تأثير التغذية بمستخلص القرنفل على التليف الكبدي في فئران التجارب

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Abstract

Liver fibrosis is a common serious disease in Egypt due to high incidence rate of infectious on liver diseases hepatitis. This study aims to extract Clove *(Syzygium aromaticum)* and its effect on liver fibrosis induced by CCl_4 in male rats. Adults male Wistar albino rats (140–180g) were randomly divided into six groups (10 rats each) as follows: group (1) normal rats act as control, group (2) rats oral administration of clove extract (CE) low dose (20 mg/kg) for 6 weeks, group (3) rats oral administration of clove extract (CE) high dose (40 mg/kg) for 6 weeks, group (4) rats those were subjected to injection intraperitoneal (IP) at a dose (5.0 mg/kg twice weekly) of CCl_4 for 6 weeks, group (5) rats CCl_4–induced liver fibrosis those were treated with oral (CE) low

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dose (20 mg/kg/day) for 6 weeks and group (6) rats CCl_4-induced liver fibrosis those were treated with oral (CE) high dose (40 mg/kg/day) for 6 weeks. the results revealed that CE succeeded to decline the liver fibrosis-induced by CCl_4; there were evidenced by the significant reduction of Liver enzymes, urea, creatinine, total cholesterol, triglycerides, LDL, glucose coupled with marked improvement in serum albumin, total protein and HDL. So this study recommended touse clove in diets for it's many benefits.

Keywords *Clove*, Antioxidant, Liver and kidney , Lipids profile, Rats

INTRODUCTION

Herbal medicinal products have been documented as a significant source for discovering new pharmaceutical molecules that have been used to treat serious diseases. Many plants species have been reported to have pharmacological activities attributable to their phytoconstituents such are glycosides, saponins, flavonoids, steroids, tannins, alkaloids, terpenes, ***(Gaber et al., 2020)***

Clove (*Syzygium aromaticum*) is an aromatic plant widely cultivated in tropical and subtropical countries. It is a herbal medicine and its active constituent *Syzygium aromaticum* L. ***(José et al., 2021)***, is also known as known by different vernacular names in different languages. It is known as qaranful (Arabic), Karamfil (Bulgarian) and Dingxiang (Chinese). It is an evergreen

tree with sanguine flowers belonging to the family *Myrtaceae* that grows in tropical climates and has been widely used in Chinese traditional medicines for over 2000 years ***(Sahid et al., 2017& Bijoy and Suparna, 2021).***

Clove is rich in volatile compounds and antioxidants such as eugenol, beta-caryophyllene, and alpha-humulin. It has been employed for centuries as a food preservative and medicine because of its antimicrobial and antioxidant properties ***José et al., (2021)*** also It is a common spice used as a flavoring and natural preservative in food industries due to its rich phenolic constituents like eugenol and eugenol acetate. These phenolic constituents possess enormous antioxidant activities, anti-glycation, antinociceptive and antimicrobial properties which enhance the bioactivities of clove spices. Several in vitro and in vivo assays have been conducted to ascertain the potential of clove spices as well as their cytotoxicity. Due to its beneficial effects, clove spices have been applied for use in agriculture as insecticides, anesthesia, antioxidants ***(Solomon et al., 2021).***

Clove powder is used as a good dietary source of Mg, Na, Ca, K, P, S, Fe and Zn. Earlier reports in literature also revealed the presence of various macro and micronutrients in clove bud powder. ***Prinewill-Ogbonna et al.,(2019)*** revealed that macro nutrient sodium (Na) was (61.6 mg/100 g), K(111.6mg/100g) and Ca (117.5mg/100g) while phosphorus P(1.6mg/100g). ***Pu et al.,(2016)*** reported according to Subramanian this was followed by calcium (5040mg/kg). Calcium (Ca) is important for regulating various cellular activities such as hormonal action, cellular

mortality, muscle function and blood clotting . It also helps in the absorption of dietary vitamin B, formation of teeth and bones. The liver is a critical organ in the human body that is responsible for an array of functions that help support metabolism, immunity, digestion, detoxification and vitamin storage among other functions. It comprises around 2% of an adult's body weight. The liver is a unique organ due to its dual blood supply from the portal vein (approximately 75%) and the hepatic artery (approximately 25%) ***(Arjun et al., 2022).*** There for Liver fibrosis is a dynamic pathological condition that can be slowed down in its initial phases. Without proper clinical management of fibrosis, progressive liver damage may lead to cirrhosis and ultimately to liver failure or primary liver cancer ***(Debanjan et al., 2020).***

Chronic liver disease occurs worldwide irrespective of age, sex, region or race. Cirrhosis is a final outcome of variant liver diseases characterized by fibrosis and architectural distortion of the liver with the development of regenerative nodules and can have varied clinical manifestations and complications (***Devadas et al., 2017).***

This study aims to show the impact of feeding with *clove* extract on liver fibrosis in experimental rats.

Materials and methods

Materials

Plant materials

Clove (Syzygium aromaticum) was obtained from the local supplier (Abd El-Rahman Harraz, Bab El-Khalk zone, Cairo, Egypt).

Chemicals

Carbon tetrachloride (CCL_4), olive oil and ethanol were obtained from Sigma Aldrich (**St. Louis, MO, USA**).

Methods

Preparation of clove extract

Clove (*Syzygium aromaticum*) was dissolved in 95% ethanol (1:10 w/v); each 1g sample had been dissolved in 10ml of solvent and extracted on a shaker at 150rpm for 24h at room temperature. Mixtures were then filtered through a sterile layer of gauze to remove any solid plant materials and then through Whatman No. 1 filter paper. The filtrate was concentrated via an evaporation process which was carried out by using a rotary evaporator at 35–40°c., and then the extract was stored at −20 °C until further use ***(Suleiman and Anas 2017)***.

Chemical methods

Determination of the gross chemical composition of clove

Moisture, ash, protein, crude fat and crude fiber were determined according to the method outlined by ***(A.O.A.C., 2010)***.

Carbohydrate content

The total carbohydrate content of the studied clove extract sample was calculated by difference 100 – (ash + protein+ fat + fiber) according to the method described in ***(A.O.A.C., 2010)***. The caloric value was calculated according to the methods of ***Seleet, (2010)***

Determination of minerals content of clove

The total content of minerals was carried out using a mixture of perchloric acid/ nitric acids ($HClO_4$/ HNO_3) according to (Inductively Coupled Plasma Emission Spectrometry) the minerals, Calcium (Ca) and Potassium (P) were determined using the ICP (ICAP6200) according to ***(Isaac and Johnson, 2002).*** Sodium (Na) content was estimated using flame photometry (jenway PFP_7) according to the procedure reported by ***(A.O.A.C., 2010).***

Antioxidant of clove extract

Determination of total phenolic content (TPC)

The content of total phenolic compounds in the extracts was estimated according to ***Jayaprakasha et al., (2003).***

Determination of clove extract radical scavenging activity (RSA)

The capacity of antioxidants in the extracts to quench DPPH radical was determined using the method of ***Nogala-Kalucka et al., (2005)***

Experimental design

sixty adult male Wistar albino rats (*Rattus norvegicus*), weighing (140-180g) were obtained from the Animal Colony, National Research Centre, Giza, Egypt; the rats were kept in suitable plastic cages and maintained on free access to food and water for a week before starting the experiment for acclimatization; they received human care in compliance with the standard institution's criteria for the care and use of experimental rats according to ethical committee of Faculty of Science, Al-Azhar University, Assuit, Egypt; however, this study was approved by the

same ethical committee. After the rats were acclimatized to experimental room conditions, they were divided randomly into six groups (10 rats each). Group (1): Comprised of normal healthy rats as a control group, group (2): Comprised of rats who were subjected to oral administration of (*Clove* extract) (CE) low dose (20 mg/kg/day) alone, group (3): Comprised of rats those were subjected to oral administration of (CE) high dose (40 mg/kg/day) alone, group (4): Comprised of rats those were subjected to injection (IP) at a dose of (5.0 mg/kg twice weekly) of CCL_4 , group (5): Comprise of CCL_4–induced liver fibrosis rats those were treated with oral of low dose (20 mg/kg/day) CE and group (6): Comprise of CCL_4–induced liver fibrosis rats those were treated with oral of high dose (40 mg/kg/day) CE.

Blood sampling

At the end of the treatment period, rats fasted overnight, and following anesthesia with diethyl ether, 3–5 ml blood samples were withdrawn from the retro–orbital plexus using heparinized–sterile glass capillaries. Each blood sample was coolly centrifuged at 3000 rpm for 15 minutes and the serum was separated and stored at –80°C until biochemical determinations as soon as possible.

Biochemical determinations

Serum glucose was determined according to the method described by ***Young, (2001)***.

Serum triglycerides, total cholesterol, HDL–c and LDL–c were determined according to ***Cole et al., (1997), Artiss and***

Zak, (1997), Lopes-Virella et al., (1977) & Wieland and Seidel, (1983) ; respectively.

Serum proteins, albumin and globulin were determined according to the method described by ***Henry (1964)*** *and* ***Dumas (1971)***

Serum ALT , AST, ALP, GGT and LDH were determined according to ***Schumann & Klauke, (2003), Moss and Henderson (1999),*** **IFCC, (1983), *Trinder, (1969)*** **and *Tietz et al., (1983)*** ; respectively.

Serum urea, uric acid and creatinine were determined according to the method described by ***Husdan and Rupoport, (1969), Trinder, (1969) and*** **Chaney *et al.*, (1960)** ; respectively.

Statistical analysis

Statistical analysis was carried out according to **Steel and Torrie, (1960).**

Results and discussion

Gross chemical composition and caloric values of clove on a dry weight basis (g/100g)

Table (1): Gross chemical composition and caloric values of clove on dry weight basis (g/100g)

Sample	Moisture %	Ash %	Protein %	Crude Fat %	Crud Fiber %	Total Carbohydrate (g)	Caloric values K.cal/100 g
Clove	11.27±1.12	6.0±0.86	7.36±1.53	20.89±2.2	10.76±0.96	54.99±4.51	437.41±6.23

- Carbohydrates were calculated by difference.

- Mean of three replicates

The data in **Table (1)** revealed that moisture, ash, proteins, crude fat and crude fiber were 11.27%, 6.0%, 7.36%, 20.89% and 10.76% while, total carbohydrate and caloric value were 54.99% and 437.41K. ca/100g. The results agree with ***Adebisi et al., (2021)*** moisture, Crude fiber, ash and Total Carbohydrates (13.29±.01), (11.07±.01), (4.95±.10) and (61.92±.02) ; respectively. ***Abd ulameed et al., (2022)*** report that ash 7.23% , crude protein 7.53% and caloric values (437.41K.cal/100g) . The clove data in this study are not in agreement with those obtained by ***Al Jobair , (2022)*** obtained that proximate analysis on clove were dry moisture (6.26 ± 0.1), ash (1.08 ± 0.01)crude protein (10.62 ± 0.1), crude fat 4.48%, crude fiber (15.48 ± 0.2), carbohydrate (65.31 ± 0.4) . Variations in moisture contents, ash, protein, crude fiber, crude fat and carbohydrate due to several factors can these compositions such as climate and growing and postharvest management and processing conditions.

Minerals content of clove (mg / 100g)

100g) / Table (2): Minerals content of clove (mg

Sample	Ca mg/100g	Na mg/100g	P mg/100g
Clove	8.05± 1. 16	2,65± 0.96	2.23± 0.86

– Mean of three replicates

The data given in **Table (2)** revealed that minerals content of clove recorded in Ca (8.05 mg/100g) , Na (2.63mg/kg) and P (2.25 mg/100g). These results agree with ***Prinewill–Ogbonna et al.,(2019)*** reported that Ca (8.04 mg/100g), Na (2.44 mg/100g) and P (2.25 mg/100g).

Antioxidant of clove extract

Table (3):Clove extract yield (%), total phenolic content (TPC) and radical scavenging activity (RSA)

Parameter	Yield (%)	TPC (mg/g)	RSA (%)
Clove extract	16.3±0.12	45.4±0.41	75.4±6.1

The yield of 16.3% , total phenolic content (TPC) of 45.4mg/g and radical scavenging activity (RSA) 75.4% of the clove extract (CE) are shown in **Table (3)**. The results agree with ***Bushra et al., (2016)*** who showed that CE exhibited an appreciable amount of total phenolics between 22.80 and 115.33 GAE mg/100g. Also, ***Mohamad et al., (2018) and Bastaki et al., (2017)*** showed that clove extract exhibited the highest

scavenging activity by inhibiting 77.2%. The reason is due to total phenolic acids are capable of removing free radicals, chelating metal catalysts; activating antioxidant enzymes, reducing α-tocopherol radicals, and inhibiting oxidases. Mostly a direct relationship has been found between total phenolics and the antioxidant activity of plant extracts and fruits indicating that the phenolic compounds are the major contributor towards imparting antioxidant attributes to plants. The multiple biological activities of medicinal plants indicate their potential as a source of functional foods and nutraceuticals ***Singh et al., (2009).***

Body weight gain

Table (4): Effect of clove extract (CE) on body weight gain in rats(g/100g)

The same colum, means with different superscript letters are significantly different **at (p≤0.05)** .

The effect of carbon tetrachloride (CCl_4) and clove extract (CE) on the rats' body weight is shown in **Table (4)**, the recorded results showed that rats treated with (CCl_4) had a significant decrease in body weight ; while rats treated with clove extract alone showed a non-significant changes increase in body weight when all were compared with the rats of the control group. In addition, when rats treated with (CCl_4) companies with clove extract low and high dose showed a significant increase in body weight when compared with the animal group treated with CCl_4 alone, reflecting the protective potential of the extract. After administration of the *Syzygium*

Parameter / Groups	Body weight gain (g/100g)
Group (1) control	58.0 ± 12.1^{A}
Group(2) CE (20 mg/kg)	57.2 ± 8.4^{A}
Group(3) CE(40 mg/kg)	58.8 ± 7.1^{A}
Group(4) CCL_4 (0.5 mg/kg)	31.5 ± 7.7^{C}
Group(5) CCL_{4+} CE(20 mg/kg)	43.8 ± 16.1^{E}
Group(6) CCL_{4+} CE(40 mg/kg)	53.9 ± 4.0^{E}

aromaticum extract to rats previously intoxicated by CCl_4, recording results showed increased body weight gain, the improvement of body weight gain suggested the beneficial effect of eugenol and there are some scientific reports supporting its effective body stimulant ***(Halder et al., 2011& Anbu and Anuradha, 2012).***

Effect of clove extract on the lipid profile of rats

Table (5): Effect of clove extract on the lipid profile of rats

The same colum, means with different superscript letters are significantly different **at ($p\leq0.05$).**

The obtained data showed that rats orally treated with CE (20 & 40 mg /kg) for six weeks illustrated the non-significant change in total cholesterol, triglycerides, high dense lipoprotein-cholesterol and low dense lipoprotein-cholesterol; respectively. In contrast, the rats intoxicated with CCl_4 (0.5 mg/kg/weekly) only for a similar period showed a significant increase of total cholesterol, triglycerides, and low dense lipoprotein-cholesterol matched a significant decreased of high dense lipoprotein-cholesterol, when all were compared the control group. On the other hand, the rats treated with CCl_4 in combination with CE (20 & 40 mg /kg) showed a significant decrease in serum total cholesterol and triglycerides, matched with increased low density lipoprotein- cholesterol consecutively, when both were compared to the group with CCl_4 only **Table (5)**. The current study revealed that CCl_4 disrupted lipid metabolism by increasing TC, TG, LDL-c

Parameters / Groups	(T.C) (mg/dl)	TG. (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)
Group (1) control	64.0±3.3^{A}	90.1±5.2^{A}	43.6±0.3^{A}	23.5±1.3^{A}
Group(2) CE (20 mg/kg)	64.0±3.8^{A}	87.4±6.4^{A}	45.9±1.1^{A}	212±0.9^{A}
Group(3) CE(40 mg/kg)	70.4±1.2^{A}	87.9±2.1^{A}	43.9±0.4^{A}	23.0±1.5^{A}
Group(4) CCL_4 (0.5 mg/kg)	171.9±8.3^{C}	182.9±4.7^{C}	35.3±1.7^{C}	93.5±4.4^{C}
Group(5) CCL_4+ CE(20 mg/kg)	93.1±6.1^{B}	108.1±7.1^{B}	42.8±0.8^{B}	68.1±2.9^{B}
Group(6) CCL_4+ CE(40 mg/kg)	84.2±9.6^{B}	101.3±7.4^{B}	43.1±1.1^{B}	60.6±4.3^{B}

and lowering HDL-c and these findings matched with the previous work of (***Shaban et al., 2022***). Hypercholesteremia after CCl_4 administration may be attributed to hindering the β-oxidation of fatty acids (***Mahmoodzadeh et al., 2017***) and activation of the esterification process of lipid (***Mesalam et al., 2021***). On the same hand, hypertriglyceridemia is ascribed to the capability of CCL_4 to transfer the acetate into hepatocytes ***(Weber et al., 2003***) and hinder the action of lysosomal lipase enzyme ***(Marimuthu et al., 2013).***This study exposed elevation in the activity of transaminases after CCL_4 administration and that was allied to hyperlipidemia which interferes with liver function and also out flow of liver cytosolic content into the circulation due

to oxidative stress of CCL_4 that leads to imbalance between antioxidant and prooxidant system ***(Xu et al., 2021).*** These results were in accordance with the previous findings reported by (***Haddar et al., 2021***). Furthermore, clove *(Syzygium aromaticum)* extract significantly ameliorated hyperlipidemia and oxidative stress induced by CCl_4 resistance in rats ***(Sharma et al., 2012***). The observed anti-lipidemic and antioxidant properties could be attributed to the presence of certain phytochemicals such as saponins and flavonoids which have been shown to possess antilipidemic and antioxidant activities. Eugenol, for example, isolated from *Syzygium aromaticum* was demonstrated to possess anti-lipidemic, antioxidant, and anti-inflammatory properties (***Lakshmi and Manasa 2021 & Batiha et al., 2020***). Hence, in hyperlipidemia conditions, consumption of S. aromaticum may help to reduce oxidative stress and even cleanse the blood vessels of lipid deposits, thereby slowing disease progression.

Effect of clove extract on total protein, albumin, globulin and A/G ratio in rats.

Table (6): Effect of (CE) on total protein, albumin, globulin and A/G ratio in rats.

Parameters / Groups	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Ratio A/G
Group (1) control	6.6 ± 0.09^{A}	3.5 ± 0.04^{A}	3.1 ± 0.06^{A}	1.1 ± 0.02^{A}
Group(2) CE (20 mg/kg)	6.8 ± 0.10^{A}	3.83 ± 0.32^{A}	3.0 ± 0.06^{A}	1.1 ± 0.06^{A}
Group(3) CE(40 mg/kg)	6.9 ± 0.08^{A}	4.0 ± 0.10^{A}	3.0 ± 0.07^{A}	1.1 ± 0.03^{A}
Group(4) CCL_4 (0.5 mg/kg)	5.8 ± 0.19^{C}	3.0 ± 0.10^{C}	2.7 ± 0.10^{C}	0.9 ± 0.01^{C}
Group(5) CCL_4+ CE(20 mg/kg)	6.7 ± 0.34^{D}	3.8 ± 0.2^{D}	2.8 ± 0.20^{D}	1.3 ± 0.18^{D}
Group(6) CCL_4+ CE(40 mg/kg)	6.9 ± 0.16^{D}	4.1 ± 0.10^{D}	3.1 ± 0.31^{D}	1.3 ± 0.09^{D}

The same colum, means with different superscript letters are significantly different **at ($p\leq0.05$)**

The data revealed that rats orally treated with CE (20 & 40 mg/kg) for 6 weeks recorded non-significant changes in serum total protein and albumin levels. In contrast, the rats intoxicated with CCl_4 (0.5 mg/kg/weekly) only for a similar period showed a significant decrease, when all were compared to normal rats. Moreover, the rats treated with CCl_4 in combination with CE extract (20 & 40 mg/kg) significant increases in total protein and Albumin levels when all were compared to the corresponding

values of CCl_4 treated rats **Table (6).**These results are consistent with the previously mentioned data of ***(Tsai et al., 2017)***. It is known the liver is the main organ involved in protein biosynthesis, chiefly albumin. Although CCl_4 induces hypoproteinemia, hyperalbuminemia was reported in our study and that may be due to the liver is not the only site of albumin creation, it can be synthesized in extrahepatic tissues such as the intestine, kidney pancreas, brain, and reproductive organs (***Shevtsova et al., 2021***). In harmony with our findings ***(Ojeaburu and Oriakhi 2021)*** demonstrated significant elevation of serum albumin in CCl_4 rat model. Clove extract has been reported in previous studies as one of the strongest antioxidants, even higher than some synthetic antioxidants like BHT or butylatedhydroxyanisole (***Misharina & Samusenko, (2008) and Wei et al., 2010***). The strong activity of clove can be due to the presence of eugenol, the main constituent of this essential oil, which is known to have antioxidant activity and a protective effect against oxidative damage induced by CCl_4 (***Wei et al.,2010).***

Liver function:

Table (8): Effect of clove extract (CE)on serum ALT, AST, ALP, GGT and LDH (U/L) in rats

The same colum, means with different superscript letters are significantly different **at ($p \leq 0.05$)**.

The obtained data showed that rats orally treated with CE (20 & 40 mg/kg) for six weeks recorded non-significant changes in serum ALT, AST, GGT and ALP activities. In contrast, the rats intoxicated with CCl_4 (0.5 mg/kg/weekly) only for a similar period

Parameters / Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	LDH (U/L)
Group (1) control	94.9 ± 7.2^{A}	168.8 ± 14.6^{A}	11.84 ± 3.2^{A}	29.9 ± 3.8^{A}	1583.4 ± 121.4^{A}
Group(2) CE (20 mg/kg)	89.8 ± 9.5^{A}	169.8 ± 36.6^{A}	10.7 ± 1.7^{A}	29.6 ± 3.8^{A}	1390 ± 80.6^{A}
Group(3) CE(40 mg/kg)	89.3 ± 13.1^{A}	161.3 ± 48.5^{A}	10.9 ± 2.6^{A}	30.2 ± 6.7^{A}	1309 ± 164.2^{A}
Group(4) CCL_4 (0.5 mg/kg)	244 ± 16.7^{C}	300.3 ± 11.4^{C}	44.6 ± 6.0^{C}	69.1 ± 7.6^{C}	3251.6 ± 251.7^{C}
Group(5) CCL_4+ CE(20 mg/kg)	128.3 ± 39^{E}	180.3 ± 28.04^{E}	17.6 ± 2.4^{E}	44.2 ± 5.5^{E}	1652 ± 203.7^{E}
Group(6) CCL_4+ CE(40 mg/kg)	142.8 ± 11.7^{E}	200.8 ± 8.9^{E}	15.8 ± 2.8^{E}	41.7 ± 5.2^{E}	1554 ± 302.2^{E}

showed a significant increase, when all were compared to normal rats. Moreover, the rats treated with CCl_4 in combination with CE extract showed significant decreases in ALT, AST, GGT and ALP activities levels when all were compared to the corresponding

values of CCl_4 treated rats **Table(7)**. In recent years and due to a lot of environmental stressors exogenous or endogenous, the liver becomes easily supposed to damage which may lead to acute or chronic liver disorders. CCL_4 is one of the hazardous poisonous chemicals that causes tissue destruction as it initiates secretion of reactive oxygen species (ROS) in all body tissues once a human or animal subjected to a single overdose or multiple low doses of it (***Shahat et al., 2022***). *Clove (Syzygium aromaticum)* supplementation was found to cause significant suppression of these activities, indicating its inhibitory effect on hepatotoxicity. Though clove essential oil has already been reported to effectively lower liver toxicity markers in CCl_4 induced hepatotoxicity (***Al-Okbi et al., 2014***), Many researchers report the plant extract contain chemical components can inter with the metabolism process and reported the role of certain flavonoids, triterpenoids and steroids in hepatoprotection against hepatotoxins. The presence of these certain compounds in *Syzygium aromaticum* is the reason of the protective effects of rat's liver (***Abd Al-azem et al., 2019***).

Effect of clove extract on urea, creatinine and uric acid (mg/dl) in rats

Table (9): Effect of clove extract (CE) on urea, creatinine and uric acid (mg/dl) in rats.

The same colum, means with different superscript letters are significantly different **at ($p \leq 0.05$)**.

The results of this study showed that rats orally treated a on significant change in with CE for six weeks recorded a n serum creatinine, urea and uric acid. In contrast, the rats intoxicated with CCl_4 (0.5 mg/kg/weekly) only for a similar period showed a significant increase in kidney function, when all were compared to normal rats. In addition, the rats treated with CCl_4 in combination with CE (20 & 40 mg/kg) showed significant decrease in serum creatinine, urea and uric acid when both were compared to that combination with CCl_4 only **Table (9)**. The present study revealed that chronic administration of CCl_4 caused marked impairment in renal function alongside significant oxidative stress in the kidney. Serum creatinine, urea and uric

Parameters / Groups	**Urea (mg/dl)**	Creatinine (mg/dl)	**Uric acid (mg/dl)**
Group (1) control	54.3 ± 2.2^{B}	0.64 ± 0.9^{B}	5.1 ± 0.17^{B}
Group(2) CE (20 mg/kg)	49.7 ± 4.2^{AB}	0.66 ± 0.03^{B}	4.9 ± 0.2^{B}
Group(3) CE(40 mg/kg)	45.0 ± 2.0^{B}	0.68 ± 0.05^{B}	4.5 ± 0.7^{B}
Group(4) CCL_4 (0.5 mg/kg)	89.5 ± 3.6^{C}	1.92 ± 0.14^{C}	6.3 ± 0.5^{C}
Group(5) CCL_4+ CE(20 mg/kg)	64.8 ± 2.6^{A}	0.92 ± 0.03^{A}	4.13 ± 0.31^{A}
Group(6) CCL_4+ CE(40 mg/kg)	57.3 ± 4.2^{A}	0.83 ± 0.05^{A}	4.6 ± 0.2^{A}

acid concentrations were significantly higher in CCl_4-treated rats. On the other hand, traditional use indicates that clove has several therapeutic properties ***(Dashti and Morshedi, 2009)***. So the major component of this herb which has been shown to have a pharmacologic effect is a eugenol ***(Gang et al, 2001).*** The current results clearly indicated that clove oil treatment a significant decrease in kidney markers (urea, uric acid and creatinine) compared with the CCl_4 group. Moreover, it succeeded to induce an improvement in kidney function. Our results are in agreement with the works of ***(*Abdel Wahhab and *Aly, 2005).***

Conclusion

In conclusion, this study demonstrates that the effect of (CE) on serum glucose level, lipids profile, liver enzymes activities and kidney functions of rats feeding on (CE) was investigated. The obtained results observed that (CE) caused a reduction in serum glucose evels and significantly decreased serum lipids profile. Treatment with (CE) at different levels caused improvement in liver and kidney enzymes activities compared the control group. Therefore, it is recommended to take it daily.

References:

A.O.A.C (2010): Official Methods of Analysis of AOAC International 18^{th} edition, Published by AOAC International, Maryland, 20877-2417, U.S.A

Abd Al-azem, D. ; Karim, H. and Sahar, A.A. (2019): The protective effects of *Syzygium aromaticum* essential oil extract against methotrexate induced hepatic and renal

toxicity in rats, Journal of Pure and Applied Microbiology, 13(1)

Abd ulameed, H. T. ; Ojomu, O. M. and Fajinmi, I. O.(2022): Comparative study on proximate analysis of *Syzygium aromaticum* bud and hunteria umbellata seed in management of diabetes mellitus, International Journal of Chemistry Studies, 6(1): 26-28.

Abdel-Wahhab, M.A. and Aly, S.E.(2005): Antioxidant property of nigellasativa (black cumin) and *Syzygium aromaticum* (clove) in rats during aflatoxicosis, J. Appl. Toxicol.,25(3):218-223

Adebisi , A.A. ; Olumide, M.D. and Akintunde, A.O.(2021): Nutritive value and phytochemical screening of turmeric and clove as a potential phyto-additive in livestock production, Nigerian J. Anim. Sci., 23 (2): 142-152.

Al Jobair ,O. M. (2022): Physicochemical, nutritional, and sensory quality and storage stability of cookies: effect of clove powder, International Journal of Food Properties, 25(1): 1009-1020.

Al-Okbi, S. Y. ; Mohamed, D. A. ; Hamed, T. E. and Edris, A. E. (2014): Protective effect of clove oil and *eugenol microemulsions* on fatty liver and dyslipidemia as components of metabolic syndrome, Journal of Medicinal Food, (17): 764-771.

Anbu, S. and Anuradha, C. (2012): Protective effect of eugenol against alcohol-induced biochemical changes in rats,

International Journal of Research in, Biotechnology and Biochemistry, 2(2): 13-18.

Arjun, K. ; Ekrem, Y. ; Chase, J. and Faiz, T.(2022): Physiology, liver, harold E. (2011): anatomy of the liver, BASIC SCIENCE Elsevier Ltd., 29(12): 589- 592.

Artiss, J. and Zak, B. (1997): Measurement of cholesterol concentration, in: N. rifai, G. R. warnick and M. H. dominiczak, Eds., Handbook of Lipoprotein Testing, AACC Press, Washington, 99-114.

Bastaki, M. ; Farrell, T. ; Bhusari, S. ; Pant, K. and Kulkarni, R. (2017): Lack of genotoxicity in vivo for food color additive tartrazine, Food Chem. Toxicol, (105): 278-284.

Batiha, G.E. ; Alkazmi, L.M.; Wasef, L.G.; Beshbishy, A.M.; Nadwa, E.H. and Rashwan, E.K. (2020): *Syzygium aromaticum L. (Myrtaceae)*: traditional uses, bioactive chemical constituents, pharmacological and toxicological activities, Biomolecules, (10): 202.

Bijoy, K.D. and Suparna, S.M. (2021): Potential of clove and its nutritional benefits in physiological perspective, International Journal of Physiology, Nutrition and Physical Education , 6(1): 103-106.

Bushra, S. ; Farooq, A. ; Muhammad, M. ; Maryam, A. and Sidra, I. (2014): In vitro antimutagenic, antioxidant activities and total phenolics of clove (*Syzygium aromaticum L.*) seed extracts, J. Pharm. Sci., 27(4): 893-899.

Chaney, A. L. ; Marbach, C.P. and Fowcett, J. K. (1960): A colorimetric method for determination of blood urea concentration, J. Clin. Chem., (8) 130-135.

Cole, T.G. ; Klotzsch, S.G. and Namara, J.M. (1997): Measurement of triglyceride concentration. in: rifai, N.; warnick, G.R. and dominiczak, M.H., (Eds.), handbook of lipoprotein testing. Washington: AACC Press,115-126

Dashti,R. M. and Morshedi, A. H. (2009): The effects of *Syzygium aromaticum* (clove) on learning and memory in mice, Asian J .Trad. Med., 4(4).

Debanjan, D. ; Jacopo, B. ; Tatiana, K. and David, A.(2020): Mechanisms of liver fibrosis and its role in liver cancer, National Library of Medicine, 245(2): 96-108.

Devadas, A. ; Srividya, R. ; Kumar, S. ; Sindhu, S. and Ganesan, M.(2017): A study on hyponatremia in decompensated liver diseasl , Journal of Medical Science And Clinical Research, 5 (4): 19832-19836.

Dumas, B.T. (1971): Diagnostic reagent kit for in vitro determination of total protein and albumin in serum (Code No25931), Clinical Chemistry Acta, 31, 87-96.

Gaber, El. B. ; Luay, M. A. ; Lamiaa, G. W. ; Amany, M. B. ; Eman, H. N. and Eman, K. R.(2020): *Syzygium aromaticum L.* (*Myrtaceae*): traditional uses, bioactive chemical constituents, pharmacological and toxicological activities, Biomolecules, (10):102.

Gang, D. ; Wang, J. ; Dudareva, N. ; Nam, K. ; Simon, J.; Lewinsohn, E. and Pichersky, E. (2001): An investigation

of the storage and biosynthesis of phenylpropenes in sweet basil. Plant Physiol. , 125(2):539–555.

Haddar, A. ; Feriani, A. ; Hamed, M. ; Sila, A. and Ellouz-Chaabouni, S. (2021): Preventive effect of Bacillus mojavensis levan against carbon tetrachloride and cisplatin toxicity: in vivo study, Environ Sci Pollut Res Int, 28(36), 50117-50126.

Halder, U. ; Bagchi, P. ; Chattopadhyay, S. ; Dutta, D. and Chawla-Sarkar, M. (2011): Cell death regulation during influenza a virus infection by matrix (M1) protein: a model of viral control over the cellular survival pathway, Cell Death Dis., (2): 197

Henry, R.J. (1964): Clinical chemistry; principles and techniques. 2nd Ed. harper and publishers, New York, Philadelphia.

Husdan, H. and Rupoport, A. (1969): Estimation of creatinine by jaffes reactions comparison of three method, Clin. Chem., (138) 459-470.

IFCC (1983): Methods for the measurement of catalytic concentration of enzymes (Part5). ; IFCC, methods for alkaline phosphatase, J. Clin. Chem. Clin. Biochem., (21):731-748.

Isaac, R.A and Johnson, W.A. (2002): Elemental analysis of plant tissue by plasma emission spectroscopy, collaborative study, J. Assoc. off. Anal. Chem., 68(3): 499.

Jayaprakasha, G. ; Tamil Selvi, A. ; Sakariah, K. (2003): Antimicrobial and antioxidant activities of grape(*Vitis vinifera*) seed extracts, Food Res Int., (36):117-22.

José, N. H. ; Gustavo, A. C. ; Moisés, M. V. and Hugo, E. A. (2021): Clove essential oil (*Syzygium aromaticum L. Myrtaceae*): extraction, chemical composition, food applications, and essential bioactivity for human health, J Agric Food Chem, (21): 22-26.

Lakshmi, V. and Manasa, K. (2021): Various phytochemical constituents and their potential pharmacological activities of plants of the genus Syzygium, Am. J. Pharm. Tech. Res., (11): 68-85.

Lopes-Virella, M. ; Stone, P. ; Ellis, S. ; Colwell, J. (1977): Cholesterol determination in high density lipoproteins separated by three different methods, Clinical Chemistry, (23): 882

Mahmoodzadeh, Y. ; Mazani, M. and Rezagholizadeh, L. (2017): Hepatoprotective effect of methanolic Tanacetum parthenium extract on CCL_4-induced liver damage in rats. Toxicol Rep, (4): 455-462.

Marimuthu, S. ; Adluri, R. ; Rajagopalan, R. and Menon, V. (2013): Protective role of ferulic acid on carbon tetrachloride-induced hyperlipidemia and histopathological alterations in experimental rats, Journal of basic and clinical physiology and pharmacology, 24(1): 59-66.

Mesalam, N. M. ; Aldhumri, S. A. ; Gabr, S. A. ; Ibrahim, M. A. ; Al -Mokaddem, A. K. and Abdel- Moneim, A. E.

(2021): Putative abrogation impacts of ajwa seeds on oxidative damage, liver dysfunction and associated complications in rats exposed to carbon tetrachloride, Mol Biol Rep, 48(6): 5305-5318

Misharina, T. ; Samusenko, A. (2008): Antioxidant properties of essential oils from lemon, grape fruit, coriander, clove, and their mixtures, Appl. Biochem. Microbiol (44):438-442.

Mohamad, T.A. ; Esther, N.M. and Hany, A.E.(2018): Clove (Syzygium aromaticum) and honey extracts significantly reduce inflammatory cytokines and liver function enzymes in experimental rats fed on carbon tetrachloride (CCl_4), Journal of Radiation Research and Applied Sciences, 11(4): 416-422.

Moss, R. and Henderson, A. (1999): Clinical enzymology. In: burtis, C.A. and ashwood, E.R., Eds., tietz textbook of clinical chemistry, 3rd Edition, Saunders, Philadephia, 617-677.

Nogala-Kalucka, M. ; Siger, A. ; Lampart-Szczapa, E. and Hoffman, A.(2005): Antioxidant activity of phenolic compounds of selected cold-pressed and refined plant oils, Rośliny Oleiste - Oilseed Crops, (2) :26.

Ojeaburu, S. and Oriakhi, K.(2021): Hepatoprotective, antioxidant and, anti-inflammatory potentials of gallic acid in carbon tetrachloride-induced hepatic damage in Wistar rats, Toxicology Reports, (8): 177-185.

Prinewill-Ogbonna, I.L. ; Ogbonna, P.C. and Ogujiofor, I.B. (2019): Proximate composition, vitamin, mineral and biologically active compounds levels in leaves of mangifera indica (*Mango*), persea americana (Avocado pea), and annona muricata (Sour sop), J. Appl. Sci. Environ. Manage, (23): 65-74.

Pu, F. ; Chen, N.; Xue, S. (2016): Calcium intake, calcium homeostasis and health, Food Sci. Hum. Wellness, (5): 8-16.

Sahid, H. ; Rafia, R. ; Ayesha, M. and Asma, B . (2017): Clove: a review of previous species with multiple uses ,International journal of chemical and biological science, (11):129-133.

Schumann, G. and Klauke, R. (2003): New IFCC reference procedures for the determination of catalytic activity concentrations of five enzymes in serum: preliminary upper reference limits obtained in hospitalized subjects, Clinica Chimica Acta, (327) 69-79.

Seleet, R.(2010): The analysis of nutrient in foods, academic Press. Inc. London. GB.

Shaban, N. Z. ; Aboelsaad, A. M. ; Awad, D.S, ; Abdulmalek, S. A. and Shaban, S. Y. (2022): Therapeutic effect of dithiophenolato chitosan nanocomposites against carbon tetrachloride-induced hepatotoxicity in rats, Environ Sci Pollut Res Int., 29(6): 8487-8502.

Shahat, A. A. ; Hassanein, H. D. ; Ullah, R.M. ; Alqahtani, A. S. ; Husseiny, H. A. ; Kowalczyk, A.H. ; Farrag, A.R. ;

Yang, S. H. (2022): Renoprotective and cardioprotective potential of moricandia sinaica (Boiss.) against carbon tetrachloride-induced toxicity in rats, Evidence-Based Complementary and Alternative Medicine, 1-12.

Sharma, A.; Bharti, S.; Kumar, R.; Krishnamurthy, B.; Bhatia, J.; Kumari, S. and Arya, D. (2012): *Syzygium* cumini *ameliorates* insulin resistance and β-cell dysfunction via modulation of PPAR, dyslipidemia, oxidative stress, and TNF-α in type 2 diabetic rats, J. Pharmacol. Sci., (119): 205-213.

Sharma, R.N. ; Tikka, S. K. ; Bhute, A. R. and Bastia, B. K. (2022): N-acetyl cysteine in the treatment of cannabis use disorder: A systematic review of clinical trials, Addictive Behaviors, (129): 107.

Singh, A. ; Dhamanigi, S. and Mohammed, A. (2009): Anti-stress activity of hydro-alcoholic extract of *Eugenia caryophyllus* buds (clove), Indian J. Pharmacol. (41): 28-31.

Solomon, I.A. ; Ademola, E. A. ; Oluwakemi, O. I. and Anthony, T. I. (2021): Clove (*Syzygium aromaticum*) spices: a review on their bioactivities, current use, and potential application in dairy products, Journal of Food Measurement and Characterization volume, (15): 3419-3435.

Steel, R.G.D. and Torrie, J.H. (1960): Principles and procedures of statistics with special reference to the biological sciences, McGraw Hill, New York, 187-287

Sulaiman, A. and Anas, M. (2017): Quantitative determination of nutritional and antinutritional composition of clove (*Eugenia caryophllata*), Dairy. Vet. Sci. J., (3): 1-3.

Tietz, N.W. ; Burtis, C.A. ; Duncan, P. ; Ervin, K. ; Petitclerc, C.J. ; Rinker, A.D., Shuey, D. and Zygowicz, E.R. (1983): A reference method for measurement of alkaline phosphatase activity in human serum, Clinical Chemistry, (29): 751-761.

Trinder, P. (1969): Enzymatic determination of glucose in blood serum, Annals of Clinical Biochemistry, (6): 24.

Tsai, J. C. ; Chiu, C. S. ; Chen, Y. C. ; Lee, M. S. ; Hao, X. Y. ; Hsieh, M. T. ; Kao, C. P. and Peng, W. H. (2017): Hepatoprotective effect of coreopsis tinctoria flowers against carbon tetrachloride-induced liver damage in mice, BMC Complement Altern Med, 17(1): 139-148.

Weber, L. ; Boll, M. and Stampfl, A. (2003): Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model, Critical reviews in toxicology, 33(2): 105-136.

Wei, A. ; Shibamoto, T. (2010): Antioxidant lipoxygenase inhibitory activities and chemical compositions of selected essential oils, J. Agric. Food Chem (58): 7218-7225.

Wieland, H. and Seidel, D. (1983): A Simple specific method for precipitation of low density lipoproteins, Journal of Lipid Research, (24): 904-909.

Xu, Y. ; Xie, L. ; Tang, J. ; He, X. ; Zhang, Z. ; Chen, Y. ; Zhou, J. ; Gan, B. and Peng, W. (2021): Morchella

importuna polysaccharides alleviate carbon tetrachloride–Induced hepatic oxidative Injury in mice, Front Physiol, 12.

Young, D.S. (2001): Effects of disease on clinical lab tests. 4th Edition, AACC, Washington DC.

تأثير التغذية بمستخلص القرنفل على التليف الكبدي في فئران التجارب

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الملخص

تليف الكبد مرض خطير شائع في مصر بسبب ارتفاع معدل الإصابة بأمراض الكبد . تهدف هذه الدراسة الي استخلاص القرنفل ومعرفه تأثيره ضد تليف الكبد الناجم عن رابع كلوريد الكربون في ذكور الفئران. تم تقسيم ذكور الفئران ويستر البيضاء البالغة (١٤٠–١٨٠ جم) بشكل عشوائي إلى ست مجموعات (١٠ فئران لكل مجموعه) على النحو التالي: مجموعة (١) فئران طبيعية تعمل كمجموعة ضابطه ، مجموعة (٢) فئران تتناول عن طريق الفم مستخلص القرنفل جرعة منخفضة (٢٠ مجم / كجم) لمدة ٦ أسابيع ، المجموعة (٣) فئران تتناول عن طريق الفم مستخلص القرنفل بجرعة عالية (٤٠ مجم / كجم) لمدة ٦ أسابيع ، المجموعة (٤) مجموعه فئران تعرضت للحقن بالبطن بجرعه (5.0مجم / كجم مرتين أسبوعياً) من رابع كلوريد الكربون لمدة ٦ أسابيع ، المجموعة (٥) فئران حقنت برابع كلوريد الكربون عولجت بجرعة منخفضة عن طريق الفم (٢٠ مجم / كجم / يوم) لمدة ٦ أسابيع ومجموعة (٦) فئران حقنت برابع كلوريد الكربون عولجت بجرعة عالية عن طريق الفم (٤٠ مجم / كجم / يوم) لمدة ٦ أسابيع. أظهرت النتائج أن مستخلص القرنفل نجح في تقليل تليف الكبد الناجم عن رابع كلوريد الكربون ؛ تم إثبات ذلك من خلال الانخفاض الكبير في انزيمات الكبد واليوريا والكرياتينين والكوليسترول الكلي والدهون الثلاثية و الكولسترول منخفض الكثافة والجلوكوز إلى جانب التحسن الملحوظ في الألبومين والبروتين الكلي والكولسترول عالي الكثافة. لذا توصي الدراسة باستخدام القرنفل في الغذاء لما له من فوائد عديدة.

الكلمات المفتاحية

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