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HEPATOPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT FROM THE STEM BARK OF SYNEDRELLA NODIFLORA ON CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY IN SWISS ALBINO RATS

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Keywords:

Synedrella nodiflora, Carbon tetrachloride, Hepatotoxicity, Serum Bilurubin, Alaninenamino transferase, Aspartate aminotransferase

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ABSTRACT: The ethanol extracts of stem bark of Synedrella nodiflora belonging to the family of Asteraceae was studied for hepatoprotective activity against Swiss Albino Rats with liver damage induced by carbon tetrachloride (CCl₄). It was found that the ethanol extract of Synedrella nodiflora at a dose of 250 mg/kg body weight exhibited significant protective effect by lowering the serum level of alanine aminotransferase (ALT) or serum Glutamate Pyruvate Transferase (SGPT), and 100 mg/kg body weight exhibited significant protective effect by lowering both the serum levels of alanine aminotransferase (ALT) or serum Glutamate Pyruvate Transferase (SGPT), aspartate aminotransferase (AST) or serum glutamate oxaloacetate transaminase (SGOT). The highest hepatoprotective activity was observed for ethanol extract of Synedrella nodiflora at a dose of 250 mg/kg body weight, and the reduction of serum level of ALT, AST and serum bilirubin were 60.918 \pm 0.7936, 81.479 \pm 2.85039 and 0.5205 ± 0.0249 respectively. Since, result of biochemical studies of blood sample of carbon tetrachloride treated rats showed significant increase in the level of serum enzyme activities, reflecting the liver injury caused by CCl₄ and blood sample from the animals treated with the ethanol extract of Synedrella nodiflora showed significant decrease in the level of serum marker, indicating the protection of hepatic cells, the extract of above plant could afford significant dose-dependent protection against CCl₄ induced hepatocellular injury.

INTRODUCTION: The liver is the second largest organ in the body, and is often seen as the most important one. The liver receives a dual blood supply with about 20% of blood coming from the hepatic artery and 80% from the portal circulation.



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The blood flow to the liver is around 20 to 25% of the total cardiac output. Toxins, infectious agents, medications, and serum inflammatory mediators may result in a diverse range of disease processes, leading to loss of normal histological architecture, reduced cell mass, and loss of blood flow.

Consequently, functional liver capacity may be lost. Efforts have been made to search for effective hepatoprotective agents. However, no effective hepatoprotective therapies are available until now. Therefore, the prevention of liver diseases has a great significance both in theory and in practice ¹.

Herbal medicines are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological and medicinal activities, higher safety margins and lesser costs². Modern drugs have very little to offer for the alleviation of hepatic ailments, whereas most important representatives of phytoconstituents used for liver diseases chiefly on regional basis include drugs like silymarin (Silybum marianum) and catechin (Anacardium occidentalis) in Europe, Glycyrrhizin (Glycyrrhiza glarbra) in Japan and chizandrins (Schizandra chinesis) in China ³. Herbal drugs have gained importance and popularity in recent years because of their safety, efficacy, and cost-effectiveness. The association of medical plants with other plants in their habitat also influences their medicinal values in some cases. One of the important and well-documented uses of plant products is their use as hepatoprotective agents. Hence, there is an ever-increasing need for safe hepatoprotective agent ⁴.

Carbon tetrachloride (CCl₄) is used as a model to study hepatotoxic effects and causes liver damage through a number of mechanisms. Liver cell injury induced by carbon tetrachloride involves the metabolism of carbon tetrachloride initially to trichloromethyl free-radical by the mixed function oxidase system of the endoplasmic reticulum. It is postulated that secondary mechanism slinks carbon tetrachloride metabolism to the widespread disturbances hepatocyte function. These in mechanisms could involve secondary generation of toxic products arising directly from carbon tetrachloride metabolism or peroxidative degeneration of membrane lipids. The possible involvement of radicals species such trichloromethyl (CCl₃), trichloromethyl peroxy (OOCCl₃), and chlorine (Cl) free radicals, as well as phosgene and aldehydic products of lipid peroxidation, as toxic intermediates are discussed. Data do not support the view that an increase in cytosolic free calcium is important in the toxic action of carbon tetrachloride or bromotrichlorotetrachloride-induced methane. Also, carbon inhibition of very low density lipoprotein secretion hepatocytes result is not a of elevated levels of cytosolic free calcium ⁵.

The plant *Synedrella nodiflora* (locally known as Dudia) belongs to the family Asteraceae. Extract of

leaves and steam exhibit potential antimicrobial activity ⁶. Ethanol extracts of the show the antioxidant activity ⁷. Thus, the objective of the present study was designed to test the hepatoprotective activity of the extract of plant material of above-specified plant against carbon tetrachloride-induced liver damaged in rats.

Medicinal Value of Examined Plant:

- Synedrella nodiflora var. pendula leaves have antidiarrheal, analgesic activities of methanolic extract ⁸.
- Various parts of *Synedrella nodiflora* var. pendula has Antimicrobial activity ⁹.
- It has anti-hyperglycemic effect against sucrose loading induced hyperglycemia ¹⁰.

The liver is a vital organ present in vertebrates and some other animal. It has a wide range of function. including detoxification, synthesis protein and production of biochemical's necessary for digestion. The liver is necessary for survival; there is currently no way to compensate for the absence of liver function long term, although liver dialysis can be used short term.

This organ plays a major role in metabolism and has some functions in the body, including glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production, and detoxification. It produces bile, an alkaline compound which aids in digestion *via* the emulsification of lipids. The liver's highly specialized tissues regulate a wide variety of high-volume biochemical reaction, including the synthesis and breakdown of small and complex molecules, many of which are necessary for vital function ¹¹.

MATERIALS AND METHODS:

Materials:

Collection and Identification of the Plant Sample: Fresh and mature steam were collected from a reliable source and identified by a taxonomist. An accession number was given by herbarium for confirming actual classification Table 1.

Scientific name	Local name	Accession number	Collection	
Synedrella nodiflora	Dudia	37501	National Botanical Garden, Dhaka, Bangladesh	

Extraction: The stems were collected from the national botanical garden, Dhaka, Bangladesh, during February 2010. Then the steams were washed with water and sun dried for 10 days. The dried steam was then pulverized into coarse powder in a grinding machine. 200 mg of powder was extracted in 500 ml of cold ethanol for 5 days. The solvent was filtered, squeezed off and evaporated by keeping it at room temperature and then crude extract was obtained. The percentage of yield of the crude methanol extract is given in the following **Table 2.**

TABLE 2: EXTRACTION OF STEM BARK OF SYNEDRELLA NODIFLORA

Scientific name	Used part	% of yield
Synedrella nodiflora	stem bark	10%

Animal: The experiment was carried out on Swiss albino rats. The rats were aged between the 2.5-3 month of both sex and weighted between 100-105 gm (average weight 100 gm). The rats were obtained from ICDDR, b animal house of Dhaka, Bangladesh.

They were fed on standard laboratory diet were allowed to drink water. They were housed in a well-ventilated cage at room temperature in hygienic condition under natural light and dark schedule in the laboratory.

Drug and Chemicals: A drug used in the experiment were followed:

- Carbon tetrachloride (CCl₄)
- The crude extract of a plant

Reagents:

- Sorbitol
- 0.9% NaCl solution

Equipments:

- Syringe with needle
- Surgical blade
- Scissor
- Forceps
- Centrifuge tube

Preparation of Sample: Preparation of Crude Extracts (Drug) Suspension:

 In case of dose 250 mg/kg body weight of rat 500 mg of crude extract was taken and the suspension was prepared by adding with 5 ml sorbitol so that every 0.25 ml of suspension contains 25 mg of crude extract.

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 In case of dose 100 mg/kg body weight of rat 200 mg of crude extract was taken and the suspension was prepared by adding with 2 ml sorbitol so that every 0.10 ml of suspension contains 10 mg of crude extract.

METHODS:

Study of Hepatoprotective Activity: The liver is especially sensitive to carbon tetrachloride. In mild cases, the liver becomes swollen and tender, and fat builds up inside the organ. In cases, liver cells may be damaged or destroyed, leading to a decreased in liver function. Carbon tetrachloride (CCl₄) is frequently used as a chemical inducer for experimental liver cirrhosis. It is metabolized by the drug metabolizing enzyme of cytochrome p450 to highly reactive trichloromethyl free radical (OOCCl₄).

Free radical attack membrane lipid and protein and this cause destruction of microsomes, mitochondria, and nuclei of the hepatocytes and thus the hepatocytes are damaged ¹².

Induction of Hepatotoxic Rats by CCl₄: Acute toxicity studies were performed according to the Organization for Economic Co-Operation and Development (OECD)-423 guidelines ¹³.

Hepatoprotective was induced by an intraperitoneal administration of single dose of CCl₄ (3 ml/kg) which was evidenced by a significant increase of serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), ATP, Bilirubin & cholesterol ¹⁴.

Experimental Design: ¹⁵ The hepatoprotective activity of *Synedrella nodiflora* extract was determined by using carbon tetrachloride-induced hepatotoxic rat model. After three days of

acclimatization, the rats were divided into four groups each comprising of five rats and treatment was done for 3 days.

Group 1: Normal control which received neither the sample nor CCl₄.

Group 2: Control group which received only a single intraperitoneal dose of CCl₄ injection.

Group 3: SN-250 group, this group consists of 5 rats, which received sample solution of crude ethanol extract of *Synedrella nodiflora* at a dose of

250 mg/kg and a single dose of CCl₄ i.p. This group is called the experimental group.

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Group 4: SN-100, this group consists of 5 rats, which received sample solution of crude extract of *Synedrella nodiflora* at a dose of 100 mg/kg and a single dose of CCl₄ i.p. This group is called the experimental group.

Administration of the Test Samples: The suspensions of the test sample (250 mg/kg & 100 mg/kg body wt.) were administered to rats 1 h, 24 h, and 48 h after injection 16 .

TABLE 3: DOSAGE REGIMEN FOR EACH GROUP OF RATS USED FOR HEPATOPROTECTIVE SCREENING

Study group	No. of Rats	Drug/Drug administration	Dose/kg body Weight	Route of administration
G-1	5	No drug		
G-2	5	CCl_4	3 ml/kg	Intraperitonial
G-3	5	CCl ₄ + Ethanol extract	3 ml/kg + 250 mg/kg	Intrapertonial
G-4	5	CCl ₄ + Ethanol extract	3 ml/kg + 100 mg/kg	Intraperitonial

Assessment of the Hepatoprotective Study: Four ways can assess hepatoprotective activity:

- Functional study
- Biochemical study
- Morphological study
- Histological study

In our present study, we have assessed the activity biochemically.

Biochemical Study:

Measurement of the Biochemical Parameter of Blood: After 72 h of drug treatment, blood was collected from each animal for enzyme estimation by cutting the carotid artery at the neck.

Collection of Serum From Blood: Blood sample was collected in a previously labeled centrifuging tube and allowed to clot at room temperature. Serum was separated by centrifugation of 3000 rpm for about 15 min. The cleared straw colored in vials with the help of micropipette and stored at -4 °C.

Biochemical Examination:

Parameter Studies: The following biochemical parameters were determined for the assessment of hepatoprotective activity.

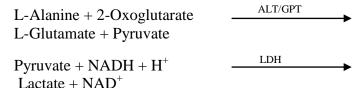
 Serum Alanine Aminotransferase (ALT) or serum glutamate pyruvate transaminase (SGPT).

- Serum Aspartate Aminotransferase (AST) or serum glutamate oxaloacetate transaminase (SGOT).
- Serum bilirubin.

Test Principle:

Test Principle of ALT/GPT BR: Alanine aminotransferase (ALT/GPT) catalyzes the transfer of the amino group from alanine to oxoglutarate with the formation of glutamate and pyruvate. The latter is reduced to lactate by lactate dehydrogenase (LDH) in the presence of reduced nicotinamide adenine dinucleotide (NADH).

The reaction is monitored kinetically at 340 nm by the rate of decrease in absorbance resulting from the oxidation of NADH to NAD⁺, proportional to the activity of ALT present in the sample.



This test has been formulated according the standarized method described by IFCC. Clin Chem Lab Med 2002; 40(7): 718-724

Test Principle of AST/GOT BR: Aspartate aminotransferase (AST/GOT) catalyzes the transfer of the amino group from aspartate to oxoglutarate with the formation of glutamate and oxaloacetate.

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The latter is reduced to malate by malate dehydrogenase (MDH) in the presence of reduced nicotinamide adenine dinucleotide (NADH).

The reaction is monitored kinetically at 340 nm by the rate of decrease in absorbance resulting from the oxidation of NADH to NAD⁺, proportional to the activity of AST present in the sample.

This test has been formulated according to the standardized method described by IFCC. Clin Chem Lab Med 2002; 40(7): 718-724.

Test Principle of Bilirubin: Bilirubin is converted to colored azobilirubin by diazotized sulfanilic acid and is measured photometrically. Of the two bilirubin fractions in serum -bilirubin-glucuronide and free bilirubin which is bound to albumin-only the former reacts directly, while free albumin reacts after being displaced from protein by an accelerator. The difference of two measurements total bilirubin (with accelerator), and direct (without accelerator) bilirubin enables the calculation of indirect bilirubin. The terms «direct» and «indirect» bilirubin refers exclusively to the reaction characteristics in the presence or absence of an accelerator or solubilizer and is only approximate equivalents of the two bilirubin fractions.

RESULTS AND DISCUSSION:

Biochemical Results: The biochemical parameters of the experimental group (G-3 and G-4) were compared with CCl₄ treated group (G-2). A

comparison was also made between the normal untreated group (G-1), and the CCl₄ treated group (G-2). The change in the biochemical parameters among the different group was tabulated and tested statistically to demonstrate the significance of the study.

Effects of Acute Administration of Carbon Tetrachloride (CCl₄) on Serum Transaminases (ALT, AST), Bilirubin Level in Rats: Here, a single intraperitoneal dose of CCl₄ (3 ml/kg body weight) was given to the standard group. The following observations were made:

- **I. Serum Alanine Aminotransferase Level** (ALT): Drug treatment produced an increase in serum ALT level in CCl_4 treated group. The mean value of serum ALT levels in control and CCl_4 treated group was 49.47 ± 2.376 , 88.173 ± 5.46381 U/L respectively. The increase in serum ALT level in CCl_4 treated group was highly significant as compared to the control group, which did not take CCl_4 . The results are shown in **Table 4**.
- II. Serum aspartate aminotransferase level (AST): The mean value of serum AST levels in control and CCl₄ treated group was 58.779 ± 0.0947 , 152.48 ± 0.0948 U/L respectively. The increase in serum AST level as compared to the control group was highly significant. The results are shown in **Table 4**.
- III. Serum Bilirubin Level: The mean value of serum bilirubin levels in control and CCl_4 treated group was 0.488 ± 0.024495 , 0.8055 ± 0.020082 respectively. The increase in serum bilirubin level as compared to the control group was highly significant. The results are shown in **Table 4**.

TABLE 4: SHOWING THE EFFECTS OF (CCL₄) ON SERUM TRANSAMINASE (AST AND ALT), BILIRUBIN LEVEL IN RATS

Group	No. of rats	Treatment	Mean serum ALT/SGPT level (U/L) ± SEM	Mean serum AST/ SGOT level (U/L) ± SEM	Mean serum bilirubin level (mg/dl) ± SEM
1	5	Normal	49.47 ± 2.376	58.779 ± 0.0947	0.488 ± 0.024495
2	5	Control	88.173 ± 5.46381	151.32 ± 0.094713	0.8055 ± 0.02082

Control = Normal food and water only; Standard = Single dose of CCl₄ 3ml/kg body weight i.p; SEM = Standard error mean. ↑ = Increase in mean serum level.

Effects of CCl₄ and CCl₄ Plus Crude Ethanolic Extract of *Synedrella nodiflora* on Serum Transaminase (ALT and AST), Bilirubin Levels in Rats: Here, a single intraperitoneal dose of CCl₄

(3 ml/kg body weight) was given along with the suspension of ethanol extract (250 mg/kg and 100 mg/kg body wt.). The following observations were recorded.

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I. Serum Alanine Aminotransferase level (ALT) The mean value of serum ALT levels Fig. 1 in CCl_4 treated groups were 88.173 ± 5.46381 and CCl_4 plus crude ethanolic extract treated groups at a different dose (250 mg/kg and 100 mg/kg body wt.) were 60.988 ± 0.7936 and 73.757 ± 1.2970 respectively. The decrease in serum ALT level in ethanolic extract treated group at a 250 mg/kg and 100 mg/kg body wt. was significant compared to CCl_4 treated group. The results are shown in **Table 5**.

II. Serum Aspartate Aminotransferase level (AST): Drug treatment produced a decreased in serum AST level **Fig. 2** in CCl₄ plus crude ethanolic extract treated group. The mean value of serum AST levels in CCl₄ and CCl₄ plus crude ethanolic extract treated group at different doses of

(250 mg/kg and 100 mg/kg body wt.) were 152.48 \pm 0.094 and 81.479 \pm 2.85039, 99.81 \pm 4.98894 respectively. The decrease in serum AST level in CCl₄ plus ethanolic extract treated group at a 100 mg/kg body wt. was significant compared to CCl₄ treated group. The results are shown in **Table 5**.

III. Serum Bilirubin Level: Drug treatment produced a decrease in serum Bilirubin level Fig. 3 in CCl₄ plus crude ethanolic extract treated group. The mean value of serum Bilirubin levels in CCl₄ and CCl₄ plus crude extract treated group at different doses (250 mg/kg and 100 mg/kg body wt.) were 0.8055 ± 0.02082 and 0.5205 ± 0.0249 , 0.6385 ± 0.0237 respectively. The decrease in serum bilirubin level in CCl₄ plus ethanolic extract treated group at a 100 was significant compared to the treated group. The results are shown in **Table 5.**

TABLE 5: EFFECT OF CCl₄ AND CCl₄ PLUS CRUDE ETHANOLIC EXTRACT OF SYNEDRELLA NODIFLORA ON SERUM TRANSAMINASES (ALT, AST), BILIRUBIN LEVELS IN RATS

Group	No. of	Treatment	Mean serum AST/SGOT	Mean serum ALT/SGPT	Mean serum Bilirubin
	rats		$level(U/L) \pm SEM$	$level(U/L) \pm SEM$	level (mg/dl) \pm SEM
1	5	No drug	58.779 ± 0.9479	49.47 ± 2.376	$0.488 \pm .024495$
2	5	CCl_4	152.48 ± 0.947136	88.173 ± 5.46381	0.8055 ± 0.02082
3	5	CCl ₄ +ethanol extract	81.479 ± 2.85039	60.918 ± 0.7936	0.5205 ± 0.0249
4	5	CCl ₄ +ethanol extract	99.81 ± 4.98894	73.757 ± 1.2970	0.6385 ± 0.02327

Statistical analysis was conducted through one way ANOVA and post hoc Dunnett's test.

The CCl_4 = single dose of CCl_4 , 3ml/kg body wt.

Ethanol extract (G-3) = Thrice dose of 250mg/kg body wt i.p.

Ethanol extract (G-4) = Thrice dose of 100mg/kg body wt i.p.

Graphical Presentation of Biochemical Parameters of Tested Groups:

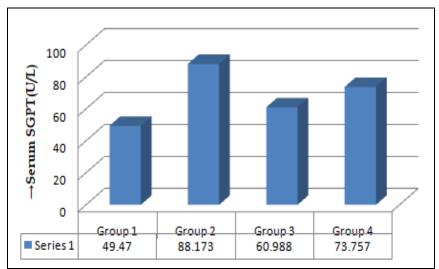


FIG. 1: COMPARISON OF SGPT LEVEL WITH DIFFERENT GROUP

Where, G-1 = Control or untreated group, G-2 = Standard or CCl_4 treated group, G-3 = Thrice dose of ethanol extract 250 mg/kg body wt. i.p. G-4 = Thrice dose of ethanol extract 100 mg/kg body wt. i.p.

 $[\]uparrow$ = Increase in mean serum level.

 $[\]downarrow$ = Decrease in mean serum level.

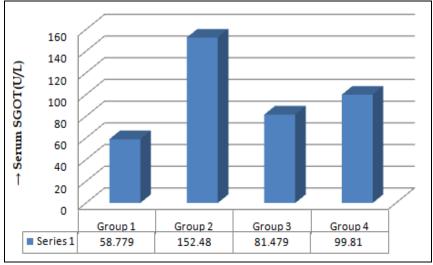


FIG. 2: COMPARISON OF SGOT LEVEL WITH DIFFERENT GROUP

Where, G-1 = Control or untreated group, G-2 = Standard or CCl_4 treated group, G-3 = Thrice dose of ethanol extract 250 mg/kg body wt. i.p., G-4 = Thrice dose of ethanol extract 100 mg/kg body wt. i.p.

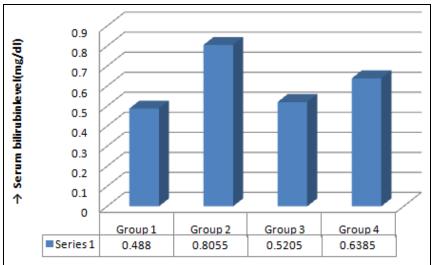


FIG. 3: COMPARISON OF SERUM BILIRUBIN LEVEL WITH DIFFERENT GROUPS

Where, G-1 = Control or untreated group, G-2 = Standard or CCl_4 treated group, G-3 = Thrice dose of ethanol extract 250 mg/kg body wt. i.p., G-4 = Thrice dose of ethanol extract 100 mg/kg body wt. i.p.

DISCUSSION: Literature review revealed that various chemical and biological investigations were carried out with this plant. Liver damage induced by CCl₄ is a commonly used model for the screening of hepatoprotective drugs. The rise in the serum level of ALT, AST and bilirubin has been attributed to the damaged structural integrity of the liver, because they are cytoplasmic in location and released into circulation after cellular damages.

When rats were treated with carbon tetrachloride it induces hepatotoxicity by metabolic activation, therefore it selectively causes toxicity in liver cells maintaining semi-normal metabolic function. Carbon tetrachloride is metabolically activated by cytochrome p-450 dependent mixed oxidase in the

endoplasmic reticulum to from trichloromethyl free radical (CCl₃) which combined with cellular lipids and proteins in the presence of oxygen to induce lipid per-oxidation. These result in a change of structure of the endoplasmic reticulum and another membrane, loss of metabolic enzyme activation, reduction of protein synthesis and loss of glucose-6-phosphatase activation, leading to liver injury.

In the pharmaceutical study, *in-vivo* hepatoprotective effect of *Syndrella nodiflora* extract had been investigated against carbon tetrachlorideinduced rats at 250 mg/kg body wt. and 100 mg/kg body wt. normal rat group showed no changes in their behavior and in all biochemical parameter **Table 4.2.**

rats that give their supreme sacrifice for the

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In carbon tetrachloride treated group it was observed that a significant increased of blood serum levels of SGPT (88.173 ± 5.46381 , p<0.05), SGOT (151.32 ± 0.0947 , p<0.05) and bilirubin (0.8055 ± 0.02082 , p<0.05) compare to normal rats **Table 4.1**. For an experimental group, the plant extract was administered at a dose 250 and 100 mg/kg body wt. for three days after administration of carbon tetrachloride (3 ml/kg body wt.) at the first day. After 72 h all experimental rats were sacrificed and various biochemical parameters were determined.

Treatment with *Syndrella nodiflora* ethanol stem bark extract recovered the injured liver to normal after 72 h at a dose of 250 and 100 mg/kg body wt, which indicate that *Syndrella nodiflora* has antihepatotoxicity effect. The highest hepatoprotective activity was observed for ethanol extract of *Syndrella nodiflora* at a dose of 250 mg/kg body wt. and the reduction of serum level of ALT, AST and serum bilirubin were 60.918 ± 0.7936 , 81.479 ± 2.85039 and 0.5205 ± 0.0249 respectively. A decrease in serum bilirubin after treatment with the extract in the liver damage model indicated the effectiveness of the extract in the normal functional status of the liver 16 .

The literature review also revealed that methanol extract of *Syndrella nodiflora* stem bark has significant hepatoprotective activity against Diclofenac-induced hepatotoxicity ¹⁷. The present biochemical analysis of said extract also showed a significant hepatoprotective activity in carbon tetrachloride-damaged liver cells.

CONCLUSION: From the overall result of the biochemical parameters, it could be inferred that ethanolic stem bark of *Synedrella nodiflora* showed the significant hepatoprotective activity, but appropriate effective concentration needs to be fixed. Further study on this plant could be extended for the isolation and structure determination of the hepatoprotective principle or principles.

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CONFLICT OF INTEREST: Nil

palliation of human sufferings.

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