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Nephroprotective Effect of Clove (*Syzygium aromaticum*) Extract on Carbon Tetrachloride Induced Nephrotoxicity in Albino Rats

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Abstract
Exposure to carbon tetrachloride (CCL4) inhibits acute and continual renal injuries besides oxidative stress in rats. The present investigation was carried out in an attempt to assess the nephroprotective potential of clove (*Syzygium aromaticum*) extract (CE) towards CCL4 precipitated nephrotoxicity. Forty adult male Wister albino rats weighing 140-180g and divided into four groups, each group (10) rats. Group (1): normal rats served as the negative control group; group (2): rats implemented with CE (50 mg/kg/day) orally; group (3): rats injected with CCL4 (0.5 mg/kg/ two times weekly) intraperitoneal, and group (4): rats inflamed with CCL4 and treated with CE. After six weeks, the results revealed that CE and CCL4 minimized the CCL4-induced renal deterioration; this was evidenced by the significant reduction in urea, creatinine, uric acid, TNF-α, IL-1β, and Na, as well as kidney MDA. This is matched with a marked enhancement in serum calcium, K, and CD4 levels and kidney GSH and CAT levels. In conclusion, Clove (*Syzygium aromaticum*) extract can be as promising as nephroprotection in opposition to CCL4 nephrotoxicity through their antioxidant and radical scavenging activities.

Keywords: Nephrotoxicity, Kidney Function, Phenolic Compounds, Antioxidant Enzyme in Tissue

Introduction
The kidney plays an important role in the removal of toxic substances from the body through filtration and excretion. It is highly vulnerable to toxic injuries caused by drugs and toxins because of high blood flow and involved cellular transport systems that cause the accumulation of these compounds within nephron epithelial cells [1,2]. The incidence of renal failure is currently increasing at an alarming rate, which is marked by loss of the kidney's ability to excrete waste, collect urine, preserve electrolytes, and maintain fluid

balance, as well as a high morbidity and mortality rate [3]. Many synthetic drugs used to treat liver and kidney diseases are ineffective and can cause severe side effects. Considering these limitations, new drugs derived from natural sources are being studied to determine their safety and efficacy [4-6].

There is still much to learn about the etiology of renal impairment brought on by carbon tetrachloride (CCL4). It could be caused by how well the liver is functioning, or renal damage could arise irrespective of hepatic events [7]. Since CCL4 frequently causes oxidative stress [8], one may anticipate that it will also cause nephrotoxicity. CCL4 is a clear liquid with a sweet scent that does not naturally exist but can be noticed at low concentrations [9]. Through the metabolic activation of those substances to a highly reactive oxygen species, exposure to diverse compounds, such as a number of environmental contaminants and medications, can harm cells (ROS).

One of the main contributors to cell membrane deterioration that results in a variety of pathological circumstances is thought to be free radical-induced lipid peroxidation [10]. In addition to being utilized as a degreaser and cleaning agent in households, factories, and dry-cleaning facilities for textiles, CCL4 was also used as a precursor to propellants and refrigerants. Most of its usage is currently prohibited due to its extreme toxicity and detrimental effects [11]. As the body's primary site of metabolism, the liver, kidneys, and lungs are the primary organs where CCL4 damages cells the most [12]. CCL4 is a strong environmental hepatotoxic, as shown by studies from both our group and other researchers [13].

Due to the existence of advantageous chemical elements in them, herbal treatments have been utilized for treating various diseases for more than 4,000 years. The main therapeutic properties of plants are their phytochemical components, which have a specific pharmacological effect when administered to humans. Phytochemicals, which are naturally occurring and have their own defensive mechanisms and protective ions against many diseases, are found in medicinal plants, leaves, vegetables, and fruits [14].

Syzygium aromaticum L., or clove, is an aromatic plant that is frequently grown in tropical and subtropical regions and is high in antioxidants and volatile chemicals like eugenol, beta-caryophyllene, and alpha-humulin [15]. It also has numerous beneficial and therapeutic functions. It is used to reduce pain and regulate stomach distension, coughing, diarrhea, dyspepsia, flatulence, and gastrointestinal spasm [16,17]. S. aromaticum also has strong antimutagenic [18], anti-inflammatory [19], antioxidant [20], and antithrombotic [21] properties. S. aromaticum's antibacterial and antifungal properties have just come to light [17,22]. The objective of this study was to be conducted to examine the possible modulatory

effects of Clove (*Syzygium aromaticum*) ethanolic extract (CEE) against kidney toxicity, injury, induced by carbon tetrachloride.

Materials & Methods

Chemicals

Carbon tetrachloride and olive oil was obtained from Sigma Aldrich (St. Louis, MO, USA). According to [23], rats were given intraperitoneal (IP) injections of carbon tetra chloride diluted in olive oil twice a week at a concentration of 0.5 mg/Kg body weight.

Plant materials and extraction

Clove (*Syzygium aromaticum*) was become received from a local supplier (Abd El-Rahman Harraz, Bab El-Khalk zone, Cairo, Egypt); then Clove the extract of dry powdered seeds turned into executed in step with changed technique of [24]; plant material became dissolved in 95% ethanol (1:10 w/v); every 1g pattern have been dissolved in 10 ml of solvent and extracted on shaker at 150 rpm for 24 h at room temperature. After that, combos have been filtered the use of Whatman No. 1 clear out paper and a sterile layer of gauze to remove any final stable plant matter. The extract turned into saved at -20 °C till it become required after the filtrate became focused through rotary evaporation using (Rotavapor® R-300) it at 35 to 40 °C.

Determination of total extract yield

In order to calculate the yield, the mixed extracts have been placed to a quick-match spherical backside flask with a recognized weight (W1), freeze dried, then weighted again (W2). The yield turned into then calculated the usage of the subsequent formula:

$$Extract\ yield\ (g/\ g\ crude\ herb) = (W2 - W1)/W3$$

Where,

- W1 is the weight of clear and dry quick fit flask in grams,
- W2 is the weight of the flask after lypholization in grams
- W3 is the weight of the crude powdered herb in grams

Determination of total phenolic content

By combining 10 ml of acetone and water (6:4 v/v) with five mg of the extract, the phenolic content material of the CE changed into decided. Then, 0.8 ml of sodium carbonate answer (7.5%) and 1.0 ml of the Folin-Ciocalteu reagent (10 fold diluted) had been mixed with 0.2 ml of the sample. Using a Cary a hundred UV-Vis spectrophotometer, the absorbance at 765 nm turned into decided after half-hour at room temperature. Using a general curve, phenolic chemical substances have been envisioned as catechin equivalents [25].

DPPH radical scavenging activity

As formerly mentioned, [26], the capacity of antioxidants in CE to quench DPPH radicals changed into ascertained. In this procedure, to rate a 200 ppm concentration, a number of the crude extract changed into dissolved in methanol. Methanol turned into used to dilute this answer from 0.2 ml to four ml, and the equal solvent turned into then used to feature 1 ml of the DPPH answer (6.09 × 10-5mol/L). After 10 minutes of standing, the mixture's absorbance was measured at 516 nm. The reference sample (blank) contained 4 ml of methanol and 1 ml of DPPH solution. The proportion of radical scavenging activity was determined using measurements done in triplicate and the following equation:

$$\text{RSA } (\%) = \left(\frac{\text{A}_{\text{control sample}} - \text{A}_{\text{sample extract}}}{\text{A}_{\text{control sample}}} \right) * 100$$

Experimental design

Forty adult male albino rats (140-180g) have been received from the animal colony, National Research Centre; The rats have been housed beneath Neath temperature manage (25±1°C) and mild manage (12/12h mild/darkish cycle) earlier than to the experiment. They had free access to food and water for a week. They acquired care from humans in accordance with the institution's norms for the managing and use of experimental animals, as in step with the protocols encouraged through the Al-Azhar University's Ethics Committee for the Faculty of Science in Assuit.

The rats had been randomly separated into four groups, every which includes 10 rats, once they had turn out to be acquainted with the settings with inside the experimental room. Group (1) blanketed healthful manage rats orally acquired 0.5 mL water for consecutive six weeks, group (2) included healthy rats orally ingested with Clove extract (50 mg/kg/day) for consecutive six weeks, group (3) protected inflamed rats with Carbon tetrachloride (0.5 mg/kg/weekly) for 6 weeks, and group (four) inflamed rats with carbon tetrachloride and handled with Clove extract (50 mg/kg/day) for 6 weeks.

Blood sampling

At the quit of remedy period, every rat became weighed earlier than being starved for the complete night. Blood samples had been taken from the retro-orbital plexus after diethyl ether inhalation anesthesia; complete blood specimens have been cool-centrifuged at 3000 rpm for 10 minutes to split the sera; the sera have been then divided into aliquots and saved at -80°C till biochemical assays had been made. The rats were slaughtered shortly after blood was collected, and each rat's kidney was removed. For biochemical analyses, after being cleansed with saline and dried, the kidney was wrapped in aluminum foil.

Biochemical determinations

Utilizing reagent kits bought from DiaSys Diagnostic System GmbH, Germany, the levels of serum urea, creatinine, uric acid, calcium, sodium, and potassium were measured according to the methods of [27-30] using spectrophotometric methods.

Inflammatory cytokines

Using reagent kits purchased from Sino Gene Clon Biotech Co., Ltd., No. 9 BoYuan Road, YuHang District 311112, Hang Zhou, China, serum concentrations of tumor necrosis factor-alpha (TNF-α) and interleukin-1beta (IL-1β) CD4 were measured using the ELISA technique (Dynatech Microplate Reader Model MR 5000) [31].

Estimation of oxidative stress markers

The kidney's Glutathione (GSH), Catalase (CAT), and MDA activities were determined according to the methods of [32-34] using reagent kits obtained from Biodiagnostic, Dokki, Giza, Egypt.

Statistical analysis

According to [35], the post hock (Tukey) multiple comparisons test at $p \leq 0.05$ was employed after one way analysis of variance (ANOVA) to compare means. This was done with the help of the statistical analysis system (SAS) computer program; copyright (c) 1998 by SAS Institute Inc., Cary, North Carolina, USA.

Results and discussion

Total phenolic content and radical scavenging activity of Clove extract

The yield, total phenolic content (TPC), and radical scavenging activity (RSA) of the clove extract (CE) are represented in figure (1).

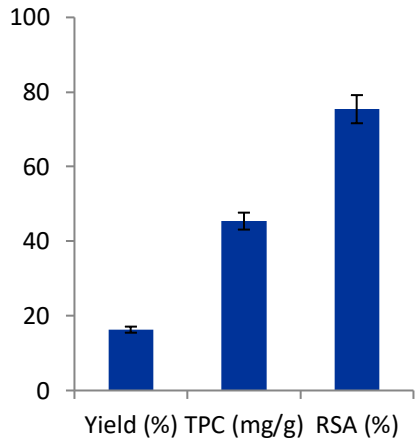


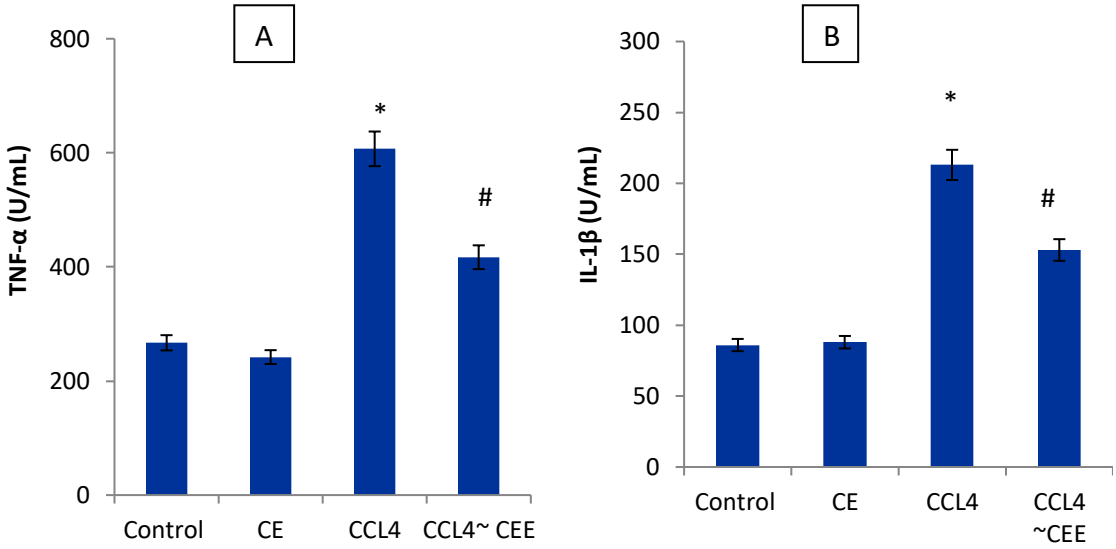
Figure (1): Yield (%), total phenolic content (mg /g) and radical scavenging activity (%) of dry powdered of Clove extract.

It is obvious to see that the yield of clove extract has the lowest (TPC) and greatest (RSA) values. The best source of phenolic chemicals, including eugenol, eugenol acetate, and gallic acid, is clove, which has a lot of promise for use in food, medicine, cosmetics, and agriculture [17]. Due to its extensive use in the fragrance, cosmetic, health, and food industries, clove essential oil has drawn a lot of attention. The biological activity of clove essential oil is relevant to human health, and it contains antibacterial, antioxidant, anti-inflammatory, anti-cytotoxic, and anesthetic effects [20,36,37]. At least 50% of the chemical is eugenol, which is the main one. Eugenyl acetate, -caryophyllene, and humulene make up the final 10–40%. It has lately been investigated in disorders linked to inflammation and oxidative stress and also exhibits notable antioxidant action. Due to the antioxidant activity's ability to scavenge free radicals from hydroxyl anions, superoxide anions, and lipid peroxides, reactive oxygen species (ROS) were reduced. As a lead for therapeutic research, -caryophyllene sparked interest [15,38].

Effect of CE and CCL_4 on serum TNF-α & IL1β and CD_4 levels

The obtained results demonstrated that the TNF-α and IL1β levels in the CCL4 group were significantly higher than those in the control group, whereas CD4 levels were significantly lower.

It's interesting to note that giving CCL4 rats CE improved all inflammatory cytokines to within normal ranges as it markedly reduced TNF-α and IL1β levels while markedly raising CD4 levels in comparison to CCL4 rats (Figure 2 a - c).



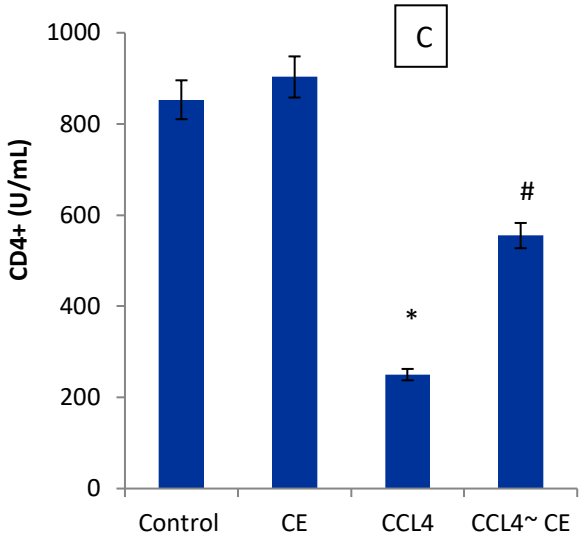


Figure (2a – c): Effect of CE and CCL4 on serum TNF-α & IL1β and CD4 levels.

One of the intricate functions of the immune system, inflammation is characterized by redness, discomfort, swelling, and warmth [39]. It happens in reaction to pathogens, irritants and damaging stimuli.

The negative side effects of traditional anti-inflammatory medications cleared the path for the development of secure substitutes. Herbal medicine is one such alternative, although it is not frequently favored due to its short shelf life and instability.

Traditional medical systems like Siddha and Ayurveda use botanical extracts with metal ions to enhance the quality of herbal therapy. According to reports, CCL4 hepatotoxicity is caused by the cytochrome P450 system's biotransformation into trichloromethyl free radical (CCl3), which easily interacts with molecule oxygen to form trichloromethyl peroxy radical [40].

Effect of CE and CCL_4 on serum urea, creatinine, uric acid and potassium in albino rats

The data in table (1) show that comparing the levels of urea, creatinine, uric acid, and potassium in the CCL4 injection group to the control group's normal values revealed significant increases in these substances. When compared to normal rats, the treatment with CE alone had no appreciable effects on these parameters. The CCL4 induced alterations in the aforementioned measures were dramatically reduced by administering CE to the CCL4 group.

Table (1): Effect of CE and CCL4 on serum urea, creatinine, uric acid and potassium in albino rats.

	Control	CE	CCL_4	CCL_4 with CE
Urea (mg/dl)	54.3±2.2	49.7±4.0	89.5±3.6*	64.8±2.0#
Creatinine (mg/dl)	0.64±0.09	0.66±0.03	1.92±0.14*	0.92±0.03#
Uric acid (mg/dl)	5.1±0.7	4.9±0.25	6.39±0.5*	4.1±0.31#
Potassium (mmol/L)	9.7±0.97	9.61±0.4	12.3±0.53*	8.3±0.94#

Each value represents the mean ±SD

* Means there is a significant difference compared with the control group at ($p \leq 0.05$)

Means there is a significant difference compared with the CCL4 group at ($p \leq 0.05$)

Over the past few years, oxidative stress has assumed a much larger role as a cofactor in cellular failure, including kidney impairment [41,42]. We can speculate that publicity to H2O2 can also additionally boom the superoxide anion and the unsafe hydroxyl (OH) radical, ensuing in glomerular dysfunction [43] and an growth with inside the serum concentrations of creatinine, blood urea, and uric acid [44]. A range of inflammatory responses, including cytokines, may become active in response to H2O2 exposure [45]. As a result, a variety of harmful renal damage may develop, followed by a decline in glomerular function, which may raise kidney biomarkers.

Effect of CE and CCL_4 on serum sodium and calcium levels in albino rats

The result showed that a significant decrease in sodium and calcium levels between the CCL4 group and the control group.

It's interesting to note that administering CE to CCL4 rats increased sodium, albumin, and calcium levels in a manner that brought them closer to normal levels compared to CCL4 rat's data in table (2).

Table (2): Effect of CE and CCL4 on serum sodium and calcium levels in albino rats

	Control	CE	CCL_4	CCL_4 with CE
Sodium (mmol/L)	134.4±7.8	140.6±7.2	117±1.76*	130±1.0#
Total calcium (mg/dl)	11.8±0.27	11.8±0.52	9.2±0.79*	11.1±0.43#

Each value represents the mean ±SD

* Means there is a significant difference compared with the control group at ($p \leq 0.05$)

Means there is a significant difference compared with the CCL4 group at ($p \leq 0.05$))

Dietary fiber, vitamins C and K, fatty acids, and manganese are all abundant in cloves. They are a great source of calcium and magnesium as well. Eugenol, a phenol, makes up the majority of the volatile oil [46]. In vitro studies have shown clove to have strong antioxidant properties [47], which lowers oxidative stress in the body [48]. Clove and

cardamom therapy successfully lowered serum levels of liver enzymes. This is explained by the antioxidant properties of clove and cardamom, which have phenolic compounds that have the ability to scavenge free radicals [49].

Effect of CE and CCL_4 on kidney malondialdehyde (MDA), glutathione (GSH) and catalase (CAT) concentrations in albino rats

Results in Table (3) show the various therapies on the levels of kidney glutathione reduced (GSH), catalase (CAT), and MDA in the animals. When compared to the control group, CCL4-induced nephrotoxicity dramatically increased kidney MDA levels while considerably lowering CAT and GSH levels. Consuming CE alone has no discernible impact on these variables. When compared to the CCL4 group, the CCL4 animals who received CE displayed large kidney MDA level reductions along with considerable increases in GSH and CAT.

Table (3): Effect of CE and CCL4 on kidney malondialdehyde (MDA), glutathione (GSH) and catalase (CAT) concentrations in albino rats.

		Control	CE	CCL_4	CCL_4 with CE
Kidney	**MDA** (µmol/g tissue)	8.6±1.0	8.3±1.4	24.8±1.9*	11.21±1.4#
	GSH (nmol/g tissue)	267±42	280±26	126±7.2*	209±10.6#
	CAT (U/g tissue)	39.06±1.36	40.9±0.52	17.8±0.66*	32.2±1.0#

Each value represents the mean ±SD

* Means there is a significant difference compared with the control group at ($p \leq 0.05$)

Means there is a significant difference compared with the CCL4 group at ($p \leq 0.05$)

MDA is a direct byproduct of lipid peroxidation, which occurs when radicals assault the unsaturated fatty acid-based cell membrane. MDA concentration is a marker for the degree of lipid peroxidation and a proximate indicator of the degree of cell damage [50]. Therefore, the level of oxygen free radical metabolism and the degree of tissue damage during IBV infection can be determined by measuring MDA, SOD, and GSHPx.

Conclusion

According to the current study, clove extract is a good source of antioxidants and phenolic compounds. Therefore, it reduces MDA and GSH while also reducing all inflammatory cytosine, sodium, and calcium levels in albino rats. As a result, using clove has various advantages.

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التأثير الوقائي لمستخلص القرنفل على السمية الكلوية المستحثة برابع كلوريد الكربون في جرذان الالبينو

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الملخص العربي

التعرض لرابع كلوريد الكربون يؤدي إلى حدوث حالات حادة ومزمنة مثل الإصابة الكلوية بالاضافة إلى الإجهاد التأكسدي في الجرذان. لذا كان الهدف من البحث هو التحقق من التأثير الوقائي لمستخلص القرنفل الناجم عن السمية الكلوية برابع كلوريد الكربون، وقد اجريت الدراسة على أربعون من ذكور الجرذان الألبينو الويستر التي تزن (140 ـ 180جم) حيث تم تقسيم الجرذان إلى أربع مجموعات كل مجموعة بها (10) جرذان، المجموعة الأولى جرذان سليمة استخدمت كمجموعة ضابطة سالبة، المجموعة الثانية تضمنت الجرذان التي تتغذى على مستخلص القرنفل (50 مللجم / كجم / يوم) بالفم، المجموعة الثالثة تضمنت جرذان تحقن برابع كلوريد الكربون (0,5 مللجم / كجم مرتين أسبوعياً) بالبطن، المجموعة الرابعة مجموعة مصابة برابع كلوريد الكربون وتعالج بمستخلص القرنفل. بعد ستة أسابيع أظهرت النتائج أن مستخلص القرنفل قلل من التدهور الكلوي الذي يسببه رابع كلوريد الكربون، ويظهر هذا كدليل من خلال الإنخفاض الكبير في مستويات اليوريا والكرياتينين وحمض البوليك TNF-α ، IL-1β و +Na وكذلك MDA للكلى. كما يتطابق ذلك مع تحسن ملحوظ في مستويات الكالسيوم ومستوى CD4 في الدم ومستويات GSH و CAT في الكلى، في الختام مستخلص القرنفل يمكن أن يكون واعداً لحماية الكلى ضد سمية رابع كلوريد الكربون من خلال أنشطة الكسح الجذري والمضاد للأكسدة.

الكلمات المفتاحية: السمية الكلوية، وظائف الكلى، المركبات الفينولية، مضادات الأكسدة في الأنسجة،