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**Research Article**

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**Phenolic Content, Antioxidant and Hepatoprotective Activities of Sabah *Hoya coronaria* Blume**Muhammad Dawood Shah<sup>1</sup>, Senty Vun-Sang<sup>2</sup>, Mohammad Iqbal<sup>2\*</sup><sup>1</sup> *Borneo Marine Research Institute, Universiti Malaysia Sabah, Kota Kinabalu, Jalan UMS 88450, Sabah, Malaysia; dawoodshah@ums.edu.my*<sup>2</sup> *Biotechnology Research Institute, Universiti Malaysia Sabah, Kota Kinabalu, Jalan UMS 88450, Sabah, Malaysia; sentyvunsang2707@gmail.com; miqbal@ums.edu.my***\*Corresponding author: [miqbal@ums.edu.my](mailto:miqbal@ums.edu.my)**Received 16 August 2023 | Reviewed 05 September 2023 | Accepted 27 September 2023 | Published 15 October 2023  
Doi: <https://10.51200/jtbc.v20i.4656>**ABSTRACT**

A common challenge that regularly results from oxidative stress is hepatic damage. This condition is characterised by a gradual progression from steatosis to chronic hepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma. The study proposed to assess the antioxidant activity and efficacy of *Hoya coronaria* aqueous extract in preventing CCl<sub>4</sub>-induced hepatic damage in rats. The DPPH technique was used in the study to assess the extract's antioxidant properties. The rats received dosages of 125 and 250 mg/kg body weight of *H. coronaria* extract for 14 days, followed by CCl<sub>4</sub> exposure. After two weeks, the rats were euthanised for analysis. The results indicated that the extract showed significant antioxidant potential and decreased the impact of CCl<sub>4</sub> on hepatic damage markers such as serum aspartate transaminase and alanine transaminase. Moreover, it increased hepatic reduced glutathione and various antioxidant enzymes while reducing malondialdehyde formation induced by CCl<sub>4</sub>. Additionally, the histopathological analysis demonstrated that *H. coronaria* extract protected the liver against fatty degeneration and necrosis induced by CCl<sub>4</sub> toxicity. These outcomes suggest that *H. coronaria* extract could be used to prevent ROS-related hepatic damage.

**Keywords:** Antioxidant enzymes; Hepatoprotection; *Hoya coronaria*; Medicinal plants; oxidative stress**Introduction**

Liver injury is a common condition that, in the majority of cases, is caused by oxidative stress and is defined by a gradual development from steatosis to chronic hepatitis, fibrosis, cirrhosis, and cancer (Kodavanti et al., 1989). Many

factors can cause liver damage, such as pollutants, radiation, chemicals, alcohol, medicines, malnutrition, and infection (Meng et al., 2020). Around 2 million people die from liver diseases each year around the globe, including one million from cirrhosis complications and one million from viral hepatitis and hepatocellular cancer, and developing countries carry the highest burden (Cainelli, 2012; Asrani et al., 2019). Many currently available drugs used for liver disease treatments suffer various limitations, such as side effects, low bioavailability, stability, and selectivity. It is vital to look for novel natural medications with the best possible effectiveness, stability, selectivity, and safety (Singh et al., 2016; Ma et al., 2019; Alnuqaydan et al., 2022).

Medicinal plants attract attention in being introduced in therapeutics for treating liver damage due to various antioxidant compounds (Shah et al., 2015a; 2017; Singh et al., 2016; Venmathi Maran et al., 2022). The natural antioxidant compounds modulate oxidative stress by reducing lipid peroxidation and elevating antioxidant enzymes (Singh et al., 2016; Alnuqaydan et al., 2022; Venmathi Maran et al., 2022). *Hoya coronaria* is a medicinal plant and belongs to the family Asclepiadaceae. It is a beautiful evergreen plant with a star-shaped large flower arising from its green stem. The plant is found in the moist habitats of Malaysia, Indonesia, the Philippines and other Southeast Asian countries (Milow et al., 2017; Rahayu & Fakhurrozi, 2020). In Malay, it is known as 'Akar Setebal' (MyBIS, 2023), while in Kadazan/Dusun language (Sabah Native), it is known as 'Wida' (Kulip, 1997). The leaves of the plants are crushed and applied for the treatment of wounds and cuts by the local people, the Orang Asli, in Kampung Bawong, Perak, West Malaysia (Samuel et al., 2010) and for pancreatitis treatment by the local people in Sabah, East Malaysia (Kulip, 1997). However, no scientific investigations have been conducted on the antioxidant activity and hepatoprotective properties of *H. coronaria*.

This study aims to evaluate the potential of *H. coronaria* as a hepatoprotective agent by investigating its antioxidant activity and chemopreventive effects against CCl<sub>4</sub>-induced oxidative stress and hepatic dysfunction in rats.

## Materials and Methods

### Chemicals

Oxidized glutathione, thiobarbituric acid, trichloroacetic acid, dithionitrobenzoic acid, 1-chloro 2,4 dinitrobenzene, hydrogen peroxide, folin-Ciocalteu reagent (FCR), sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>), sodium chloride (NaCl), sodium nitrate (NaNO<sub>3</sub>), aluminium chloride (AlCl<sub>3</sub>), sodium hydroxide (NaOH),

2, 2-diphenyl-2-picrylhydrazyl (DPPH), gallic acid, catechol, and ferric chloride (FeCl<sub>3</sub>) were purchased from Sigma Aldrich (St. Louis, MO, USA). Alcohol, acid alcohol, blue buffer, eosin, haema-toxylin, xylene and DPX mounting medium for histological assessment were purchased from Leica Biosystem (Wetzlar, Germany).

### ***Plant Leaves Preparation***

*H. coronaria* leaves were harvested from the Papar district (5.48943°N, 115.80992°E) of Sabah, Malaysia. Institute for Tropical Biology and Conservation (IBTP), Universiti Malaysia Sabah experts identified the collected leaves. After cleaning with distilled water, the leaves were dried in an oven at 37°C and ground into powder. To prepare the extract, 100 g of the powder was boiled in distilled water (1:10 ratio) on a stirring hot plate for 10 minutes. After cooling for an hour at room temperature, the decoctions were filtered with a strainer to remove significant residues and then filtered once more with Whatman No. 1 filter paper. The resulting filtrate was freeze-dried and stored in a tube at -80°C for further investigation, as Venmathi Maran et al., (2021) described.

### ***Total Phenolic Content (TPC)***

The total phenolic content was determined using the Folin-Ciocalteu method. In this method, 200 ml of plant extract was mixed with 1.5 ml of Folin-Ciocalteu's reagent (1:10) and left at room temperature for 5 minutes. Next, 1.5 ml of sodium carbonate (60 g/l) was added, and the mixture was incubated in the dark at room temperature for 90 minutes. The absorbance at 725 nm was measured against a blank using a spectrophotometer. Gallic acid was used as a standard to determine the phenol content, expressed as gallic acid equivalents (GAE) per gram of material. The test was repeated thrice, and the obtained absorbance was averaged (Velioglu et al., 1998).

### ***2, 2-Diphenyl-2-picrylhydrazyl (DPPH) Assay***

The antioxidant activity of the extract was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to Hatano et al., (1988) method. The extract was dissolved in dimethyl sulfoxide (DMSO) at a 5 mg/ml concentration and combined with DPPH radicals ( $6 \times 10^{-5}$  mol/L) in an ethanol solution. The experiment was carried out in triplicate, with ascorbic acid as the control. The following formula was used to compute the percentage of radical scavenging activity: % RSA = [(Control absorbance - Sample absorbance)/Control absorbance] × 100.

### ***Experimental Protocol***

The animal experiment was treated ethically following the university's standards and federal legislation for animal experimentation (Animal Ethics Committee (AEC): UMS/IP7.5/M3/4-2012). Male Sprague-Dawley rats weighing 120-150 g and aged 8-12 weeks were obtained from the Animal House Health Campus at Universiti Sains Malaysia (USM). Animals were acclimatised for one week in an animal room on a 12-hour light-dark cycle and had ad libitum access to food and water. CCl<sub>4</sub> was mixed with corn oil (1:1) to prepare a 1.2 ml/kg body weight dose. In comparison, the *H. coronaria* extract was suspended in distilled water and administered to the rats through gastric gavage needles at 125 and 250 mg/kg body weight dosages. Twenty-four adult male rats were randomly distributed into four groups of six rats each: The first group was given saline; the second group was given CCl<sub>4</sub> (1.2 ml/kg b.w.); the third group was given *H. coronaria* (125 mg/kg b.w.) with CCl<sub>4</sub> (1.2 ml/kg b.w.); and the fourth group was given *H. coronaria* (250 mg/kg b.w.) with CCl<sub>4</sub> (1.2 ml/kg b.w.). CCl<sub>4</sub> treatment was given on the 13th and 14th days of the experimental period.

All of the rats were sacrificed 24 hours after the last CCl<sub>4</sub> treatment. Blood was taken through cardiac puncture with sterile disposable needles, and serum was recovered by centrifuging at 2000xg for 15 minutes. The animals' livers were quickly removed and cleansed with cold saline (0.85% w/v) to remove any unwanted debris. A small percentage of the liver tissues were maintained in a 10% neutral buffered formalin solution for histopathological studies, while the rest of the tissues were stored at -80 °C for biochemical study.

### ***Determination of Serum Biochemistry***

The enzyme activities of serum transaminases (ALT and AST) were investigated using the Reflotron1 technology.

### ***Preparation of Post-Mitochondrial Supernatant (PMS) from Liver***

Sample livers (10% w/v) were homogenised in phosphate buffer (0.1 M, pH 7.4) and centrifuged at 3000 rpm for 20 minutes at 4 °C to remove debris from the nuclei. The resulting supernatant was centrifuged at 10,000 rpm for 30 minutes at 4 °C, and the resulting PMS was utilised to measure various biochemical parameters.

### ***Biochemicals Assay***

Lipid peroxidation in the liver was done following the method of Buege and Aust by measuring the rate production of thiobarbituric acid reactive substances (TBARS) and expressed as MDA equivalents (Buege & Aust, 1978). Reduced GSH

was determined according to the method of Jollow *et al.* (1974). Glutathione peroxidase (GPx) activity was observed according to the procedure of Mohandas *et al.*, (1984). Catalase (CAT) activity was conducted using the method of Claiborne (1985). Glutathione reductase (GR) activity was performed following the method of Carlberg and Mannervik (1975). Glutathione S-transferase (GST) activity using CDNB as a substrate was estimated following the method of Habiq *et al.*, (1974), while quinone reductase (QR) activity was assessed following the method of Benson *et al.*, (1980) as modified by Iqbal *et al.*, (1999).

#### ***Histopathological Examination***

Liver tissues were fixed in a 10% phosphate-buffered formaldehyde solution, embedded in paraffin wax, and sliced into 4  $\mu\text{m}$  sections. The sections were stained with haematoxylin and eosin (H&E) and mounted on permanent slides. Photomicrographs were taken while inspecting the slides under a microscope equipped with photography tools.

#### ***Determination of Protein***

Bicinchoninic acid and a protein assay kit (BCA1) were used to measure the total protein level, with bovine serum albumin as the reference.

#### ***Statistical Analysis***

The mean  $\pm$  SD was used to express the results, and statistical comparisons were performed using ANOVA (one-way analysis of variance). Levene's test was used to determine variance homogeneity, and data analysis was carried out using the computer program SPSS (Release 17.0, SPSS). A significant difference was considered when the p-value was less than 0.05.

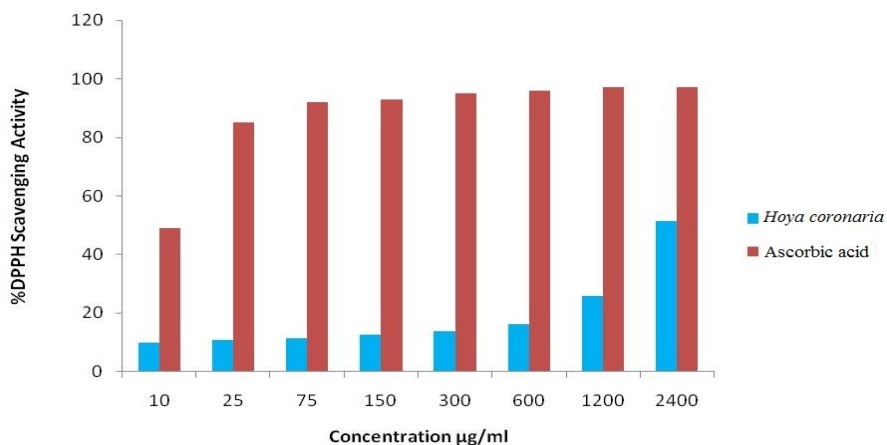
## **Results**

#### ***Total Phenolic***

The total phenolic content of *H. coronaria* aqueous extract was  $78.75 \pm 2.28$  mg/g, expressed in gallic acid equivalent (GAE)/mg of extract.

#### ***Effect of H. coronaria on DPPH Radical Scavenging***

Figure 1 illustrates the concentration-dependent increase in the DPPH scavenging capacity of the *H. coronaria* extract, ranging from 10 to 2400  $\mu\text{g}/\text{mL}$ . The extract's  $\text{EC}_{50}$  value was calculated to be 2240  $\mu\text{g}/\text{mL}$ .



**Figure 1.** Free radical protection activity (DPPH) of *H. coronaria* at different concentrations. Each bar represents the mean  $\pm$  SD (n=3).

### *Effects of H. coronaria on Lipid Peroxidation and GSH*

**Table 1** shows the impact of *H. coronaria* on malondialdehyde (MDA) and GSH. The level of hepatic MDA in CCl<sub>4</sub>-intoxicated rats increased significantly ( $p > 0.05$ ) by 56.3% compared to the control group. However, pre-treatment with *H. coronaria* extract ( $p < 0.05$ ) reduced liver MDA levels significantly by 19.3% and 30.6% in a dose-dependent manner. In addition, administration of CCl<sub>4</sub> alone significantly ( $p < 0.05$ ) reduced GSH levels in the liver by 64.6%. The pre-treatment of *H. coronaria* extract caused a significant ( $p < 0.05$ ) increase in the level of hepatic GSH by 42.2% and 98.1% in a dose-dependent manner.

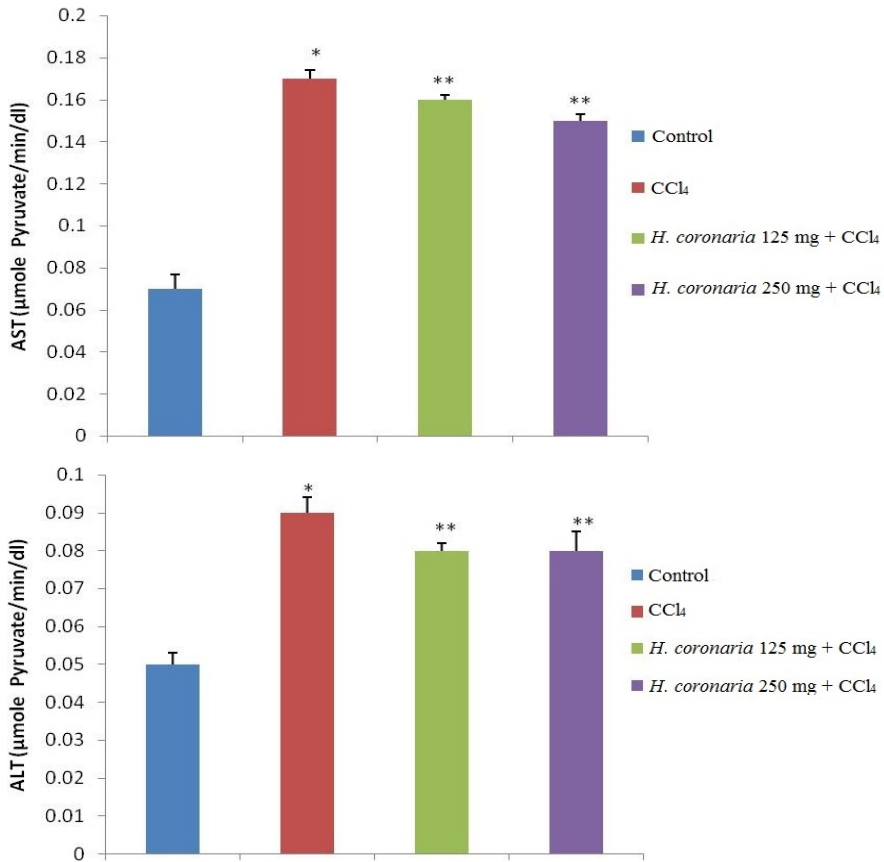
**Table 1.** Effect of *H. coronaria* on CCl<sub>4</sub>-induced changes in hepatic lipid peroxidation and reduced glutathione.

Groups	LPO (nmol/g tissue)	GSH ( $\mu$ mol/g tissue)
Control	14.90 $\pm$ 0.6	3.08 $\pm$ 0.18
CCl <sub>4</sub>	23.32 $\pm$ 0.8*	1.09 $\pm$ 0.05*
<i>H. coronaria</i> 125 mg + CCl <sub>4</sub>	18.80 $\pm$ 0.4**	1.55 $\pm$ 0.09**
<i>H. coronaria</i> 250 mg + CCl <sub>4</sub>	16.15 $\pm$ 0.4**	2.16 $\pm$ 0.06**

Each value represents the mean  $\pm$  SD of six animals (n = 6).

\* Indicate a significant difference from the control group ( $p < 0.05$ ).

\*\* Indicate significant difference from CCl<sub>4</sub> alone treated group ( $p < 0.05$ ).



**Figure 2.** Hepatoprotective effect of *H. coronaria* on CCl<sub>4</sub>-induced hepatotoxicity: serum transaminases. Each bar represents the mean  $\pm$  SD of six animals (n = 6).

\* Indicate a significant difference from the control group ( $p < 0.05$ ).

\*\*Indicate significant difference from CCl<sub>4</sub> alone treated group ( $p < 0.05$ ).

### ***Effects of H. coronaria on CCl<sub>4</sub>-Induced Hepatotoxicity***

The levels of serum transaminases (aspartate aminotransferase (AST) and alanine aminotransferase (ALT)) significantly ( $p < 0.05$ ) increased in the CCl<sub>4</sub>-treated group compared to the control group. However, pre-treatment with an extract of *H. coronaria* significantly ( $p > 0.05$ ) reduced the elevation of serum transaminases, indicating its potential to inhibit the elevated levels of these enzymes in rats intoxicated with CCl<sub>4</sub> (Figure 2).

### ***Effects of H. coronaria on Antioxidant Enzymes***

**Table 2** presents the effects of *H. coronaria* extract on antioxidant enzyme activities. The activities of CAT, GPx, GR, QR, and GST were significantly reduced by exposure to CCl<sub>4</sub> (37%, 31%, 43%, 75%, and 42%, respectively). However, pre-treatment with *H. coronaria* extract dose-dependently increased the activities of these enzymes and resulted in significant elevation ( $p < 0.05$ ) of CAT (12% and 41%), GPx (7% and 24%), GR (22% and 47%), QR (47% and 88%), and GST (13% and 55%).

**Table 2.** Hepatoprotective effects of *H. coronaria* on the hepatic antioxidant profile of rats intoxicated with CCl<sub>4</sub>.

Groups	CAT	GPx	GR	QR	GST
Control	328.61 ± 13.1	34.77 ± 1.9	35.09 ± 0.24	8.59 ± 0.1	35.09 ± 0.66
CCl <sub>4</sub>	207.34 ± 16.9*	23.92 ± 0.6*	20.32 ± 0.21*	2.07 ± 0.1*	20.32 ± 0.55*
<i>H. Coronaria</i> 125 mg + CCl <sub>4</sub>	231.17 ± 13.7*	25.52 ± 0.5**	22.98 ± 0.23*	3.60 ± 0.2**	22.98 ± 0.54**
<i>H. Coronaria</i> 250 mg + CCl <sub>4</sub>	292.97 ± 9.1**	29.67 ± 0.6**	31.67 ± 0.38*	8.04 ± 0.3**	31.67 ± 0.44**

The antioxidant enzyme activities are presented in **Table 2** as follows: catalase (CAT) (nmol H<sub>2</sub>O<sub>2</sub> consumed/min/mg protein), glutathione peroxidase (GPx) (nmol NADPH oxidized/min/mg protein), glutathione reductase (GR) (nmol NADPH oxidized/min/mg protein), quinone reductase (QR) (nmol dichloroindophenol reduced/min/mg protein), and glutathione-S-transferase (GST) (nmol CDNB conjugate formed/min/mg protein). Each bar represents the mean ± SD of six animals (n = 6).

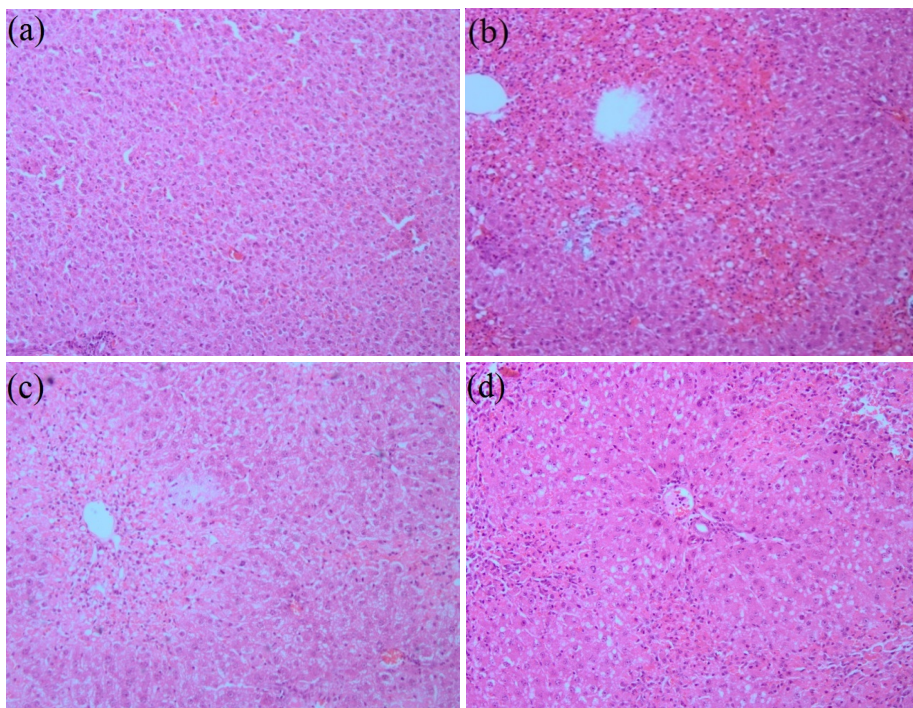
\* Indicate a significant difference from the control group ( $p < 0.05$ ).

\*\* Indicate significant difference from CCl<sub>4</sub> alone treated group ( $p < 0.05$ ).

### ***Effects of H. coronaria on Histopathology***

The CCl<sub>4</sub>-treated rats exhibited histological changes such as increased necrosis, blood vessel congestion, fatty degeneration and derangement of hepatocytes, as shown in **Figure 3**. However, the administration of aqueous extracts of *H. coronaria* significantly improved these changes, indicating that *H. coronaria* exposure reduced the hepatic injury induced by CCl<sub>4</sub>.





**Figure 3.** Haematoxylin and eosin (H&E) photomicrographs of histopathological changes in rat livers. The images show (a) a control liver, (b)  $\text{CCl}_4$  intoxicated, (c) *H. coronaria* (125 mg/kg b.w. +  $\text{CCl}_4$ ) and (d) *H. coronaria* (250 mg/kg b.w. +  $\text{CCl}_4$ ). All images are shown at a magnification of X20.

## Discussion

The findings of this study suggest that the water extracts derived from *H. coronaria* leaves can protect the liver against  $\text{CCl}_4$ -induced damage in rats by regulating oxidative stress. Our studies have revealed that *H. coronaria* extract exhibits significant antioxidant activity, as demonstrated by its ability to scavenge the stable, accessible radical DPPH dose-dependent manner. The extract contains  $78.75 \pm 2.28$  mg/g of total phenolics expressed as gallic acid equivalent (GAE, mg/g of extract), suggesting that phenolics are essential components of the extract. Literature suggests that the antioxidant activity of plant extracts is often attributed to their phenolic content, which can act as free radical scavengers, hydrogen donors, singlet oxygen quenchers, and metal ion chelators (Dillard & German, 2000; Yao et al., 2010; Hossain & Nagooru, 2011; Aryal et al., 2019).

The liver is responsible for detoxifying the body, and damage from toxic chemicals can lead to increased levels of serum transaminases, enzymes that are released into the bloodstream. Serum transaminases are commonly utilised as indicators of liver disease due to their sensitivity and reliability (Li et al., 2015; Vun-Sang et al., 2022). Consistent with previous studies, the activities of serum ALT and AST were elevated in the CCl<sub>4</sub> intoxicated group (Group 2) compared to the normal saline-treated group (Group 1) (Li et al., 2015; Vun-Sang et al., 2022). Following CCl<sub>4</sub> administration, liver damage can lead to increased membrane permeability and changes in transport function, resulting in the release of cytoplasmic hepatic enzymes like ALT and AST into the bloodstream (Li et al., 2015; Vun-Sang et al., 2022). In groups 3 and 4, where rats were pre-treated with *H. coronaria* extract and then treated with CCl<sub>4</sub>, the activities of ALT and AST were dose-dependently reduced compared to group 2, suggesting the potential of the extract in mitigating liver damage. This effect could be attributed to the plant's anti-inflammatory and antioxidant properties (Singh et al., 2016) and possible hepatic damage repair (Vun-Sang et al., 2022).

The P450 system biotransforms CCl<sub>4</sub>, specifically cytochromes CYP2E1, CYP2B2, and perhaps CYP3A, to generate the highly reactive metabolites CCl<sub>3</sub>• and CCl<sub>3</sub>OO• (Recknagel et al., 1989; Weber et al., 2003). These metabolites attack polyunsaturated fatty acids, leading to lipid peroxidation and the production of malondialdehyde (MDA) in liver cells, which is a significant factor in the development of diseases (Ohkawa et al., 1979; Vaca et al., 1988; Recknagel et al., 1989; Weber et al., 2003). Our results indicate that the levels of MDA produced in the CCl<sub>4</sub> intoxicated group (Group 2) were higher than the normal group (Group 1). However, treatment with *H. coronaria* extract at doses of 125 and 250 mg/kg body weight in groups 3 and 4, respectively, led to a reduction in MDA levels compared to group 2, indicating the potential of the extract to protect cell membranes from free radicals. The extract was found to reduce lipid peroxidation in a dose-dependent manner, consistent with previous studies (Shah et al., 2015b; Gnanaraj et al., 2016; Amzar & Iqbal, 2017).

Treatment with CCl<sub>4</sub> reduced the level of reduced glutathione (GSH), which is a crucial non-enzymatic antioxidant in the body that protects against oxidative damage. This finding is consistent with previous studies (Shah et al., 2015b; Gnanaraj et al., 2016; Amzar & Iqbal, 2017), which suggest that the decline in GSH level may be attributed to increased utilisation of cells to scavenge the free radicals caused by CCl<sub>4</sub>. However, we observed that *H. coronaria* extract could significantly increase the levels of GSH in the liver, possibly by scavenging reactive oxygen species and preventing oxidative damage.

Antioxidant enzymes, including glutathione reductase (GR), catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST) and quinone reductase (QR), are vital for safeguarding cells against oxidative damage (Li et al., 2015; Vun-Sang et al., 2022). CCl<sub>4</sub> metabolism into trimethyl free radicals through cytochrome P450 leads to increased oxidative stress (Recknagel et al., 1989; Weber et al., 2003), resulting in a reduction in the activity of antioxidant enzymes in the CCl<sub>4</sub>-intoxicated group compared to the normal saline-treated group 1. Our research demonstrates that the *H. coronaria* extract can increase the activity of various antioxidant enzymes, including GR, CAT, GPx, GST, and QR, relative to the CCl<sub>4</sub>-intoxicated group 2. This improvement in antioxidant enzyme activity is most likely due to the extract's ability to scavenge reactive oxygen species and protect against oxidative damage, as also seen in previous studies (Shah et al., 2015b; Gnanaraj et al., 2016; 2017; Amzar & Iqbal, 2017).

Histopathological studies involve examining morphological changes in liver tissue under microscopic examination to assess the effect of *H. coronaria* extract. In rats treated with CCl<sub>4</sub>, the liver section exhibited sinusoidal dilation, inflammatory infiltration, necrosis of hepatocytes, and steatosis (fatty acid formation). Most of these alterations were significantly decreased when animals were pre-treated with *H. coronaria* extract. The histopathological findings confirmed the biochemical results and indicated that *H. coronaria* extract prevented liver cell necrosis, fatty infiltration, and fibrosis, all of which are markers of liver damage. These histopathological changes are consistent with previous studies that have demonstrated the protective effects of phytochemicals in plant extracts against liver damage caused by CCl<sub>4</sub> (Shah et al., 2015b; Gnanaraj et al., 2016; 2017; Amzar & Iqbal, 2017).

## Conclusions

According to the study, *H. coronaria* aqueous extract exhibited significant antioxidant activity and protection against CCl<sub>4</sub>-induced hepatic damage in rats. The extract improved hepatic GSH and other antioxidant enzyme levels. The histopathological study showed that the liver was protected by *H. coronaria* extract from fatty degeneration and necrosis induced by CCl<sub>4</sub> toxicity. The results suggest that *H. coronaria* extract may be helpful in minimising liver damage caused by ROS. More study is required to examine the extract's safety and effectiveness in humans and identify the potential bioactive compounds responsible for the extract's protective effects.

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## Conflict of interest statement

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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