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Research Article

Modulatory Potential of *Corriandrum sativum* on Experimentally Induced Hepatic Injury in ICR Mice: A Biochemical and Histopathological Investigation

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ABSTRACT
Ayurvedic medical practitioners in Sri Lanka use several locally available medicinal plants either as single drugs or in combination with other plants in the treatment of liver diseases. *Corriandrum sativum*, a glabrous herb emitting a strong odour when rubbed, is used for colds, influenza, bilious complaints, liver disorders and fever. It is also used as a diuretic, tonic and aphrodisiac. The aim of this study is to investigate the hepatoprotective activities of the aqueous extract of *Corriandrum sativum* seeds against CCl_4 and acetaminophen induced hepatotoxicity in ICR mice. Hepatotoxicity was induced by the administration of a single intraperitonial dose of CCl_4 (0.5 mL kg^{-1} CCl_4 in olive oil) and single oral dose of acetaminophen (300 mg/kg in saline) after a 16 h fast. *Corriandrum* extract (0.9 g kg^{-1}) was used on pre and post-treatment basis. Both pre and post-treatment decreased the CCl_4 mediated increase in serum enzyme activities (ALT, AST, ALP) and increased the reduced glutathione concentration in the liver significantly. A significant improvement was also observed in a majority of serum enzymes and reduced glutathione concentration in acetaminophen treated mice. Histopathological studies provided supportive evidence for the biochemical analysis in both CCl_4 and acetaminophen treated mice. The ability of the plant extract to protect the liver against changes mediated by carbon tetrachloride and acetaminophen confirmed that the plant possesses anti-hepatotoxic properties against CCl_4 and acetaminophen induced liver damage in ICR mice.

Keywords: *Corriandrum sativum*, Carbon tetrachloride, Acetaminophen, Hepatoprotective, Histopathology

INTRODUCTION

Many drugs derived from plants and used in modern medicine were developed by ethnomedical leads followed by ethno pharmacological studies. There are more than 100 drugs of known structure used in allopathic medicine that were extracted from higher plants. Furthermore, in the past two decades, due to a variety of reasons such as dissatisfaction with modern medications for the treatment of many chronic and deadly disease conditions, resistance built up by microbes to modern drugs and disabilities during aging, there has been a remarkable increase in the interest in herbal remedies demonstrated by the general public of most developed countries[1]. Viral hepatitis is the most common cause of liver inflammation and hepatitis B is the most common viral hepatitis worldwide, affecting approximately 10% of the adult population in endemic areas and causing approximately 780,000 deaths per year worldwide. In countries like United States, hepatitis (HCV) has become the most common viral hepatitis since the widespread vaccination of Hepatitis B. An estimated 130 to 170 million people worldwide are chronically infected with hepatitis C[2,3]. Both hepatitis B and hepatitis C are treated with IFN-α, often in combination with other antiviral drugs. Side effects reported makes it questionable to use IFN-α in large numbers of patients, and it makes it difficult to use prolonged maintenance therapy to suppress HBV[4]. Also, combination therapy costs approximately 10000 – 18000 USD per year. Although Interferon, nucleotide analogue combination therapy has been the standard of care in developed countries regardless of which genotype of HCV the patient was infected with, the overall SVR (Sustained Viral Response) rate is 50–60% with this treatment. The search for new drugs continues to improve the SVR rates in patients infected with certain HCV genotypes as well for those who do not respond well for the combination therapy[5]. Since most of these advanced therapies are not affordable to the patients in the developing world, the search for new therapeutics remains a higher priority. Plant drugs are known to play a vital role in the management of liver disorders in Sri Lanka. Ayurvedic medical practitioners in Sri Lanka use several locally available medicinal plants either as single drugs or in combination with other plants in the treatment of liver diseases. *Corriandrum sativum*, a glabrous herb emitting a strong odour when rubbed, is a native of Palestine, Syria and Greece. *Corriandrum* plants are now cultivated in India and Sri Lanka. The fruit is used with

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dry ginger as decoction for colds, influenza and fever. It is also used as a diuretic, tonic and aphrodisiac. *Corriandrum* is also used for dyspepsia, bilious complaints and liver disorders[6]. Carbon tetrachloride (CCl_4) is certainly the best known example of a chemical whose hepatotoxicity is presumably the consequence of the formation of free radicals. Histopathological changes observed in CCl_4 toxicity are similar to that of viral hepatitis so that hepatotoxicity of CCl_4 has probably been more extensively studied than that of any other hepatotoxin. Carbon tetrachloride is metabolized by the cytochrome P450 enzyme system in the liver, which forms a trichloromethyl radical responsible for lipid peroxidation[7]. Acetaminophen (APAP) is a safe and effective analgesic at therapeutic doses. However, APAP received at an excessively high dose can cause severe liver injury and even acute liver failure (ALF)[8]. The main mechanism of APAP-induced hepatotoxicity is due to the formation of a reactive metabolite N-acetyl-p-amino-benzoquinone imine (NAPQI), which in turn depletes glutathione (GSH) and binds to a variety of cellular mitochondrial proteins. In this study, the aqueous seed extract of *Corriandrum sativum* was tested for hepatoprotective activity against liver damage induced by carbon tetrachloride and acetaminophen in ICR mice. Assessment of liver function was performed by the determination of its specific serum markers as well as the reduced glutathione concentration in the liver homogenate. The study is also supported by a histopathological investigation of liver damage.

MATERIALS AND METHODS

Experimental animals

Healthy male ICR mice, 6-8 weeks old and weighing 30-35 g, were allowed free access to water and pelleted food *ad libitum*. All animals were fasted for 16 h before administration of the hepatotoxin. All protocols used in this study were approved by the ethics committee of the University of Ruhuna, Sri Lanka, guided by the CIOMS international guiding principles of biomedical research involving animals.

Chemicals

Acetaminophen was a gift from the Sri Lanka Pharmaceutical Manufacturing Corporation. 5, 5'-dithiobis (2-nitrobenzoic acid) was purchased from Sigma (St. Louis, Missouri). N-Acetylcysteine (NAC) was obtained from the Teaching Hospital, Karapitiya, Galle, Sri Lanka. Diagnostic kits for serum alanine aminotransferase (ALT, EC 2.6.1.2), aspartate aminotransferase (AST, EC 2.6.1.1) and alkaline phosphatase (ALP, EC 3.1.3.1) were purchased from Randox (UK). 5, 5'-Dithiobis (2-nitrobenzoic acid) was purchased from Sigma (St Louis, MO). All other reagents were commercially available and of reagent grade.

Preparation of the plant extract

Seeds of *Coriandrum sativum* were purchased commercially. The sample was authenticated by comparison with the herbarium specimen preserved at the National Herbarium in the Botanical Gardens, Peradeniya, Sri Lanka. A voucher specimen (UoR/BIOCHEM/RH/05) was deposited at the Department of Biochemistry, University of Ruhuna, Sri Lanka. The normal therapeutic dose of humans extrapolated to mouse was used[9]. 2.625 g of *Corriandrum* seeds were refluxed in 30 mL of distilled water for 1 h and concentrated to 20 mL. Extraction was done daily prior to the administration of the plant extract to mice. Each mouse was administered a dose of 0.9 g kg^{-1} orally by gavage. The extract was prepared daily from the dried plant material.

Induction of liver damage

Hepatic injury was induced in mice by the administration of either CCl_4 or Acetaminophen. All animals were fasted for 16 hours before administration of the hepatotoxin. CCl_4, 0.5 mL/ kg in olive oil (CCl_4: olive oil was 1:10) was injected intraperitonially. Acetaminophen was dissolved in saline and heated to 60°C to obtain a homogenous solution. 300 mg / kg was administered orally by gavage.

Treatment of animals

Control groups

Mice were divided into two groups of 10 animals in each. The first group served as the normal control group and received distilled water orally by gavage. The second group was treated with the *Corriandrum* extract alone for 7 days. Animals were killed 7 days after the administration of the plant extract.

Carbon tetrachloride-induced hepatotoxicity

Mice were randomly divided into six groups (groups 3-8) of 10 animals in each. A single intraperitonial dose of CCl_4 was injected (0.5 mL/kg in olive oil, CCl_4: olive oil 1:10) in each animal after a 16 h fast. In groups 3 and 4 the animals were killed 24 h and 4 days, respectively, after the administration of CCl_4. Animals in group 5 were administered *Coriandrum sativum* extract half an hour after the administration of a single dose of CCl_4 and were killed 24 h later. The same procedure was carried out for group 6 but instead of killing after 24 h, they were given the extract alone for a further two days at 24 h intervals (post-treatment). They were killed on the fourth day. Groups 7 and 8 were administered the *Coriandrum sativum* extract daily for seven days and on the seventh day a single dose of CCl_4 was injected half an hour after the administration of the plant extract. The mice were killed after 24 h and 4 days, respectively.

Acetaminophen induced hepatotoxicity

Mice were randomly divided into four groups (groups 9-12) of 20 animals each. 300 mg/kg of acetaminophen (dissolved in saline and heated at 60˚C) was administered orally after a 16 h fast. Group 9 was given acetaminophen alone and killed 4h later. Group 10 received the same dose of acetaminophen and half an hour later 500 mg/kg of NAC was given orally. The mice were killed 4 h later. In the group 11, *Corriandrum sativum* extract was administered instead of NAC. *Coriandrum sativum* was administered for 7 days in group 12 and on the seventh day acetaminophen was administered half an hour after the administration of the plant extract. Animals were killed 4h later.

Determination of liver enzyme concentrations

A combination of the methods of Reitman and Frankel[10], and Schmidt and Schmidt[11], were used for the determination of alanine aminotransferase (ALT, EC 2.6.1.2) and aspartate aminotransferase (AST, EC 2.6.1.1) concentrations. Serum alkaline phosphatase (ALP, EC 3.1.3.1) concentration was measured using an optimized standard method according to the recommendations of the Deutsche Gesellschaft fur Klinische Chemie[12]. All assay kits were purchased from Randox laboratories Ltd, UK.

Determination of reduced glutathione content

A liver section was homogenized and used for the determination of the liver reduced glutathione (GSH) content. The method of Jollow *et al*[13] as described in Sedlak and Lindsay[14] was used. The method was based upon the development of a relatively stable yellow colour when 5, 5'dithiobis-2-nitrobenzoic acid (Ellman reagent) reacts with reduced glutathione and other sulfhydril compounds.

Histopathological assessment of liver damage

Liver tissues were excised, weighed and a section of the liver was fixed in 10% buffered formalin for histopathological assessment of liver damage.

Histological sections of the formalin-fixed liver tissue were stained with haematoxylin and eosin.

Statistical analysis

The results were evaluated by one-way analysis of variance and Tukey's multiple comparison test. A probability (*P*) value of less than 0.05 was considered significant.

RESULTS

Figure 1 and 2 summarize the effect of *Corriandrum* extract on serum enzyme levels and liver reduced glutathione level against CCl_4 induced hepatotoxicity. A significant increase (P<0.001) in the activities of serum enzyme levels and a decrease (P<0.001) in liver reduced glutathione occurred within 24 h of exposure of mice to a single dose of CCl_4. In *Corriandrum* treated mice, a significant improvement was observed in all the parameters 24 h after the administration of CCl_4 compared to the results observed 4 days later. Pre-treatment showed a faster recovery and improved the serum enzyme levels of ALT, AST, ALP and liver reduced glutathione level by 26.97, 45.12, 67.29 and 106.32 percent respectively 24 h after the administration of CCl_4. Histopathological examination of the liver tissue

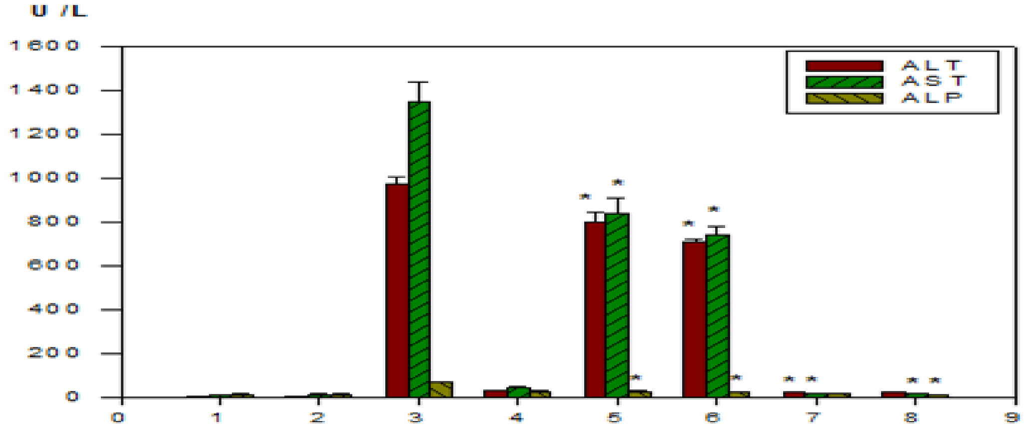

Figure 1: Effect of *Corriandrum* and CCl_4 on serum enzyme levels of ALT, AST

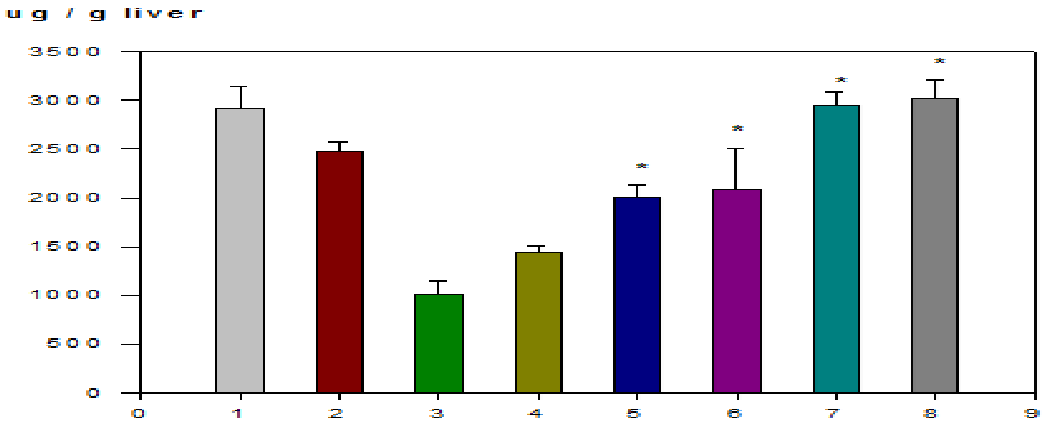

Figure 2: Effect of *Corriandrum* and CCl_4 on liver reduced glutathione level

Fig 1 & 2: n=10 mice in each group. Group 1: Normal control group, treated with distilled water; Group 2: *Corriandrum* (0.9 g/kg, p.o) for 7 days .Group 3: a single dose of carbon tetrachloride (0.5 ml/kg in olive oil, ip) and sacrificed 24 h later; Group 4: a single dose of carbon tetrachloride (0.5 ml/kg in olive oil, ip) and sacrificed 4 days later; Group 5: Post-treatment with *Corriandrum*, sacrificed 24 h later; Group 6: Pre-treatment with *Corriandrum*, sacrificed 24 h later; Group 7: Post-treatment with *Corriandrum*, sacrificed 4 days later; Group 8: Pre-treatment with *Corriandrum*, sacrificed 4 days later. Results are given as mean ± S.E.M.

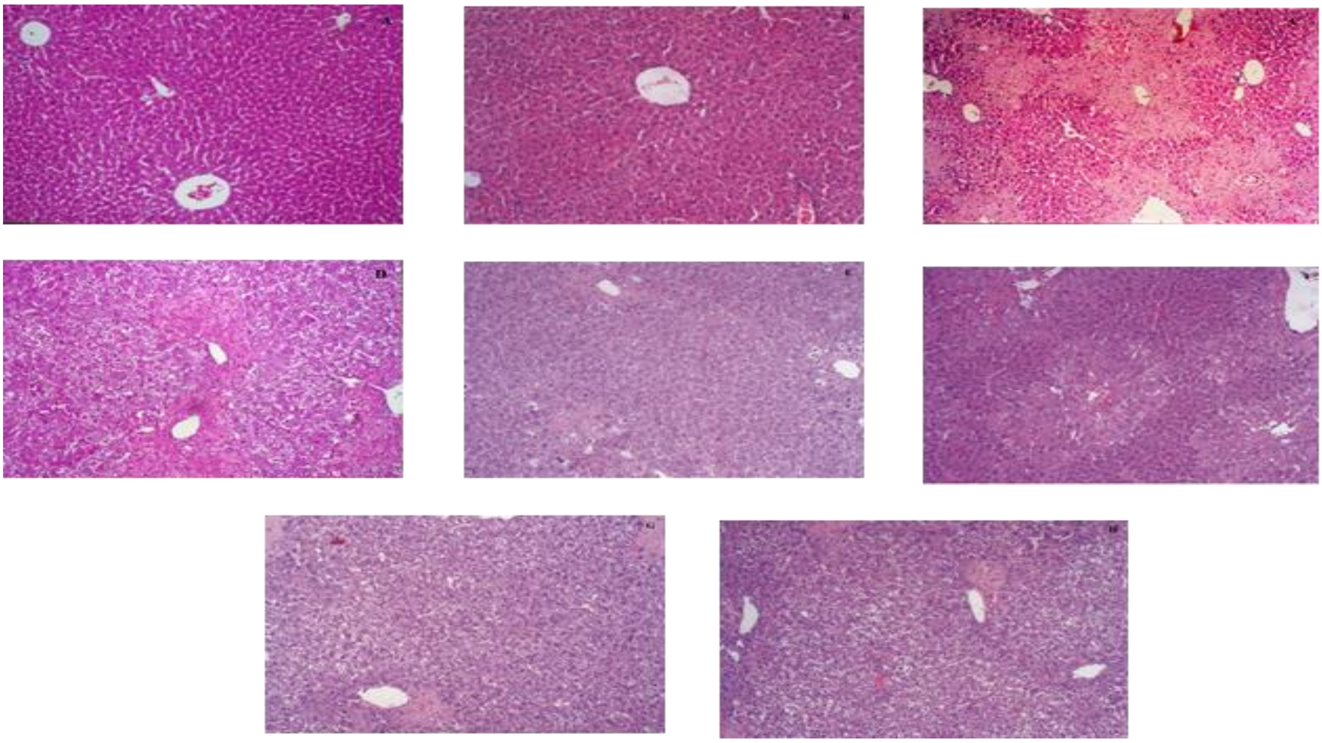

Figure 3: Liver Histopathology of mice sacrificed 24 h and 4 days later. A: Normal control, B: *Corriandrum* control, C: CCl_4 control (24 h), D: CCl_4 control (4 days), E: *Corriandrum* post-treated (24 h), F: *Corriandrum* pre-treated (24 h), G: *Corriandrum* post-treated (4 days), H: *Corriandrum* pre-treated (4 days)

provided supportive evidence for the biochemical analysis (Fig 3). Microscopically, liver slices from control animals stained with haematoxylin and eosin showed normal parenchymal architecture with cords of hepatocytes, portal tracts and terminal veins without noticeable alterations (Fig 3A). Liver sections of mice challenged with CCl_4 alone 24 h after the administration of CCl_4 showed mainly centrilobular necrosis with focal fatty changes and ballooning degeneration in the surviving hepatocytes (Fig 3C). The areas of necrosis were less in mice 4 days after the administration of CCl_4 (Fig 3D). Histopathologically, areas of necrosis were less in *Corriandrum* pre-treated mice (Fig 3E) compared to the CCl_4 control group, 24 h after the administration of CCl_4. Only a focal necrosis was visible in the post-treated group (Fig 3F). However, pre-treatment (Fig 3G) was better compared to the post-treatment (Fig 3H) 4 days later. *Corriandrum* control group showed the normal parenchymal architecture (Fig 3B). As shown in figures 4 and 5, the activities of serum ALT, AST and ALP 4 h after the administration of acetaminophen alone were significantly increased ($P<0.001$). In addition, Liver reduced glutathione levels were significantly decreased ($P<0.001$) compared to the normal control. *Corriandrum* pre-treated mice showed better results compared to post-treated mice. In the post treated group, only serum ALT level and AST level were improved significantly. Compared to the plant treated groups, N-Acetyl cysteine showed a faster recovery. All serum enzyme levels were decreased significantly and the liver reduced glutathione level was increased significantly compared to the plant treated groups. Macroscopically, liver appeared dark and congested in acetaminophen intoxicated mice.

Histologically, the liver showed confluent necrosis with vacuolation and ballooning degeneration in the surviving hepatocytes (Fig 6 C). There was no significant difference between the post-treatment (Fig 6F) and the pre-treatment (Fig 6E) in *Corriandrum* treated mice four hours later. Smaller areas of necrosis were still visible in the plant-treated groups compared to the acetaminophen control. Vacuolation and congestion together with necrosis were visible in both pre and post-treated mice.

DISCUSSION

Liver is one of the most important organs in the body, with a remarkable capacity to fully regenerate after significant hepatic tissue damage. It is involved in many exocrine and endocrine functions such as synthesis, storage and metabolism[15]. In the present study, two human conditions of liver damage were simulated in mice using acetaminophen and carbon tetrachloride (CCl_4). These are commonly used models for the screening of hepatoprotective drugs. Out of the two chemicals, carbon tetrachloride and acetaminophen, carbon tetrachloride induced damage is histologically similar to viral hepatitis in humans[16]. CCl_4 intoxication is a widely used experimental model for the induction of liver injury. The highly hepatotoxic metabolites, namely, trichloromethyl radicals ($CCl_3\cdot$ and $CCl_3O_2\cdot$) are generated during the metabolic activation of CCl_4 by the cytochrome P-450 system in the liver. These radicals have a central role in the initiation of lipid peroxidation, inflammation, and

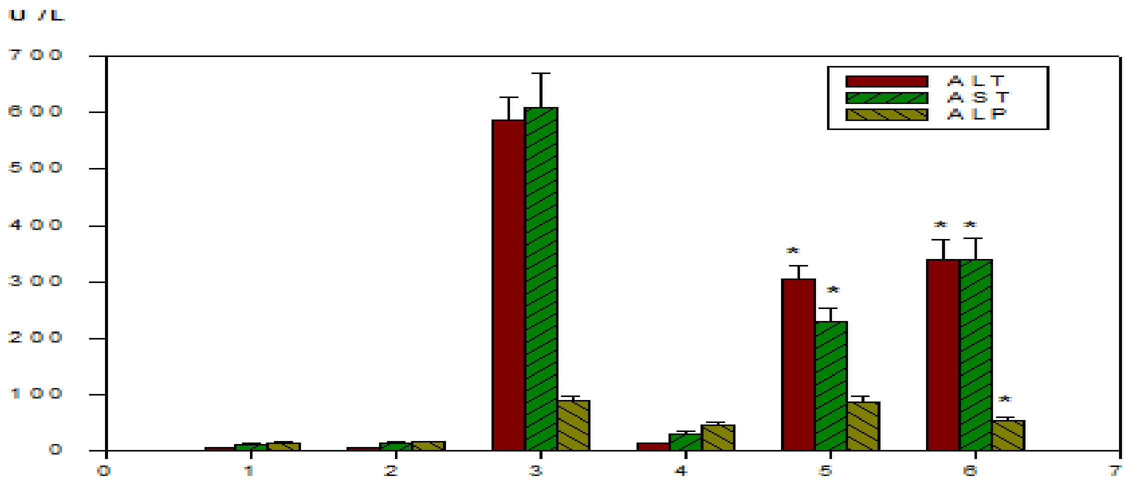

Figure 4: Effect of *Corriandrum* and acetaminophen on serum enzyme levels of ALT, AST and ALP

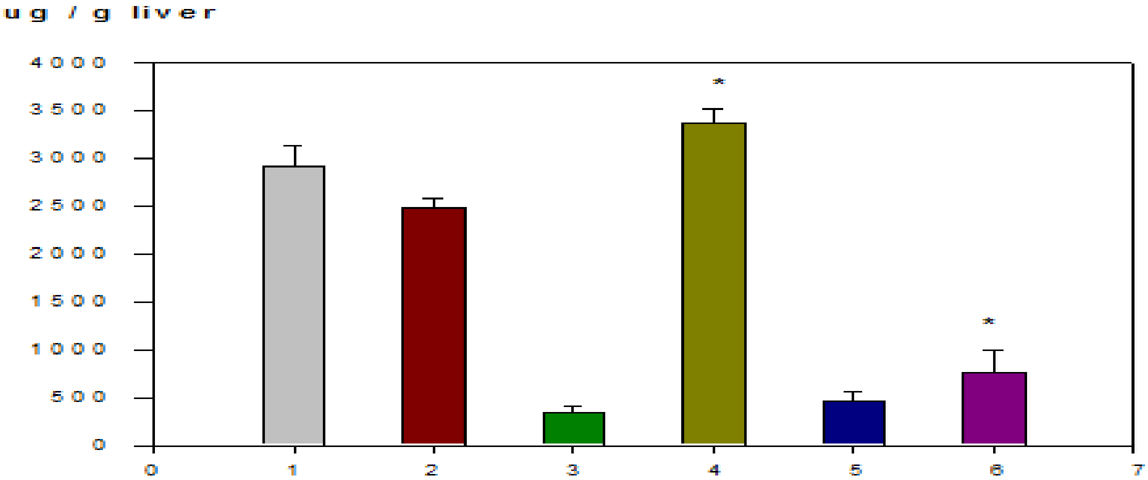

Figure 5: Effect of *Corriandrum* and acetaminophen on liver reduced glutathione level

Fig 4 & 5: n=20 mice in each group. Group 1: Normal control group, treated with distilled water; Group 2: *Corriandrum* control (0.9 g/kg, p. o) for 7 days .Group 3: a single dose of acetaminophen (300 mg/kg in saline, orally) and sacrificed 4 h later; Group 4: a single dose of acetaminophen + N-acetyl cysteine (500 mg/kg) and sacrificed 4 h later; Group 5: Post-treatment with *Corriandrum*, sacrificed 4 h later; Group 6: Pre-treatment with *Corriandrum*, sacrificed 4 h later; Results given as mean ± S.E.M.

fatty changes of the liver[17]. Furthermore, CCl_4 intoxication is associated with oxidative stress since the $CCl_3\cdot$ and $CCl_3O_2\cdot$ radicals alter the antioxidant status of the liver by deactivating the hepatic antioxidant enzymes including superoxide dismutase, glutathione peroxidase, glutathione reductase and Glutathione-S-transferase [18]. Trichloromethyl radicals also react with the sulfhydryl groups of GSH leading to its deactivation. In the present study, treatment with CCl_4 markedly increased the levels of AST, ALT, and ALP in blood. The leakage of the marker enzymes into the blood was associated with marked necrosis, loss of hepatic architecture, hydropic degeneration, fatty changes, Kupffer cell hyperplasia, central vein congestion, and infiltration of the liver by lymphocytes[18]. Acetaminophen is a common analgesic and antipyretic drug which is safe at therapeutic doses. Many studies demonstrated the induction of necrosis in hepatocytes by the administration of high doses of acetaminophen in animals. After the administration of high doses of acetaminophen, it is extensively metabolized into N-acetyl-p-benzoquinoneimine (NAPQI) which depletes GSH and leads to hepatotoxicity[19]. Acetaminophen was also shown to inhibition of cellular proliferation, induction of oxidative stress, lipid peroxidation, depletion of ATP levels, and alteration of Ca^{+2} homeostasis. All of these changes are considered potentially fatal to the cell[20]. To evaluate liver injury, concentrations of biochemical markers (ALT, AST, and ALP activity) are measured[21]. In our study, the hepatotoxicity due to CCl_4 and acetaminophen was confirmed by elevated levels of biochemical parameters like ALT, AST, ALP. A significantly high serum enzyme activity of ALT and AST, 974.82 and 1347.4 U/L ($P<0.001$, Fig 1) were observed 24 h after the administration of CCl_4 and 588.12 and 609.37 ($P<0.001$, Fig 4) 4 h after the administration of acetaminophen. This can be explained by the fact that hepatic cells contain a host of enzymes and possess a variety of metabolic

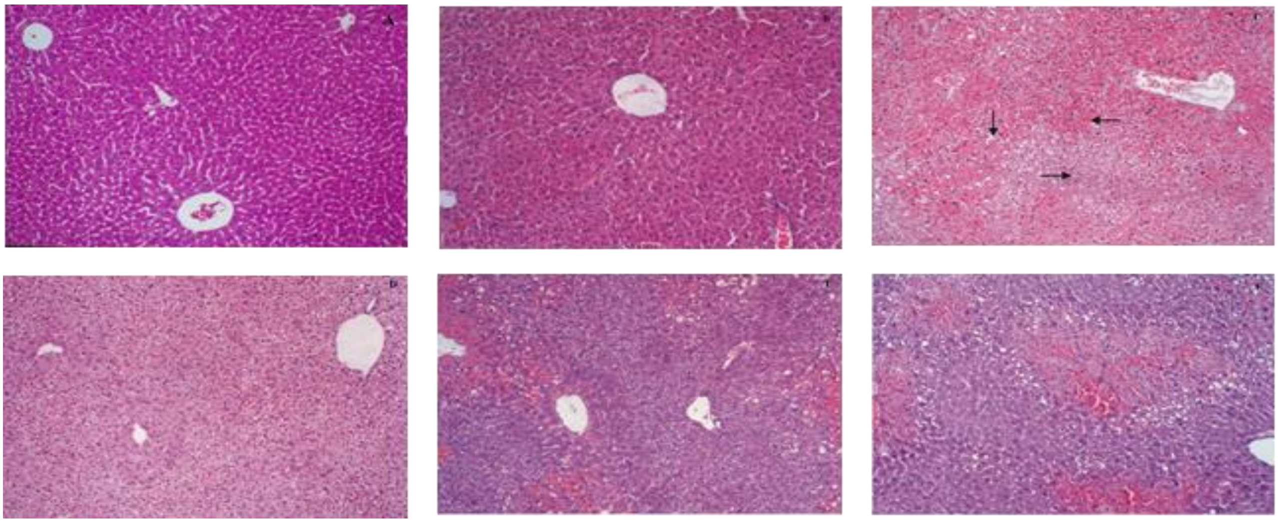

Figure 6: Liver histopathology of mice sacrificed 4 h later, A: Normal control, B: *Corriandrum* control, C: Acetaminophen control (24 h), D: Acetaminophen + NAC (positive control), E: *Corriandrum* post-treated (4 h), F: *Corriandrum* pre-treated (4 h),

activities. ALT was found in higher concentrations in cytoplasm and AST particularly in mitochondria. The rise in the ALT is usually accompanied by an elevation in the levels of AST, which play a vital role in the conversion of amino acids to keto acids. In hepatotoxicity, the transport function of liver cells is disturbed, causing leakage of plasma membrane[19], therefore resulting in leakage of these enzymes leading to an increase in their serum level.

The increased level of ALT and AST in acetaminophen-induced liver injury is an indicator of cellular leakage and loss of membrane integrity of liver cells[19]. The elevated serum level of alkaline phosphatase is due to its increased synthesis by bile canaliculi cell lining in response to the increased biliary pressure and cholestasis[20]. Serum ALP activity was increased significantly ($P<0.001$) to 68.54 (Fig 1) and 89.58 (Fig 4) respectively in CCl_4 and acetaminophen treated mice. GSH is one of the most abundant tripeptide, non-enzymatic biological antioxidant present in the hepatocytes, which is a key component of the overall antioxidant defense system that protects the membrane protein thiols of hepatocytes from deleterious effects of reactive oxygen metabolites such as hydrogen peroxide and superoxide radicals[22]. The decline of GSH level in the CCl_4 treated group might be due to its utilization by the excessively generated quantity of free radicals in the hepatocytes leading to hepatic injury. However, the subsequent recovery in rats treated with *Corriandrum* extract might be due to *de-novo* GSH synthesis or GSH regeneration (GSSG to GSH), or both[22]. In the present study, the liver GSH level was decreased significantly ($P<0.001$) to 1015.16 and 346.16 µg /g liver respectively in CCl_4 (Fig 2) and acetaminophen (Fig 5) control groups compared to 2916.04 µg /g liver in the normal control group. Modulation of cellular thiol pool has been used as a potential therapeutic strategy against APAP hepatotoxicity. Currently, the best therapeutic option to prevent progression to liver failure of an overdosed patient is administration of N-acetylcysteine (NAC). Hence, NAC was chosen as the positive control in this study. N-acetyl cysteine treatment effectively restored the depleted levels of these nonenzymic antioxidants. NAC could significantly interfere with the pathophysiology of free radical producing drug induced oxidative stress[23]. Wong et al. have reported the ability of NAC in regulating GSH concentration and thus protect liver damage from reactive metabolites formed from CCl_4. Increase in GSH levels could also contribute to the recycling of other antioxidants such as vitamin E and vitamin C[24]. A comparison of the hepatoprotective activity of plant extract treated groups with N-acetyl cysteine, the widely used antidote for acetaminophen poisoning, showed that under the experimental conditions used, plant extracts were not as effective as N-acetyl cysteine. Serum enzyme activities of ALT, AST and ALP in NAC treated mice were reduced by 97.8, 94.9 and 46.9 percent compared to the acetaminophen treated group. Histopathological examination also provided supportive evidence for the results obtained from the enzyme analysis. Microscopically, liver slices from control animals stained with haematoxylin and eosin showed normal parenchymal architecture with cords of hepatocytes, portal tracts and terminal veins without noticeable alterations (Fig 3A). Liver sections of mice challenged with carbon tetrachloride alone showed mainly centrilobular necrosis with ballooning degeneration in the surviving hepatocytes (Fig 3B). Macroscopically, the liver appeared dark and congested in acetaminophen intoxicated mice. Histologically, the liver showed confluent necrosis with vacuolation and ballooning degenation in the surviving hepatocytes (Fig 6C). Massive centrilobular congestion is an important feature of acetaminophen induced hepatotoxicity in mice that precedes the appearance of necrosis and results from alterations to hepatocytes and their relationship to sinusoidal lining cells. Congestion results from the accumulation of red blood cells within endocytic vacuoles and the space of disse, which collapses the original sinusoidal lumens[25]. The histopathological observations showing a faster regeneration of hepatic cells in mice, seem to suggest the possibility of the plant extract being able to condition the hepatic cells to a state of accelerated regeneration thus decreasing the leakage of ALT, AST and ALP into the circulation.

In the present study, both preventive and curative effects of the plant extracts were evaluated. Ayurvedic practitioners often prescribe these preparations to the whole household when there is a hepatitis patient in the home. Pre-treatment was designed to find out the scientific basis of this practice. In the present study, it was observed that *Corriandrum* plant extract alone did not increase the serum enzyme activities of ALT, AST and ALP significantly compared to the normal control group (Fig1 and 4). The significant reduction in serum AST and ALT levels observed in *Corriandrum* treated mice (prophylactic and curative groups) indicates hepatoprotective potential which may be due to cell membrane stabilization, repair of damaged hepatic tissue and/or antioxidant activity of the extract. Since the toxicity is enhanced by factors that cause GSH depletion, enhanced NAPQI formation or reduction in the antioxidative capacity of the liver, it could be suggested that the partial hepatoprotection afforded by *Corriandrum* extract may be ascribed to the opposing action of one or more of these factors. Increased GSH level in mice pre-treated with plant extract may result from the enhancement of either *de novo* GSH synthesis or GSH regeneration or both. As a consequence of the action of plant extracts in GSH metabolism, hepatic GSH level can be sufficiently maintained to counteract the increased formation of free radicals as in the case of carbon tetrachloride and acetaminophen induced toxicity. Our study suggested a significant protective effect of *Corriandrum sativum* extract against CCl_4 and acetaminophen-induced hepatotoxicity. *Corriandrum sativum* extract may exerts this protection through amelioration of lipid peroxidation by its scavenging activity of free radicals and enhancement of the antioxidant defense system by replenishment of reduced glutathione stores in the liver.

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