RESEARCH ARTICLE

Hepatoprotective and Antioxidant Activity of Stem and Leaves Parts of *Cissus woodrowii* (Stapf ex Cooke) Santapau

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Received: 18th December, 2023; Revised: 10th January, 2024; Accepted: 17th May, 2024; Available Online: 25th June, 2024

ABSTRACT

The contemporary investigation aimed to assess the hepatoprotective potential of *Cissus woodrowii* (Stapf ex Cooke) Santapau against carbon tetrachloride-induced hepatotoxicity. Through evaluating antioxidant levels in liver tissues and blood marker enzymes, it was observed that administration of the plant extract at doses of 200 and 400 mg/kg significantly mitigated liver damage caused by carbon tetrachloride. The study found that carbon tetrachloride-induced reductions in total protein, catalase, glutathione (GSH), and superoxide dismutase (SOD) levels, while increasing levels of alkaline phosphatase, alanine aminotransferase, total bilirubin, lipid peroxidation, and aspartate aminotransferase. However, rats treated with varying doses of the plant extract exhibited restoration towards normal levels of serum marker enzymes and antioxidants, contrasting with those solely exposed to carbon tetrachloride. These findings collectively suggest that *C. woodrowii* (Stapf ex Cooke) Santapau possesses antioxidant properties and exerts a hepato-protective effect against CCl_4-induced liver impairment in rats.

Keywords: *Cissus woodrowii*, Histopathology, Biochemical parameters, Carbon tetrachloride, Lipid peroxidation, Antioxidant.

International Journal of Pharmaceutical Quality Assurance (2024); DOI: 10.25258/ijpqa.15.2.05

How to cite this article: Patil PN, Gilhotra RM, Sharma S, Wadkar KA. Hepatoprotective and Antioxidant Activity of Stem and Leaves Parts of *Cissus woodrowii* (Stapf ex Cooke) Santapau. International Journal of Pharmaceutical Quality Assurance. 2024;15(2):588-594.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Natural medicines have garnered a lot of interest from both wealthy and poor nations due to their little and non-existent side effects.[1] Research from the World Health Organization (WHO) indicates that a large segment of the universal population relies on herbal remedies to alleviate or control serious health problems.[2] There are 350 species in the genus *Cissus*, which is part of the Vitaceae family and is used in traditional medicine all over the world to cure a wide range of illnesses.[3,4] This particular plant, *Cissus woodrowii*, is a part of the Vitaceae family and inhabitant of hilly areas of Maharashtra (Pune, Kolhapur). It features tall bushes with huge, broadly orbicular leaves and stems that are either straight or slightly inclined. Lamina 20–30 X 20–25 cm and petioles 15 to 25 cm are the dimensions that are used to identify these leaves; however, it is possible that these measurements pertain to the basal leaves, and our herbarium specimens typically contain the distal leaves, which are typically smaller.[5] Historically, animals have been treated with its roots as an anticancer. To alleviate rheumatic discomfort, the stem paste is applied topically.[6,7] A popular name for Woodrow's grape tree is *C. woodrowii* (Stapf ex Cooke) Santapau.[8] Since all other members of the Vitaceae family are woody lianas, this particular species of *Cissus* stands out taxonomically as a shrub-like plant.[9] The research work on *C. woodrowii* (Stapf ex Cooke) Santapau can proceed with reference of some plants like *C. pallid, C. pteroclada hayata,* likewise of Vitaceae family.

MATERIALS AND METHODS

Chemicals

Silymarin was purchased from Micro Labs in India, while thiobarbituric acid was acquired from E-Merck in India, and the remaining chemicals were sourced from Sisco Research Laboratory, Mumbai, India.

Collection and Extraction

The hilly region of Kolhapur in India is where the fresh stem and leaves of *C. woodrowii* (Stapf ex Cooke) Santapau were harvested. Pulverized and shade-dried were the stem and leaves. Subsequently, 2.5 L of ethanol (95% v/v) were used as

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solvents while 1-kg was packed into a soxhlet apparatus and heated continuously for 8 hours. The desiccator was used to dry the extract after vacuuming it. For each day of our experiment, we mixed small amounts of the crude extract with 5% gum acacia without cleaning them first.

Animals

Male and female Wistar rats weighing between 200 and 250 g were purchased in Pune and employed throughout the entire study. They were provided with a standard laboratory diet and unrestricted access to water. Following approval from the Institutional Animal Ethics Committee (IAEC), the study was conducted under the reference IAEC/Sangli/2022-23/4.

Antioxidant Enzyme Activity Assay

Preparation of tissue homogenate

Centrifuge the mixture at 1500 rpm for 10 minutes after precisely weighing the tissue (1 gm) and adding nine times the amount of PBS (0.1 M, pH 7.4) in accordance with weight-to-volume ratio of 1:9. To test for lipid peroxidation (LPO), superoxide dismutase (SOD) and catalase (CAT) activity, and other parameters, collect the supernatant.

Superoxide dismutase

Following the dilution protocol outlined in reference 5, SOD activity was evaluated using quercetin as the substrate. A 1-mL test solution was prepared by combining 0.08 mmol/L EDTA and 0.1 mol/L sodium phosphate buffers in equal proportions (pH 7.8). The diluted tissue sample was then mixed with distilled water and EDTA-sodium phosphate buffer (1:1000), followed by the addition of the assay mixture. Spectrophotometric readings were taken at 406 nm to measure the increase in absorbance caused by quercetin oxidation at 0 and 20 minutes. Distilled water served as a blank in place of the tissue sample.[10,11]

Catalase

The method of assaying CAT activity was 1-mL of 4% ammonium molybdate and 2 mL of 0.03% H_2O_2 solution were combined to initiate the reaction. An enzyme is considered to have one unit of catalase activity when it can remove 1-µmol of H_2O_2 per minute from 1-g of tissue. The spectrophotometric measurement of the hydrogen peroxide breakdown in the reaction mixture was taken at 410 nm.[12]

Lipid peroxidation assay

Following the protocol outlined in the reference[7] the homogenized tissues were tested for lipid peroxidation using the TBARS method. This technique creates a colored adduct that can be detected spectrophotometrically by reacting TBA with malondialdehyde (MDA), a byproduct of lipid peroxidation, in an acidic and high-temperature environment. The following steps were taken in brief: 2 mL of distilled water were added to 0.1 mL of tissue sample, 1-mL of TBA reagent, 1-mL of trichloracetic acid, and then, after 10 minutes of heating in a boiling water bath, butanol was added. We centrifuged the mixture for 10 minutes once it cooled. The samples were compared to a blank by determining the absorbance in the organic phase at 532 nm.[13]

Acute toxicity studies

Using (OECD standards 423), we assessed the acute oral toxicity (AOT) of *C. woodrowii* extracts in wistar rats. We evaluated the animals for death up to 48 hours after administering a single dose of extracts; they were fasted for 12 hours before the experiment. The dose for the subsequent animal was set according to OECD guideline 423, taking into account short-term toxicity. Acute and short-term toxicity investigations informed the determination that 2000 mg/kg body weight was the optimal dose for preclinical research.[14,15]

Hepatoprotective activity

Length of the body and its organs loss of both body and organ weight suggests damage to the organs. Alterations of organs caused by chemicals were marked. Traditional methods for determining a drug's toxicity in toxicological studies have involved comparing the organ weights of animals given the drug and those given a placebo.[16,17] Body weight and organ (liver) weight were found to differ considerably between CCl_4 treated group and the normal control group, according to the data given. Rats' body and organ weight were less affected by CCl_4 when exposed to an aqueous and ethanolic extract of *C. woodrowii*.

Every group of three rats was randomly assigned to one of seven categories.

- *Group I control (vehicle)*

For six days in a row, they were given once a day of saline water (5 mL/kg, parenterally).

- *Group II (CCl4 induced)*

For six days in a row, they were given a single daily dose of saline water (5 mL/kg, parenterally).

- *Group III (standard)*

Continually administered a single daily dose of the usual medicine silymarin (100 mg/kg p.o.) for 6 hours straight.

- *Group IV (Test -I)*

Taken orally once daily for six days in a row, the stem ethanolic extract of *C. woodrowii* (Stapf ex Cooke) Santapau had a dose of 200 mg/kg.

- *Group V (Test –II)*

Taken orally once daily for six days in a row, the stem ethanolic extract of *C. woodrowii* (Stapf ex Cooke) Santapau (400 mg/kg) was administered.

- *Group VI (Test –III)*

Taken orally once daily for six days in a row, ethanolic extract of *C. woodrowii* (Stapf ex Cooke) Santapau leaves had a dosage of 200 mg/kg body weight.

- *Group VII (Test –IV)*

Taken orally once daily for 6 days in a row, ethanolic extract of *C. woodrowii* (Stapf ex Cooke) Santapau leaves (400 mg/kg).

Seventh day of the trial, all groups from II to VII were given a single dosage of CCl_4 in olive oil (1:1 v/v, 1-mL/kg i.p.). Every group of animals had their food and water intake tracked throughout the experiment. On day one, we noted the rat's body weight. By day eight, we had computed the mean body weight. To learn how the medication affected the rats' typical anatomy and physiology, we also measured their liver and kidney weights.

The formula was used to compute the organ index: (kidney or liver weight/body weight) × 100%.

Sample collection after 24 hours of the last treatment on the eighth day, each rat was individually slaughtered by cervical decapitation. To obtain clear serum, centrifuged at 3000 rpm for 10 minutes after being collected into dry, sterile tubes. Rapid removal of the liver and kidney was followed by rinsing with cold saline, blotting, and subsequent weighing. Histopathological analysis and the MDA assay both make use of freshly harvested liver tissue.

Assessment of liver function

Following the methods described, biochemical parameters were examined. Following liver removal, morphological alterations were noted. Antioxidant experiments, including those involving LPO, SOD, and catalase, utilized 10% of the liver homogenate. For histological examinations, a section of liver was embedded in 10% formalin.

Histopathological studies

Separate liver samples were taken for histological analysis after blood was drained and before being cleaned with normal saline. After 48 hours of fixing in 10% buffered neutral formalin, the materials were prepared to be fixed with bovine solution for 6 hours. A 5 mm thick paraffin sections were subjected to an alcohol-xylene series treatment and then hematoxylin and eosin staining. Microscopical evaluation of histopathology changes was performed on the sections.[18,19]

RESULTS

There were steroids, flavonoids, saponins, alkaloids, triterpenes, and polyphenolic chemicals found in preliminary phytochemical research. Studies of acute oral toxicity tracked the mortality rate of animals treated with the extract for up to 48 hours. Up to a dosage of 2000 mg/kg, the extract was showed no death. In Table 1 and Figure 1 we can see how *C. Woodrowii* affected the serum marker enzymes. Liver damage was indicated by significantly higher blood ALT, ALP, in addition to overall bilirubin levels and a decrease in protein intensities in CCl4-treated mice. *C. woodrowii* extract significantly inhibited CCl_4-induced hepatotoxicity at dosages ranging from 200 to 400 mg/kg. The CCl_4-treated rats exhibited a notable ($p < 0.001$) rise in LPO levels as determined by the thiobarbituric acid response. The increase in LPO level was almost completely reduced ($p < 0.001$) through treatment using *C. woodrowii* (400 mg/kg). Table 2 and Figure 2 show that *C. woodrowii* had an impact that was similar to the standard medication silymarin.

Table 3 and Figure 2 show related to control group, amount of SOD, CAT, and GSH in liver tissue was considerably declined ($p < 0.001$) after CCl_4 treatment. A notable rise in SOD, CAT, and GSH was observed in rats with ethanolic extract of *C. woodrowii* at dosages of 200, 400 mg/kg, in comparison to rats cured using CCl_4. Rats with CCl_4 had significantly lesser ranges of antioxidant enzymes in their livers compared to those treated with silymarin.

DISCUSSION

Among the several hepatotoxins employed in the research of liver diseases in experimental settings, carbon tetrachloride stands out. The active metabolite of CCl_4 is primarily responsible for its hepatotoxic effects. The polyunsaturated fatty acid-rich endoplasmic reticulum membrane lipids are subjected to peroxidative destruction. Lipid peroxidation is one mechanism by which CCl_4 degrades biomembranes, which is why it is harmful to the liver. This is shown by the fact that serum marker enzyme levels fall. In order to assess liver damage caused by CCl_4, it is usual practice to determine enzyme levels, such as ALT.[21,22] Upon cell death or membrane breakdown, enzymes are released into circulation, making their serum levels quantifiable. Similar to how viral hepatitis, myocardial infarction, or muscular damage can all lead to increased levels of, these levels also indicate liver illness. This damage catalyzes the conversion of alanine to pyruvate and glutamate. As a result, it is a more accurate metric for identifying liver damage since it is tissue-specific. When the levels of certain enzymes in the bloodstream are high, it means that the liver's cell membranes are leaking and not functioning properly. The activity of hepatic cells is correlated with total protein, bilirubin, and serum ALP levels. A rise in biliary pressure clues to a rise in synthesis.[23]

Table 1: Outcome of extracts on CCl_4-induced hepatotoxicity in rats

Treatment	*Dose*	*SGOT (U/L)*	*SGPT (U/L)*	*ACP(U/L)*	*ALP (U/L)*
Control	1-mL/kg	56.66 ± 32.14	74.66 ± 24.44	1.71 ± 0.55	4.50 ± 0.90
Disease control	1-mL/kg	329.66 ± 50.50	485.33 ± 48.88	8.86 ± 1.62	14.30 ± 0.68
Silymarin	100 mg/kg	70.00 ± 20.00	181.33 ± 24.44	2.83 ± 0.14	5.09 ± 0.34
C. woodrowii stem ethanol extract	200 mg/kg	103.33 ± 45.09	208.00 ± 27.71	3.88 ± 0.28	5.29 ± 1.18
C. woodrowii stem ethanol extract	400 mg/kg	143.33 ± 51.31	234.66 ± 75.61	4.00 ± 1.73	6.46 ± 0.58
C. woodrowii leaves ethanol extract	200 mg/kg	210.00 ± 50.00	341.33 ± 24.44	4.08 ± 0.38	8.42 ± 0.90
C. woodrowii leaves ethanol extract	400 mg/kg	270.00 ± 105.83	549.33 ± 66.61	4.83 ± 1.75	9.01 ± 0.90

Table 2: Result of *C. woodrowii* liver weight in CCl_4-induced hepatotoxicity in rats

Treatment	*Body weight (g)*		*Liver weight mean (g)*
	Before	*After*	
Normal control	112 ± 6.23	115 ± 5.65	06.00 ± 0.5
Disease control	119 ± 8.34	125 ± 5.05	13.66 ± 0.23
Silymarin	113 ± 12.12	118 ± 6.50	7.66 ± 0.05
C. woodrowii stem ethanol extract	122 ± 7.65	127 ± 5.65	8.66 ± 0.34
C. woodrowii stem ethanol extract	124 ± 4.65	129 ± 5.35	11.00 ± 0.26
C. woodrowii leaves ethanol extract	132 ± 5.65	134 ± 4.35	10.6 ± 0.16
C. woodrowii leaves ethanol extract	130 ± 7.65	135 ± 4.45	10.33 ± 0.12

After CCl_4 was given, enzyme levels like ALT, ALP, and ACP were considerably high ($p < 0.001$), while total protein levels were considerably lower compared to control group. When given in a way that depended on the dose, along with silymarin at a dose of 100 mg/kg, these enzyme levels improved considerably again ($p < 0.001$). One possible explanation for the extract's ability to reverse CCl_4-induced liver damage is that it stabilizes cell membranes, which in turn prevents intracellular enzyme leakage. That hepatic parenchyma heals and hepatocytes regenerate, bringing serum transaminase levels back to normal, is consistent with the generally held belief. If total protein, bilirubin, and ALP levels can be effectively controlled, it suggests that the liver cells' secretory system is starting to work better. A hepatoprotective drug's ability to mitigate a hepatotoxin's negative effects or restore normal liver physiology determines the drug's effectiveness. It appears that either the physical structure of the hepatocyte cell membrane was protected or impaired hepatic cells were regenerated because silymarin and herbal extracts were related to reduced CCl_4-induced elevated enzyme levels

in the experimental groups. An increase in liver LPO levels caused by CCl_4 indicates that lipid peroxidation is worsened, which damages tissues and prevents the antioxidant defense mechanism from preventing the creation of too many free radicals. *C. woodrowii* treatment effectively undoes these alterations. *C. woodrowii's* hepatoprotective effects are probably attributable to its antioxidant properties.[24,25]

One indicator for liver injury is a decrease in SOD enzyme activity. According to Curtis and Mortiz, SOD is a key player in the enzymatic antioxidant defense mechanism. It reduces damaging outcomes of superoxide radicals by scavenging their anion toward producing H_2O_2. The oxidative damage to the liver caused by reactive free radicals is reduced because

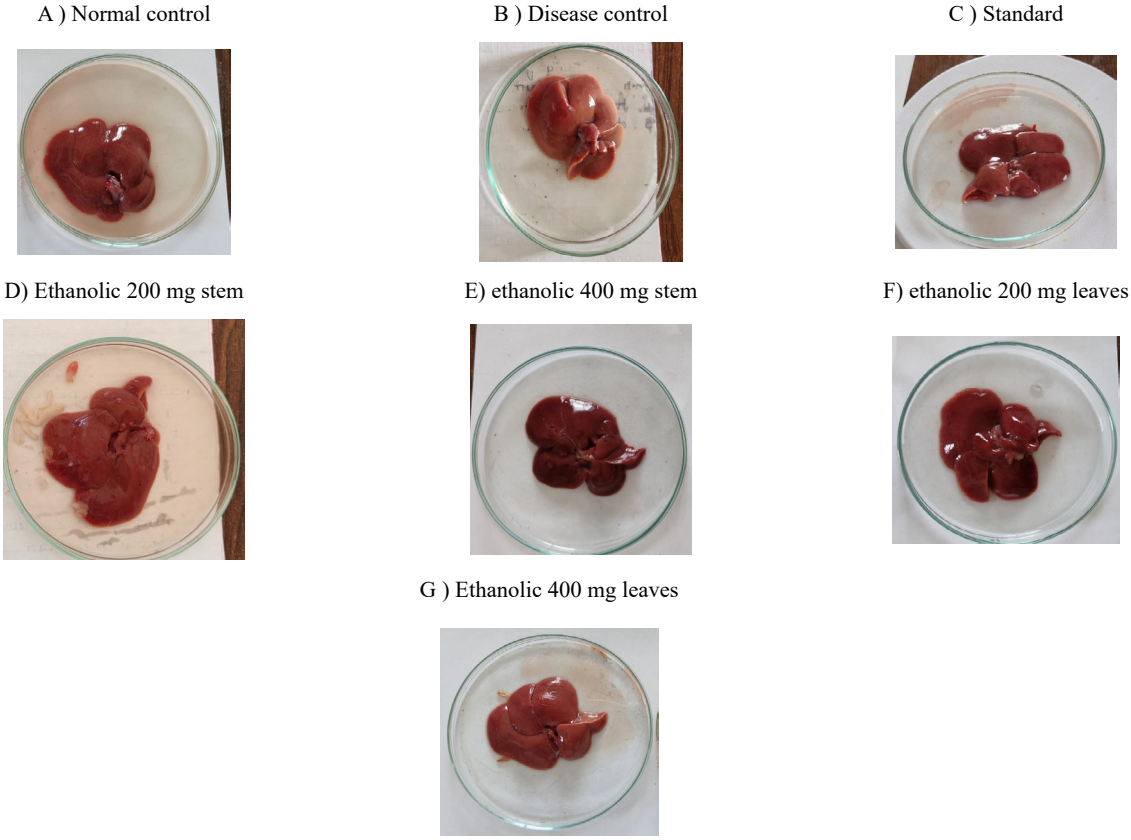
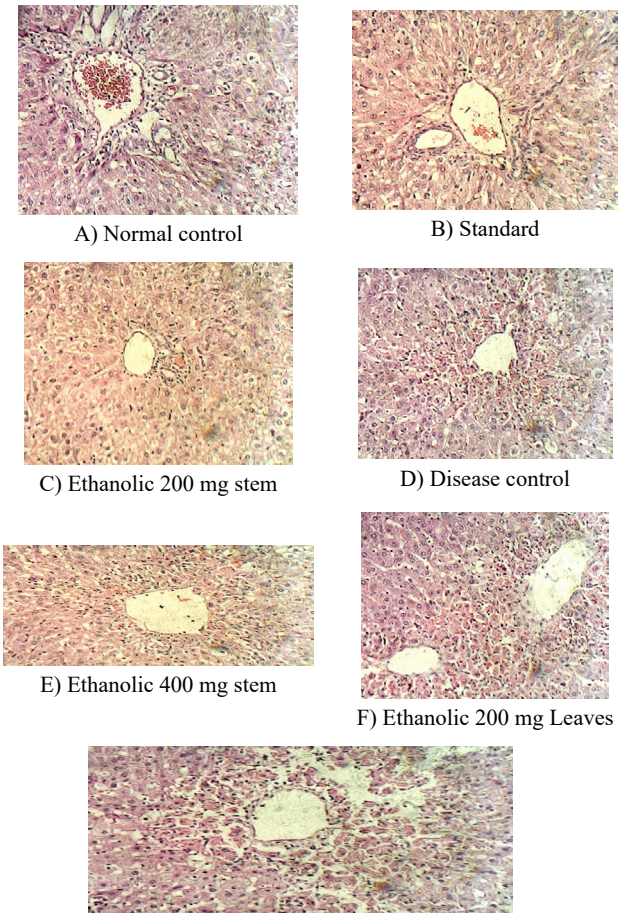


Figure 1: A: (control): Normal liver B: (CCl4 treated): displaying a large region of broken tissue, severe swelling, as well as congestion. C: (pre-treatment with silymarin): less inflammation and deterioration. D: (pre-treatment of *C. woodrowii* stem ethanolic extract dose of 400 mg/kg): decreased inflammation degenerative alterations. E: (pre-treatment of *C. woodrowii* stem ethanolic extract dose of 400 mg/kg): decreased inflammation degenerative changes. F (pre-treatment of *C. woodrowii* leaves aqueous extract dose of 400 mg/kg): inflammation decreased, deterioration occurred.

Table 3: Effect of *C. woodrowii* on antioxidants intensities in CCl_4-induced hepatotoxicity in rats

Treatment	*Dose (mg/kg)*	*MDA moles MDA/ mg Proteins*	*SOD U/mg of protein*	*Catalase U/mg of protein*	*GSH U/mg of protein*	*TP (g/dl)*
Control	-	± 0.014	250.00 ± 0.001	± 0.005	0.419 ± 0.052	0.16 ± 0.055
Disease Control	1-mL/k g	252.58 ± 0.035	100.00 ± 0.012	3.199 ± 0.029	0.066 ± 0.011	0.08 ± 0.022
Silymarin Standard	100 mg/kg	66.77 ± 0.058	225.00 ± 0.006	4.225 ± 0.030	0.102 ± 0.020	0.11 ± 0.032
C. woodrowii stem ethanolic extract	200 mg/kg	223.54 ± 0.073	150.00 ± 0.029	3.951 ± 0.017	0.183 ± 0.035	0.11 ± 0.032
C. woodrowii stem ethanolic extract	400 mg/kg	148.06 ± 0.12	200.00 ± 0.001	3.712 ± 0.011	0.191 ± 0.026	0.11 ± 0.032
C. woodrowii leaves ethanolic extract	200 mg/kg	162.58 ± 0.043	175.00 ± 0.005	4.259 ± 0.024	0.294 ± 0.040	0.18 ± 0.045
C. woodrowii leaves ethanolic extract	400 mg/kg	66.77 ± 0.023	200.00 ± 0.005	3.746 ± 0.005	0.176 ± 0.055	0.09 ± 0.014



A) Normal control B) Standard C) Ethanolic 200 mg stem D) Disease control E) Ethanolic 400 mg stem F) Ethanolic 200 mg Leaves G) Ethanolic 400 mg leaves

Figure 2: Histopathological studies A: (control): a slice of the liver showing normal architecture and structure. B: (CCl_4 treated): displaying a large region of broken tissue, severe swelling, as well as congestion. C: (pre-treatment with silymarin): less inflammation and deterioration. D: (pre-treatment of *C. woodrowii* stem ethanolic extract dosage of 400 mg/kg): abridged inflammation, degenerative alterations. E: (pre-treatment of *C. woodrowii* leaves ethanolic extract dose of 400 mg/kg): inflammation reduced, deterioration occurred. CVC stands for "central vein." F (pre-treatment of *C. woodrowii* leaves aqueous extract dose of 400 mg/kg): less inflammation and deterioration.

C. woodrowii produces a substantial increase in hepatic SOD activity. The enzyme antioxidant catalase (CAT) is present in every tissue of an animal, with the liver and red blood cells exhibiting the highest levels of activity.[26,27] CAT neutralizes hydrogen peroxide and shields tissues from hydroxyl radicals, which are extremely reactive. Thus, several harmful effects owing to superoxide radical and hydrogen peroxide absorption may occur if CAT activity is reduced. As with silymarin, the gold standard hepatoprotective medication, a greater dose (500 mg/kg) raises CAT levels. The liver produces glutathione and several other non-enzymatic biological antioxidants.[28,29] It preserves the thiols found in membrane proteins and eliminates free radical species. In rats treated with CCl_4, a lower GSH level is linked to an increase in lipid peroxidation. The levels of GPx and GST were markedly and dose-dependently elevated after *C. woodrowii* administration. In hepatocytes, CCl_4 caused centrilobular necrosis and extensive vascular degeneration. Mild degenerative alterations and the absence of centrilobular necrosis were observed in subjects treated with ethanolic and aqueous extracts of *C. woodrowii* stem and leaves at varying doses, suggesting that the plant had hepatoprotective properties. The pathophysiology of the majority of diseases has been linked to processes mediated by free radicals. In addition to its free radical scavenging activity, *C. woodrowii* appears to protect rats in contradiction of CCl_4-imparted hepatotoxicity by reducing lipid peroxidation and rising levels of antioxidant enzymes. Flavonoids are present in the *C. woodrowii* extract, according to preliminary phytochemical investigations. Flavonoids protect the liver. Flavanoids may be responsible for *C. woodrowii's* antioxidant and hepatoprotective effects.[29,30]

CONCLUSION

As per results of *C. woodrowii* plant we conclude that this plant, with some phytoconstituents like flavonoids potential to give Hepatoprotective activity that can be utilised for further formulation related to hepatoprotective formulation from extraction of phytochemicals from this plant.

ACKNOWLEDGMENT

The authors acknowledge expressing our sincere gratitude to Dr. Shitalkumar S.Patil, Principal, Dr. J. J. Magdum Pharmacy College, Biocyte Laboratory Sangli, Maharashtra and Suresh Gyan Vihar University management for continuous support.

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