

In vivo Pharmacological Effectiveness of Heat-treated Cucumber (*Cucumis sativus L.*) Juice against CCl₄-induced Detoxification in a Rat Model

Vivek Kumar Bajpai¹, Ji-Eun Kim², Yong-Ha Park^{1*}, Sun Chul Kang^{2,*}

¹Department of Applied Microbiology and Biotechnology, Yeungnam University, Gyeongsan, Gyeongbuk 712-714, SOUTH KOREA.

²Department of Biotechnology, College of Engineering, Daegu University, Gyeongsan, Gyeongbuk 712-714, SOUTH KOREA.

ABSTRACT

Background: Detoxification of heavy metals such as carbon tetrachloride (CCl₄) poisoning has become a major focus of researchers. **Objectives:** In this study, heat-treated cucumber juice was assessed for its pharmacological effect on protection of carbon tetrachloride (CCl₄)-induced acute liver and kidney damages. **Methods:** Initially, during detoxification of CCl₄, the rats of all groups except the control group (without treatment) were injected with a single intraperitoneal dose of 2.5 mL CCl₄/kg with 50% concentration (1:1 ratio; v/v in olive oil). Additionally, CCl₄-treated rats were also dosed with saline, 10 mg/kg, 100 mg/kg, and 500 mg/kg cucumber juice for 1, 3 and 5 days once in a day after 6 h from CCl₄ administration. **Results:** As a result, the best concentration of cucumber juice was noted to be 10 mg/kg, which showed remarkable protective effect on body weight (212-217 g), enzymatic activities of aspartate aminotransferase (AST; 53 IU/L), alanine aminotransferase (ALT; 125 IU/L), blood urea nitrogen (BUN; 18.8 mg/dL), and creatinine content (0.65 mg/dL) as compared to control (220-232 g; 477 IU/L; 987 IU/L; 16.8 mg/dL; and 0.95 mg/dL), respectively. Moreover, histology and histomorphometry analysis of liver and kidney led to significant reduction in percentage of hepatopathy and nephropathy parameters such as hepatic (46.90 ± 2.63%) and kidney (39.86 ± 2.01) degenerative regions, number of hepatocytes (638.00 ± 47.25), tubules (327.60 ± 26.67) and glomerulus (29.20 ± 1.36). **Conclusion:** These findings suggest that heat-treated cucumber juice has a significant pharmacological effect on CCl₄-induced acute liver and kidney damages in experimental rats.

Key words: *Cucumis sativus*, Cucumber juice, CCl₄ detoxification, AST, ALT, BUN

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Correspondence:

Yong-Ha Park,
Department of Applied
Microbiology and
Biotechnology,
Yeungnam University,
Gyeongsan,
Gyeongbuk 712-714,
SOUTH KOREA.
Fax: +82-53-813-4620
E-mail: peter@ynu.ac.kr

Sun Chul Kang,
Department of Biotechnology,
College of Engineering,
Daegu University,
Gyeongsan, Gyeongbuk
712-714, SOUTH KOREA
Fax: +82-53-850-6559
E-mail: sckang@daegu.ac.kr

INTRODUCTION

Carbon tetrachloride (CCl₄) is one of the xenobiotics that has been reported to induce acute and chronic tissue injuries and is a well-established hepatotoxin.¹ It has been used extensively to study hepatotoxicity in animal models by initiating lipid peroxidation, thereby causing injuries to kidney, heart, testis and brain, in addition to liver pathogenesis.² Since, CCl₄ induces oxidative stress in a number of organ settings,³ it might be expected to contribute severe nephrotoxicity. Liver and kidney are particularly susceptible to oxidative stress due to the direct release of CCl₄ metabolites

and cytokines, which propagate inflammatory response. CCl₄ has been reported to increase serum hepatotoxicity and nephrotoxicity markers.⁴

Researches in modern medicine have led to various breakthroughs by the discovery of drugs to cure and prevent challenging ailments as well as liver and kidney disorders. However, complications of liver damage which still persist have not got curable therapies to any extent even by a single or combined treatment strategy. The liver is one of the vital organ, susceptible to repeated damage due to its versatile functions.



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Apart from carbon tetrachloride (CCl_4), other damage-causing agents include alcohol, antibiotics, and their metabolites, as well as other drugs used for the treatment of various diseases and infections.⁵ Currently, most of the commercially existing hepatoprotectives are polyherbal formulations containing more than 5-6 herbal extracts, with enhanced proportion of antioxidant compounds.⁶ However, management of liver damage by a single or precise medicament is still a leading challenge in this research area.

On the other hand, the liver is the most important organ concerned with the biochemical activities in the human body. It has great capacity to detoxicate toxic substances, and synthesizes useful metabolic biomolecules. Therefore, damage to the liver inflicted by hepatotoxic agents is of grave consequences. In view of severe undesirable side effects of synthetic agents, there is growing focus to follow the systematic research methodology and to evaluate the scientific basis for the traditional herbal medicines which are claimed to possess hepatoprotective activity.⁷ In fact, it has been shown that the trichloromethyl radical (CCl_3), which is formed by the metabolism of CCl_4 via the liver microsomal cytochrome P450 system, reacts rapidly with molecular oxygen to produce the trichloromethyl peroxy radicals. These radicals react with unsaturated fatty acids of phospholipids present in the cell membrane, initiating the lipid peroxidation in the liver cell, eventually leading to liver dysfunction.⁸ Hence, there is a huge demand for finding noble, precise and potential sources of hepatoprotective agents from natural sources.

Phytogenic agents have traditionally been used by herbalists and indigenous healers for the prevention and treatment of many diseases associated with kidney and liver injuries.⁷ Cucumber (*Cucumis sativus* L.) was selected for this work, since it is very common and cultivated throughout the world and often eaten as a raw vegetable without cooking.⁹ In the ancient Korean system of medicine, a number of plants and herbs have been indicated for detoxification of various poisonings. However, cucumber has gained plenty of research subjects being a processed foodstuff, whereas, there is no scientific evidence that cucumber has been used as a hepatic detoxifying agent so far. In addition, the fresh cucumber is nutritiously a very good source of vitamin C, calcium, potassium, and also provides some dietary fiber, vitamin A, vitamin B6, thiamin, folate, pantothenic acid, magnesium, phosphorus, copper, and manganese which may further support its role as a hepatoprotective agent.

Hence, in this study, heat-treated cucumber juice was tested to assess its pharmacological effectiveness on

CCl_4 -induced acute liver and kidney damages in experimental rats.

MATERIALS AND METHODS

Sample preparation

Cucumbers (*Cucumis sativus* L.) were purchased from an agricultural market in the Gun-Wi, Gyeongbuk, Republic of Korea, and stored at -20°C until further processing. After thawing for 2-3 h at 4°C , the cucumbers were subjected to crush and filtered with Whatman No. 2 filter paper to gain raw cucumber juice. Further, this cucumber juice was subjected to heat treatment in a water bath for 40 min at 80°C , followed by re-filtration, and freeze-drying, and stocked at -20°C until used.

Animals

Male Sprague-Dawley rats used in this study were obtained from Orient Co. Ltd., Republic of Korea. All rats were given with *ad libitum* access to standard laboratory chow and tap water, and were kept under standard conditions (temperature; $24 \pm 1^\circ\text{C}$, relative humidity; $55 \pm 3\%$ and 12 h light/dark cycle). All rats were allowed to acclimatize for 1 week prior to experimentation.

Acute toxicity test

The acute toxicity test was performed on the experimental rats using the oral route. Heat treated cucumber juice was administered at various doses, ranging from (5–500 mg/kg), to different groups of rats. The animals were observed continuously for 1 h and then at half-hourly intervals for 4 h on the first day for clinical signs and symptoms of toxicity and further up to 72 h followed by 14 days for any mortality.

Induction of hepatotoxicity by CCl_4

The rats of all groups except the control group (no treatment) were induced for liver toxicity by the intraperitoneal injection of CCl_4 (2.5 mL/kg b.w.), 1:1 diluted with 50% concentration (v/v in olive oil) for two successive days of the experiment.¹⁰

Experimental groups and detoxification of carbon tetrachloride (CCl_4)

The rats were divided randomly into groups comprising 6 rats in each group and fed the same diet throughout the experimental period. The experimental design is described as follows: CCl_4 -treated rats were also dosed with saline (CCl_4 -con), 10 mg/kg (CCl_4 -10), 100 mg/kg (CCl_4 -100), 500 mg/kg (CCl_4 -500) cucumber juice by gavage for 1, 3 and 5 days once in a day after 6 h from CCl_4 administration. Rats of each group were sacrificed on the last day (1, 3, and 5 days, respectively) for the

analyses of detoxification effect. Also, body weights of rats were measured for 5 days once in a day. For this experiment, the rats were divided into five groups and were treated as follows:

Group 1: Normal diet as a control (C); Group 2: CCl₄ treatment with normal diet and saline supplement negative control (CCl₄-con); Group 3: CCl₄ treatment with normal diet 10 mg/kg cucumber juice diet (CCl₄-10); Group 4: CCl₄ treatment with normal diet 100 mg/kg cucumber juice diet (CCl₄-100) and Group 5: CCl₄ treatment with normal diet 500 mg/kg cucumber juice diet (CCl₄-500).

Collection of blood and tissues

Rats were sacrificed under ethyl ether anesthesia on day 1, 3 and 5 after CCl₄ administration. At each time, blood was collected from the abdominal aorta and centrifuged at 3,000 x g for 30 min to obtain serum, and the serum was used for biochemical analysis immediately. Liver and kidney were also excised and rinsed with phosphate-buffered saline (PBS) for histopathological examination.

Biochemical evaluation

Determination of hepatic enzyme levels in serum

Collected blood samples were placed at room temperature for 45 min and centrifuged at 5,000 x g for 10 min at 4°C in order to separate serum, and the separated serum was analyzed for various biochemical parameters including enzymatic assays to evaluate the protective effect of cucumber juice. Enzyme activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) as well as blood urea nitrogen (BUN) and creatinine were determined using commercially available diagnostic kits with auto dry chemistry-analyzer (Spotchem™ SP-4410®, Kyoto Daiichi Kagaku Co., Ltd. Japan).

Histopathological studies

Tissue samples from liver and kidney were separated, sliced and fixed in Bouin solution (Picric acid: Formalin: Acetic acid, 15:5:1), then embedded in paraffin. Sections of 3~4 μm thickness were made using microtome and stained with hematoxylin-Eosin (H-E), and after that observed under a light microscope (Nikon, Japan), to visualize histopathological changes in the liver and kidney.

Histomorphometry

Percentage of degenerative regions in liver showing centrilobular necrosis on hepatic lobules containing necrotic debris, apoptotic cells, ballooning cells, inflammatory cells and acidophilic cells, and number of degenerative cells in centrilobular regions of hepatic parenchyma were calculated as %/mm² and N/1000 hepatocytes, respectively. In kidney parenchyma,

percentage of degenerative regions showing tubular necrosis and focal inflammatory cell infiltration, as well as the number of the vasodilated atrophic glomerulus and degenerative tubules were also calculated as %/mm², N/100 glomeruli and N/1000 tubules, respectively. The histomorphometry was conducted by using an automated image analyzer (DMI-300 Image Processing; DMI, Korea) under 100 magnifications of microscopy (Nikkon, Japan) at 5 fields (n=5), respectively.

The percentage changes between normal-control, CCl₄-control and test groups were calculated to evaluate the efficacy of cucumber juice and to detect the severity of CCl₄-intoxication, respectively, by following equations:

$$\% \text{ Changes vs CCl}_4\text{-control (\%)} = \left\{ \frac{\text{Data of test groups} - \text{Data of CCl}_4\text{-control}}{\text{Data of CCl}_4\text{-control}} \right\} \times 100$$

Statistical analysis

Data were expressed as mean ± S.E.M. and were analyzed with SPSS software, version 11.5. Differences between group means were calculated by a one-way analysis of variance (ANOVA). The results were considered statistically significant when P < 0.05.

RESULTS AND DISCUSSION

Acute toxicity test

No toxicity symptoms were recorded in the experimental rats of any of the groups. The LD₅₀ value by oral route could not be determined as no lethality was observed in the animals.

Protective effect of heat-treated cucumber juice on body weight

No animals were damaged by the administration of CCl₄ during the experimental period. Administration of CCl₄ caused a significant decrease in the body weight of rats (CCl₄-induced rats for 5 days) as compared with the control rats (Fig. 1). No significant changes were found in body weights in control and CCl₄-induced rats on day 1, whereas the remarkable decline in body weight was observed on day 2 in CCl₄-induced rats. The CCl₄-induced rats co-treated with cucumber juice (10, 100, and 500 mg/kg) for five days also gained weight during the experimental period. There was a rapid increase in body weight on day 2 at the concentration of 10 mg/kg treatment as compared to only CCl₄-induced rats. As a result, 10 mg/kg treatment was found to be the highest effective dose of enhancing the body weight for 5 days as compared to other cucumber juice treatments.

Biochemical analysis of serum enzyme levels

In this experiment, we could assess the recovery of liver injury caused by CCl₄ treatment after the administration

of cucumber juice by the estimation of the AST and ALT activities. All CCl_4 -treated groups, radically led to the marked increases in AST and ALT activities after 1 day as compared to control group, whereas low AST and ALT activities were observed for 10 mg/kg treatment group. After 3 days, AST and ALT activities of all groups were decreased (Fig. 2 & Fig. 3). The activity of these enzymes was normalized after 5 days in all groups. For 5 days, the 10 mg/kg group seems to be effective, and significantly reduced the activities of marker enzymes namely AST and ALT. Hence, the best concentration of cucumber juice noted for hepatoprotective effect was found to be 10 mg/kg. However, for the levels of blood urea nitrogen (BUN) and creatinine, there were no significant differences in all treatment groups as compared to the normal group (Fig. 4 & Fig. 5). It is well established that hepatotoxicity by CCl_4 is due to enzymatic activation to release CCl_3 radical in free state, which in turn disrupts the structure and function of lipid and protein macromolecule in the membrane of the cell organelles.¹¹ The disturbance in the transport function of the hepatocytes as a result of hepatic injury causes the leakage of enzymes from cells due to altered permeability of membrane.¹² The increased levels of AST and ALT are conventional indicators of liver injury. In the present study, it was also seen that the administration of CCl_4 elevated the levels of serum marker enzymes, AST and ALT. The CCl_4 -10 group exhibited lower levels of AST and ALT as compared to other CCl_4 -treated groups. The stabilization of serum AST and ALT levels of the CCl_4 -10 group is a clear indication of the improvement of the functional status of the liver cells. These findings can be further corroborated by histopathological studies.

Histopathological findings

Histological features of liver of CCl_4 treated rats showed vacuolated hepatocytes along with fatty deposition, necrosis and degenerative changes (Fig. 6A). Infiltration of inflammatory cells in the central vein was also evident in this experimental group. These set of changes have also been reported by other researchers following CCl_4 treatment.^{13,14} Clear focal degenerative regions consisted of necrotic changes on tubules and vasodilated atrophic glomeruli so called CCl_4 -induced nephrotoxicity as described by several researchers,⁴ were also demonstrated in all CCl_4 -dosing groups (Fig. 6B). Overall, histological analysis supported the results obtained from the serum enzyme and other biochemical parameters showing less damage in the cytoarchitecture of the liver. Similar findings were observed by Mihailovic et al.¹⁵ for the hepatoprotective effects of *Gentiana asclepiadea*.

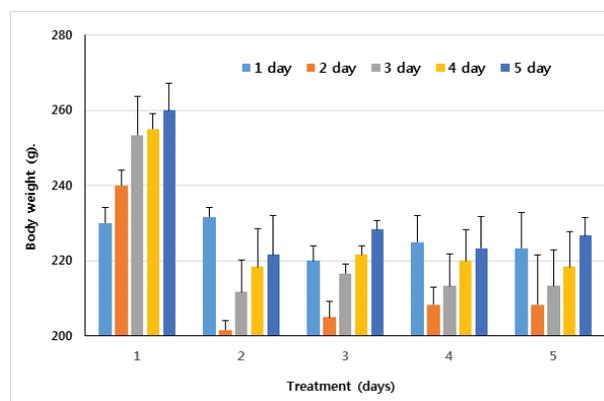


Figure 1: Effect of heat-treated cucumber juice on body weight in rats until 5 days. 1: No treatment (C); 2: Treatment of CCl_4 group (CCl_4 -control); 3: Treatment of CCl_4 and 10 mg/kg of cucumber juice (CCl_4 -10); 4: Treatment of CCl_4 and 100 mg/kg of cucumber juice (CCl_4 -100), and 5: Treatment of CCl_4 and 500 mg/kg of cucumber juice (CCl_4 -500).

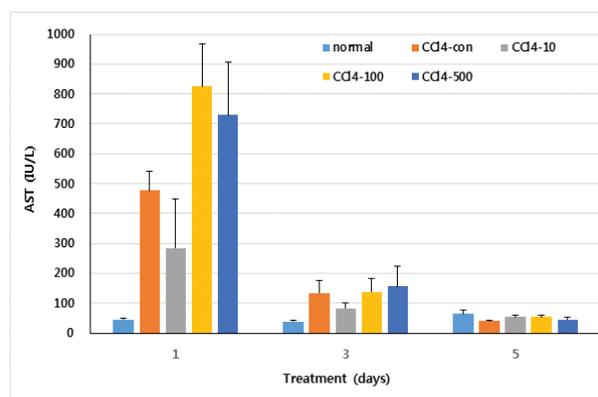


Figure 2: Effect of heat-treated cucumber juice on aspartate transaminase (AST) activity in rats on 1st, 3rd and 5th days. normal: No treatment (Control); CCl_4 -control: Treatment of CCl_4 ; CCl_4 -10: Treatment of CCl_4 and 10 mg/kg (cucumber juice); CCl_4 -100: Treatment of CCl_4 and 100 mg/kg (cucumber juice), and CCl_4 -500 Treatment of CCl_4 and 500 mg/kg (cucumber juice).

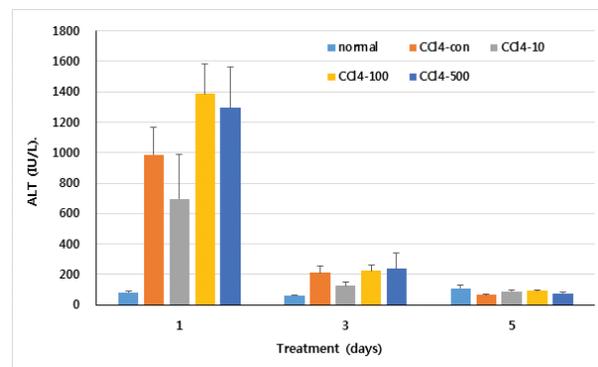


Figure 3: Effect of heat-treated cucumber juice on alanine aminotransferase (ALT) activity in rats on 1st, 3rd and 5th days. normal: No treatment (Control); CCl_4 -control: Treatment of CCl_4 ; CCl_4 -10: Treatment of CCl_4 and 10 mg/kg (cucumber juice); CCl_4 -100: Treatment of CCl_4 and 100 mg/kg (cucumber juice), and CCl_4 -500 Treatment of CCl_4 and 500 mg/kg (cucumber juice).

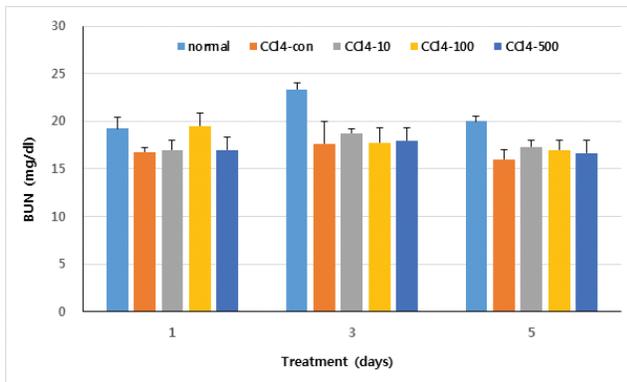


Figure 4: Effect of heat-treated cucumber juice on the levels of blood urea nitrogen (BUN) activity in rats on 1st, 3rd and 5th days. normal: No treatment (Control); CCl₄-control: Treatment of CCl₄; CCl₄-10: Treatment of CCl₄ and 10 mg/kg (cucumber juice); CCl₄-100: Treatment of CCl₄ and 100 mg/kg (cucumber juice), and CCl₄-500 Treatment of CCl₄ and 500 mg/kg (cucumber juice).

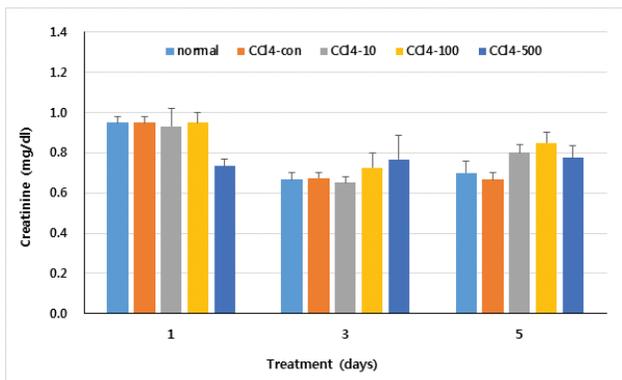


Figure 5: Effect of heat-treated cucumber juice on the levels of creatinine in rats on 1st, 3rd and 5th days. normal: No treatment (Control); CCl₄-control: Treatment of CCl₄; CCl₄-10: Treatment of CCl₄ and 10 mg/kg (cucumber juice); CCl₄-100: Treatment of CCl₄ and 100 mg/kg (cucumber juice), and CCl₄-500 Treatment of CCl₄ and 500 mg/kg (cucumber juice).

Recently, Ravichandra *et al.*¹⁶ also reported that histopathological studies of the liver intoxicant group caused fatty changes, granular degeneration and inflammation, coagulative necrosis, degenerative necrosis and bile duct hyperplasia. Coagulative necrosis is the most common type of necrosis caused by irreversible focal injury, mostly due to the sudden cessation of blood flow, ischemia. Based on the histological examinations, it was observed that there were minimal lesions with almost no presence of fatty change which hypothesized that the bioactive compounds present in cucumber juice may protect the liver from hepatic damage. Similar results were observed by Cetin *et al.*, who observed potent efficacy of hesperidin, a citrus-based flavanone glycoside on CCl₄-induced liver damage in experimental rats.¹⁷

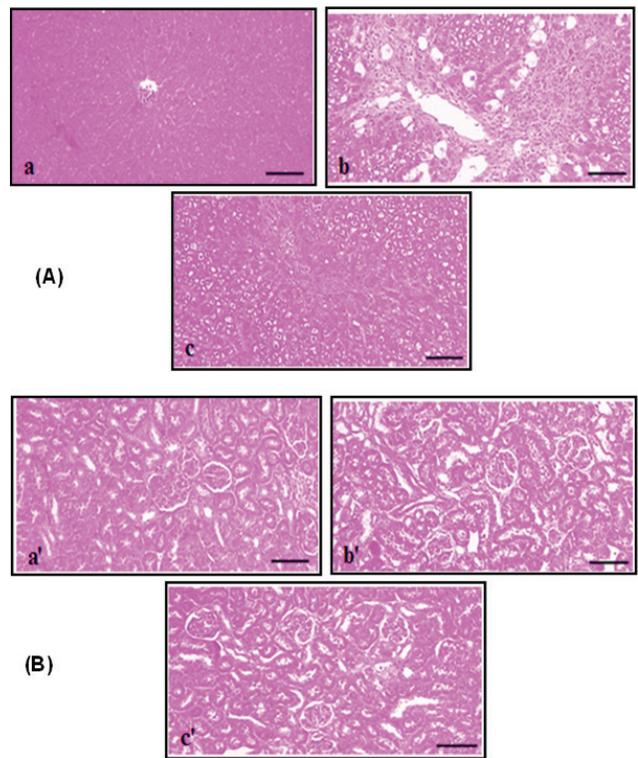


Figure 6: Histological profiles of the liver (A) and kidney (B) on day 3 in normal rats (a), CCl₄-control (b) CCl₄ and (c) CCl₄-10 + heat-treated cucumber juice group. Tissue samples were stained H-E. Scale bars = 100 μm. Normal: No treatment; CCl₄-con: Treatment of CCl₄; CCl₄-10: Treatment of CCl₄ and 10 mg/Kg concentration of cucumber juice.

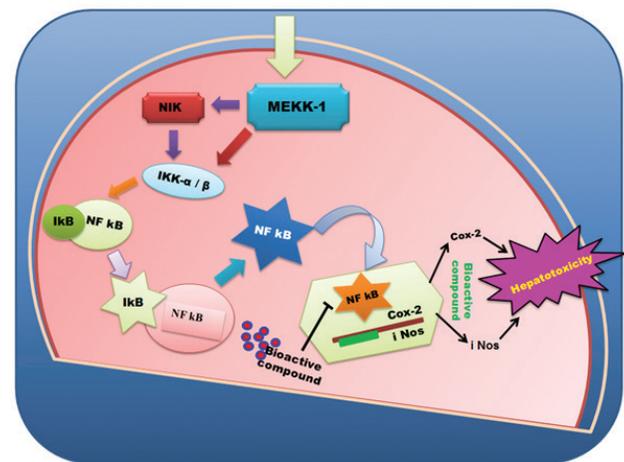


Figure 7: Proposed molecular mechanism of hepatoprotective effect of bioactive molecules and herbal drugs.

Histomorphometry observations

The changes in the percentage of degenerative regions in hepatic parenchyma, number of degenerative hepatocytes in liver and degenerative regions in kidney parenchyma, number of degenerative glomerulus and tubules in the kidney are listed in Table 1 and 2, respectively.

Table 1: Changes in the CCl₄-induced hepatopathies in CCl₄-intoxicated rats

Group	CCl ₄ -induced hepatopathies	
	Percentage of hepatic degenerative regions	Number of degenerative hepatocytes
Normal	5.90 ± 1.83	64.80 ± 19.87
CCl ₄ -con day 3	77.46 ± 3.84	834.60 ± 38.24
CCl ₄ -10 day 3	46.90 ± 2.63	638.00 ± 47.25

Normal: No treatment; CCl₄-control: Treatment of CCl₄; CCl₄-10: Treatment of CCl₄ and 10 mg/kg concentration of cucumber juice.

Table 2: Changes in the CCl₄-induced nephropathies in CCl₄-intoxicated rats.

Group	CCl ₄ -induced nephropathies		
	Percentage of kidney degenerative regions	Number of degenerative tubules	Number of degenerative glomerulus
Normal	2.66 ± 0.72	51.20 ± 12.99	5.80 ± 1.02
CCl ₄ -con day 3	52.45 ± 4.95	618.20 ± 33.29	53.60 ± 3.17
CCl ₄ -10 day 3	39.86 ± 2.01	327.60 ± 26.67	29.20 ± 1.36

Normal: No treatment; CCl₄-control: Treatment of CCl₄; CCl₄-10: Treatment of CCl₄ and 10 mg/kg concentration of cucumber juice.

Most of the CCl₄ intoxication-related histopathological and nephropathological changes were worsen on day 3 after CCl₄-dosing, and these CCl₄-related tissue damages were re-confirmed with histomorphometry (Table 1 & Table 2).

The CCl₄-related hepatopathies and nephropathies were dramatically decreased in CCl₄-10 as compared to CCl₄-control, respectively. The percentage of degenerative regions in hepatic parenchyma and a number of degenerative hepatocytes, kidney tubules and glomerulus in CCl₄-10 were significantly decreased as compared to CCl₄-control. In addition, the percentage of degenerative regions in the kidney parenchyma were also decreased in CCl₄-10 as compared to CCl₄-control.

The percentage changes of degenerative regions in hepatic parenchyma of CCl₄-control on day 3 after CCl₄ administration were found to be 1,212.81%. However, CCl₄-10 represented -39.45% changes of degenerative regions in hepatic parenchyma as compared to that of CCl₄-control. The percentage changes in the number of degenerative hepatocytes in CCl₄-control on day 3 after CCl₄ administration were found as 1,187.96%, which were reduced to -23.56% in CCl₄-10 as compared to that of CCl₄-control.

On the other hand, the percentage changes of degenerative regions in the kidney parenchyma of CCl₄-control on day 3 after CCl₄ administration were found to be 1,873.14%, which were dramatically reduced to -23.99% in CCl₄-10 as compared to that of CCl₄-control. The percentage changes in the number of degenerative kidney tubules in CCl₄-control on day 3 after CCl₄ administration were found as 1,107.42%, whereas CCl₄-10 treated group represented -39.73%

changes in the number of degenerative kidney tubules as compared to that of CCl₄-control. In addition, the percentage changes in the number of degenerative glomeruli in CCl₄-control on day 3 after CCl₄ administration were found to be 824.14%, which were reduced to -45.52% in the CCl₄-10 treated group as compared to that of CCl₄-control. Similar observations were also reported by Mihailovic *et al.*¹⁵. Biochemical tests of blood did not reveal any significant effect on the kidney, whereas histopathological examination revealed a clear effect of cucumber juice on both liver and kidney. Other histological manifestations observed were also consistent with the findings of other authors.^{10,18}

Cordiro and Kaliwal¹⁹ also reported protective effects of a *Bridelia retusa* bark extract against CCl₄-induced toxification in a mouse model using liver and kidney tissue histological parameters.¹⁹ Also, extract derived from the herbal plant, *Nigella sativa* has been found to exert hepatoprotective effects against CCl₄-induced toxification in a rat model as confirmed by remarkable healing efficacy in stomach and duodenum tissues.²⁰ It was observed that the content of cucumber juice treatment increased the regenerative and reparative capacity of the liver and kidney at the same time. These results suggest that the effective compounds present in the cucumber juice could act as protective agents against CCl₄-induced liver and kidney damage, leading to normal functioning of liver and kidney with minimal cell disturbance.

Strategy for possible molecular mechanism

Recent findings on the molecular mechanisms underlying the hepatoprotective action of various herbal molecules

indicate that these molecules can suppress certain specific pro-inflammatory genes whose expression has been shown to be regulated by various transcriptional factors. Generally, bioactive phytochemicals abolish the expression of COX-2 and iNOS proteins in the liver, suggesting that these compounds may play an important role not only in alleviating liver inflammation but also probably protect the body system from liver cancer. NF- κ B, a ubiquitous and heterodimer transcription factor, residing in the cytoplasm is translocated to the nucleus upon activation which induces gene transcription.²¹ The transcriptional factor NF- κ B has been shown to induce the expression of more than 200 genes, including COX-2 as well as play a central role in the regulation of inflammatory proteins such as COX-2 and iNOS.²¹ Pre-treatment of experimental animals with bioactive phytochemicals may abrogated the DNA binding activity of NF- κ B and AP-1 transcriptional factors induced by dimethyl nitrosamine (DMN).²² Bioactive phytochemicals, both in isolated and crude form which can suppress the transcriptional factors NF- κ B and AP-1 have significant potential in preventing the onset of cancer.²³ To support the hypothesis, estimated based on the ability of heat-treated cucumber juice to inhibit the DMN-mediated DNA binding of NF- κ B and AP-1 transcriptional factors and expression of pro-inflammatory proteins such as COX-2 and iNOS, a proposed underlying molecular mechanism of hepatoprotective effect of bioactive compounds has been depicted in Fig. 7, which may further explain the molecular basis and the possibilities of hepato-carcinogenesis and chemotherapeutic application of naturally occurring phytochemicals.²⁴

CONCLUSION

Our results show that the hepatoprotective effects of cucumber juice may be due to both an increase in the activity of the antioxidant-defense system and inhibition of lipid peroxidation possibly by the action of various biologically active phytochemicals present in cucumber juice. Moreover, significant reduction in the levels of serum enzymes AST and ALT was observed with the consumption of cucumber juice. Further, histopathological and histomorphometry results also supported the protective effect of cucumber juice in experimental animals. Hence, based on the above finding, it can be concluded that this pharmacological evaluation using cucumber juice may have great possibilities to protect the liver and kidney against toxic effects of CCl₄.

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CONFLICT OF INTEREST

None of the authors has a conflict of interest to disclose.

ABBREVIATION USED

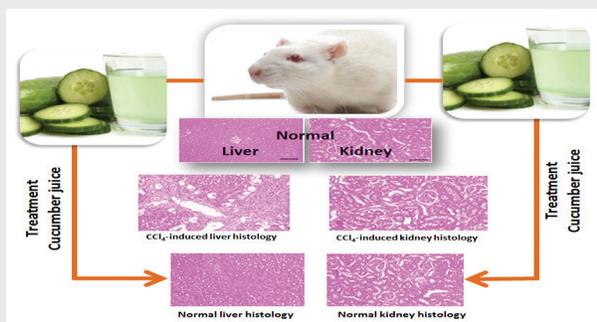
CCl₄: Carbon tetrachloride; **AST**: Aspartate aminotransferase; **ALT**: Alanine aminotransferase; **BUN**: Blood urea nitrogen; **HE**: Hematoxylin-Eosin; **RBC**: Red blood cells; **SEM**: Standard error mean; **DNA**: Deoxyribonucleic acid; **NF- κ B**: Nuclear factor-Kappa B; **iNOS**: Inducible nitric oxide synthase; **COX-2**: Cyclooxygenase-2.

REFERENCES

- Xu JY, Su YY, Cheng JS, Li SX, Liu R, Li WX. Protective effects of fullereneol on carbon tetrachloride-induced acute hepatotoxicity and nephrotoxicity in rats. *Carbon*. 2010;10:1388-96. <https://doi.org/10.1016/j.carbon.2009.12.029>.
- Khan RA, Khan MR, Sahreen S. CCl₄-induced hepatotoxicity: protective effect of rutin on p53, CYP2E1 and the antioxidative status in rat. *BMC Comp Alt Med*. 2012;8:172-8. <https://doi.org/10.1186/1472-6882-12-178>.
- Mohamed NZ, Abd-Alla HI, Aly HF, Mantawy M, Ibrahim N, Hassan SA. CCl₄-induced hepatonephrotoxicity: protective effect of nutraceuticals on inflammatory factors and antioxidative status in rat. *J Appl Pharm Sci*. 2014;4:087-100.
- Ozturk F, Ucar M, Ozturk IC, Vardi N, Batcioglu K. Carbon tetrachloride-induced nephrotoxicity and protective effect of betaine in Sprague-Dawley rats. *Urology*. 2003;62:353-6. [https://doi.org/10.1016/S0090-4295\(03\)00255-5](https://doi.org/10.1016/S0090-4295(03)00255-5).
- Wiese JG, Shlipak MG, Browner WS. The alcohol hangover. *Ann Int Med*. 2000;132:897-902. <https://doi.org/10.7326/0003-4819-132-11-200006060-00008> PMID:10836917.
- Rajashakar V, Rao EU, Srinivas P. Biological activities and medicinal properties of Gokhru (*Pedaliumpurexurex* L.). *Asian Pac J Trop Biomed*. 2012;2:581-5. [https://doi.org/10.1016/S2221-1691\(12\)60101-4](https://doi.org/10.1016/S2221-1691(12)60101-4).
- Anantha KCD, Siva RC, Manohar RA. Hepatoprotective effect of biherbal ethanolic extract against paracetamol-induced hepatic damage in albino rats. *J Ayurveda Integ Med*. 2012;3:198-203. <https://doi.org/10.4103/0975-9476.104436> PMID:23326091 PMCID:PMC3545240.
- Recknagel RO, Glende EAJ, Dolak JA. Mechanisms of carbon tetrachloride toxicity. *Pharmacol Ther*. 1989;43:139-54. [https://doi.org/10.1016/0163-7258\(89\)90050-8](https://doi.org/10.1016/0163-7258(89)90050-8).
- Heinonen-Tanski H, Sjoblom A, Fabritius H, Karinen P. Pure human urine is a good fertilizer for cucumbers. *Biores Technol*. 2005;98:214-7. <https://doi.org/10.1016/j.biortech.2005.11.024> PMID:16413181.
- Khan AA, Alzohairy M. Hepatoprotective effects of camel milk against CCl₄-induced hepatotoxicity in rats. *Asian J Biochem*. 2011;6:171-80. <https://doi.org/10.3923/ajb.2011.171.180>.
- Mujumdar AM, Upadhyaya AS, Pradhan AM. Effect of Azadirachta indica leaf extract on CCl₄ induced hepatic damage in albino rats. *Indian J Pharma Sci*. 1998;60:363-7.
- Castro JA, de Ferreyra GC, de Castro CR, Sesame H, de Fenos OM, Gillette JR. Prevention of carbon tetra chloride induced necrosis by inhibition of drug metabolism studies on the metabolism of their action. *Biochem Pharmacol*. 1974;23:295-302. [https://doi.org/10.1016/0006-2952\(74\)90420-1](https://doi.org/10.1016/0006-2952(74)90420-1).

- Amat N, Upur, H, Blazekovic B. *In vivo* hepatoprotective activity of the aqueous extract of *Artemisia absinthium* L. against chemically and immunologically induced liver injury in mice. *J Ethnopharmacol.* 2010;131:478-84. <https://doi.org/10.1016/j.jep.2010.07.023> PMID:20637853.
- Feng Y, Wang N, Ye X, Li H, Feng Y, Cheung F, Nagamatsu T. Hepatoprotective effect and its possible mechanism of *Coptidis rhizoma* aqueous extract on carbon tetrachloride-induced chronic liver hepatotoxicity in rats. *J Ethnopharmacol.* 2011;138,683-90. <https://doi.org/10.1016/j.jep.2011.09.032> PMID:21963555.
- Mihailovic V, Mihailovic M, Uskokovic A, Arambašić J, Mišić D, Stankovic V, Katanic J, Mladenovic M, Solujic S, Matic S. Hepatoprotective effects of *Gentiana asclepiadea* L. extracts against carbon tetrachloride induced liver injury in rats. *Food Chem Toxicol.* 2013;52:83-90. <https://doi.org/10.1016/j.fct.2012.10.034> PMID:23146698.
- Ravichandra VD, Ramesha C, Sridhar KA. Hepatoprotective potentials of aqueous extract of *Convolvulus pluricaulis* against thioacetamide induced liver damage in rats. *Biomed Aging Pathol.* 2013;3:131-5. <https://doi.org/10.1016/j.biomag.2013.06.005>.
- Cetin A, Ciftci O, Otlu A. Protective effect of hesperidin on oxidative and histopathological liver damage following carbon tetrachloride administration in Wistar rats. *Arch Med Sci.* 2016;12:486-93. <https://doi.org/10.5114/aoms.2015.49484> PMID:27279838 PMCID:PMC4889676.
- Sun F, Hamagawa E, Tsutsui C. Evaluation of oxidative stress during apoptosis and necrosis caused by carbon tetrachloride in rat liver. *Biochim Biophys Acta.* 2001;1535:186-91. [https://doi.org/10.1016/S0925-4439\(00\)00098-3](https://doi.org/10.1016/S0925-4439(00)00098-3).
- Cordeiro MC, Kaliwal BB. Protective role of bark extract of *Bridelia retusa* Spreng on CCl_4 induced histological toxicity in mice. *J Pharmacol Phytochem.* 2013;2:142-8.
- Danladi J, Akpulu SP, Owolagba GK, Afuwai VK, Hadiza RA, Murdakai T. Histological effect of oral administration of CCl_4 -induced stomach and duodenum damage and the protective effect of *Nigella sativa* in adult Wistar rats. *IOSR J Dental Med Sci.* 2014;13:53-8. <https://doi.org/10.9790/0853-13755358>.
- Nanji AA, Jokelainen K, Tipoe GL, Rahemtulla, A, Thomas P, Dannenberg AJ. Curcumin prevents alcohol-induced liver disease in rats by inhibiting the expression of NF-kappa B-dependent genes. *Am J Physiol Gastrointest Liv Physiol.* 2003;284:321-7. <https://doi.org/10.1152/ajpgi.00230.2002> PMID:12388178.
- Farombi EO, Shrotriya S, Surh YJ. Kolaviron inhibits dimethyl nitrosamine-induced hepatotoxicity by suppressing COX-2 and iNOS expression: NF- κ B and AP-1 as potential molecular targets. *Life Sci.* 2009;84:149-55. <https://doi.org/10.1016/j.lfs.2008.11.012> PMID:19081081.
- Aggarwal BB, Shishoda S. Suppression of the nuclear factor (B activation pathway by spice-derived phytochemicals: Reasoning for the seasoning. *Ann NY Acad Sci.* 2004;1030:434-41. <https://doi.org/10.1196/annals.1329.054> PMID:15659827.
- Fogh J, Trempe X. *Human Tumor Cells in Vitro*; Fogh, J, Ed.; Plenum Press: New York, NY, USA, 1975; pp. 115-59. <https://doi.org/10.1007/978-1-4757-1647-4> https://doi.org/10.1007/978-1-4757-1647-4_5.

PICTORIAL ABSTRACT



SUMMARY

- This study reports the pharmacological effectiveness of cucumber (*Cucumis sativus* L.) for heavy metal detoxification in a rat model.
- The cucumber juice significantly reduced level of histopathological parameters in the CCl_4 induced detoxification in animals.
- Treatment with cucumber juice exhibited significant effects on CCl_4 -induced hepatopathies in CCl_4 -intoxicated rats.
- Administration of cucumber juice also exhibited significant effects on CCl_4 -induced nephropathies in CCl_4 -intoxicated rats, as compared to CCl_4 -control.
- Eventually cucumber juice evoked a significant protective effect on liver and kidney induced by CCl_4 .

About Authors



Dr. Vivek Kumar Bajpai: Is working as a Foreign Assistant Professor in the Department of Applied Microbiology and Biotechnology, Yeungnam University, Republic of Korea. He has published more than 100 peer reviewed research/review articles and patent in international journals of scientific repute. He has been serving as an Associate Editor to one of the world's leading journals BMC Complementary and Alternative Medicine, Frontiers in Microbiology and academic editor to PLoS ONE.



Dr. Sun Chul Kang: Is working as a professor in the Department of Biotechnology, Daegu University, Republic of Korea. He has published more than 150 peer reviewed research articles in international journals. He has registered more than 10 national and international patents and edited several academic books in aspect of biotechnology. He has been visiting scientist in number of international universities. He is holding the position of Editor-in-Chief for Journal of Agricultural Chemistry and Environment.

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