 micrograms per milliliter) decreased the survival of MCF-7 cell lines by 81 % compared to DOX. This is consistent with the microscopic image. This indicates that LGE has a more considerable inhibiting impact than DOX)300 micrograms per milliliter (. This suggests that LGE may have the potential to mitigate oxidative stress in human cells,

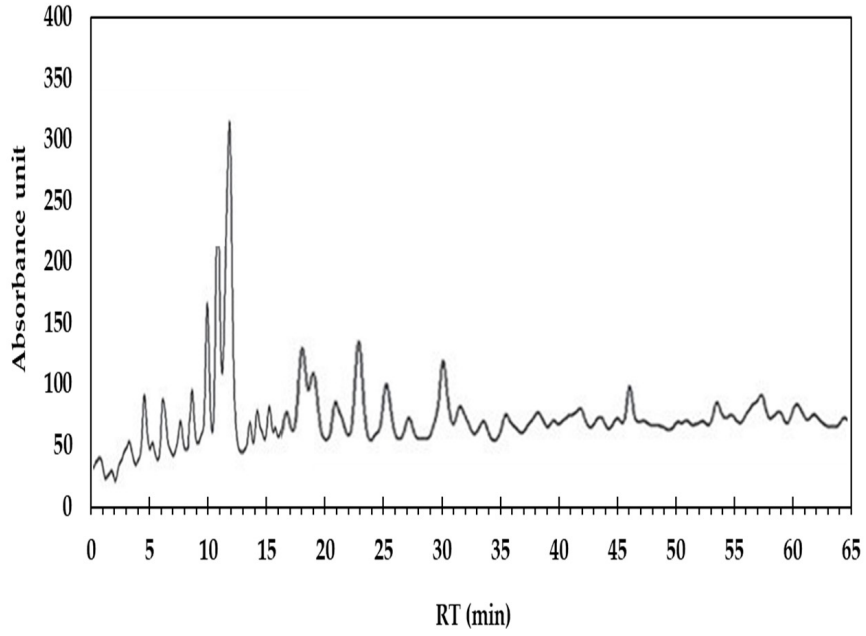

Figure 2. Chromatographic profile of polyphenolic compounds obtained from lemongrass ethanolic extract.

in agreement with Pan et al. (2022), who studied the influence of lemongrass extract on the viability of SiHa cancer cells and VERO (kidney cells) compared to rotenone, they found that lemongrass extract maintain the population and viability of VERO cells, and release the oxidative stress caused by rotenone, while inhibited the viability of SiHa cancer cell lines higher than rotenone.

Antimicrobial

LGE has a wide spectrum antimicrobial activity against tested pathogenic bacteria and *Candida* (Figure 4), representing inhibition zones. The diameters (mm) of inhibition zones rose as concentration increased. The range of 9-40 mm against tested bacteria and 10-30 mm against tested *Candida*, who excelled in bacterial or fungal antibiotic zones. SA was the most susceptible bacterium to 300 µg/mL LGE (40 mm), while KP was more resistant (26 mm). On the side of *Candida,* CG was the more resistant to LGE 300 µg/mL (32 mm), followed by CA with 29 mm. Our results are correlated with Hassan et al. (2021) finding that lemongrass extracts at the concentration of 150 mg/mL have antibacterial activity against *Bacillus cereus* (35 mm). While the results of Zulfa et al. (2016) studied the antimicrobial potential of lemongrass extract against *B cereus, E. coli* O157:H7*, K. pneumoniae, S. aureus,* and *C. albicans* found that the IZDs ranged between 9-12 mm. and Ranjah et al. (2022) who found the IZDs against *S aureus* and *E.coli* were 18 and 16 mm, respectively.

Figure 5 depicts that 20 to 40 µg/mL was the least LGE concentration against bacteria and fungi. SA had the lowest MIC (20 µg/mL), while KP had the highest MIC (40 µg/mL). Zulfa et al. (2016) found that the MIC value of lemongrass extract against *B cereus, E. coli* O157:H7*, K. pneumoniae, S. aureus,* and *C. albicans* ranged between 80-630 µg/mL.

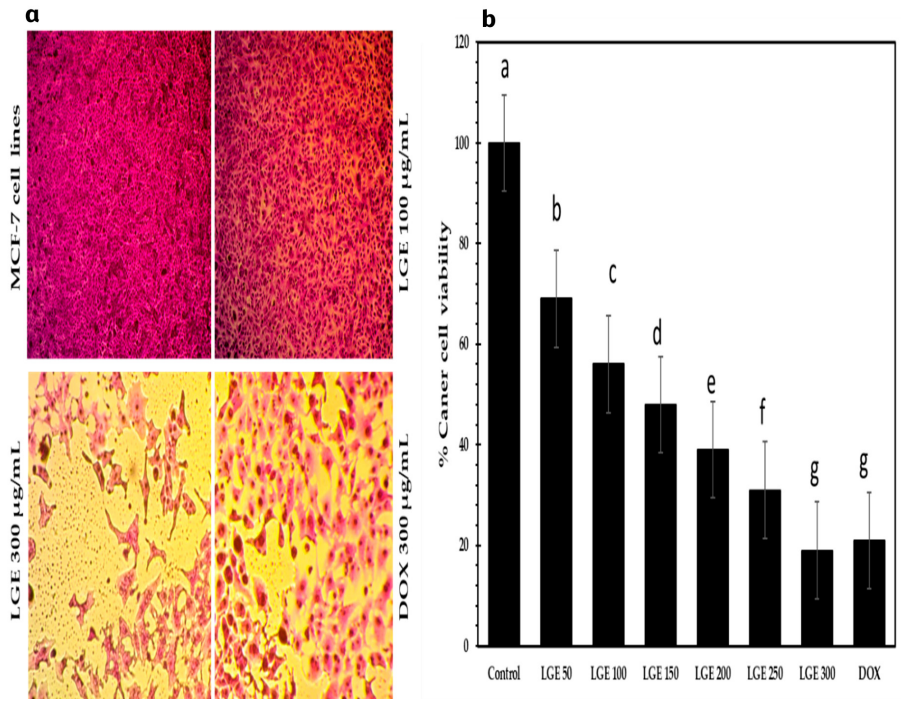

Figure 3. (a) Microscopic image of the effect of varying levels of LGE on the viability of MCF-7 cancerous cells. (b) A histogram shows the percentage of viable breast cancer cells after being treated with different concentrations of LGE, compared to doxorubicin.

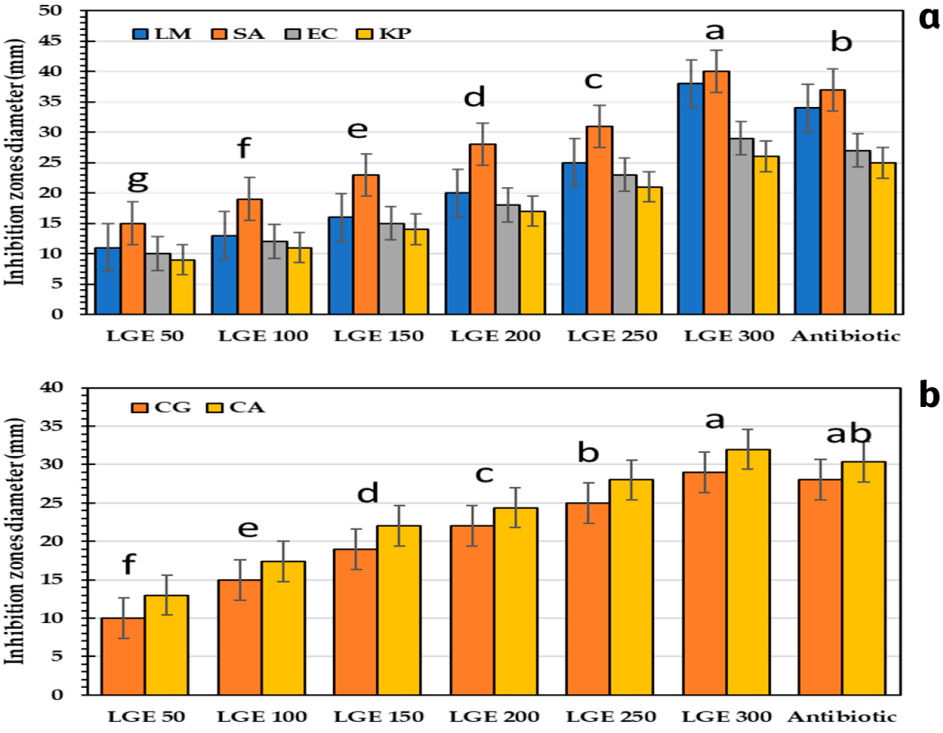

Figure 4. Diameters of the inhibition zones of LGE at various doses against harmful microorganisms (a) and Candida (b) compared to standard (levofloxacin and clotrimazole).

Antiviral activity

Figure 6a shows that lemongrass extract at concentrations (50-300 µg/mL) successfully inhibited the binding between the SARS-Cov-2 spike and ACE-2 in a concentration-dependent manner. The 50 µg/mL concentration inhibited the binding by 20 %; the inhibition rate increased to 75 % in LGE 300 µg/mL with no significant differences about AC384 (p=0.15).

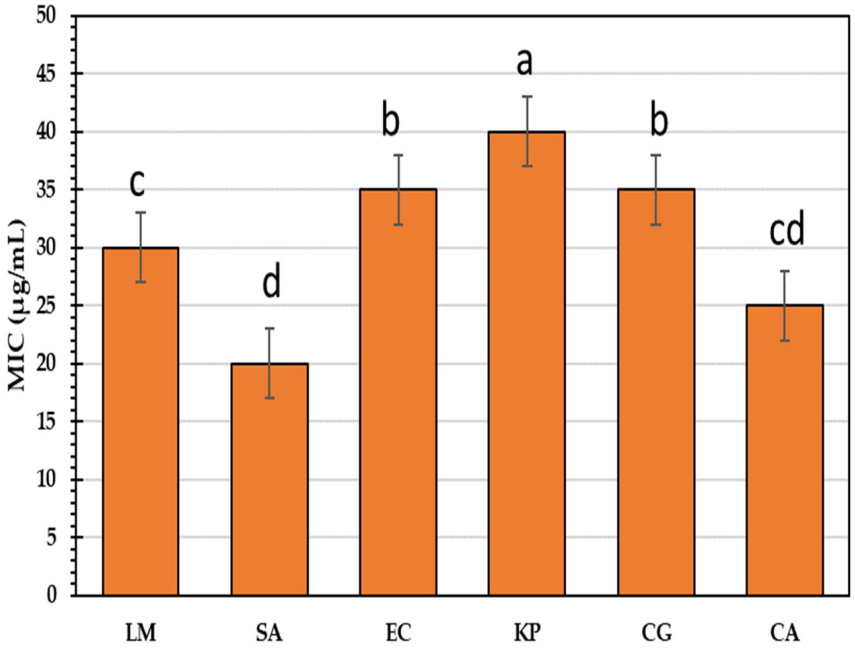

Figure 5. The minimum inhibitory concentration of LGE against tested bacteria and fungi.

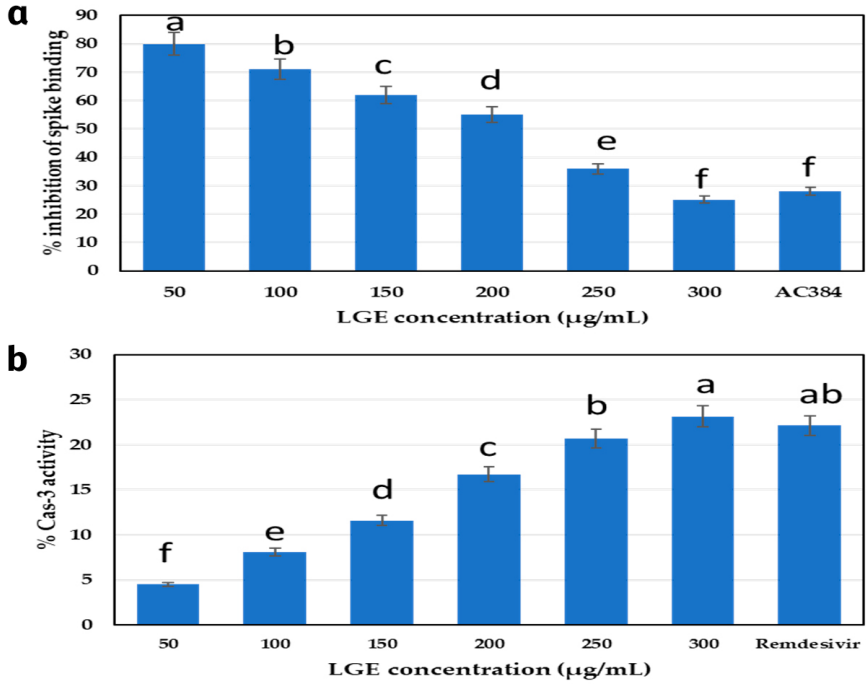

Figure 6. (a) Antiviral activity of lemongrass extract against COVID-19 virus compared to AC384. (b) Cas-3 activity as proinflammatory cytokines indicates the role of LGE in enhancing immunity.

In Figure 6b, the activity caspase-3 increased with increasing LGE concentration, indicating the role of LGE in enhancing immunity, which plays a significant role in facing COVID-19. The activity of Cas-3 rose from 4.5 in LGE 50 to 23.15 % in LGE 300 with no sense of the chemical drug Remdesivir.

SARS-CoV-2 enters cells through binding to receptors. This virus's glycoprotein will connect to the cellular ACE-2 protein (Zhang et al. 2020). In SARS-CoV, Protein S is a significant predictor of host cell entrance. Viral and host variables contribute to SARS-CoV infection (Li et al. 2020). An insufficient immune response leads to viral multiplication and tissue damage, but an overactive immunological

response can also induce tissue harm (Bao et al. 2020). However, no effective medicine or vaccine has yet been discovered to combat SARS-CoV-2. However, efforts must be made to sustain the immune system before this virus successfully infiltrates cells and triggers uncontrolled proinflammatory cytokines (Zumla et al. 2020). Due to this, several researchers have investigated the viability of plants as antiviral agents. The Cymbopogon genus, which includes *Cymbopogon nardus* (*C. nardus*) as a natural plant, has flavonoids, phenols, and tannins as its primary constituents (Simanjuntak 2020). Plant flavonoids, phenols, tannins, and vitamins can function as antimicrobial, anti-inflammatory, and antioxidative agents and immunomodulators that affect immunological response (Pramudya & Wahyuningsih 2019). Earlier research indicates that the influence of lemongrass extract (*C. citratus*) on the blood profile of broiler chickens can increase the amount of blood cells.

Growth performance

The FW and BWG were significantly (p<0.05) affected by lemongrass treatments (Table IV). When compared to the positive control group, which had FW (265.4 g) and BWG (15.51 g), the rats treated with 300 mg/kg of lemongrass leaf ethanol extract had the best FW (293.1 g) and BWG (31.66 g), respectively. The increased FW and BWG in challenged rats treated with lemongrass leaf extract could be attributed to lemongrass's high contents of minerals, vitamins, and antioxidants, which may protect cells from free radical damage (Burri et al. 2017, El-Zahar et al. 2021a). In concurring with our findings, (Mohamed et al. 2017) reported that the use of lemongrass leaf extract in rat diets enhanced (P≤0.05) weight gain and improved nutritional status when compared to the control negative ones. Dietary herbal plant oil significantly improved broiler chickens' body weight and gain (Elewa et al. 2023). Also, Rahman et al. (2022) found an increase in broiler's importance when diet supplemented with lemongrass leaves powder. Also, lemongrass extract and essential oil exhibit antioxidant and acetylcholinesterase inhibitory effects. Lemongrass combined with spearmint herbs has significantly improved broiler performance, liver health, serum and meat zinc, and iron concentrations (Rahman et al. 2022).

Impact of LGE on the oxidative stress and the lipid profile in CCl_4-rats

The lipid profile, including triglycerides, cholesterol, and LDL, was elevated by CCl_4-induced oxidative stress, whereas HDL levels were reduced. Four weeks of pretreatment with *Cymbopogon citratus* leaf extract reduced LDL, TG, and cholesterol levels while increasing HDL levels dose-dependent and meaningfully (Table V). The rats were given 300 mg/k-1 of *Cymbopogon citratus* leaf extract,

Table IV. Body weight gain of rats treated with lemongrass leaf extract (mean±SD).

Group	Performance parameters		
	IW (g)	FW (g)	BWG (g)
NC.	247.8±5^{a}	307.1±5^{a}	33.90±5^{a}
PC.	229.8±2^{b}	265.4±2^{c}	15.51±2^{c}
LG _100	225.5±5^{bc}	290±4^{b}	28.8±1.5^{b}
LG_ 300	223.1±4^{c}	293.1±4^{b}	31.66±4^{ab}

Initial weight IW, Final weight FW, NC non-treated rats (negative control); PC CCl_4-induced hepatotoxicity in rats (positive control); LG_ 100 CCl_4-rats treated with 100mg/Kg leaf ethanolic extract of body weight; LG_ 300 CCl_4-rats treated with LGE (300mg/Kg body weight). The different lowercase letters indicate significant differences.

which had the lowest TC level (71.6 mg/dl). In comparison, the CCl_4-induced hepatotoxicity rats had the highest total cholesterol level (135.2 mg/dl), the PC group exhibited the most significant levels of TG and LDL (118.6 and 85.3 mg/dl, respectively) compared to the LGE group (78.6 and 24.1 mg/dl, respectively). NC group had the least HDL-c concentration (25.40 mg/dl) compared to the other groups. However, the rats treated with lemongrass leaf extracts significantly increased HDL-c content (37.3 and 34.20 mg/dl, respectively).

Compared to groups treated with *Cymbopogon citratus* leaf extract, Serum cholesterol levels, triglycerides, and LDL-c increased significantly ($P<0.05$), and there was a drop in HDL-c in the positive control. Positive control causes oxidative stress, elevating reactive oxygen species generation. A rising amount of scholarly literature offers a wealth of first-hand information. ROS can harm cells by oxidizing key biological components like membrane lipids, proteins, and DNA (Burri et al. 2017, Said et al. 2019). The study's results indicated that it improved the lipid profile because lemongrass leaves contain phenolics, potent antioxidants (El-Zahar et al. 2021b, Said et al. 2019). According to Nambiar & Matela (2012), Ranjah (2019), the ethanolic extract of fresh leaves of *Cymbopogon citratus* has a hypocholesterolemic impact.

In vivo effects of LGE on liver markers and kidney functions

The alteration of physiological indices can affect characteristics related to health (Emam et al., 2023). All biochemical markers assessed in rats treated with CCl_4 presented a considerable rise ($p<0.05$) compared to the other groups. However, the liver and kidney markers were reduced in *Cymbopogon citratus* extract-treated rats for 4-weeks before intoxication with CCl_4 (Table IV). In addition, the total protein, albumin, and GSH levels in the serum of CCl_4-rats (G2) were lower than those of the negative control group (Table VI). The administration of *Cymbopogon citratus* ethanolic extract (100 and 300 mg/kg^{-1} body weight) (groups 3 and 4, respectively) significantly ($p<0.05$) reduced the concentrations of the parameters relative to that of the CCl_4 -treated rats. The bioactive compounds in lemongrass leaf can help to improve liver function by reducing inflammation, protecting the liver cells from damage, and promoting the regeneration of new liver cells (El-Zahar et al. 2021a, b, Kouassi et al. 2017).

Rahim et al. (2014) stated a significant reduction ($p<0.05$) in the elevated levels of ALT and AST in serum blood and liver homogenates was evidence that lemongrass prevented liver damage induced by H_2O_2 injection, as well as an increase in total protein and albumin levels. Concerning MDA, it can

Table V. The impact of LGE on the lipid profile and oxidative stress in CCl_4-rats (mean±SD).

Experimental groups	Parameters			
	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
NC	67.4 ± 2^{c}	77.5 ± 2^{c}	36.2 ± 2^{ab}	16.7 ± 2^{c}
PC.	135.2 ± 5^{a}	119.2 ± 5^{a}	25.4 ± 5^{c}	85.4 ± 5^{a}
LG_100	77.1 ± 5^{bc}	84 ± 3^{b}	37.3 ± 2^{a}	24.9 ± 2^{b}
LG_300	72.6 ± 4^{b}	79.6 ± 4^{bc}	34.2 ± 4^{b}	23.5 ± 4^{b}

NC non-treated rats (negative control); PC CCl_4-induced hepatotoxicity in rats (positive control); LG_ 100 CCl_4-rats treated with 100mg/Kg leaf ethanolic extract of body weight; LG_ 300 CCl_4-rats treated with 300mg/Kg ethanolic leaf extract of body weight. The different lowercase letters indicate significant differences.

Table VI. Impact of lemongrass leaf extract on liver and oxidative stress indicators in oxidative stress rats(mean±SD).

Groups	Parameters					
	AST(u/l)	A.L.T. (u/l)	T.P. (g/dl)	T.A. (g/dl)	G.S.H. (nmol/ml)	M.D.A. (nmol/ml)
N.C.	35.6 ± 2^{c}	43.8 ± 2^{c}	6.14 ± 0.3^{ab}	3.82 ± 0.2^{a}	57.5 ± 2^{a}	46.36 ± 3^{c}
PC	81.6 ± 5^{a}	88.2 ± 5^{a}	5.61 ± 0.2^{c}	2.94 ± 0.7^{c}	40.2 ± 2^{c}	67.9 ± 5^{a}
LG_100	45.1 ± 3^{bc}	53.6 ± 2^{b}	5.98 ± 0.5^{b}	3.34 ± 0.3^{b}	51.2 ± 3^{b}	51.52 ± 3^{b}
LG_300	40.2 ± 4^{b}	50.6 ± 4^{b}	6.22 ± 0.7^{a}	$3.68\pm0.3a^{b}$	56.3 ± 3^{a}	49.22 ± 4^{b}

NC non-treated rats (negative control); PC CCl_4-induced hepatotoxicity in rats (positive control); LG_ 100 CCl_4-rats treated with 100mg/Kg leaf ethanolic extract of body weight; LG_ 300 CCl_4-rats treated with 300mg/Kg ethanolic leaf extract of body weight. The different lowercase letters indicate significant differences.

have been found that the positive control was 67.9 µmol/L, which is considered the highest mean value of MDA in contrast with the control negative ones, which reported the smallest value (46.36 nmol/ml). There is a significant decrease in rats treated with 300 mg/kg lemongrass leaf extract with 51.22 nmol/ml. MDA profoundly damages cell membranes, changing their structure and function (Nair & Nair 2013, Saenthaweesuk et al. 2017, Said et al. 2019). MDA formation and buildup can lead to oxidative processes, inhibition, and cytotoxicity. MDA is a tumor promoter and a co-carcinogen (Koc et al. 2003).

Unlike the control, the CCl_4 revealed a significant increase ($p< 0.05$) in kidney markers (creatinine and urea), as seen in Table VII. While the LGE rats had a substantial drop in creatinine and urea levels compared to the positive control group, this reduction was not observed in the positive control group. Creatinine and urea levels of several rat groups were lowered in the rat group administered 300 mg/kg of lemongrass leaf extract as a negative control compared to PC. (Rahim et al. 2014), Reducing creatinine, urea, and MDA relative to a positive control group confirmed these findings. Because of the lemongrass leaf's relatively high natural antioxidant substance, treated rats compared to CCl_4- rats, lemongrass leaf extract significantly decreased renal function indicators (creatinine and urea). These natural antioxidants can lower serum urea and creatinine through uricosuric potential clearance or enhance renal blood flow. Furthermore, a potent antioxidant can reduce oxidative stress and inflammation in body cells, lowering the production and uric acid concentration (Said et al. 2019, Ullah et al. 2013).

Histopathological alterations in hepatic and renal tissues

Histological analysis of organs was used to assess hepatic damage and the extent of histological alterations in several groups of rats, as shown in Table VIII and Figures 7 and 8. The results of the histological study match those of the biochemical analysis. Histopathological examination of liver tissue from treated rats with lemongrass leaf extract revealed a significantly improved performance. Kupffer cells are present in hepatocytes with moderate invasion and edema in the portal vein's inflammatory cells, a decrease in necrosis, and lipid alterations, as illustrated in Figure (7). When histopathologically examined, the CCl_4-induced rats' livers showed fatty changes with necrosis in liver cells, significant fatty and inflammatory alterations, vascular congestion, and mild fibrosis.

Table VII. Effect of lemongrass leaf extract on kidney function parameters in oxidative stress rats(mean±SD).

Groups	Parameter	
	Creatinine (mg/dl)	Urea (mg/dl)
NC	0.39 ± 0.12^{c}	16.6 ± 0.2^{c}
PC	0.82 ± 0.15^{a}	27 ± 0.5^{a}
LG_100	0.55 ± 0.14^{b}	19.2 ± 0.4^{b}
LG_300	0.46 ± 0.12^{bc}	17.4 ± 0.4^{bc}

NC non-treated rats (negative control); PC CCl_4-induced liver toxicity in rats (control positive); LG_ 100 oxidative stress rats treated with 100mg/Kg leaf ethanolic extract of body weight; LG_ 300 oxidative stress rats treated with 300mg/Kg ethanolic leaf extract of body weight. The different lowercase letters indicate significant differences.

Table VIII. Effect of lemongrass leaf extract on histological changes in mice with oxidative stress.

Organ	Lesions	Control	CCl_4	LG_100	LG_300
Kidney	Necrotic glomeruli	–	+++	+	–
	Necrotic renal tubules	–	+++	+	–
	Hemorrhages	–	+++	+	–
	Congested	–	+	+	+
	Casts formation	–	–	+	+
	Regenerative attempts	–	–	+	+
	Inflammatory cells infiltrations	–	+	–	–
	Fibrosis	–	++	+	–
	Cystic dilated renal tubules	–	–	–	–
Liver	Portal fibrosis	–	+++	+	–
	Hepatocellular degeneration	–	+++	+	–
	Congested blood vessels	–	+++	+	–
	Inflammatory cells infiltrations	–	++	+	–
	Kupffer cells hyperplasia	–	–	+	+
	Regenerative attempts (adipocytes)	–	–	+	+

The lesion scores system is designed as (– =No alteration 0%, += Mild alteration 25-30%, ++=Moderate alteration 35-65%, +++=Severe or advanced alteration up to 70%).

The liver parenchyma of rats given *Cymbopogon citratus* leaf extract (100 and 300 mg/kg/day) as an oral treatment showed a statistically significant improvement, with no cytoplasmic degradation in undamaged liver hepatocytes (Figure 7). Sadek et al. (2018) and Saenthaweesuk et al (2017) collaborated on these research results to estimate the safety limits of wild plant extracts at various doses, in which histological changes in the hepatic, renal, and cardiac tissues were evaluated. Compared to the negative control, the groups treated with wild plant extracts had no histological abnormalities in the liver organ. As demonstrated in Table VIII and Figure 8, renal injury was also

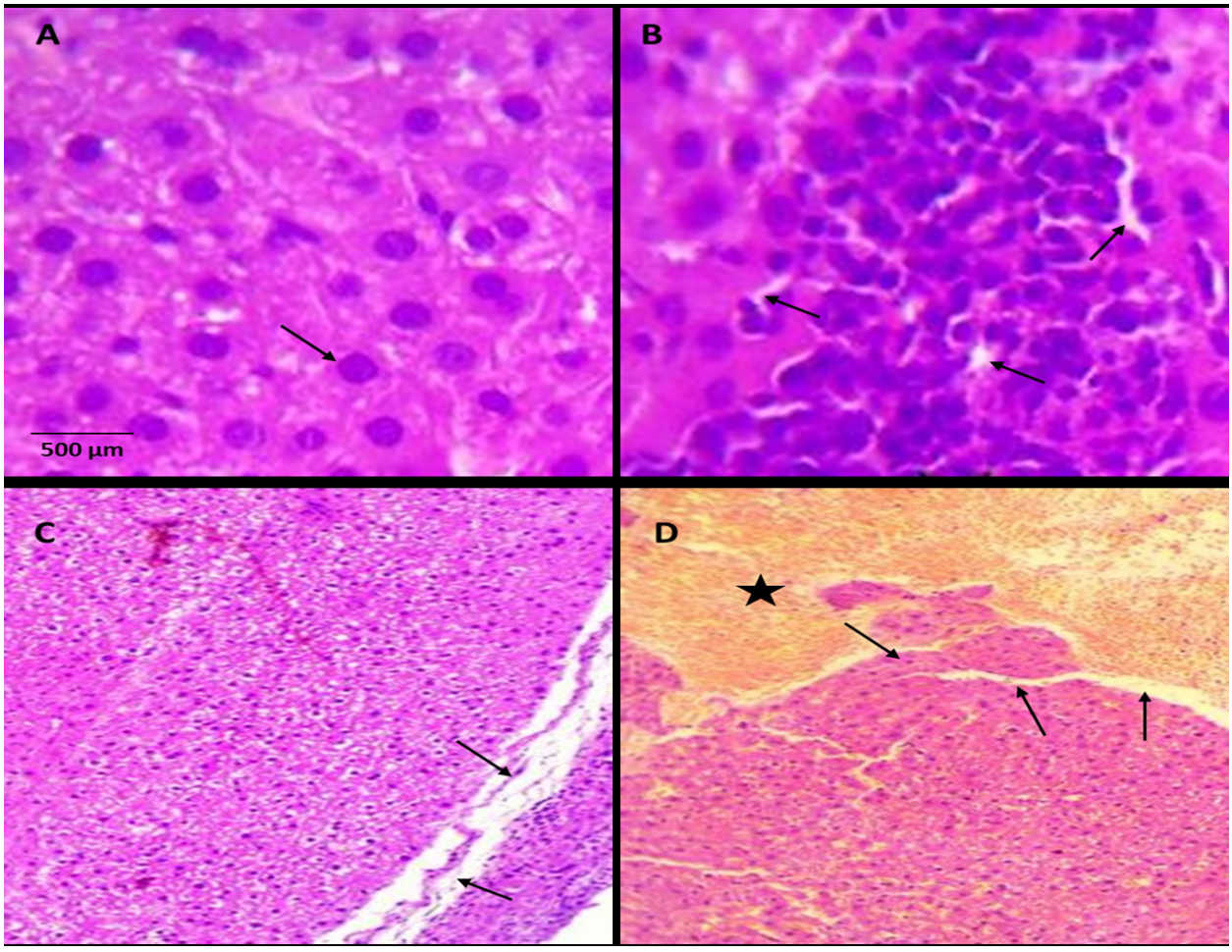

Figure 7. Microscopic examination of liver tissues depicts *Cymbopogon citratus* effect on CCl_4-induced hepatic injury in rats. (a) Normal liver tissue (negative control) (showing normal hepatic parenchymal structure). (b) Hepatic tissue of hepatotoxicity rats (control positive ones), demonstrating portal vein engorgement with blood and lymphocytosis (star), encircled by edema (wide star) and assemblages of the inflammatory cell) (c) Lemongrass 100mg/kg DW pretreatment rats (showing normal hepatic portal trade (circle) comprised of artery, vein, and bile duct) (d) Administration of rats with lemongrass 300mg/kg DW (interstitium fibrous bridge (arrows) with hepatic cells sinusoids and cords).

assessed through histological analysis of the organ and the degree of histological changes in the various groups of rats. On histological examination, the control group of rat kidney sections exhibited normal glomeruli, interstitial tubules, and blood vessels.

The kidney tissue slices from CCl_4-treated rats revealed glomerular crowding, capillary tuft and tubule vacuolization, and shedding of the kidney tubular lining. Figure 8 shows that rats administered silymarin had tubular necrosis, glomerular tuft vacuolization, regenerative and desquamation vacuole-like structures, and regenerative and desquamation vacuolization in kidney sections (Figure 8). Kensarah & Azzeh (2012) suggested that *Cymbopogon citratus* administration significantly protected against gentamicin-induced changes in BW, serum creatinine, creatinine clearance, serum uric acid, serum electrolytes, urinary volume, urinary protein, urinary lactate dehydrogenase, urinary alkaline phosphatase, and renal damage. In agreement with our results, Sadek et al. (2018), Said et al. (2019)

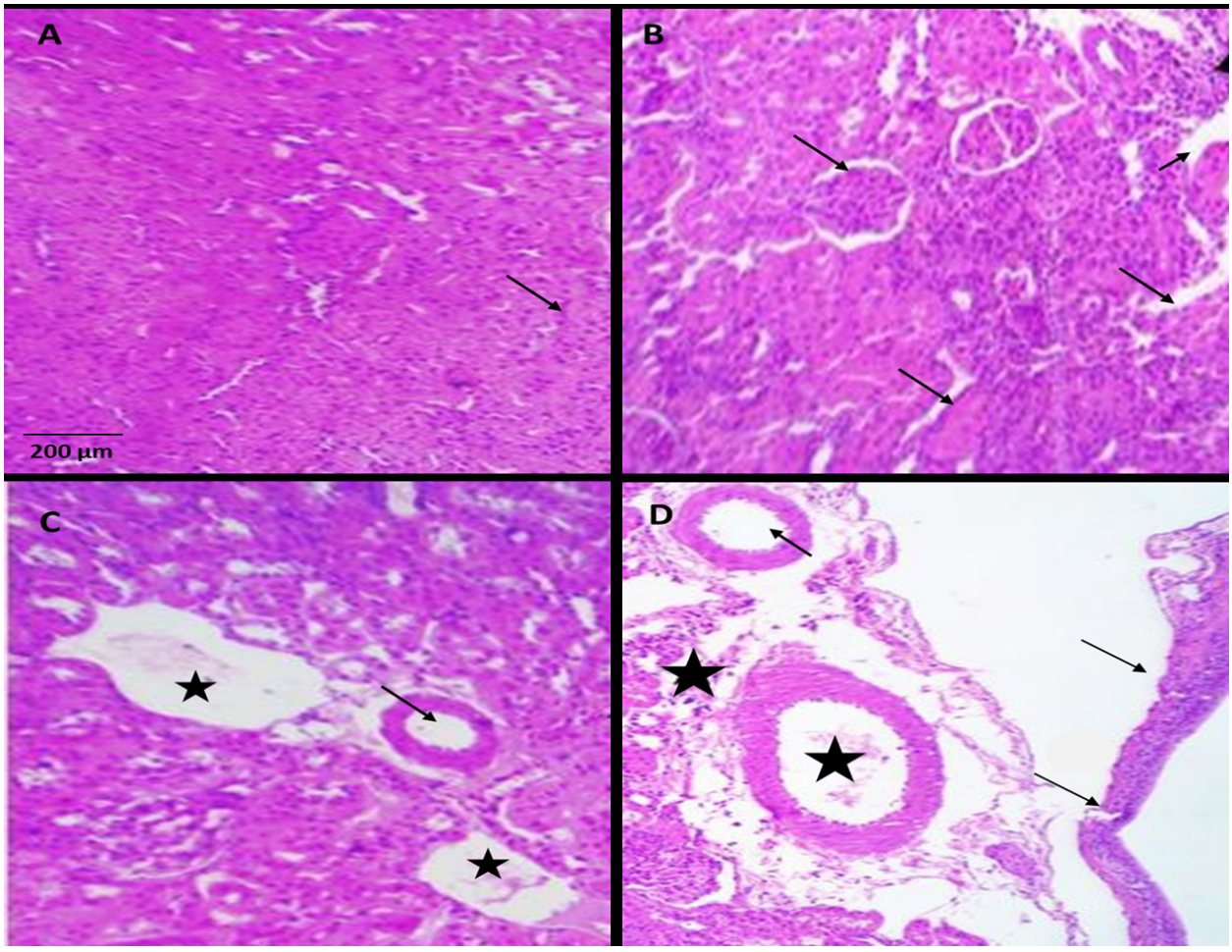

Figure 8. Microscopic examination of kidney tissues depicting *Cymbopogon citratus* effect on CCl_4-induced hepatic injury in rats: (a) Normal kidney tissue (negative control) (displaying typical tubules and glomeruli in the kidney). (b) Hepatotoxicity rat kidney tissue (positive control group) (exhibiting necrotic glomerular tufts with basement membrane thickening, periglomerular fibrous bands, and interstitial bleeding.) (c) Lemongrass 100mg/kg DW pretreatment rats (typical renal architecture except for lobulated glomerular tufts, partially thickened basement membrane, and periglomerular-extravasated erythrocytes) (d) Lemongrass 300mg/kg DW administration to rats (showing that the structure of the kidneys is almost normal, but there are a few casts in the tubular lumina).

reported that the use of lemongrass extract in rat diets improved ($p \leq 0.05$) kidney performance in rats affected by oxidative stress and improved the shape of the kidney glomeruli and kidney tubules.

CONCLUSIONS

Cymbopogon citratus leaf extract has antioxidant, anticancer, and antimicrobial potential; the LGE treatment reduced CCl_4-induced biochemical and histomorphological alterations. This nephrotoxicity preventive effect of *Cymbopogon citratus* could be correlated to antioxidative substances like phenolics and flavonoids, which reduce oxidative danger and restore normal physiological function. The results are reliable and back up the use of *Cymbopogon citratus* to treat a variety of liver and renal disorders. It could be stated that taking lemongrass leaf extract as a bioactive supplement is useful

in preserving a good oxidative status, positively reflecting overall health. In conclusion, *Cymbopogon citratus* extract can reduce oxidative stress injury in the hepatic tissue.

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