The Possible Synergistic Role of Phytic Acid and Catechin in Ameliorating the Deteriorative Biochemical Effects Induced by Carbon Tetrachloride in Rats

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Abstract: Carbon tetrachloride (CCl4)-induced hepatotoxicity in rats was used to assess the effect of catechin, phytic acid and their combination on the indexes of rats cirrhosis. Liver profile enzymes, oxidative status and histological examinations revealed that catechin and phytic acid significantly arrested progression of hepatic cirrhosis induced by CCl4. Reduced activities of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and cholesterol level, were detected while the levels of total protein and albumin were increased. Electrophoretic separation of LDH isoenzymes revealed a marked reduction in LDH 4 & 5 isoenzymes by combined treatment with both phytic acid and catechin. The development of CCl4-induced hepatic cirrhosis altered the redox state resulting in decreased hepatic glutathione (GSH), increased serum nitric oxide (NO) levels and the formation of liver lipid peroxidative products which were partially normalized by treatment with catechin and its combination with phytic acid, respectively. Moreover, P53 was increased significantly with catechin while a marked reduction in sialic acid level was noted post phytic acid treatment. Histological examinations showed that the combination of both phytic acid and catechin nearly returned collagen fiber distribution to normal pattern. These results demonstrate that administration of phytic acid together with catechin may be useful in the treatment and prevention of hepatic cirrhosis on the experimental level.

Key words: Cirrhosis, Catechin, Phytic acid, Synergism, P53, CCl4

INTRODUCTION

Cirrhosis or chronic liver failure is most widely spread in developing countries and is considered the seventh leading cause of death[17]. CCl4 is an important service as a model substance to elucidate the mechanism of action of hepatotoxic effects such as fatty degeneration, fibrosis, hepatocellular death, and carcinogenicity. CCl4 is activated by cytochromes to form trichloromethyl radical (CCl3·), which can bind to nucleic acid, protein and lipid impairing crucial cellular processes such as lipid metabolism, resulting in fatty degeneration (steatosis). Adduct formation between (CCl3·) and DNA is an initiator of hepatic cancer. This radical can also react with oxygen to form trichloromethylperoxy radical (CCl3OO·), a highly reactive species which initiates the chain reaction of lipid peroxidation[12]. Considering the hazards of treatment failure, drug resistance and heavy costs associated with current hepatic therapy, medicinal plants have attracted interest of many researchers in this field[15]. Catechin has been reported to possess numerous biological activities including antimutagenic, antibacterial, hypocholesterolemic, antioxidant, antitumor and cancer preventive activities[3]. Histological and hepatic hydroxyproline examination revealed that catechin significantly arrest progression of hepatic fibrosis induced by CCl4. Catechin caused a significant amelioration of liver injury (reduced activities of serum alanine aminotransferase and aspartate aminotransferase) and partially normalized hepatic reduced glutathione and lipid peroxidative products. These results reflect that administration of catechin may be useful in the treatment and prevention of hepatic cirrhosis[44]. Moreover, catechin ameliorates CCl4-induced liver injury by down-regulation of TNF, iNOS synthesis, and the decrease of lipid peroxidation. In hepatic cell carcinoma. ELISA showed that catechin significantly increased the expression of p53 and p21/WAF1 protein, and this contributed to cell cycle arrest[23]. Tea polyphenols have no known toxicity. Thus, they represent a promising strategy to prevent liver injury associated with endotoxemia, especially in alcoholic liver disease and liver failure[3].

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Phytic acid (Inositol Hexaphosphate) (IP6) possesses a broad range of bioactivities. It has been long used as safe natural antioxidant by removing active oxygen in the body and suppressing lipid peroxide production. It absorbs excess iron ions, enhancing immunity and has an effect on heart disease, liver dysfunction, dermatitis and anticancer actions. It is known further to prevent kidney stone formation and cholesterol deposition. The remarkable affinity of IP6 for iron totally inhibits this metal’s ability to catalyze the formation of hydroxyl radicals which cause oxidation and cancer. It also promotes the breakdown of collagen. There is reasonable hope that this treatment may affect not only the progression of the disease, but may also reverse preexisting fibrosis, as demonstrated for CCl₄-induced cirrhosis in the rat[32]. A recent study done discovered that phytic acid and green tea synergize to significantly reduce the number of preneoplastic lesions. Again, this points to the general principle that two or more natural agents are more effective together[39]. Based upon the previous, the present study has been planned to identify the synergistic effect of both phytic acid and catechin in ameliorating hepatotoxicity induced by carbon tetrachloride in rats by monitoring liver profile, redox status, P53& sialic acid levels as well as histological examination hoping that this study will beneficially protect CCl₄ factories and dry clean employers or patients suffering from liver diseases.

**MATERIALS AND METHODS**

**Materials:** All chemicals used were of high analytical grade, products of Sigma (USA) and Randox (United Kingdom). Catechin and phytic acid were imported from Sigma. Carbon tetrachloride was obtained from El-Nasr Chemical Industries Company.

**Animals:** Animals care and handling were done according to the guide lines issued by the world health organization, Geneva Switzerland and the ministry of scientific research (Cairo – Egypt). The inbred Swiss male albino rats from a stock purchased from the animal house (National Research Center, Cairo – Egypt), weighing 100-120 gm were selected from an inbred colony maintained under the controlled conditions of temperature (23±2°C), humidity (50±5%) and light (14 and 10 h of light and dark, respectively). The animals were provided with sterile food and water ad libitum. Eight animals were each housed in polypropylene cages containing sterile paddy husk (procured locally) as bedding. This study was approved by the Institutional Animal Ethical Committee.

**Experimental Design:** The present study includes eight groups where each group consisted of 10 rats.

- The first group served as normal control.
- The second group was treated with phytic acid (1 ml of 0.3 % / K g body weight suspended in gum accacia orally daily for 2 weeks[39]).
- The third group was treated with catechin (10 mg/Kg body weight orally daily for 2 weeks[37]).
- The fourth group received combination of phytic acid and catechin.
- The fifth group was treated with carbon tetrachloride single doses (1 mg / K g body weight[29]) as a model for liver cirrhosis.

Treated groups that contained 3 sub-groups pretreated with CCl₄ (1P) then received the previous mentioned doses 24 hrs post CCl₄ injection.

- The first group received phytic acid.
- The second group received catechin.
- The third group received a combined dose of phytic acid and catechin.

After 2 weeks animals were sacrificed and blood was withdrawn from retro-orbital sinus, serum was separated using centrifuge (SIGMA Labozenrifugen 2K15) for 10 minutes at 4000 rpm and stored at -20 till processing.

Liver tissues were collected and divided into two parts the first was for biochemical measurements while the other was preserved at 10% formalin for histological examinations.

**Biochemical Analysis:**

**Serum Determinations:**

\( I_a \) Serum Determination of Aminotransferases (AST and ALT) activities: These were determined according to the method of Reitman and Frankel[30]. Diagnostic kits were purchased from Randox company (United Kingdom); following the instructions of the manufacturer. AST and ALT activities were measured by monitoring the concentration of oxaloacetate or pyruvate hydrazones formed with dinitrophenylhydrazine (DNP) in alkaline medium at wave length 540 nm using Shimshduz UV recording spectrophotometer UV 240.

\( I_b \) Determination of Alkaline Phosphatase (ALP) Activity: This was an optimized standard method according to the principle of Kochmer and Moss[22]. Diagnostic kits were obtained from Randox.
Fig. 1: Control group showing normal hepatocytes (Masson’s strichrome stain (MTC) X400).

Fig. 2: CCl₄ displayed highly damaged portal tracts enclosed with thick loose collagen fibers (MTC X400).

Fig. 3: CCl₄ group treated with phytic acid showing congested hepatic arteries, hepatocytes having basophilic hyaline cytoplasm highly arranged in strands (MTC X400).

Fig. 4: CCl₄ group treated with catechin arteries, displays damaged hepatocytes with pyknotic nuclei and vasoulated cytoplasm. Blood capillaries filled with hemolized blood (MTC X400).

Fig. 5: CCl₄ group treated with phytic acid and catechin showing the pattern of collagen fiber distributions with great similarity to that of control (MTC X400).

Histopathological Examinations: company (United Kingdom); following the instructions of manufacturer. Alkaline phosphatase acts upon AMP-buffered sodium thymolphthalein monophosphate. The addition of an alkaline reagent stops enzyme activity and simultaneously develops a blue chromogen, which is measured colorimetrically at wave length 590 nm.

(Iₕ) Determination of Lactate Dehydrogenase Activity and Electrophoretic Separation of its Isoenzymes: These were determined according to the method previously explained by Henderson[18] and Dietz[19] respectively and the activity was measured at wave length 340 nm.

(I₆) Determination of Total Proteins: This was done according to the procedure previously recorded by Wray et al.[40]. Total proteins react with
Lane1: control group.
Lane2: reference control heart.
Lane3: control phytic acid group.
Lane4: control catechin group.
Lane5: control phytic acid and catechin group.
Lane6: CCl4 group.
Lane7: CCl4 + phytic acid group.
Lane8: CCl4 + catechin group.
Lane9: CCl4 + phytic acid + catechin.

Bradford reagent to give a blue complex which is measured colorimetrically at wavelength 594 nm.

(1) Serum Determination of Albumin Level: This was done according to Doumas et al.[7]. Diagnostic kits were purchased from Randox company (United Kingdom); following the instructions of the manufacturer. In a buffered solution, bromocresol green forms with albumin, a green colored complex, its intensity is proportional to the amount of albumin present in the sample which was measured at wave length of 630 nm.

(1) Serum Determination of Total Cholesterol (TC) Level: This was done according to the procedure previously explained by Roeschlaul et al.[32]. Diagnostic kits were purchased from Randox company (United Kingdom); following the instructions of the manufacturer. Cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-amino antipyrene in the presence of phenol and peroxidase at wave length 500 nm.

(1) Serum Determination of Nitric Oxide Level: Promega's Griess Reagent system Griess[16], is based on the chemical reaction between sulfanilamide and N-1-naphthylethylenediamine dihydrochloride (NED) under acidic phosphoric acid condition to give coloured azo-compound which can be measured colourimetrically at wave length 520-550 nm.

(1) Determination of Sialic Acid Level: Serum sialic acid was determined using Ehrlich’s reagent, according to the method of Werner and Odin[40] and Waters et al.[41]. Sialic acid, a class of important ketoses that contain nine carbon atoms are acetylated derivatives of neuraminic acid (2-Keto-3,5-dideoxy-D-2-nonalulosonic). This can react with p-dimethylaminobenz aldehyde to give marked color which can be read spectrophotometrically at wave length 565 nm.

ELISA Determination of P53: P53 was determined according to the method of Hirao et al.[19] using ABC Diagnostics kit. Using enzyme linked immunosorbent assay (ELISA) depending on antigen – antibody reaction at wave length 400 nm.

Liver Tissue Homogenate Determinations:

(1) Determination of Glutathione Content: Glutathione was estimated according to the method of Moron et al.[26]. The analysis involved the extraction of GSH with metaphosphoric acid and then its reaction with dithiobisnitrobenzoic acid (DTNB) to form a yellow colour which was measured at wave length 412 nm.

(1) Determination of Liver Lipid Peroxides Level: Lipid peroxides were estimated using thiobarbituric acid as described by Ohkawa et al.[27]. This method depends on measuring the small amounts of free serum malondialdehyde (MDA) in nmol/mL, formed during the process of lipid peroxidation at wave length 532 nm.

Histopathological Investigations: A small sample of liver was fixed in 10% buffered formalin and processed in paraffin wax for collagen content. Briefly, Sections (5µm) of liver stained with Masson’s trichrome stain were mounted in neutral dixterene dibutyl phthalate xylene (DPX) and then differentiated in 0.5% acetic acid. Light microscopic examination were made at 40, 100, 200 and 400 magnifications. At least three different sections were examined in each sample of liver. The pathologist evaluating these sections was unaware of the groups when assessing the histology[13].

Statistical Analysis: Results are presented as mean ±SD for the animals in each experimental group. Significant difference between groups is analyzed by one – way ANOVA combined with post – hoc (LSD – SPSS computer programs) to compare between more than 2 means. The level of significance was considered to be (P<0.05).

Table (1) demonstrates the elevation in serum liver enzymes activities post CCl4 injection compared to normal control group. Administration of phytic acid
RESULTS AND DISCUSSIONS

Table 1: Different parameters measured in CCl4 group as a cirrhotic model and the therapeutic action of phytic acid, catechin and their combination in comparison to their controls.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>CCl4</th>
<th>CCl4 + Phytic</th>
<th>CCl4 + Catechin</th>
<th>CCl4 + Phytic + Catechin</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (U/L)</td>
<td>25.63±0.259</td>
<td>91.55±0.075</td>
<td>46.17±0.280</td>
<td>43.40±0.184</td>
<td>41.77±0.140</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>32.36±0.204</td>
<td>70.28±0.176</td>
<td>52.18±0.161</td>
<td>50.30±0.142</td>
<td>45.27±0.097</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>86.47±0.099</td>
<td>105.69±0.086</td>
<td>64.24±0.211</td>
<td>101.59±0.186</td>
<td>103.18±0.145</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>200±30.72</td>
<td>1112±150.31</td>
<td>380.76±50.25</td>
<td>770.52±140.24</td>
<td>190.38±29.52</td>
</tr>
<tr>
<td>Total protein(mg/mL)</td>
<td>116.75±0.165</td>
<td>54.59±0.161</td>
<td>75.28±0.175</td>
<td>87.14±0.125</td>
<td>99.78±0.187</td>
</tr>
<tr>
<td>Albumin(gm/dL)</td>
<td>3.54±0.181</td>
<td>3.37±0.113</td>
<td>3.45±0.146</td>
<td>3.54±0.127</td>
<td>3.47±0.195</td>
</tr>
<tr>
<td>Cholesterol(mg/L)</td>
<td>66.59±0.196</td>
<td>68.15±0.117</td>
<td>53.34±0.169</td>
<td>59.94±0.207</td>
<td>58.24±0.170</td>
</tr>
<tr>
<td>GSH (mmol/gm tissue)</td>
<td>3.90±0.097</td>
<td>1.75±0.133</td>
<td>2.04±0.116</td>
<td>2.60±0.314</td>
<td>2.91±0.200</td>
</tr>
<tr>
<td>LPOO(nmol/mL/gm tissue)</td>
<td>2.36±0.178</td>
<td>3.05±0.164</td>
<td>1.92±0.12</td>
<td>2.21±0.093</td>
<td>1.83±0.10</td>
</tr>
<tr>
<td>NO (µmol)</td>
<td>50.28±0.147</td>
<td>78.11±0.095</td>
<td>60.29±0.142</td>
<td>55.34±0.105</td>
<td>44.31±0.198</td>
</tr>
<tr>
<td>Sialic acid(mg/dL)</td>
<td>116.63±0.240</td>
<td>183.20±0.155</td>
<td>111.57±0.161</td>
<td>150.14±0.055</td>
<td>133.32±0.171</td>
</tr>
<tr>
<td>P53 (U/mL)</td>
<td>4.2025±0.046</td>
<td>3.901±0.015</td>
<td>3.926±0.073</td>
<td>4.1357±0.09</td>
<td>4.115±0.095</td>
</tr>
</tbody>
</table>

Results were expressed as mean ± SD and significant difference according to control group at P<0.0001, ANOVA showed a highly significant difference between all groups at P<0.0001. But P53 at P<0.001.

and catechin either alone or in combined form produced a significant reduction in the previous enzymatic activities. However, the combination of both phytic acid and catechin produced the most significant reduction in ALT, AST and LDH levels. While phytic acid alone reduced significantly ALP level to the normal level compared to CCl4 intoxicated group at P<0.0001. Administration of CCl4 also resulted in a significant reduction in total protein, albumin, GSH and P53 levels and a marked elevation in cholesterol, LPOO, NO and sialic acid levels in comparison to control group. Combination of both phytic acid and catechin caused the most significant elevation in total protein and GSH levels and a marked reduction in LPOO and nitric oxide levels at P<0.0001 in comparison to groups treated with either phytic acid or catechin. However, catechin alone markedly elevated albumin and P53 levels to the normal values compared to CCl4 intoxicated group at P<0.0001 and P<0.001 respectively. While phytic acid caused the most significant reduction in both cholesterol and sialic acid levels compared to CCl4 intoxicated group at P<0.0001.

RESULTS AND DISCUSSIONS

Results: Separation of the five LDH isoenzymes by Gel electrophoresis showed that the combination of both phytic acid and catechin reduced significantly LDH 4&5 which was markedly increased post CCl4 injection. These isoenzymes are specific markers for liver disease in comparison to reference control (heart). Discussion: In the present study, 24h after CCl4 injection, ALT, AST, ALP and LDH serum levels were remarkably increased. As previously suggested that the hepatocytes were acutely injured, cell membrane integrity was altered and the enzymes in hepatocytes leaked out[12]. However, after treatment with phytic acid and catechin the pathological increases in ALT, AST, ALP and LDH were significantly reduced as shown in chart (1). These results reflect that phytic acid and catechin had a protective effect against hepatocyte injury and this comes in line with those of Xiao-hui[44] and[12] who reported the protective effect of catechin and phytic acid respectively against CCl4 induced liver cirrhosis. Electrophoretic separation of LDH isoenzymes revealed a significant elevation in these isoenzymes post CCl4 injection specially LDH 5 isoenzyme indicating liver dysfunction this is confirmed with that previously reported by Osypiew[28] while a significant reduction of the previous isoenzymes was observed after the treatment with the collective combination of phytic acid and catechin this seems to be coincide with Miyagawa[25] and Begoña[1]. Serum albumin is the predominant serum protein, which reflects the synthetic function of the liver. Data of the present study reflected that CCl4 intoxication produced a significant reduction in both protein and albumin serum levels compared to control group this is
coincides with that of Vazques et al.,[37] who observed a reduction in protein synthesis after \( \text{CCl}_4 \) treatment and in full agreement with Rothschild et al.,[33] who observed that when the liver has been chronically damaged, albumin may be low. In the present study administration of both phytic acid and catechin together produced a significant increase in protein level while catechin alone showed the most significant elevation in albumin level as shown in chart(2). This coincides with that of Silvina[36] who observed that catechin showed a protective capacity of 70-90% in preventing SH-groups oxidation specially in albumin. Also cholesterol level was markedly increased in carbon tetrachloride treated groups compared to control group. This is in agreement,
with that of Farooq et al.\(^\text{[10]}\) who observed an alteration in cholesterol level after carbon tetrachloride injection. Phytic acid produced the most significant reduction in cholesterol level as shown in chart (4). This may be explained on the basis that phytic acid has a strong ability to chelate multivalent metal ions especially zinc, calcium, and iron. Alternatively, its ability to chelate minerals has been reported to have some protective effects. Such as decreasing iron-mediated free radical formation and lowering serum cholesterol, triglycerides and lipid peroxide in experimental animals\(^\text{[45]}\).

The free radicals and its triggered lipid peroxidation were involved in the main mechanisms by which carbon tetrachloride injured hepatocytes. Lipid peroxide elevated level could reflect the degrees of lipid peroxidative injury in hepatocytes. GSH, a peroxide scavenger, could eliminate superoxide anion and hydrogen peroxide. The content of GSH reflects the ability against peroxidation\(^\text{[14]}\). NO\(^-\) can maintain tissue protective reactions as well as pro-oxidant effects. NO\(^-\) reacts with superoxide radical (O\(^2^-\)) resulting in peroxynitrite which is a strong oxidant that reacts with thiols and initiates lipid peroxidation\(^\text{[24]}\).

In this study, GSH in liver homogenate of the cirrhotic model group was remarkably reduced, reflecting that the potency of antioxidation in injured cells was altered. However, liver lipid peroxides and serum nitric oxide levels were markedly elevated post \(\text{CCL}_4\) intoxication. Phytic acid and catechin inhibited the increase in lipid peroxide, nitric oxide and increased GSH levels as shown in chart (3). These results seem to be highly coincide with that of Chen et al.\(^\text{[2]}\) and Rady\(^\text{[31]}\) who reported the strong antioxidative effects of each of catechin and phytic acid respectively. This is attributed to the increase in glutathione-S-transferase activity, inhibiting edema, hyperplasia, free radical scavenging, blocking tumor promoter-induced inhibition of intercellular communication and enhancing cell-mediated immunity. While phytic acid chelates multivalent metal ions especially zinc, calcium, and iron leading to reduction in iron-mediated free radical formation and lowering serum lipid peroxide and the synthesis of nitric oxide synthase (which is stimulated by calcium) in experimental animals\(^\text{[20]}\).

The increase in sialic acid level was considered as a marker for cell lysis\(^\text{[5]}\). \(\text{CCL}_4\) intoxicated group showed the most significant elevation in sialic acid level. This observation was previously reported by Kishore et al.\(^\text{[21]}\) who investigated Sialic acid level in the livers of control and \(\text{CCL}_4\) treated rats. In the present study phytic acid showed the most significant reduction in sialic acid level as shown in chart (4). These results are in parallel with that of Mahmoud et al.\(^\text{[24]}\) who reported that Phytic acid is an anti-neoplastic agent, and administration of phytic acid during tumorogenesis, is associated with biochemical changes including enhancement of apoptosis, inhibition of oxidative stress and decreasing sialic acid and NO levels.

The tumor suppressor gene p53 is expressed in response to DNA damage; its protein product blocks cells in the G phase of the cell cycle. This gives cells additional time to repair their DNA damage so its called honest cell guard. However, it may trigger apoptosis if damage is too high\(^\text{[29]}\). ELISA technique in the present study revealed that P53 level was significantly reduced in \(\text{CCL}_4\) intoxicated group. This result may be explained on the basis that \(\text{CCl}_4\) acts as a tumor promoter on genetically initiated cells through increasing the intracellular concentration of reactive oxygen species and promoting tissue necrosis/regeneration and cell proliferation and/or may be due to p53 mutant allele. Our results concerning p53 was in accordance with that of Farazi et al.\(^\text{[3]}\) who previously noticed a significant fraction of hepatocarcinomas arises in alcoholic liver cirrhosis model through increasing the intracellular concentration of reactive oxygen species and promoting tissue necrosis/regeneration and cell proliferation. They also reconfirmed the scientific fact indicating the ability of tumor suppressor p53 to restraints the expansion of carcinogen-initiated cells by inducing cell cycle arrest and apoptosis; accordingly, p53-deficient mice develop spontaneous and chemically induced neoplasms at a much higher frequency than normal mice.

The present study revealed that the administration of catechin alone resulted in the most significant improvement in P53 serum level as shown in chart (4). These results may be due to the highly antioxidative power of catechin previously proved by Chen et al.\(^\text{[2]}\).

Our results are also parallel with Kuo & Lin\(^\text{[23]}\) who observed the ability of catechin to inhibit the proliferation of hepatic cell carcinoma by inducing apoptosis through significant increase in the expression of p53 and p21 W AF 1 protein, thus blocking cell cycle progression in the G phase.

Histological examinations in this study showed that \(\text{CCL}_4\) intoxicated group revealed a highly damaged portal tracts enclosed with thick loose collagen fibers. This is coincide with that of Favari\(^\text{[21]}\) who observed that treatment with CCl4 resulted in a marked elevation.
in collagen content about four-fold and histological examination of liver samples showed that collagen increase distorted the normal liver architecture. The results of this work showed that the combination of both phytic acid and catechin nearly returned the pattern of collagen fiber distribution to normal this is parallel to that of Farazi\cite{9} and Fox and Eau Elbert\cite{12} who observed improvement in collagen contents post catechin and phytic acid treatment respectively and their ability to promote the breakdown of collagen.
Conclusion: From the previous results it was obvious that the combination of both phytic acid and catechin showed the most significant improvement in each of ALT, AST activities, total protein, GSH, LPOO and NO levels. While phytic acid alone improved the levels of ALP, sialic acid and cholesterol. However, catechin showed the most significant improvement in albumin and P53 levels. Histological examinations showed that phytic acid in combination with catechin nearly returned the pattern of collagen fiber distribution to normal. It was obvious that the antioxidant effect of phytic acid was synergized by addition of catechin and have a big role in relieving oxidative stress, scavenging free radicals and inhibiting lipid peroxidation. Moreover it is better recommended a collective combination of both phytic acid and catechin during the course of liver disease or CCl4 factories and dry clean workers to accelerate the improvement of liver functions so the addition of these antioxidants natural products will decrease the need for high doses of drugs and may also shorten the period of treatment. A prospective combination of both phytic acid and catechin will by time cancel the need for other loading drugs and protects the liver from this metabolic burden to much of prescribed drugs.

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