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Evaluation of the ameliorative role of ginger (*Zingiber officinale*) extract against carbon tetrachloride-induced hepatotoxicity in male albino rats

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ABSTRACT

Background: One well-known hepatotoxin that is frequently used to cause acute toxic liver injury in a variety of experimental animals is carbon tetrachloride (CCl4). There have been suggestions that ginger (Zingiber officinale) offers great promise in alleviation of many diseases**. Aim:** The purpose of this study was to evaluate the ameliorative role of ginger extract (GE) against the liver damage induced by CCL4 in male rats. **Material &methods:** Thirty-two male adult albino rats were used in this study. The rats were divided into four groups (N=8): control group, GE-supplemented group (200mg /kg b.wt daily for consecutive 35 days), CCL4-treated group (2mg/kg b.wt/twice weekly for 5 weeks), and CCL4-intoxicated group followed by treatment with GE. **Results**: GE for consecutive 35 days significantly reduced the adverse effects of CCL4 toxicity on the levels of liver enzymes (ALT, AST, and ALP), total bilirubin, total protein, and albumin. The altered liver tissue antioxidants (SOD, CAT, and GPx) and MDA were significantly ameliorated on treatment with GE. Additionally, the liver histopathological alterations induced by CCL4 were markedly recurred on treatment with GE. **Conclusion:** Ginger extract has a potential ameliorative role against CCL4-induced adverse biochemical and histological alterations in the liver of male rats.

Keywords: Zingiber officinale, ginger, carbon tetrachloride**,** CCL4, hepatotoxicity.

INTRODUCTION

The liver is one of the dynamic organs that are essential for the metabolism of macromolecules, the production of bile acids, and the process of removing circulating toxins (**Zheng et al., 2022).** Multiple factors, such as receiving repeated doses of medication, receiving medical treatment, and being exposed to harmful substances in the environment are all linked to liver injury **(Attallah et al., 2022).** Untreated liver damage frequently develops into cirrhosis, malignancy, and liver fibrosis **(Kermanizadeh et al., 2022).**

Carbon tetrachloride (CCL4), a colorless chlorinated hydrocarbon, is used in the degreasing process and is incorporated into the construction of fire extinguishers and refrigerants **(Aramjoo et al., 2022).** CCL4, however, is a non-flammable environmental toxin; therefore repeated exposure causes cell death and necrosis **(Popoola et al., 2022**). CCl4 is a member of the group of hepatotoxins that only work after metabolic activation. Cytochrome p450 enzymes, primarily CYP2E1, metabolize it in the endoplasmic reticulum (ER) to produce the extremely reactive trichloromethyl radical (CCl3•). The

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extremely reactive trichloromethyl peroxyl radical (CCl3OO•), which is produced when CCl3• reacts quickly with oxygen, combines with lipids to produce products of lipid peroxidation. Polyunsaturated fatty acids (PUFA) of the ER and mitochondria are vulnerable to free radical oxidation by the free radicals. One of the primary mechanisms of CCl4-induced liver damage is lipid peroxidation driven by free radicals **(Weber et al., 2003).**

Recently several researchers focused on the use of medicinal plants as an alternative source for treatment or alleviation of several diseases instead of the usual drugs. *Zingiber officinale* (ginger) is an aromatic spice that has been used for thousands of years in culinary and medicinal applications all over the world **(Li et al., 2019).** Over 400 components have been identified in ginger; nevertheless, its primary constituents include carbohydrates, lipids, and volatile oils. Volatile oils that include zingerone, shogaols, and gingerols with 6-gingerol serving as the primary pungent component, are responsible for the flavor and odor of ginger **(Li et al., 2019)**. Ginger, *Zingiber officinale*, has been utilized in Asian medicine for 6000 years and is considered an effective treatment for several diseases. In addition to its culinary applications, it has been used to relieve nausea associated with travel and during pregnancy. It also functions as an antioxidant, antibacterial, and anti-fungal effect (**Park et al., 2014; Thomson et al., 2014**). Previous reports have shown that gingerol, the active ingredient of ginger, has anti-inflammatory and analgesic activities (**Young et al., 2005**). Also, it has been shown that *Zingiber officinale* rhizomes have an antioxidant action and can protect against free radicals (**Masuda et al., 2004)** hence its anti-inflammatory and anticancer activities (**Lee et al., 2008**). Moreover, ginger rhizome was found to reduce cholesterol biosynthesis in the liver (**Verma et al., 2004).** Accordingly, this work aims to evaluate the potential ameliorative role of ginger rhizome extract against CCl4-induced liver damage in male rats.

MATERIALS & METHODS

1. Chemicals

Carbon tetrachloride (CCl4) was purchased from Sigma, Aldrich (USA). It is a colorless liquid with a "sweet" smell that can be detected at low levels. The rest of the chemicals were purchased from Biodiagnostic, Cairo, Egypt.

2. Preparation of Ginger Extract (GE)

One kilogram of *Zingiber officinale* rhizomes was purchased from the local market of Jazan city. The rhizomes were washed and peeled, cut into small pieces, and dried for seven days. The dried ginger rhizomes were grinded using an electric blender to powder. The powder was macerated in distilled water (125 g/1000 ml) for 12 hours at room temperature and then filtered to obtain the final aqueous extract (**Kamtchoving et al., 2002).**

3. Experimental animals

Thirty-two male Wistar albino rats weighing 160-180 g were used in this study. The animals were kept in wire-bottomed cages in a room under standard conditions of illumination with a 12-hour light-dark cycle at 25 ± 1°C and 50% relative humidity, and provided with tap water and a balanced diet of *libitum*. After acclimatization for one week, the rats were randomly divided into four groups as follows (N=8):

Control group: they were given olive oil (2ml/kg b.wt/2 times/week; I.P).

Ginger extract group: the rats were supplemented orally with ginger extract (200 mg/kg b.wt for consecutive 35 days **(El-Sharaky et al., 2009).**

CCL4-treated group: the rats were treated with CCl4 dissolved in equal volume of olive oil (1:1) (2 ml/kg/2 times/week; I.P for five weeks) (**Hassan et al. 2016**)

CCl4&GE-treated group: the rats were treated with CCl4 for 35 days followed by treatment with GE for consecutive 35 days by the same doses in group2&3.

4. Sample collection and tissue preparation

At the end of the experimental period (5 weeks for groups 1-3 and 10 weeks for group 4), the rats were anesthetized and the blood was collected in glass

tubes. The serum was separated by centrifugation at 3000 rpm for 10 min and stored at −80°C bending biochemical analysis. The animals were dissected and the whole liver was removed immediately, washed in normal saline, and cut into two halves. One half was fixed in 10% neutral buffered formalin for histological and immuno-histochemical investigation and the other half was kept frozen for estimation of antioxidants.

5. Investigated parameters

5.1. Measurement of serum AST, ALT, ALP, total bilirubin, total protein and albumin

The activities of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were assayed using commercially available diagnostic kits (Sigma Diagnostics (I) Pvt. Ltd., Baroda, India). Total bilirubin was measured with a spectrophotometer at 600 nm (**Steven**, **1996).** Total protein was measured at 540 nm with a spectrophotometer **(Doumas et al, 1981).** Measurement of albumin was measured with a spectrophotometer at 630 nm (**Gustafsson, 1976)**.

5.2. Determination of CAT, SOD, GPx, and MDA activities in the liver tissues

The activity of catalase (CAT) in the liver tissue was estimated by using a rat CAT ELISA Kit **(**Cat No. MBS2600683) purchased from MyBioSource Company. Superoxide dismutase (SOD) activity in liver tissues was determined based on the ability of the enzyme to inhibit nitroblue tetrazolium (NBT) reduction by superoxide (**Kakkar et al., 1984).** Glutathione peroxidase (GPx) was assessed in the liver homogenates using commercially available kits (Biodiagnostic, Egypt) based on the manufacturer's instructions (**Paglia and Valentine, 1967**). Malondialdehyde (MDA) was measured according to the method of **Ohkawa et al. (1979)** using commercial kits (Biodiagnostic, Cairo, Egypt). The colorimetric absorbance was determined at 532 nm. Specific activity was presented as nmol/mg protein.

5.3. Histological technique for hematoxylin and eosin stain

The formalin-fixed liver was dehydrated with an ascending ethanol series, cleared with xylene, and embedded in paraffin. A 5-6 µm thick section of the liver was obtained, stained with hematoxylin and eosin **(Bancroft & Gamble, 2008).** The obtained sections were investigated under a bright field light microscope and microphotographed.

5.4. Immunohistochemical Demonstration of Nuclear Factor Kappa B (NF-κB).

Four µm thick sections of liver were prepared from each group. Sections underwent deparaffinization and rehydration, and H2O2 in methanol was used to inhibit endogenous peroxidase activity. Sections were pre-treated in a microwave with citrate buffer (pH 6.0) followed by treatment with a monoclonal antibody against nuclear factor kappa B (NF-κB) at room temperature (Thermo Scientific, USA, dilution 1:100). UltraVision detection System (Thermo Scientific) was used as follows: sections were incubated with biotinylated goat anti-polyvalent, then with streptavidin peroxidase and finally with DAB plus chromogen. Slides underwent a hematoxylin counterstain. The amount of cell immunopositivity was evaluated by examining the slides under a light microscope.

Statistical analysis:

Data are expressed as mean± standard error {n=8 per group} statistical analysis one way ANOVA followed by post hoc test means in the same row with different superscript (*) are significantly different when $p<0.05$ * significant at value <0.05, **significant at p-value<0.01 and ***significant p-value < 0.001 in comparison with control.

RESULTS

1. Body weight changes

The results of the present work revealed that the mean body weight of CCl4-treated rats was significantly lowered ($P<0.01$) than the control however in the CCl4-treated group that supplemented with ginger extract, the mean body weight showed non-significant changes compared with the control (Figure 1).

2. Changes in the activities of serum AST, ALT, ALP, total bilirubin, total protein, and albumin

The obtained results revealed that the levels of serum ALT, ALP, AST, total bilirubin, total protein, and albumin for control and ginger groups of rats were localized in the normal standard range among normal albino rats. On the other hand, a highly significant increase in the levels of serum AST, ALT, ALP, and total bilirubin (P<0.01) was recorded among CCl4-treated rats if compared with control, while the levels of total protein and albumin appeared significantly lowered (P<0.001) than control. Post-supplementation of GE to CCl4 treated rats have successfully restored the altered liver functions near to the normal values as in control (Figure 1)

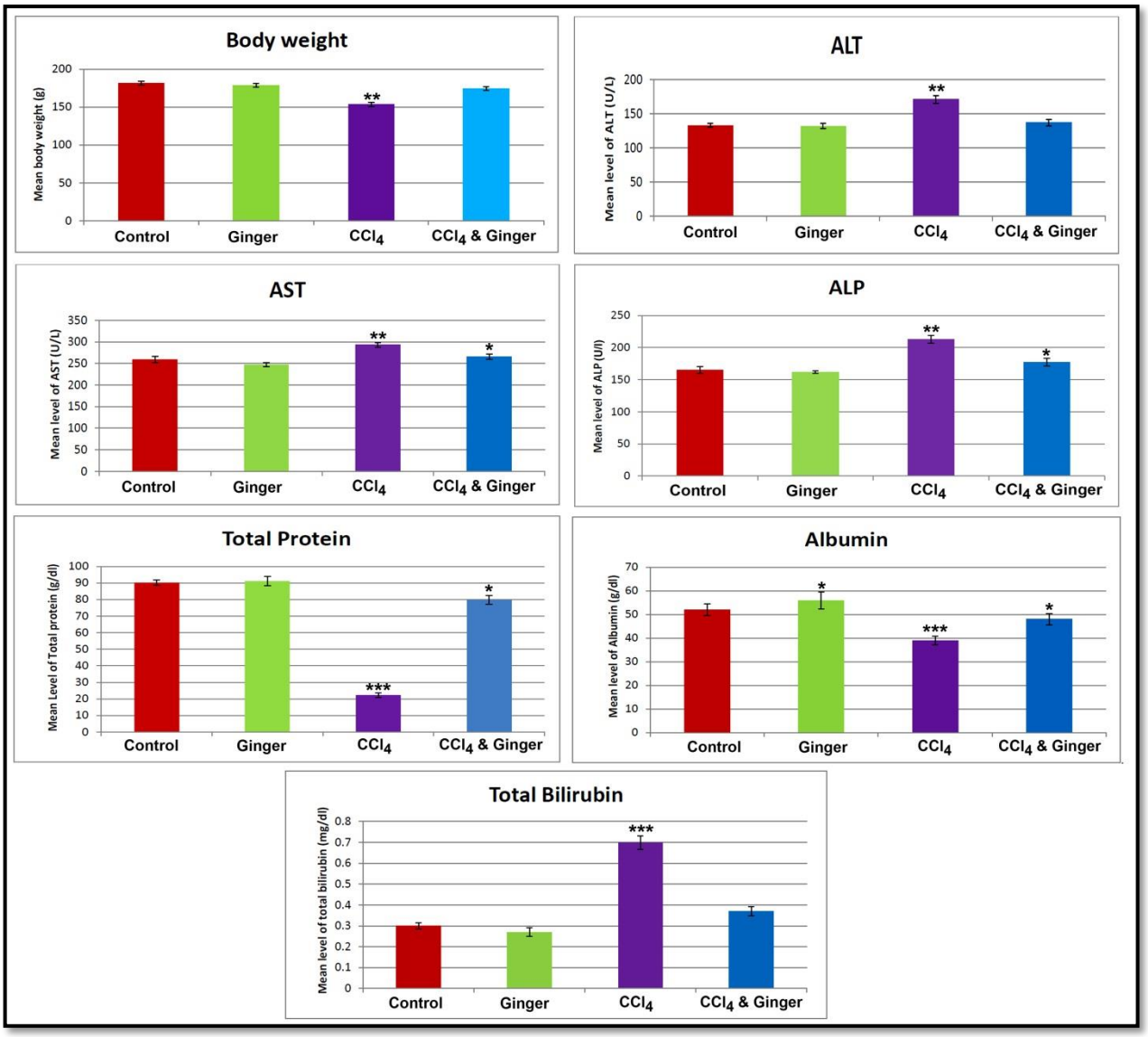

Fig.1: The mean body weight and serum levels of AST, ALT, ALP, total protein, albumin, and total bilirubin among the different studied groups.

*Data are expressed as mean± standard error {n=8 per group} statistical analysis one way ANOVA followed by post hoc test means in the same row with different superscript (*) are significantly different when p<0.05 * significant at value <0.05, **significant at p- value<0.01 and ***significant p-value < 0.001 in comparison with control*

3. Changes in the levels of CAT, SOD, GPx, and MDA in liver tissues

In GE-treated rats, the levels of liver tissue SOD, CAT, and GPx showed no significant change with the control while the level of MDA appeared significantly lower (P <0.05) than the control. In CCl4-treated rats, the levels of SOD, CAT, and GPx appeared significantly lower (P <0.001) than control while the level of MDA appeared significantly higher than (P <0.001) control. In CCl4-treated rats post-supplemented with GE for consecutive 35 days, the levels of SOD, CAT, and GPx were successfully increased while the level of MDA significantly declined if compared with CCl4 treated group but still showed low significance (P <0.05) than the control (Figure 2).

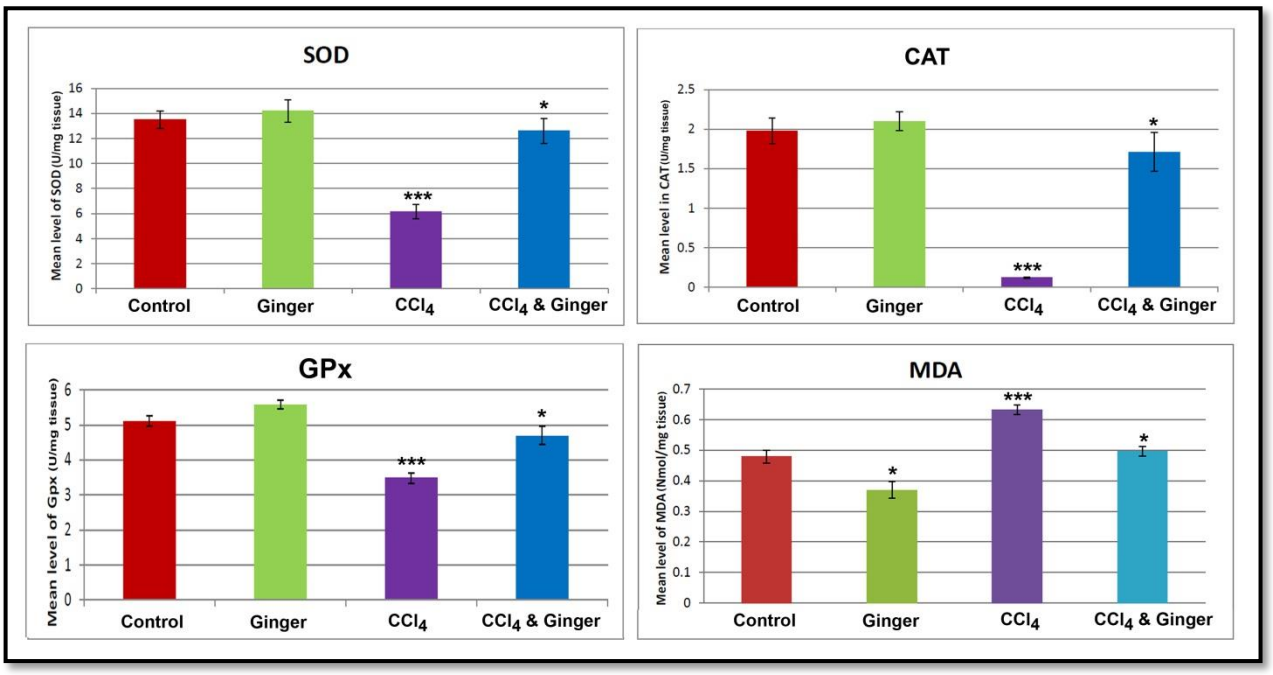

Fig.2: The mean levels of SOD, CAT, GPx, and MDA in the liver tissue among the different studied groups.

*Data are expressed as mean± standard error {n=8 per group} statistical analysis one way ANOVA followed by post hoc test means in the same row with different superscript (*) are significantly different when p<0.05 * significant at value <0.05, **significant at p- value<0.01 and ***significant p-value < 0.001 in comparison with control*

4. Histological observations

The liver sections from control and GE-supplemented rats appeared with normal histological architecture (Figure 3A&B). On the other hand, the liver section from CCl4-treated rats showed remarkable histopathological signs. These signs included congested central and hepatic portal veins, dilated blood sinusoids, pyknotic and vacuolated hepatocytes, excessive Kupffer cells, and infiltrated cells around the portal area (Figure 3C). In rats treated with CCl4 followed by treatment with GE, most of the liver histopathological signs caused by CCL4 markedly disappeared (Figure 3D).

5. Immunohistochemical observation of nuclear factor *kappa*-B (NF-κB).

The liver sections from control and GE-treated rats displayed negative to very weak reactions for NF-κB antibody (Figure 4A&B) however, a strong positive expression for NF-κB was noticed in the liver section from cCl4 treated rats (Figure 4C). On treatment with GE to CCL4-treated rats, the immune reactivity for NF-κB appeared moderately expressed (Figure 4D)

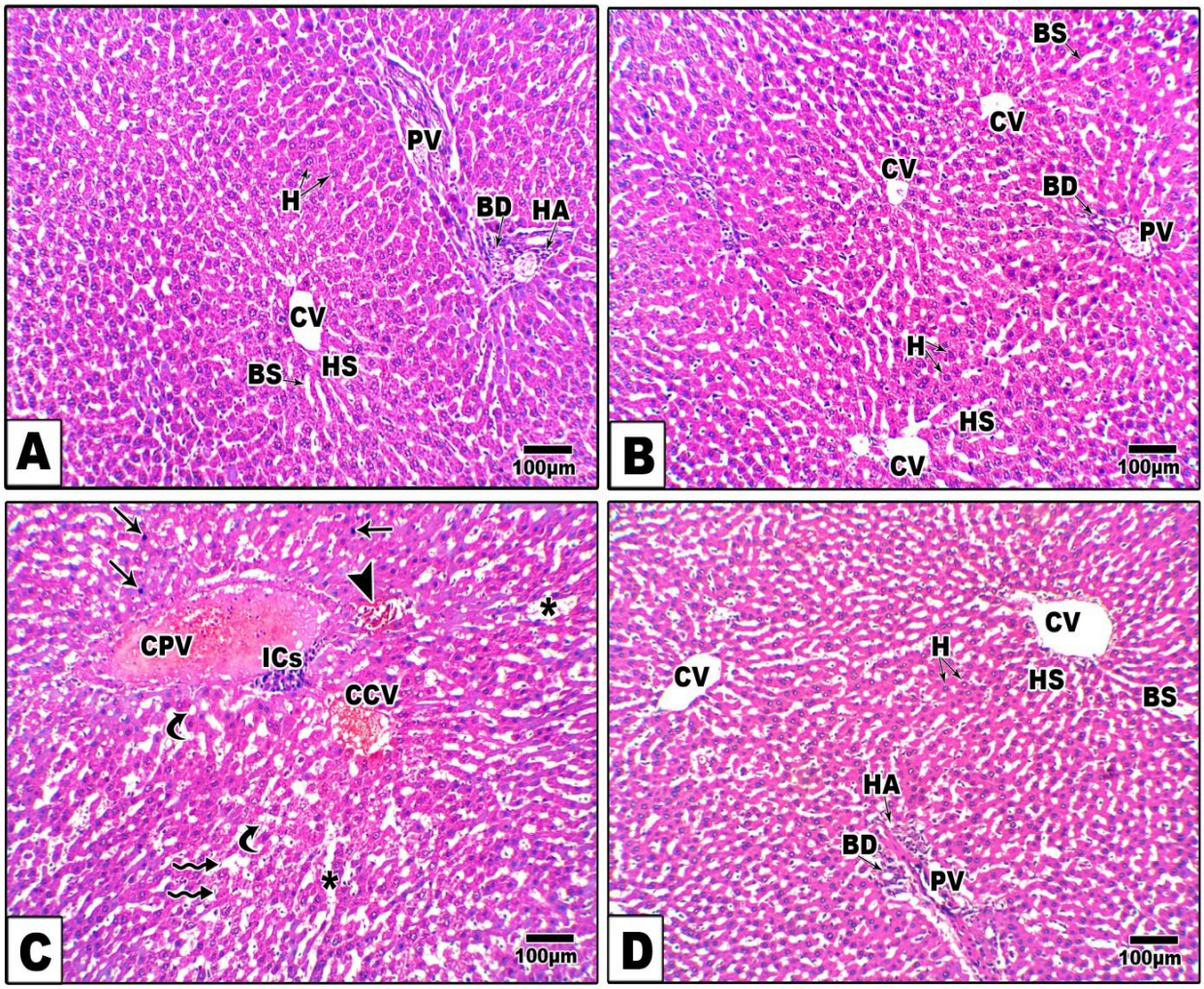

Fig.3: Photomicrograph of histological sections through the liver of the control group (image A), GE group (image B), CCl4-treated group (image C), and CCl4&GE group (image D). In the control and GE groups, the liver sections appear histologically normal. In CCl4-treated rats, the liver section showed congested portal, central veins, arteriole (arrowhead), pyknotic (straight arrows) and vacuolated (curved arrows) hepatocytes, dilated blood sinusoids(asterisks), excessive Kupffer cells (wavy arrows) In CCl4 &GE group of rats the liver sections showing remarkable amelioration that tend to be more or less as control. (*Stain: Hx&E Scale bar=100µm*).

Abbreviations: Blood sinusoids(BS), Bile ductule (BD), Central vein(CV), congested portal vein (CPV), congested central vein(CCV), Hepatocytes(H), Hepatic strands (HS), portal vein (PV), Hepatic arteriole(HA), and infiltrated cells (ICs).

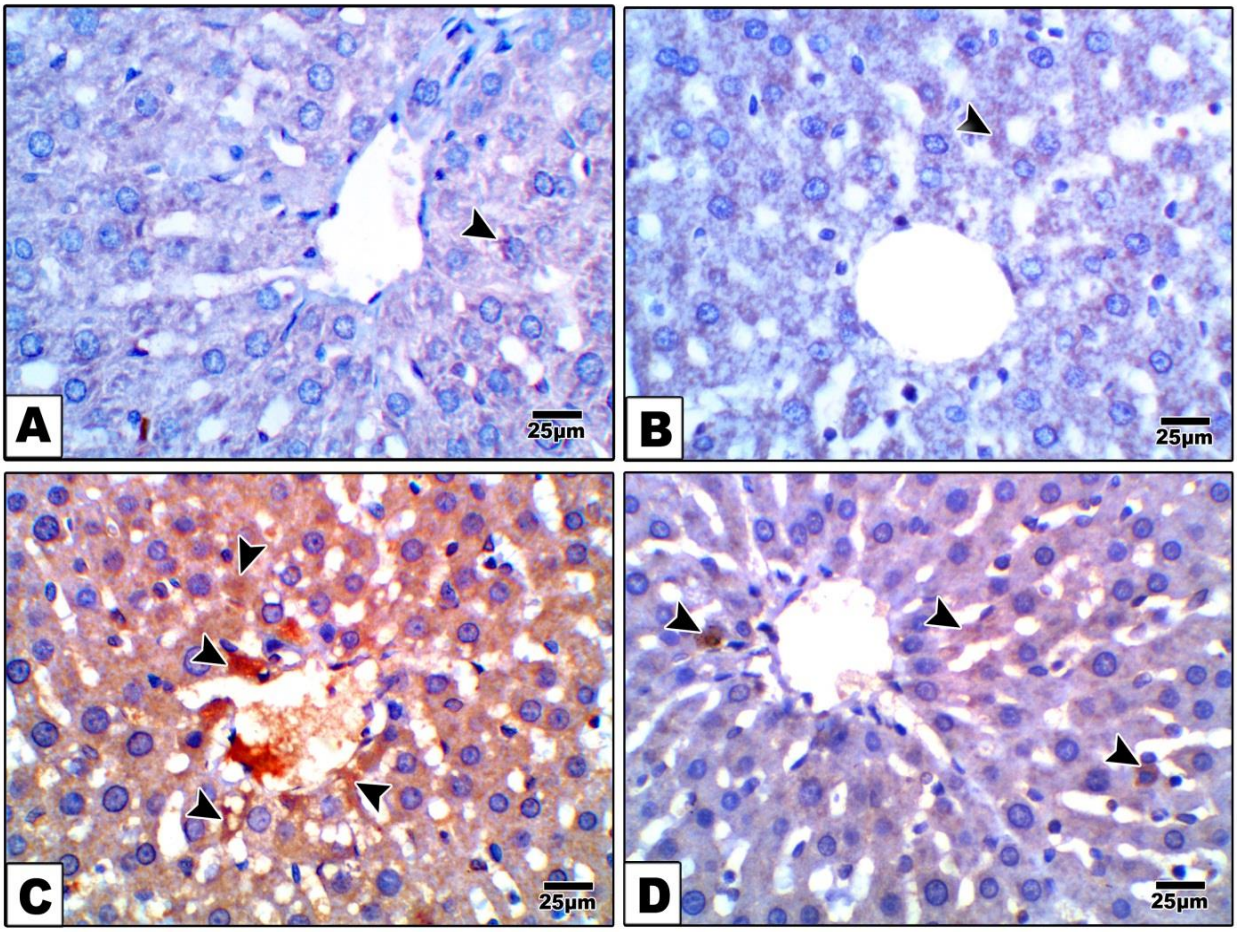

Fig.4: Photomicrograph of paraffin-embedded sections through the liver of control (Panel A**)**, GE (Panel B**)**, CCl4 (Panel **C),** and CCl_4 and GE (Panel D**)** rats stained with NF-κB antibody. In control and GE groups, the liver sections appear negatively or very weakly stained with NF-κB antibody however, in CCl4-treated rats, the liver section appears strongly stained. In CCl4 and GE-treated rats, the liver section showed moderate expression for NF-κB antibody. (*Stain: NF-κB antibody, Scale bar=25 µm*).

DISCUSSION

Several chemical agents are known to induce liver injury. It is believed that this chemicals-induced hepatic injury is especially accompanied by oxidative stress and imbalance between the production and elimination of free radicals **(Castro and Freeman, 2001**). Carbon tetrachloride (CCl4) is one of these chemicals that have a powerful hepatotoxic agent **(Manna et al., 2006; Botsoglou et al., 2008**). It is thought that dietary plants can protect tissues against the damaging effect of oxidative stress (**El-Neweshy et al., 2013; El-Sayed et al., 2015).** Accordingly, this study attempted to evaluate the ameliorative role of ginger extract against the hepatotoxicity induced by CCL4 in male rats.

The results of the present work revealed a remarkable significant decrease in the final body weight of CCL4-treated male rats, however on treatment with GE the body weight was restored near to the normal control. This result goes parallel with the finding of **Domenicali et al.(2009)** who recorded a significant reduction in body

weight and low food intake after inhalation of rats to CCL4 for one week. The reduction of the body weight in CCL4-treated rats might be in part attributed to CCL4-liberated metabolites which cause loss of appetite. The data are concerned with the obvious restoration of body weight after supplementation with ginger extract following **Mahmoud& Elnour (2013)** who declared that ginger extract has a powerful role in maintaining body weight through quick and easy assimilation of some nutrients found in the bloodstream and also through regulation of liver and renal functions.

The obtained results revealed a significant increase in the levels of serum liver enzymes, (AST, ALT, and ALP) as well as total bilirubin while a remarkably significant decrease in total protein and albumin in CCL4-toxicated rats. These results are in harmony with previous studies (**Ismail & Al-Nahari, 2009; Patrick-iwuanyanwu et al., 2010**). According to **Grajeda-Cota et al. (2004),** CCl4 is a hazardous chemical that can harm hepatic cell membranes, reducing their viability and increasing enzyme leakage as a result. Observations of the present study revealed that CCl4 induced a significant increase in the leakage of AST, ALT, and ALP enzymes into the serum. These enzymes are found in the cytoplasm of the cell and are released into the bloodstream when the cell membrane is disrupted (**Lin &Huang**, **2000**). Previously it was confirmed that the presence of excess enzymes outside the cell is considered a sign of hepatic cell damage (**Kamel et al., 2010)** and this is consistent with the obtained results of histopathological studies in this study. The data concerned with the significant increase of bilirubin level in CCL4-intoxicated rats go parallel with the finding of **Ojeaburu and Oriakhi (2021)** who found significant elevation in the level of bilirubin in rats treated with a single dose of CCL4.

Treatment with GE in CCL4-intoxicated rats significantly reduced the increased serum ALT, AST, and ALP activities when compared with CCL4-intoxicated rats. **Shati and Elsaid (2009)** recorded that, the water extracts of ginger have a potential role in amelioration of disrupted liver functions induced by CCl4. **Badr et al. (2016)** indicated that GE treatment leads to a significant reduction in sera ALT and AST activities in hepatic tumor-induced mice. Another similar study revealed that GE has a potential role in the reduction of liver enzymes elevated in lead-intoxicated rats **(Farag et al., 2010)**.

Oxidative stress markers like MDA increase because of excess liberation free radicals **(Khan et al., 2020)**. MDA is a pronounced stress marker responsible for liver damage. Tissue antioxidant levels are reduced under oxidative stress, and the increasing quantity of reactive oxygen species impairs membrane phospholipids **(Conde et al., 2022).** In this study, CCl4 intoxicated rats revealed a significant increase of MDA compared with control, however, MDA level was markedly declined on treatment with GE. **Ojeaburu and Oriakhi (2021)** reported a significant increase in the level of MDA in rats injected with a single dose of CCL4. Furthermore, studies have shown that CCl4 metabolites interact with polyunsaturated fatty acids to create covalent adducts with proteins and lipids, which set off a series of events that lead to lipid peroxidation, cell membrane breakdown, and liver damage (**Szymonik-Lesiuk et al., 2003)**. Similarly, there was a remarkable decline in the SOD, CAT, and GPx levels due to hepatic injury induced by CCl4. This may be related to the antioxidant enzymes being consumed as a result of the oxidative stress caused by CCl4 (**Szymonik-Lesiuk et al., 2003).**

In this study, the treatment with GE in CCL4-intoxicated rats significantly restored the levels of antioxidants enzymes near to the control value but did not rich to the ideal, whereas, the levels of liver tissues SOD, CAT, and GPx significantly elevated while the level of MDA significantly declined if compared with CCL4-intoxicated rats. A similar study reported by **Lebda et al. (2012)** revealed that ginger can decrease the elevated hepatic MDA level caused by paracetamol in rats. The results of this investigation imply that ginger may mitigate oxidative stress by reducing lipid peroxidation in the liver that has been treated with CCL4. This result concurred with the findings of **Sakr et al. (2011)** who

confirmed that ginger contains a higher concentration of flavonoids with potent antioxidant properties. The rats treated with ginger showed decreased MDA levels and elevated antioxidant enzymes, indicating a reduction in peroxidative damage. These results align with research suggesting that ginger may protect against liver toxicity caused by CCL4. This effect may be mediated by the free radicals scavenging activity of ginger (**Lamfon, 2011).**

In the current work, deleterious histological changes were recorded in the liver sections of CCL4-intoxicated rats. Such changes included congested hepatic veins, pyknotic and vacuolated hepatocytes, dilated blood sinusoids, and a relative increase in Kupffer cells. The obtained results go parallel with the findings of previous reports (**Ismail & Al-Nahari, 2009; Adewale et al., 2014)**. **Patrick-iwuanyanwu et al. (2010**) found that administration of CCl4 can cause deleterious alterations in the hepatic structural integrity. The process by which CCl4 can cause histological lesions in the liver through its metabolite, trichloromethyl radical (CCl3), was explained by **Weber et al. (2003).** To be more specific, CCL3 interacts with proteins, lipids, and nucleic acids to disrupt important cellular functions that lead to reduced protein levels and changed lipid metabolism (fatty degeneration). Additionally, CCL3 undergoes additional metabolism to produce trichloromethylperoxy radicals (CCl3OO•), which then cause polyunsaturated fatty acids to be destroyed by lipid peroxidation. Hence, there is reduced membrane permeability across cellular compartments and widespread, inflammatory liver damage. Furthermore, considerable liver cell damage was seen by **Prakash et al. (2008)** in rats given a single dosage of CCl4, and they linked this damage to elevated AST and ALT levels. **Sidhu et al. (2014)** added that hepatic necrosis and inflammation are mainly attributed to the liberation of enzymes from the cytoplasm to the serum.

Treatment of rats with GE post intoxication with CCl4 attenuated the histopathological changes induced by CCL4 in the liver. This finding agrees with the results of previous studies **(Patrick-Iwuanyanwu et al., 2007, Nasri et al., 20123).** The potential of ginger extract to prevent atrazine-induced liver and kidney damage in mice was investigated by **EL-Shenawy et al. (2011).** Their findings demonstrated that giving ginger extract intraperitoneally for two weeks while concurrently administering atrazine significantly reduced the levels of serum AST, ALT, ALP, and tissue lipid peroxide, hence improving the structural integrity of the liver. The antioxidant and therapeutic properties of ginger extract may be caused by its high concentrations of polyphenolic and flavonoid components **(Hassan et al., 2016; Danwilai et al., 2017).**

Conclusion

Based on our findings, treatment of male rats with carbon tetrachloride at a dose of 2 ml/kg b.wt twice/week for consecutive five weeks was implicated in the induction of oxidative stress on the liver via elevations of liver enzymes (ALT, AST, and ALP) and total bilirubin and reduction liver tissue antioxidants (SOD, CAT, and GPx). Post-treatment with ginger rhizome extract (200 mg/kg.b.wt) daily for consecutive 35 days successfully ameliorated the deleterious biochemical and histological changes induced by CCL4. These findings confirm the strong antioxidant, anti-inflammatory, and cytoprotective roles of ginger in CCL4-intoxicated rats. More work is needed to evaluate the mechanism of action of ginger extract.

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Conflict of interest

All authors declared that there were no conflicts of interest

REFERENCES

Adewale O.B, Adekeye A.O, Akintayo C.O, Onikanni A, Sabiu Saheed. (2014): Carbon tetrachloride (CCl_4)-induced hepatic damage in experimental Sprague Dawley rats: Antioxidant potential of Xylopia aethiopica.The Journal of Phytopharmacol. 3(2): 118-123

Aramjoo, H., Mohammadparast-Tabas, P., Farkhondeh, T., Zardast, M., Makhdoumi, M., Samarghandian, S., & Kiani, Z. (2022).

Protective effect of Sophora pachycarpa seed extract on carbon tetrachloride-induced toxicity in rats. BMC Complement Med Ther, 22(1), 76-88.

Attallah, N. G. M., Mokhtar, F. A., Elekhnawy, E., Heneidy, S. Z., Ahmed, E., Magdeldin, S., Negm, W. A., & El-Kadem, A. H. (2022). Mechanistic Insights on the In Vitro Antibacterial Activity and In Vivo Hepatoprotective Effects of Salvinia auriculata Aubl against Methotrexate-Induced Liver Injury. Pharmaceuticals (Basel), 15(5), 1-26.

Badr, M.O., Sakr, S.A., Abd-Eltawab, H.M. (2016). Ameliorative effect of ginger extract against pathological alterations induced in mice bearing solid tumors. Journal of Bioscience and Applied Research 2(3), 185-196.

Bancroft JD, Gamble M (2008): Theory and practice of histological techniques. Elsevier Health Sciences.

Botsoglou NA, Taitzoglou IA, Botsoglou E, Lavrentiadou SN, Kokoli AN, Roubies N. (2008): Effect of long-term dietary administration of oregano on the alleviation of carbon tetrachloride-induced oxidative stress in rats. J. Agric. Food Chem., 15: 6287-6293.

Castro, L. and Freeman, B. (2001): Reactive oxygen species in human health and disease. Nutrition, 17: 161–165.

Conde de la Rosa, L.; Goicoechea, L.; Torres, S.; Garcia-Ruiz, C.; Fernandez-Checa, J.C. (2022). Role of oxidative stress in liver disorders.Livers, 2, 283–314.

Danwilai K, Konmun J, Sripanidkulchai B, and Subongkot S, "Antioxidant activity of ginger extract as a daily supplement in cancer patients receiving adjuvant chemotherapy: a pilot study," Cancer Management and Research, vol. Volume 9, pp. 11 –18, 2017.

Domenicali M, Caraceni P, Giannone F, Baldassarre M, Lucchetti G, Quarta C.(2009): A novel model of CCl4-induced cirrhosis with ascites in the mouse. J Hepatol . 51(6): 991-999.

Doumas, B. T. Bayse D., Borner K., Cart R. J., Peters T. , Schaffer R.(1981). "A Candidate Reference Method for the Determination of Total Protein in Serum: Development and Validation," Clinical Chemistry, Vol. 27, No. 10, pp. 1642-1650.

El-Neweshy MS, El-Maddawy ZK, El-Sayed YS. (2013). Therapeutic effects of date palm (Phoenix dactylifera L.) pollen extract on cadmium-induced testicular toxicity. Andrologia 45:369–78.

El-Sayed YS, Lebda MA, Hassinin M, Neoman SA. (2015). Chicory (Cichorium intybus L.) root extract regulates the oxidative status and antioxidant gene transcripts in CCl4-induced hepatotoxicity. PLoS One 10:e0121549

El-Sharaky AS, Newairy AA, Newairy AA, Kamel MA, Eweda SM.(2009). Protective effect of ginger extract against bromobenzene-induced hepatotoxicity in male rats. Food Chem Toxicol., 47: 1584-1590.

EL-ShenawyNS, El-Ahmary B, A. Al-Eisa R. (2011). Mitigating Effect of Ginger against Oxidative Stress Induced by Atrazine Herbicides in Mice Liver and Kidney. J Biofertil Biopestici.2 (2):1-7.

Farag AGA, Elhalwagy MEA, Farid HEA. (2010). Effect of ginger supplementation on developmental toxicity induced by fenitrothion insecticide and/or lead in albino rats. Pestic Biochem Physiol 97: 267–74.

Grajeda-Cota, P.; Ramírez-Mares, M. V. and González de Mejía, E. (2004). Vitamin C protects against in vitro cytotoxicity of cypermethrin in rat hepatocytes. Toxicology in Vitro, 18: 13–19.

Gustafsson J. E. C., (1976):"Improved Specificity of Serum Albumin Determination and Estimation of Acute Phase Reactant by the Use of Bromocresol Green Reaction," Clinical Chemistry, Vol. 22, No. 5, pp. 616-622.

Hasan IH, **El-Desouky MA, Hozayen WG, Abd el Aziz GM. (2016).** Protective Effect of Zingiber

Officinale against CCl4-Induced Liver Fibrosis Is Mediated through Downregulating the TGF-β1/Smad3 and NF-κB/IκB Pathways. Pharmacology.97(1-2):1-9.

Ismail H A., Al-Nahari H Y. (2009): The Protective Effect of Ginger (Zingiber Officinale) On Some Biochemical Parameters In Rats. Egypt. J. Exp. Biol. (Zool.), 5: 411 – 417.

Kakkar PS, Das B, Viswanathan PNA (1984): Modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys, 21:130–132**.**

Kamel, H H., Abd-El-Rahman A H, Ahmed WMS, Mohamed A H. (2010). Protective Effect of Some Antioxidants against Ccl4-Induced Toxicity in Liver Cells from BRL3A Cell Line. J. of Americ. Sci.,6(10):992-1003.

Kamtchoving P, Mbongue GY, Fndio T, Dimo H, Jatsa B. (2002). Evaluation of androgenic activity of *Zingiber officinale* and *Pentadiplandra brazzeana* in male rats. Asian J Androl 4 (4): 299-301.

Kermanizadeh, A., Valli, J., Sanchez, K., Hutter, S., Pawlowska, A., Whyte, G., Moritz, W., & Stone, V. (2022). Particulate and drug-induced toxicity assessed in novel quadruple cell human primary hepatic disease models of steatosis and pre-fibrotic NASH. Arch Toxicol, 96(1), 287-303.

Khan, S.; Rahman, M.M.; Kabir, F.; Nahar, K.; Mamun, F.; Lasker, S.; Subhan, N.; Hossain, M.H.; Nahar, L.; Sarker, S.D.; et al. (2020). Trichosanthes dioica Roxb. prevents hepatic inflammation and fibrosis in CCl4-induced ovariectomized rats. Clin. Nutr. Exp. 33, 1–17.

Lamfon, H.A. (2011). Protective effect of ginger (Zingiber officinale) against metalaxyl induced hepatotoxicity in albino mice. Journal of American Science 7(6), 1093- 1100.

Lebda, M.A., Taha, N.M., Korshom, M.A., Mandour, A.A., EL- Morshedy, A.M. 2012. Biochemical effect of ginger on some blood and liver parameters in male Newzeland rabbits. Online Journal of Animal and Feed Research 2, 197-202

Lee S. H. Cekanova M. Baek S. J. Multiple mechanisms are involved in 6-gingerol-induced cell growth arrest and apoptosis in human colorectal cancer cells. Mol. Carcinog. 2008;47(3):197–208.

Li H, Liu Y, Luo D, Ma Y, Zhang J, Li M, Yao L, Shi X, Liu X, Yang K. 2019. Ginger for health care: an overview of systematic reviews. Complement Ther Med. 201945:114-123.

Lin CC, Huang PC. (2000): Antioxidant and hepatoprotective effects of Acathopanax senticosus. Phytother. Res., 14:489-494.

Mahmoud R.H., Elnour W.A. (2013): Comparative evaluation of the efficacy of ginger and orlistat on obesity management, pancreatic lipase and liver peroxisomal catalase enzyme in male albino rats European Review for Medical and Pharmacological Sciences17: 75-83.

Manna P, Sinha M, Sil PC. (2006): Aqueous extract of Terminalia arjuna prevents carbon tetrachloride induced hepatic and renal disorders. BMC. Complement. Altern. Med., 6: 33.

Masuda Y. Kikuzaki H. Hisamoto M. Nakatani N.(2004). Antioxidant properties of gingerol related compounds from ginger. BioFactors. 2004;21(1–4):293–296.

Nasri H, Mortazavi M, Ghorbani Aet al.(2012). "Oxford-MEST classification in IgA nephropathy patients: a report from Iran, " Journal of nephropathology, 1(1)31:42.

Nasri H, Nematbakhsh M, Ghobadi S, Ansari R, Shahinfard N, RafieianKopaei M .(2013). Preventive and curative effects of ginger extract against histopathologic changes of gentamicin-induced tubular toxicity in rats. Int. J. Prev. Med. 4(3):316-321.

Ohkawa H, Ohishi N, Yagi K. (1979): Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem, 95:351–358.

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

Paglia D. E. and Valentine W. N. J. (1967): Lab. Clin. Med., 70, 158–169.

Park GH, Park JH, Song HM, Eo HJ, Kim MK, Lee JW, Lee MH, Cho KH, Lee JR, Cho HJ, Jeong JB.(2014). Anti-cancer activity of Ginger (Zingiber officinale) leaf through the expression of activating transcription factor 3 in human colorectal cancer cells, BMC Complement, BMC Complementary Altern. Med., 14, 8.

Patrick-iwuanyanwu KC, Wegwu MO, Okiyi JK. (2010): Hepatoprotective effects of African locust bean and negro pepper in CCl4 induced liver damage on Wistar albino rats. Int.J. Pharmacol., 6(5):744-749**.**

Patrick-Iwuanyanwu KC, Wegwu MO, Ayalogu EO. (2007): Prevention of CCl4-induced liver damage by ginger, and vitamin E. Pak. J. Biol. Sci., 4: 617-621.

Popoola, A. B., Ademilusi, E. O., Adedeji, T. G., & Fasanmade, A. A. (2022). Effect of silymarin on blood coagulation profile and osmotic fragility in carbon tetrachloride induced hepatotoxicity in male Wistar rats. Toxicology Reports, 9, 1325-1330.

Prakash O, Singh GN, Singh RM, Mathur SC, Bajpai M, Yadav S. (2008): Protective effects of herbal formula against carbon tetrachloride-induced hepatoxicity. Int.J. Pharmacol., 4: 282-286.

Sakr, S.A., Hawazen, L.A., Amina, E.E. (2011). Ginger (Zingiber officinale) extract ameliorates metalaxyl fungicide induced nephrotoxicity in albino mice. African Journal of Pharmacy and Pharmacology 5(2), 104-112.

Shati AA, Elsaid FG. (2009): Effects of water extracts of thyme (Thymus vulgaris) and ginger (Zingiber officinale Roscoe) on alcohol abuse.Food Chem.Toxicol. 47(8):1945-1949.

Sidhu, I.P.S., Bhatti, J.S., Bhatti, G.K. (2014). Modulatory action of melatonin against chlorpyrifos induced hepatotoxicity in Wistar rats. Asian J Med Sci 2, 123-131.

Steven, C. K.(1996): "Alanine and Aspartate Aminotransferase, Principle and Usage," 3rd Edition, In: J. T. James and R. Jenifer, Eds., Liver Function, in Clinical Chemistry Theory, Analysis and Correlation, M. Mosby, London: 504-527.

Szymonik-Lesiuk S, Czechowska G, Stryjecka-Zimmer M, Słomka M, Madro A, Celiński K, Wielosz M.(2003). Catalase, superoxide dismutase, and glutathione peroxidase activities in various rat tissues after carbon tetrachloride intoxication. J Hepatobiliary Pancreat Surg. 10(4):309-15.

Thomson M, Corbin R, Leung. L **2014**. Effects of ginger for nausea and vomiting in early pregnancy: a metal-analysis, J. Am. Board Fam. Med., 2014, 27(1), 115–122

Verma S. K. Singh M. Jain P. Bordia A. (2004). Protective effect of ginger, *Zingiber officinale* Rosc. on experimental atherosclerosis in rabbits. Indian J. Exp. Biol. 2004;42 (7):736–738.

Weber L.W and Boll, A. 2003. Stamp, Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model, CRC Crit. Rev. Toxicol. 33105–136.

Young H. Y. Luo Y. L. Cheng H. Y. Hsieh W. C. Liao J. C. Peng W. H.(2005). Analgesic and anti-inflammatory activities of [6]-gingerol. J. Ethnopharmacol.;96 (1–2):207–210.

Zheng, W. V., Li, Y., Cheng, X., Xu, Y., Zhou, T., Li, D., Xiong, Y., Wang, S., & Chen, Z. (2022). Uridine alleviates carbon tetrachloride-induced liver fibrosis by regulating the activity of liver-related cells. J Cell Mol Med, 26(3), 840-854.