Ameliorative effects of *Asparagus falcatus* L. and *Vetiveria zizanioides* (L.) Nash on carbon tetrachloride induced oxidative stress in mice

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The hepatoprotective and antioxidative effects of two aqueous plant extracts, *Asparagus falcatus* and *Vetiveria zizanioides*, were determined against carbon tetrachloride (CCl₄) induced oxidative stress in mice. Hepatotoxicity was induced by the administration of a single intraperitoneal dose of CCl₄ (0.5 mL kg⁻¹ CCl₄ in olive oil) after a 16 hrs fast. Aqueous extracts of the tubers of *Asparagus* and root of *Vetiveria* (0.9 gm kg⁻¹) were used on pre and post-treatment basis. The ability of plant extracts to protect the liver against changes mediated by carbon tetrachloride confirms that plants possess anti-hepatotoxic properties against CCl₄ induced liver damage. Both pre and post-treatment decreased the CCl₄ mediated increase in serum enzyme activities (ALT, AST, ALP) and increased the reduced glutathione concentration in the liver. Administration of *Vetiveria* alone improved the GSH status significantly. Glutathione reductase and glutathione S-transferase activities were increased significantly (*P* < 0.05) and lipid peroxidation and activity of glutathione peroxidase were reduced significantly in the plant extract treated groups compared to the CCl₄ control group. Histopathological studies provided supportive evidence for the biochemical analysis. The magnitude of hepatoprotective properties varied between the two plant extracts but the hepatoprotection mediated by *Vetiveria* was more potent than that of *Asparagus*.

**Keywords:** *Asparagus falcatus*, *Vetiveria zizanioides*, Hepatoprotection, Oxidative stress, Antioxidative effect, Carbon tetrachloride.

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Oxidative damage caused by reactive oxygen species (ROS) is considered as an important factor in the pathogenesis of various diseases including liver disorders, atherosclerosis, lung and kidney injury, aging and diabetes. With an estimated prevalence of about 170 million people worldwide, chronic hepatitis C is an important cause of chronic liver disease. Liver plays important functions in the maintenance, performance, and regulation of homeostasis of the body. Therefore, maintenance of a healthy liver is essential for the overall wellbeing of an individual. Liver is also a vital organ that has a crucial role in the detoxification of various xenobiotics. Excessive exposure to drugs and environmental pollutants overpowers the natural protective mechanisms of the liver that leads to hepatic injury. Liver damage is associated with cellular necrosis, plasma membrane damage, and depletion in the reduced glutathione level (GSH) and it is a widespread pathology which involves oxidative stress and a progressive evolution from hepatic steatosis, fibrosis, and even life-threatening conditions, such as liver cirrhosis and hepatocellular carcinoma. In spite of the tremendous advances in modern medicine, only a few drugs are available that offer protection to the liver from damage and help to regenerate hepatic cells. Steroids and vaccines have been used for the treatment of liver diseases; however, they have serious adverse side effects and are of limited therapeutic potential. Many natural products have recently received attention as key sources of antioxidants because they are highly active in the prevention and treatment of diseases induced by oxidative stress. Silymarin, a mixture of flavonolignans obtained from the plant milk thistle, is a popular herbal extract used as a hepatoprotective agent. However, some clinical trials have indicated that the standard doses of silymarin were ineffective in many patients with chronic liver disease. Due to the limited therapeutic options available for the treatment of liver diseases, the search for new and

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safe hepatoprotective candidates is quite apparent. Herbal medicines have been used for centuries for the treatment of several ailments. Natural products remain as important sources of lead structures for the development of many drugs. Recently, there has been a resurgence of interest in the use of natural products because of their reduced side effects when compared to synthetic drugs. Plants are considered as potential hepatoprotective agents because they contain a combination of different phytochemicals that are synergistic in their action. Hepatoprotective and antioxidant effects have been investigated in different plant extracts due to their potent antioxidant activities, such as Allium sativum L. & Cucuruma longa L., Salvia tomentosa Mill., Meconopsis integrifolia (Maxim.) Franch and Eurycoma longifolia. However, aqueous extracts of Asparagus falcatus and Vetiveria zizanioides have never been investigated against CCl₄ induced liver injury in mice. Asparagus falcatus that belongs to the family Liliaceae is a tall subscandent under shrub with terete, smooth, armed branches commonly available in Sri Lanka, other parts of tropical Asia and Africa. Aqueous decoctions of the tuberous root are used by traditional Ayurvedic practitioners in Sri Lanka for chronic congestion of the liver, jaundice, gallstones, and chronic nephritis. It also acts as a diuretic, diluent, antilithic, and aphrodisiac. Vetiveria zizanioides is a perennial herb with a branched densely tufted rootstock with long, spongy, aromatic, brown root fibers. The root acts as a bitter stomachic, carminative, cholagogue and is useful in anorexia, chronic dyspepsia, acute and chronic congestion of the liver and jaundice. Vetiveria also act as a diaphoretic and a diuretic. In the present study we aimed to investigate the hepatoprotective and antioxidant effect of Asparagus falcatus and Vetiveria zizanioides against carbon tetrachloride induced oxidative stress in mice.

**Methodology**

**Experimental animals**

Healthy male ICR mice, 6 ± 8 weeks old and weighing 30 ± 35 gm, were allowed free access to water and pelleted food *ad libitum*. All animals were fasted for 16 hrs 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**

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 chemicals were commercially available and of reagent grade.

**Preparation of the plant extract**

Tubers of Asparagus falcatus and roots of Vetiveria zizanioides plants were collected from the Galle district in the Southern province of Sri Lanka. 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 was deposited at the Department of Biochemistry, University of Ruhuna, Sri Lanka. Tubers of Asparagus falcatus and roots of Vetiveria zizanioides plants were cut into small pieces and dried at 40 °C for two days. The normal therapeutic dose of humans extrapolated to mouse was used. 2.625 gm of the dried plant material was refluxed in 30 mL of distilled water for 1 hr and concentrated to 20 mL. Each mouse was administered a dose of 0.9 gm kg⁻¹ orally by gavage. The extract was prepared daily from the dried plant material.

**Treatment of animals**

**Control groups**

Mice were divided into three groups of 10 animals in each. The first group served as the normal control group and received distilled water orally by gavage. The second and third groups were treated with the Asparagus and Vetiveria extracts 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 ten groups (groups 4-13) of 10 animals in each. A single intraperitoneal dose of CCl₄ was injected (0.5 mL/kg in olive oil, CCl₄: olive oil 1:10) in each animal after a 16 hrs fast. In groups 4 and 5 the animals were killed 24 hrs and 4 days, respectively, after the administration of CCl₄. Animals in group 6 were administered Asparagus falcatus extract half an hour after the administration of a single dose of CCl₄ and were killed 24 hrs later. The same procedure was carried out for group 7 but instead of killing after
24 hrs, they were given the extract alone for a further two days at 24 hrs intervals (post-treatment). They were killed on the fourth day. Groups 8 and 9 were administered the Asparagus falcatus extract daily for 7 days and on the seventh day a single dose of CCl4 was injected half an hour after the administration of the plant extract. The mice were killed after 24 hrs and 4 days, respectively. Animals in group 10 were administered the Vetiveria zizaniodes extract half an hour after the administration of a single dose of CCl4 and were killed 24 hrs later. The same procedure was carried out for group 11 but instead of killing after 24 hrs, they were given the extract alone for a further two days at 24 hrs intervals (post-treatment). They were killed on the fourth day. Group 12 &13 were administered Vetiveria zizaniodes extract daily for seven days and on the seventh day a single dose of CCl4 was injected half an hour after the administration of the plant extract. The mice were killed after 24 hrs and 4 days, respectively.

**Determination of liver enzyme concentrations**

A combination of the methods of Reitman & Frankel as described by Tayal et al., and Schmidt & Schmidt as described by Betros & Sikaris, 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 as stated in Kpomah et al. 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 as described in Sultana et al. 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 sulfhydryl compounds.

**Estimation of lipid peroxidation**

The extent of lipid peroxidation was estimated in liver homogenates/serum by the measurement of malondialdehyde (MDA) formation using thiobarbituric acid method. Method of Okawa at al as described by Jatwa et al. was used. Malondialdehyde (MDA) is reacted with thiobarbituric acid at 95 °C and the absorbance of the pink coloured product was measured at 532 nm against a reagent blank.

**Estimation of antioxidant enzymes in the liver**

Glutathione reductase (GR, EC 1.6.4.2) and glutathione peroxidase (GPx, EC 1.11.1.9) levels were assayed in the cytosolic fraction. GR assay is based on the oxidation of NADPH to NADP+ catalysed by a limiting concentration of glutathione reductase. GPx catalyses the reduction of hydrogen peroxide to water and organic peroxides (ROOH) to the corresponding stable alcohols. (R-OH) is using glutathione as a source of reducing equivalents. Oxidized glutathione produced upon reduction of organic peroxide by cellular GPx is recycled to its reduced state by glutathione reductase. The enzyme activity was determined by measuring the disappearance of NADPH at 340 nm and was expressed as nmol of NADPH oxidized per minute per mg protein. Glutathione-S-transferase (GST, EC 2.5.1.18) activity was measured by the method as described by Ansar et al. Assay is based on the conjugation of 1-chloro, 2, 4, dinotrobenzene (CDNB) with reduced glutathione producing a dinitrophenyl thioether and chloride ion. Product formation is accompanied by the appearance of an absorption band at 340 nm.

**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**

Table 1 summarizes the effect of aqueous plant extracts of Asparagus falcatus and Vetiveria zizanioides on serum enzyme levels and liver reduced glutathione level against CCl4 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 hrs of exposure of mice to a single dose of CCl4. The results in Table 1 demonstrate that pre or post-treatment
and liver reduced glutathione level were improved by the administration of CCl4. Improvement in the serum ALP levels were not observed. A significant increase in liver reduced glutathione level, 24 hrs after the administration of CCl4 and post-treated groups showed a faster recovery compared to the CCl4 control group. Table 1 summarizes the effect of the plant extracts in serum enzyme levels and liver reduced glutathione with Asparagus extract can moderate CCl4-mediated alterations in serum enzyme levels and liver reduced glutathione level but the plant extract itself has no effect on these parameters in the drug control group. Serum enzyme concentrations of the pre-treated mice were reduced more than that of post-treated mice compared to the CCl4 control group. Asparagus pre and post-treated groups showed a faster recovery compared to the CCl4 control group four days after the administration of CCl4. Similar to the pattern observed in Asparagus treated mice, Vetiveria treated mice also showed a significant decrease in all serum enzyme levels of ALT, AST, ALP and a significant increase in liver reduced glutathione level, 24 hrs after the administration of CCl4 (Table 1). A significant improvement in the serum ALP levels were not observed in pre and post-treated mice, four days after the administration of CCl4. But the serum ALT level and liver reduced glutathione level were improved significantly by 23.4, 26.6 and 83.9, 61.9 %, respectively in post and pre-treated groups 4 days later. A significant increase in the liver reduced glutathione level was observed in the Vetiveria control group compared to the normal control group. Table 1 summarizes the effect of the plant extracts on the antioxidant enzyme activity and lipid peroxidation against CCl4 induced hepatocellular injury. Only GPx activities were significantly altered. A significant decrease in serum GST activities were reduced to 92.2, 21.1 and 83.2, 67.8 %, respectively, 24 hrs and 4 days after the administration of CCl4. In Asparagus and Vetiveria control group, there were no significant changes in the antioxidant enzyme levels. In the Asparagus treated mice, except the glutathione reductase level, all other enzyme levels were significantly improved 24 hrs after the administration of CCl4. GST levels were also significantly improved 24 hrs after the administration of CCl4.
increased significantly in both pre and post-treated mice four days later. *Vetiveria* extract showed a significant improvement in all three enzyme levels in post treated group 24 hrs later. But only the GST level was not increased significantly in the pre-treated group. When the formation of malondialdehyde in the liver cytosolic fraction was compared between the CCl₄ control group and the normal control group, a percentage increase in 153.3 and 163.6 were observed 24 hrs and four days after the administration of CCl₄. However, a percentage increase in 92.1 and 18.2 was observed in the serum malondialdehyde levels 24 hrs and four days after the administration of CCl₄. Malondialdehyde levels in serum were significantly reduced in *Asparagus* treated mice both 24 hrs and four days after the administration of CCl₄. Only *Vetiveria* post-treated mice four days after the administration of CCl₄ showed a significant reduction in malondialdehyde levels in the liver.

Histopathological examination of the liver tissue provided supportive evidence for the biochemical analysis (Figs. 1-3). 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. 1). Liver sections of mice challenged with CCl₄ alone showed mainly centrilobular necrosis with focal fatty changes and ballooning degeneration in the surviving hepatocytes 24 hrs after the administration of CCl₄ (Fig. 2A). The areas of necrosis were less in mice four days after the administration of CCl₄ (Fig. 3A). Although areas of necrosis were visible in mice pre-treated with *Asparagus* (Fig. 2C), 24 hrs after the administration of CCl₄, the extent of damage was less compared to *Asparagus* post-treated group (Fig. 2B). A marked reduction in necrosis was visible in *Asparagus* post-treated mice four days later (Fig. 3B). However, in the *Asparagus* pre-treated group, only a few areas of necrosis were observed four days after administration of CCl₄ but the majority of liver parenchymal architecture was normal (Fig. 3C). No deviation from the normal parenchymal architecture was observed in the *Asparagus* control group (Fig. 1B).

Areas of necrosis were markedly less in the liver tissues of mice pre-treated with *Vetiveria* (Fig. 2E) 24 hrs after the administration of CCl₄ compared to the same in *Asparagus* treated mice; But areas of necrosis were still visible in *Vetiveria* post-treated mice (Fig. 2D). A marked reduction in necrosis was again observed in the liver tissue four days after the administration of CCl₄. Pre-treatment of mice with plants extracts showed better results than the post-treatment. *Vetiveria* control group showed normal parenchymal architecture (Fig. 1C).

**Discussion**

Despite advances in modern medicine, there is no successful therapeutic approach regarding stimulation of hepatic function, liver protection or enhancement of hepatic cell regeneration. Current drugs, such as pegylated interferon-alpha (IFN-α) and ribavirin used in the treatment of hepatitis virus infection, are not

![Fig. 1 — Liver Histopathology of A: Normal control, B: Asparagus control, C: Vetiveria control](image1)

![Fig. 2 — Liver Histopathology of mice sacrificed 24 hrs later; A: CCl₄ control, B: Asparagus post-treated, C: Asparagus pre-treated, D: Vetiveria post-treated, E: Vetiveria pre-treated](image2)
effective in all patients and some of them will not tolerate this therapy. Silymarin, the most known hepatoprotective substance, has shown limitations regarding treatment of chronic liver impairment such as cirrhosis. Thus, it is imperative to identify highly effective pharmaceuticals with minimum toxicity for the treatment of hepatic disorders. Active compounds isolated from medicinal plants can be considered as potential candidates for the treatment of liver diseases.

In the present study, both preventive and curative effects of the aqueous plant extracts of *Asparagus falcatus* and *Vetiveria zizanioides* were evaluated against carbon tetrachloride. CCl₄ intoxication is a widely used experimental model for liver injury. The highly hepatotoxic metabolites, namely, trichloromethyl radicals (CCl· and CCl₃O₂·) are generated during the metabolic activation of CCl₄ by cytochrome P-450. These radicals have a central role in the initiation of lipid peroxidation, inflammation, and fatty changes of the liver. Moreover, CCl₄ intoxication is associated with oxidative stress since the CCl₃· and CCl₃O₂· radicals alter the antioxidant state of the liver by deactivating the hepatic antioxidant enzymes including SOD, GPx, GR, and GST. Trichloromethyl radicals also react with the sulfhydryl groups of GSH leading to its deactivation. The leakage of the marker enzymes into the blood is 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. In the present study, CCl₄ treatment markedly increased the levels of AST, ALT, and ALP. As demonstrated in Table 1, significant reductions in the activities of serum ALT, AST and ALP enzymes were observed in the plant extract treated groups compared with those of the toxin control group showing reduced leakage of enzymes from the hepatocytes. It may be due to the rapid regeneration of the cell membranes in the presence of active components present in the plant extracts. Pre-treatment with the plant extracts prior to administration of the hepatotoxin resulted in a significant protection of the liver compared to the post-treatment against damage mediated by hepatotoxins. In almost all plant treated groups, pre-treatment with the plant extract reduced the serum ALT, AST and ALP activities compared to post-treatment in CCl₄ intoxicated mice (Table 1).

GSH is a critical determinant of tissue susceptibility to oxidative damage and the depletion of hepatic GSH has been shown to be associated with an enhanced toxicity to chemicals including paracetamol and CCl₄. Significant impairment in hepatic GSH status associated with a substantial hepatocellular damage induced by carbon tetrachloride further suggests the determinant role of hepatic GSH in the development of carbon tetrachloride toxicity. Cell injury induced by xenobiotics occurs only if mitochondrial GSH was depleted. Upon in vitro oxidative challenge, the extent of GSH depletion measurable in tissue homogenates is determined by the balance between GSH oxidation and GSH regeneration. In the present study, the liver GSH level was decreased significantly in CCl₄ control groups but a significant increase (P < 0.05) in liver GSH was observed in *Asparagus* and *Vetiveria* treated groups of mice intoxicated with CCl₄. However, the percentage improvement was higher in four days compared to mice sacrificed 24 hrs later. This suggests the ability of the plant extracts to regenerate liver GSH stores. In the process of lipid peroxidation, malondialdehyde (MDA) is formed as a catabolic product. The adverse effect on hepatic GSH status is associated with a substantial increase in hepatic MDA level, an indirect index of lipid peroxidation. In this study, the MDA level in the liver tissue was markedly increased in response to CCl₄ intoxication, indicating oxidative damage of the liver. A percentage increase in malondialdehyde concentration by 153.3 and 163.6 were observed in liver homogenate 24 hrs and 4 days after the administration of CCl₄ (Table 1). The results
of the present study demonstrated that treatment with *Asparagus* and *Vetiveria* increased the MDA level compared to the CCl₄ control group. A significant change was observed in serum levels of MDA in all groups treated with *Asparagus* extracts. The inhibitory effect against lipid peroxidation suggests that both plant extracts could prevent the liver injury induced by free radicals along with the subsequent pathological changes in the liver.

The antioxidative defense enzymes have been suggestive of playing an important role in maintaining physiological levels of oxygen and hydrogen peroxide and eliminating peroxides generated from exposure to xenobiotics or drugs. Within cells, the highest concentrations are found in liver although glutathione peroxidase is widely distributed in almost all tissues. The elevation of GPx activity is commonly associated with oxidative stress. Glutathione reductase, a secondary antioxidant enzyme is used for the regeneration of reduced glutathione from oxidized glutathione. It has a similar tissue distribution to glutathione peroxidase. The ratio of reduced to oxidized glutathione is usually kept very high as a result of the activity of the enzyme glutathione reductase. Drug metabolizing enzymes, such as glutathione-S-transferase, work in concert with antioxidant systems by metabolizing electrophiles and xenobiotics, and some of the molecules involved in the two defense mechanisms are induced simultaneously in response to xenobiotic exposure. The cytosolic glutathione-S-transferase catalyses the conjugation reaction of GSH and electrophilic substances, and therefore, has an integral role in the detoxification of electrophilic toxicants. A percentage increase in 344.8 and 265.8 was observed in glutathione peroxidase (GPx) activity in CCl₄ intoxicated mice 24 hrs and 4 days after the administration of CCl₄, while a percentage reduction in 92.2, 21.1 and 83.2, 67.8, respectively was observed in glutathione reductase (GR) and glutathione-S-transferase (GST) activities (Table 1). All three plants significantly improved the changes mediated by the hepatotoxins on the levels of glutathione peroxidase, reductase and S-transferase. The observed rise in the glutathione peroxidase activity could be an adaptive response to high levels of free radicals formed in response to toxic insult. When GPx activity was considered pre-treatment showed better results than the post-treatment. But the pattern is to the opposite direction when the GR activity was concerned. However, there was a positive correlation between the levels of GSH and GR in plant treated groups. Since administration of plant extracts significantly increased the GR activity, it can be assumed that the active components in plants may be responsible for a reduction in the formation of peroxynitrite or hydroxyl radicals thereby minimizing the oxidative stress. Although a definite pattern is not observed in the GST concentration, all values were improved compared to respective control groups.

Histopathological studies are the gold standard also provided supportive evidence for the biochemical analysis. Although the areas of necrosis were still visible in plant extract treated mice, extent of damage was reduced compared to the CCl₄ control group. Histopathological observations suggested that the reactive oxygen species and lipid peroxidation may play an important role in hepatic fibrosis and necrosis that was observed with the loss of normal liver architecture. Toxic reactive metabolite, trichloromethyl free radical, binds covalently to macromolecules of the lipid membranes of adipose tissue and causes peroxidative degradation. As a result, fats from the adipose tissue are translocated and accumulated in the hepatocytes. The degenerative changes were shown to be minimal or absent with the plant extract treatment. This might be due to lower fat accumulation and re-establishment of the antioxidant defense system in the liver tissue through the antioxidant and hepatoprotective nature of the two plant extracts used. Results presented in this study are in accordance with a study published by Parmar et al. in which the treatment with methanolic extract of *Vetiveria zizanioides* significantly prevented the functional (thiopentone-induced sleeping time), physical, biochemical and histological changes induced by ethanol, indicating the recovery of hepatic cells. Further studies are required to determine the active components of the plants under study and the exact mechanism that underlie their protective effect against liver damage. Based on the biochemical and histopathological evidence, it can be concluded that *Asparagus falcatus* and *Vetiveria zizanioides* can be considered as having significant hepatoprotective activity and antioxidant activity can be considered as one mechanism of action where *Vetiveria zizanioides* shows the higher effect compared to *Asparagus falcatus*.

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