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Research Article
Protective Effect of *Cucumis sativus* on Carbon Tetrachloride CCl_4-induced Liver Damage in Rats

Uzuazokaro Mark-Maria Agatemor, Okwesili Fred Chiletugo Nwodo, Idakwoji Precious Adejoh and Chioma Assumpta Anosike

Department of Biochemistry, University of Nigeria, Nsukka, Enugu State, Nigeria

Abstract

Background and Objective: Research on liver diseases have been on increase and have become a global concern. *Cucumis sativus* has been reported to have anti-oxidant activity, high flavonoid content, anti-inflammatory and analgesic activity and may likely of help in the management of liver disease. The hepatoprotective effect of the homogenate of *Cucumis sativus* fruit were therefore studied and histopathological assessment of the liver damage was done. **Materials and Methods:** The fresh fruit of *Cucumis sativus* was homogenized and used for all experimental analysis. The effects of the fruit homogenate on liver function biomarkers and lipid profile in rats intoxicated with carbon tetrachloride (CCl_4) were evaluated using standard biochemical methods. Data were analyzed using SPSS and two-way ANOVA; the acceptance level of significance was $p<0.05$. **Results:** Treatment of rats with the homogenate of *Cucumis sativus* fruits significantly ($p<0.05$) decreased CCl_4-induced elevated levels of the liver enzymes and total bilirubin in the serum when compared to positive control. The homogenate also attenuated the CCl_4-induced elevation of LDL, total cholesterol and triacylglycerol concentration and ameliorated the induced depletion of HDL. Pre-treatment with *Cucumis sativus* fruit homogenate significantly improved the structure of hepatic cells. The homogenate showed a sign of liver cell protection comparable to that of the standard drug-silymarin. **Conclusion:** The results of this study demonstrated that the homogenate was effective in the prevention of CCl_4-induced hepatic damage in rats and may improve the liver integrity of consumers.

Key words: *Cucumis sativus*, liver damage, carbon tetrachloride, fruit homogenate, drug-silymarin

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Corresponding Author: Uzuazokaro Mark-Maria Agatemor, Department of Biochemistry, University of Nigeria, Nsukka, Enugu State, Nigeria

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The liver plays a pivotal role in regulating various physiological processes. It is also involved in several vital functions, such as metabolism, secretion and storage. It has great capacity to detoxify toxic substances and synthesize useful principle[1]. It helps in the maintenance, performance and regulation of homeostasis in the body. It is involved in almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction. It aids metabolism of carbohydrate, protein and fat, detoxification, secretion of bile and storage of vitamins[2]. The role played by the organ in the removal of substances from the portal circulation makes it susceptible to first and persistent attack by offending foreign compounds, culminating in liver dysfunction. These hepatotoxic agents activate some enzyme activities in the cytochrome P-450 system such as CYP2E1 leading to oxidative stress[3]. Injury to hepatocyte and bile duct cells lead to accumulation of bile acid inside liver. This promotes further liver damage.

Cucumis sativus (Cucumber) is a widely cultivated plant in the gourd family of Cucurbitaceae, which also includes important crops such as melon, water melon and squash[4]. Cucumber (*Cucumis sativus)* is used by native people to cure many illnesses in some countries. In Africa, ripe raw cucumber fruits are used as a cure for sprue, a disease that causes flattering of the villi and inflammation of the lining of the small intestine and in Indo China, cooked immature fruits are used to treat dysentery in children[5]. It is also useful in fighting constipation, as the fibre content helps to overcome the hypotony which is the cause of constipation[5]. Swapnil *et al.*[6] reported the use of *Cucumis sativus* in the treatment of patients with high blood pressure and with irritated skin as a result of sun burn. *Cucumis sativus* fruit has been reported to have anti-oxidant activity, high flavonoid content, anti-inflammatory and analgesic effect[7-9]. This study was aimed to assess the effect of the homogenate of *Cucumis sativus* fruit on CCl_4-induced hepatotoxicity in rats so as to know its efficacy in the management of liver diseases.

MATERIALS AND METHODS

Plants materials: *Cucumis sativus* fruits were obtained from Nsukka main market, Nsukka, Enugu State, Nigeria and were identified by Mr. Alfred Ozioko of Bioresources Development and Conservation Programme (BDCP) Research Centre, Nsukka, Enugu. The fruit of *Cucumis sativus* was homogenized (daily before administration) with Kenwood high speed blender and used without further dilution.

Animals: Wistar albino rats (120-200 g) were purchased from the Animal House of the Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. The animals were acclimatized to laboratory condition for 7 days before the experiments and maintained *ad libitum* on water and Grower's mash rat pellets (Pfizer Feeds, Aba) bought from Nsukka market. The guide for the care and use of laboratory animals procedures were followed in this study.

Experimental design: Thirty six Wistar albino rats acclimatized to laboratory condition were randomly divided into six groups of six animals each. They were maintained under optimal atmospheric and hygienic conditions and allowed access to both feed and water *ad libitum*.

Induction of hepatic damage: Hepatic damage was induced in the rats by the administration of CCl_4 at the dose of 1.5 ml/kg body weight (b.w.) in olive oil (1:1) every 72 h for 10 days. Details are as follow:

- Group 1 = Olive oil (5 mL kg^{-1} b.w.) only
- Group 2 = CCl_4 treated (1.5 mL kg^{-1} b.w.) only
- Group 3 = *Cucumis sativus* fruits homogenate (2 mL kg^{-1} b.w) and CCl_4 (1.5 mL kg^{-1} b.w.)
- Group 4 = *Cucumis sativus* fruits homogenate (4 mL kg^{-1} b.w) and CCl_4 (1.5 mL kg^{-1} b.w.)
- Group 5 = Standard drug-Silymarin (100 mg kg^{-1}) and CCl_4 (1.5 mL kg^{-1} b.w.)
- Group 6 = *Cucumis sativus* fruits homogenate (4 mL kg^{-1} b.w.) only

At the end of the treatment period, all the animals were fasted for 18 h, then blood samples were collected into centrifuge tubes (non-heparinised sample bottles) through rectobulba plexus in the eye. Each blood sample was allowed to clot and the serum obtained by centrifugation at 3000 rpm for 10 min. The clear serum obtained as the supernatant was then carefully aspirated with syringe and needle and used freshly for the assessment of some biochemical and liver function tests.

Assessment of liver damage: Liver damage was assessed by the estimation of serum activities of ALT and AST, using the

method of Reitman and Frankel[10] and ALP using the method of Babson *et al.*[11], serum concentration of bilirubin was estimated using the method of Jendrassik and Grof[12], total cholesterol by the method of Abell *et al.*[13], HDL by the method of Rao *et al.*[14], triacylglycerol by the method of Tietz[15] and LDL using the method of Assmann *et al.*[16]. These assays were done by using commercial kit (Randox, UK) in the experimental animals. Histopathological assessment of the liver damage was done using the method of Culling[17] by studying haematoxylin and eosin stained slides of liver tissue. Histopathological changes such as fatty changes, necrosis, vacuole, space formation and loss of cell boundaries were observed under a microscopy.

Ethical approval: All experimental protocols including the use of animal models were approved and followed the guidelines of the Faculty of Biological Sciences Ethical Committee of the University of Nigeria, Nsukka, Nigeria.

Statistical analysis: Data obtained were analyzed using Statistical Product and Service Solutions (SPSS), IBM version 20. The values were expressed as Mean±standard error of mean (SEM). Statistical analysis was performed by one way analysis of variance (ANOVA) followed by least significance difference (LSD) multiple comparison test and the acceptance level of significant was $p<0.05$ for all results. Differences between means were assessed by Duncan's test.

RESULTS

The serum biochemical parameters of the experimental groups are presented in Table 1 and 2. Rats treated with CCl_4 only showed a hepatic damage as observed by the increase level of hepato-specific enzymes as well as severe alteration in different liver parameters. There was significant increase in the serum ALT, AST, ALP, total bilirubin, LDL, total cholesterol, triacylglycerol and significant reduction in the level of serum HDL in CCl_4-intoxicated groups compared to group 1 animals.

In this study, results in Table 1 showed CCl_4 administration produced significant elevations of serum ALT, AST and ALP in other groups compared to the group 1 (normal control). However, pre-treatment of rats with 2 and 4 mL kg^{-1} b.w. of *Cucumis sativus* L. homogenate significantly decreased ALT levels in group 3 and group 4 respectively when compared to group 2, treated with CCl_4 only. While there were no significant reductions in AST and ALP enzyme activities in the group 3 pre-treated with 2 mL kg^{-1} b.w., of *Cucumis sativus* fruit homogenate, group 4 pre-treated with 4 mL kg^{-1} b.w., of the fruit homogenate gave significant decrease in AST and ALP activities as compared to CCl_4 treatment group 2.

Per oral (p.o.) administration of CCl_4 in olive oil to group 2 rats caused the concentration of total bilirubin, cholesterol, triacylglycerol and LDL to increase ($p<0.05$) significantly with respect to control group 1 as showed in Table 2. In the group 3, treated with 2 mL kg^{-1} b.w., of the homogenate before CCl_4 administration, total bilirubin, cholesterol, TAG and LDL concentration decreased significantly relative to group 2, CCl_4 treated group while there was significant elevation in HDL concentration as compared with the CCl_4 treatment group 2. Treatment of rats with 4 mL kg^{-1} b.w., of the fruit homogenate and silymarin (Standard drug) decreased the concentration of total bilirubin, cholesterol, TAG and LDL significantly ($p<0.05$) and increased the concentration of HDL significantly ($p<0.05$) relative to CCl_4 treatment group 2. These effects were doses

Table 1: Effect of *Cucumis sativus* on the liver function biomarkers of rat intoxicated with CCl_4

Group	ALT (IU L^{-1})	AST (IU L^{-1})	ALP (IU L^{-1})	TB (mg dL^{-1})
Group 1	41.00±0.71	21.00±1.15	45.84±0.42	4.13±0.34
Group 2	67.25±0.48*	40.00±2.61*	54.17±1.22*	8.00±0.53**
Group 3	50.75±1.44	38.75±3.42	51.99±0.94	5.80±0.44
Group 4	42.25±2.14	32.50±0.65	47.67±0.36	5.65±0.71
Group 5	42.75±1.65	31.75±1.65	47.03±0.19	5.43±0.37
Group 6	42.50±1.66	23.25±0.63	47.33±1.44	3.53±0.80

*p<0.01, **p<0.05, significantly different from Group 1, ALT: Alanine amino transferase, AST: Aspartate amino transferase, ALP: Alkaline phosphatase, TB: Total bilirubin

Table 2: Effect of *Cucumis sativus* on the lipid profile of rat intoxicated with CCl_4

Group	CHOL (mmol L^{-1})	TAG (mmol L^{-1})	HDL (mmol L^{-1})	LDL (mmol L^{-1})
Group 1	3.15±0.32	1.36±0.12	1.74±0.17	0.79±0.27
Group 2	6.93±0.48*	1.67±0.07	0.72±0.07*	5.45±0.55*
Group 3	4.73±0.09	1.49±0.07	1.56±0.09	2.49±0.16
Group 4	4.41±0.35	1.39±0.11	1.62±0.14	2.16±0.30
Group 5	4.17±0.16	1.32±0.22	1.57±0.15	2.00±0.20
Group 6	3.39±0.19	1.29±0.06	1.96±0.11	0.84±0.30

*p<0.01, significantly different from Group 1, TAG: Total cholesterol triacylglycerol, HDL: High density lipoprotein, LDL: Low density lipoprotein, CHOL: Cholesterol

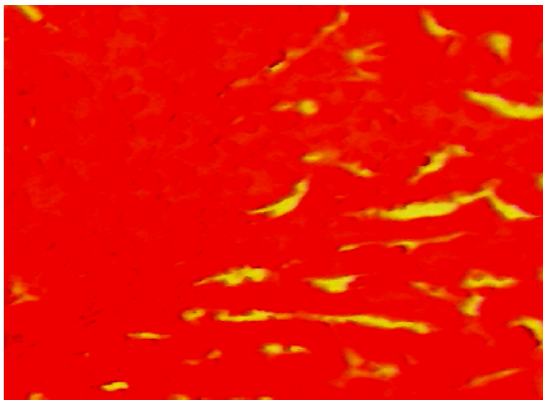

Plate 1: Photomicrograph of sections of liver administered with 5 mL kg^{-1} b.wt. olive oil (Group 1; normal control)
H and E X400

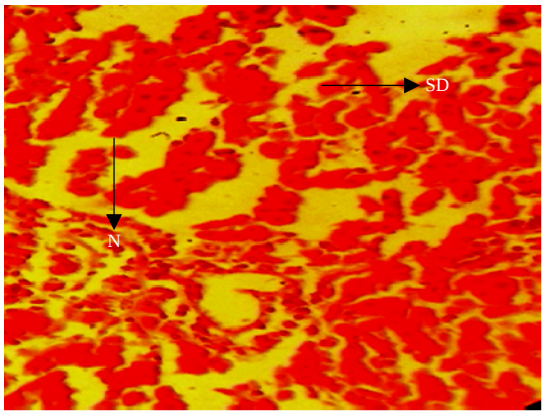

Plate 2: Photomicrograph of sections of liver from rats treated with 1.5 mL kg^{-1} b.w. of CCl_4 with severe centrilobular necrosis (N), cell infiltration, mild sinusoidal dilatation (SD) and minimal degree of lobular disarray with multiple spotty pyknosis
H and E X400

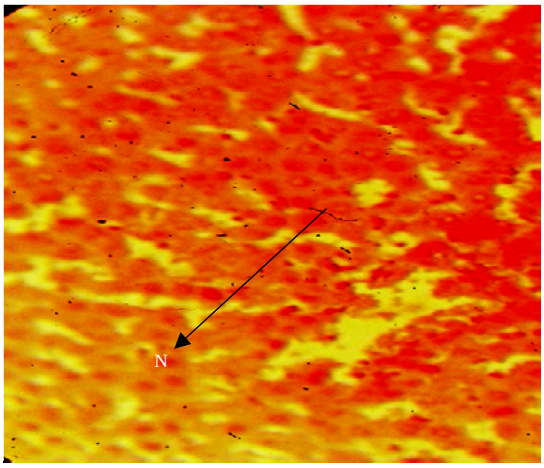

Plate 3: Photomicrograph of sections of liver from rats administered with 2 mL kg^{-1} b.w. of the homogenate of Cucumis sativus fruit and 1.5 mL kg^{-1} of CCl_4 showing severe to mild necrosis (N), moderate degree of cell infiltration.
H and E X400

dependent as pre-treatment with 4 mL kg^{-1} b.w., decreased the concentration of total bilirubin, cholesterol, TAG and LDL and elevated the concentration of HDL further relative to that of group 3 treated with 2 mL kg^{-1} b.w., group 6, treated with 4 mL kg^{-1} b.w., of *Cucumis sativus* homogenate only did not produce any significant ($p>0.05$) change in total bilirubin, cholesterol, TAG, LDL and HDL concentrations when compared with normal control group 1. From these results the degree of protection observed was maximum with higher dose of the homogenate of *Cucumis sativus* L. (4 mL kg^{-1} p.o.). These effects were doses dependent as pre-treatment with 4 mL kg^{-1} b.w., decreased the activity of ALT, AST and ALP and the concentration of total bilirubin, cholesterol, TAG and LDL and elevated the concentration of HDL further relative to that group which treated with 2 mL kg^{-1} b.w., if it is significant that per se (Group 6, only homogenate treated), the homogenate did not produce any significant ($p>0.05$) change in ALT, AST, ALP activities and total bilirubin, cholesterol, TAG, LDL and HDL concentrations when compared with normal control group. From these results the degree of protection observed was maximum with higher dose of the homogenate of *Cucumis sativus* L (4 mL kg^{-1}. p.o.).

The photomicrograph of the liver of the rats in group 1 treated with only olive oil (Plate 1) showed intact and normal liver architecture. Reference to Plate 2 Result obtained for liver section of CCl4 treated rats revealed severe centrilobular necrosis with sinusoidal dilatation (SD), multiple spotty pyknosis and severe cell infiltration when compared to that of the control (Plate 2). The photomicrograph of liver of rats treated with silymarin, group 5 and CCl_4 revealed protective normal liver architecture (Plate 3 and 4, respectively). The liver section of group 6 rats that received only the fruit effect on the liver tissue (Plate 5). Similarly, pre-treatment with Cucumis sativus homogenate 2 and 4 mL kg^{-1} b.wt. showed homogenate, 4 mL kg^{-1} b.wt. was not different from that of the control. It also showed normal liver architecture (Plate 6).

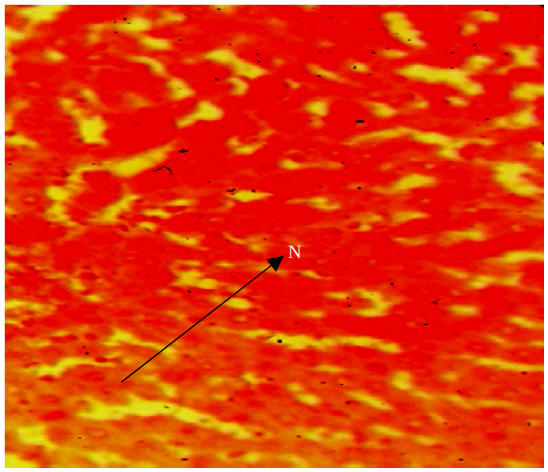

Plate 4: Photomicrograph of sections from rats administered with 4 mL kg^{-1} b.wt. of the homogenate of Cucumis sativus fruit and 1.5 mL kg^{-1} of CCl_4 showing mild centrilobular necrosis (N) and minimal degree of cell infiltration
H and E X400

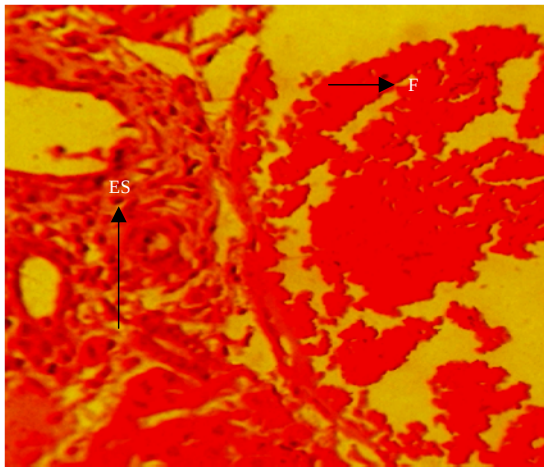

Plate 5: Photomicrograph of sections from rats treated with 100 mg kg^{-1} of silymarin and 1.5 mL of CCl_4 showing near normalization of fatty accumulation (F), eosinophilia secretion (ES) and mild cell infiltration
H and E X400

DISCUSSION

Fruits in the daily diet have been strongly associated with reduced risk for some forms of cancer, heart disease, stroke and other chronic diseases[18-19]. In this study, the effects of the homogenate of *Cucumis sativus* fruits on the liver integrity

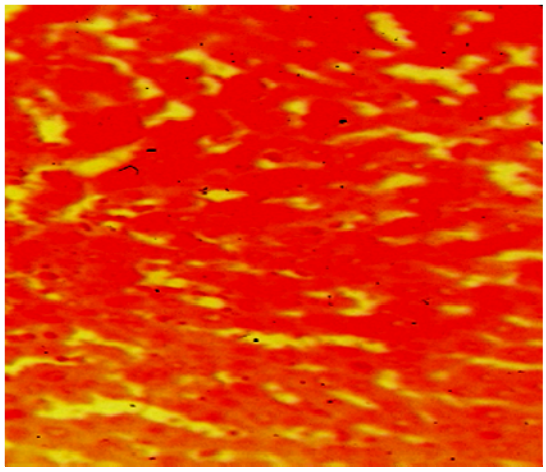

Plate 6: Photomicrograph of sections from rats administered with 4 mL kg^{-1} b.wt. of homogenate Cucumis sativus fruit showing no severe pathological change
H and E X400

were investigated using CCl_4-induced hepatotoxicity in rat models. The study revealed some pharmacological potential of *Cucumis sativus* fruits in management of liver diseases, thus improving the integrity of the liver. Carbon tetrachloride (CCl_4) challenge caused a marked rise in the serum levels of the liver enzymes alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP) of the rats used in this study demonstrating severe hepatic damage. It also caused elevated levels of total bilirubin, serum low density lipoprotein (LDL), total cholesterol, triacylglycerol (TAG) and decreased level of serum high density lipoprotein (HDL), demonstrating oxidative stress. The treatment of the animals with the homogenate of *Cucumis sativus* fruits decreased the CCl_4 induced elevated levels of the liver enzymes and total bilirubin in the serum suggesting that *Cucumis sativus* possesses anti-hepatotoxic and liver protective activities.

Bilirubin, a major breakdown product of haemoglobin rises when there is liver injury or damage leading to the discolouration of the skin known as jaundice. Elevation of total bilirubin which results from decreased uptake of and conjugation of bilirubin by the liver is caused by liver cell dysfunction which is as a result of decreased secretion from the liver[20]. Reduction of CCl_4 induced elevated total bilirubin by the homogenate of *Cucumis sativus* fruit showed a protective effect against CCl_4 induced liver toxicity. This fruit perhaps protect the liver by enhancing bilirubin uptake and conjugation by the liver and subsequent secretion into the bile ducts. The *Cucumis sativus* fruit homogenate also

assuaged the CCl_4-induced elevated levels of low density lipoprotein, total cholesterol and triacylglycerol and ameliorated the induced depletion of high density lipoprotein. CCl_4 is a well-established hepatotoxin; inducing liver injury by producing free radicals. It is activated by phase II detoxifying enzymes in liver cell endoplasmic reticulum to form trichloromethyl and peroxy trichloromethyl free radicals[21]. CCl_4-induced liver inflammation and damage can result in locally increased production of free radicals by inflammatory enzymes, as well as the release of inflammatory mediators.

Studies have shown that certain plants with antioxidants activity protect against the CCl_4-induced inflammation and impairment in hepatic function[22-23]. The efficacy of any hepatoprotective drug is essentially dependent on its capability of either reducing the harmful effects of a hepatotoxin or of maintaining the normal physiological mechanism that are unbalanced by a hepatotoxin[24]. The presence of phenolic, flavonoids and anthocyanin in the fruit homogenate of *Cucumis sativus*[9], explains its role in hepatoprotection by inhibiting the free radicals mediated damage[25]. Takeota and Dao[26] reported that flavonoids are antioxidant agents that interfere with free radical formation. The mechanism of action of flavonoids involve suppression of a wide range of reactive oxygen, nitrogen and chlorine species formation by inhibition of enzymes or by chelating trace elements involved in free radical production[27-28]. Food and fruits rich in flavonoids and other phenolic compounds have been associated with decreased risk of developing inflammatory and other related diseases[29]. Though, it is uncertain whether this protective effect is attributable to the phenols or to other agents in the diet[30], considerable data indicated that increased oxidative damage is associated with and may contribute to the development of all major age-related diseases and it has been logical to attribute the alleged protective effects of flavonoids to their antioxidant ability[31]. By extension, these effects are attributable to *Cucumis sativus* homogenate. The observations in this study correlate the earlier reports by Gopalakrishan and Kalairasi[32], that *Cucumis sativus* has significant hepatoprotective effect on paracetamol-induced hepatotoxicity. Dhande *et al.*[33] also reported the anti-hepatotoxic potential of *Cucumis sativus* on CCl_4 induced hepatotoxicity.

In this study, the homogenate of *Cucumis sativus* fruit attenuated the CCl_4-induced elevated levels of low density lipoprotein (LDL), total cholesterol and triacylglycerol and increased the level of high density lipoprotein (HDL). The reduction in total serum cholesterol and LDL observed after the administration of the homogenate of *Cucumis sativus* fruit could be attributed to its antioxidant properties, as was reported in our earlier study[9].

Han *et al.*[34] reported the presence of phytosterol in *Cucumis sativus* fruit. Phytosterols have significant hypocholesterolemic effect and cholesterol-lowering potentials[35]. Therefore, the cholesterol-lowering effect of *Cucumis sativus* fruits homogenate could also be attributed also the presence of phytosterols. Reduction of low density lipoprotein concentration may be due to the antioxidant property of *Cucumis sativus*. Antioxidants prevent LDL peroxidation and retard the accumulation[36], thereby decreasing the risk of DNA oxidative damage through lipid peroxidation. The LDL oxidation causes accumulation of fat within the artery walls, thereby clogging up the arteries and increasing the risk of atherosclerosis and cardiovascular diseases[37]. Balanced cholesterol level reduces the incidence of LDL oxidation and the associated risk of atherosclerosis and other related heart diseases. Liver injury causes the accumulation of abnormal amounts of fats, predominantly triacylglycerol in the parenchymal cells into the systemic circulation[38]. The elevated serum triacylglycerol levels observed in this study might have been partially due to lipoprotein lipase. Modest hypertriacylglycerolemia occurs in association with alcohol, virus and drug induced hepatitis[39]. The mechanism of this process may involve inhibition of lipolytic enzymes, hepatic triacylglycerol lipase and lipoprotein lipase[38].

The reduction of these enzymes may lead to decreased removal of triacylglycerol from serum and the accumulation of triacylglycerol in the tissues. Significant reduction in the levels of triacylglycerol observed in the serum of *Cucumis sativus* treated rats, shows beneficial effect of *Cucumis sativus* fruit homogenate against CCl_4-toxicity. This could be attributed to the action of pectin from the fruit[40]. Pectin, also known as pectic polysaccharide is a structural heteropolysaccharide, rich in galacturonic acid. Sudheesh and Vijayalakshmi[40] reported that the oral administration of the pectin extracted from the fruit of *Cucumis sativus* decreased the activities of glucose-6-phosphate dehydrogenase and malate dehydrogenase while it increased the activities of lipoprotein lipase and plasma LCAT (Lecithin-cholesterol acyltransferase). The depleted levels of serum high density lipoprotein (HDL) in the CCl_4-induced hepatotoxicity rats may be due hypertriacylglycerolemia induced by reactive metabolite formed during metabolism by CYP2E1, CYP2β and possibly CYP3A to form the tri-chloromethyl radical, CCl_3[41-42].

The HDL is a free radical scavenger and prevents peroxidation of beta lipoproteins[43]. Decreased HDL may be due to diminished lecithin cholesterol acyl transferase (LCAT)

activity and may also contribute to increased cholesterol level. In this study, oral administration of the homogenate of *Cucumis sativus* fruit increased the level of HDL in treated groups comparable to that of the standard drug-silymarin, thereby indicating the antihyperlipidaemic effect of *Cucumis sativus.*

Additionally, histopathological changes were observed indicating liver damage after CCl_4 administration. It has been reported by previous findings that CCl_4 causes necrosis[44]. Pre-treatment with *Cucumis sativus* fruit homogenate significantly improved the structure of hepatic cells. The homogenate showed significant protection comparable to that of standard drug-silymarin, as it was evident from the absence of necrosis and space formation. Therefore, suggested that pre-treatment with the homogenate markedly attenuated the hepatotoxicity caused by CCl_4.

CONCLUSION

The results of this study demonstrate that the homogenate was effective for the prevention of CCl_4-induced hepatic damage in rats and may improve the liver integrity of consumers.

SIGNIFICANCE STATEMENT

To the best of our knowledge, the hepatoprotective properties of the whole fruit is yet to be empirically established. Here, we show the protective effect of whole *Cucumis sativus* L. fruit homogenate on CCl_4-induced liver damage using animal models. We also examined the histology of the liver organs of the animals administered with the whole fruit homogenate. Our results highlight the potential pharmaceutical function of the whole fruit homogenate in the management of liver diseases.

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