

## RESEARCH ARTICLE

# Pharmacognostical, Hepatoprotective and Anthelmintic Evaluation of *Cuscuta reflexa* Roxb. and *Gymnema sylvestre* (RETZ.) RZ BR. EX. SM.

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Received: 18th January, 2022; Revised: 20th March, 2022; Accepted: 03rd April, 2022; Available Online: 25th June, 2022

## ABSTRACT

The present study was designed to evaluate the pharmacognostic, hepatoprotective and anthelmintic study of methanolic extracts of aerial parts of *Cuscuta reflexa* Roxb. (MECR) and *Gymnema Sylvestre* (Retz.) R. Br. ex. Sm. (MEGS). Pharmacognostical studies were observed as macroscopic & microscopic characterization of the plants. An acute toxicity test was performed using albino mice according to Organization for Economic Co-operation and Development (OECD) 425 guidelines. Hepatoprotective activity of MECR and MEGS in liver injury induced by carbon tetrachloride was performed by using albino rats. Anthelmintic activity was designed according to the standard methods on the adult Indian earthworm *Pheritima posthuma*.

Pharmacognostical study was observed the using of parts of plants through transverse section, powder microscopic and fluorescence analysis of powder studies. MECR and MEGS were showed a significant increase in all parameters for the dose of 50 and 100 mg/kg in a dose dependent manner. Anthelmintic activity showed significant effect using of 25, 50, 100 mg/kg doses of MECR and MEGS. Methanolic extracts of *C. reflexa* Roxb. and *G. sylvestre* (Retz.) R. Br. ex. Sm. have hepatoprotective and anthelmintic activity.

**Keywords:** Acute toxicity, Anthelmintic, Hepatoprotective, Pharmacognostical.

International Journal of Drug Delivery Technology (2022); DOI: 10.25258/ijddt.12.2.9

**How to cite this article:** Biswal B, Panda SK. Pharmacognostical, Hepatoprotective and Anthelmintic Evaluation of *Cuscuta reflexa* Roxb. and *Gymnema sylvestre* (RETZ.) RZ BR. EX. SM.. International Journal of Drug Delivery Technology. 2022;12(2):516-521.

**Source of support:** Nil.

**Conflict of interest:** None

## INTRODUCTION

Current drugs have been inaccessible from nature for thousands of years. Based on these isolations, pharmaceutical agents are used in predictable treatments.<sup>1</sup> Nature is a resource of medicines, and medicinal plants serve the cause of numerous powerful and prevailing drugs in different countries.<sup>2</sup> The ideal medicinal plant was not established for treating liver disease and helminthiasis disease. Natural products from plants play a significant role in the improvement of new agents to prevent the beginning of hepatotoxicity and helminthiasis.

*Cuscuta reflexa* Roxb. (Family: Cuscutaceae) is known as "Akashabela, Amarbela Kasur" in Hindi, also used in India.<sup>3</sup> This plant is a tropical and sub-tropical herb depending on the host plants to originate as a parasite weed. Antibacterial properties have been observed in the stem of *C. reflexa* Roxb. (*C. reflexa*), which is used internally to treat fever and externally in itching.<sup>4</sup> This plant also exhibits anti-cancer and anti-inflammatory activities, diuretic activity<sup>5</sup> and anti-HIV activity.<sup>6</sup>

*G. sylvestre* (Retz.) R. Br. ex. Sm. (*G. Sylvestre*) is an indigenous herb, belonging to the class dicotyledonous of the

family Asclepiadaceae.<sup>7</sup> The plant is a good basis for many bio-active substances.<sup>8</sup> It has deep roots in history, being one of the major botanicals used in the Ayurvedic system of medicine to treat conditions ranging from diabetes, and malaria to snakebites.<sup>9</sup> According to the Ethanomedical information of *G. sylvestre*, it is being used as an anti-diabetic, stomachic, stimulant, laxative, and diuretic.<sup>10</sup>

The pharmacognostic, hepatoprotective, and anthelmintic studies of MECR and MEGS are helping to establish a new drug against liver illness and helminthiasis, which is still not reported today. Hence, the present study aims to provide pharmacognostic, hepatoprotective, and anthelmintic evaluation of *C. reflexa* and *G. sylvestre*.

## MATERIALS AND METHODS

### Collection and Authentication of Plant Materials

*C. reflexa* (Convolvulaceae) and *G. Sylvestre* (Apocynaceae) aerial parts of the plant were collected in December 2020 and March 2021, respectively, from various areas of Purba Medinipur District fields of West Bengal and authenticated

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by Botanist K. Karthigeyan and Vinay Ranjan (Scientist-E, Botanical Survey of India, Kolkata, West Bengal, India), respectively. The plant's herbarium was prepared and preserved in the Central National Herbarium, Botanical Survey of India, Kolkata, West Bengal, India.

### Preparation of Extracts

The plants' dry and powdered aerial parts (110 g) were extracted with 700 mL of methanol by the Soxhlation process.<sup>11</sup> The extracts obtained were concentrated under reduced pressure and air-dried.<sup>12</sup> The concentrated extracts were further dried in desiccators,<sup>13</sup> containing fused calcium chloride. Afterward, the extracts were stored in a refrigerator at 4°C for further use.

### Preliminary Phytochemical Tests

The methanolic extracts were tested for alkaloids, glycosides, saponins, steroids, terpenoids, phenols, tannins, flavonoids, proteins and amino acids, and Pholbatannin.<sup>14-18</sup>

### Pharmacognostical Evaluation

#### Macroscopic Study

The results were studied and mentioned in the result section, the microscopical characteristics of *C. reflexa* and *G. Sylvestre*.

#### Microscopic Study

The transverse section (TS) of stem, leaf, and root were obtained by usual techniques.<sup>19-21</sup> The powder microscopic and fluorescence analysis of powdered plant parts were studied as per the standard method.<sup>22</sup>

#### Acute Toxicity Study

The acute toxicity of MECR and MEGS were performed as per OECD 425 guidelines. This test was conducted by using female non-pregnant albino mice weighing 25 to 32 g. Prior to dosing, the mice fasted overnight. The study was conducted with the prior permission of the Institutional animal ethical committee (IAEC Reg. No. 1881/PO/Re/S16/CPCSEA, Approval No. 09/IAEC/KCPT/2021-22). Experimental animals were divided into two groups, with six animals in each group. Five different doses (25, 200, 500, 2000 and 5000 mg/kg) of both the extracts were administered orally in a test group of animals. After that, the effects of toxicity were observed in the first 4 hours and thereafter once every 24 hours for the next 14 days.

### Hepatoprotective Activity

#### Animal

A total of 42 Wister rats (6 normal controls and 36 hepatoprotectives) were used in this study. The rats (150–200 g) were divided into seven groups (control 1 group, toxic 1 group, standard 1 group and test 4 group) with six animals in each group. Test groups comprise MECR 50 mg/kg, MECR 100 mg/kg, MEGS 50 mg/kg, MEGS 100 mg/kg group. The drug treatment was given orally per day.

#### Experimental Design

Hepatoprotective activity was performed with the prior permission of the Institutional animal ethical committee

(IAEC Reg. No. 1881/PO/Re/S16/CPCSEA, Approval No. 16/IAEC/KCPT/2021-22). The rats were divided into seven groups with six animals for suspending the standard drugs and methanolic extracts using gum acacia (5%) as a vehicle.

*Group I:* Normal control group received 5% aqueous gum acacia (1 mL/200g vehicle) up to 7 days by oral route and also received olive oil on the 7<sup>th</sup> at a dose of 0.12 mL/100 g.

*Group II:* Toxic control group received aqueous 5% gum acacia (1-mL/200g) for up to 7 days by oral route.

*Group III:* Standard treatment group received 0.5% silymarin 25 mg/kg (1 mL/200g) up to 7 days by oral route.

*Group IV and V:* *Cuscuta reflexa* extract (CRE) treatment group received CRE 50 mg/kg and 100 mg/kg in 5% gum acacia, respectively, by oral route up to 7 days.

*Group VI and VII:* *Gymnema Sylvestre* extract (GSE) treatment group received GSE 50 mg/kg and 100 mg/kg in 5% gum acacia, respectively, up to 7 days by oral route.

All the treatment was given via oral route daily for 7 days. On the 7<sup>th</sup> day, CCl<sub>4</sub> (Carbon tetrachloride) in olive oil (1:1 ratio) 0.25 mL/100 g was given to all groups of animals (group II to VII, except group I) by oral route to induce liver damage. Blood samples were collected from animals through the retro-orbital puncture method for biochemical examinations such as serum glutamate oxalate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALKP), total bilirubin (TB). The histopathological studies were performed by using liver samples of all animals group.

### Anthelmintic Activity

#### Earthworm's Collection

Indian earthworm *Pheritima Posthuma* was collected from Berhampur, Odisha, India, by using of water logged soils. Then washed with normal saline solution and kept in tyrode solution.

#### Experimental Design

The anthelmintic study was conducted per the standard method by using the adult Indian earthworm *Pheritima Posthuma*. Standard drug (Albendazole) and methanolic extracts (MECR and MEGS) were diluted with normal saline solution (0.9 % NaCl) and prepared at three different concentrations (25, 50 and 100 mg/mL). A normal control group received a normal saline solution as a negative control. Ten solutions (3 solutions for standard, six solutions for extracts and one solution for control) were kept in separate Petri dish and after that, six earthworms (8 cm. each) were placed in each Petri dish and observed paralysis time (no movement of worms) and death time (no movement of worms when dipped in warm water) which was recorded in terms of minutes.

### Statistical Analysis

The results were expressed as Mean ± SEM. The total variation was analyzed by one-way analysis of variance (ANOVA).

## RESULTS AND DISCUSSION

### Preliminary Phytochemical Evaluation

Alkaloids, glycosides, saponins, steroids, terpenoids, phenols, tannins and flavonoids phytoconstituents were present in the methanolic extracts of *C. reflexa* and *G. Sylvestre*.

### Pharmacognostical Evaluation

#### Macroscopic Study

*C. reflexa* stem showed related to brown colored (pale greenish-brown or greenish-yellow or blackish brown or brownish), aromatic odor and aromatic & bitter tastes. Flowers are small, bell-shaped and white to pink in color.

*G. Sylvestre* leaves showed green color; flowers are small and yellow in color, the stem is hairy and light brown color, roots are brown or yellowish-brown in color and odor is characteristics and taste is slightly bitter & astringent.

#### Microscopic Study

*C. reflexa* stem showed cuticle, epidermis, cortex, bundle cap, phloem, cambium, xylem, medullary ray, pith and vascular bundle. Powders showed phloem fibres, xylem fibres and reticulate fibre vessels. Fluorescence analysis of powder studies of *C. reflexa* showed in Table 1.

*G. Sylvestre* stem showed epidermis, pericycle, vessels, xylem, pith, phloem, endodermis, and cortex. Leaf of *G. Sylvestre* showed collenchyma, upper epidermis, lower epidermis, xylem, cortex, vascular bundle, phloem, spongy

parenchyma and palisade cells. Root showed cork, vessels, cortex, medullary rays, xylem, and phloem. Powder showed vascular bundles, lamina, and spongy parenchyma. Fluorescence analysis of powder studies of *G. Sylvestre* showed in Table 2.

#### Acute Toxicity Study

The oral acute toxicity study determines a safe dose for further experimental study in animals which can also be extrapolated to human use. *C. reflexa* and *G. Sylvestre* extracts were showed absent mortality and wellness parameters at doses of 25, 200, 500, 2000 and 5000 mg/kg body weight. The animals (test & control group) had shown normal skin, breathing, eyes, mucous membrane, behavioral pattern, salivation, sleep and blood pressure parameters. Animals were not shown tremors, lethargy, diarrhea and coma. The morphological changes in the liver, brain, and kidney are insignificant in all animal groups.

### Hepatoprotective Activity

*In-vivo* hepatoprotective activity of *C. reflexa* extract (CRE) and *G. Sylvestre* extract (GSE) were performed on Wister albino rats, including the  $\text{CCl}_4$  method. The results are mentioned in Table 3. Toxic group (group II) results showed a significant increase in serum levels of SGOT ( $340.5 \pm 5.4$ ), SGPT ( $382.2 \pm 4.8$ ), ALKP ( $426.7 \pm 4.8$ ), and total bilirubin (TB) ( $4.3 \pm 0.0$ ). The standard group (group III) showed serum levels of SGOT, SGPT, ALKP, and total bilirubin were  $118.6 \pm$

**Table 1:** Fluorescence analysis of powdered *Cuscuta reflexa* Roxb.

SL. No.	Experiments	Visible/daylight	UV light	
			254 nm	365 nm
1.	Drug powder as such	Brown	Light brown	Black
2.	Drug + 1N NaOH	Black	Dark black	Charcoal black
3.	Drug + 1M HCl	Brown	Green brown	Black
4.	Drug + Picric Acid	Black-brown	Henna color	Black
5.	Drug + 1M $\text{H}_2\text{SO}_4$	Brown	Greenish brown	Black
6.	Drug + Acetic acid	Brown	Greenish brown	Black
7.	Drug + Ammonia soln. + $\text{HNO}_3$	Golden brown	Greenish brown	Black
8.	Drug + dil. $\text{HNO}_3$	Brown	Yellowish black	Charcoal black
9.	Drug + methanol	Dark brown	Greenish brown	Black

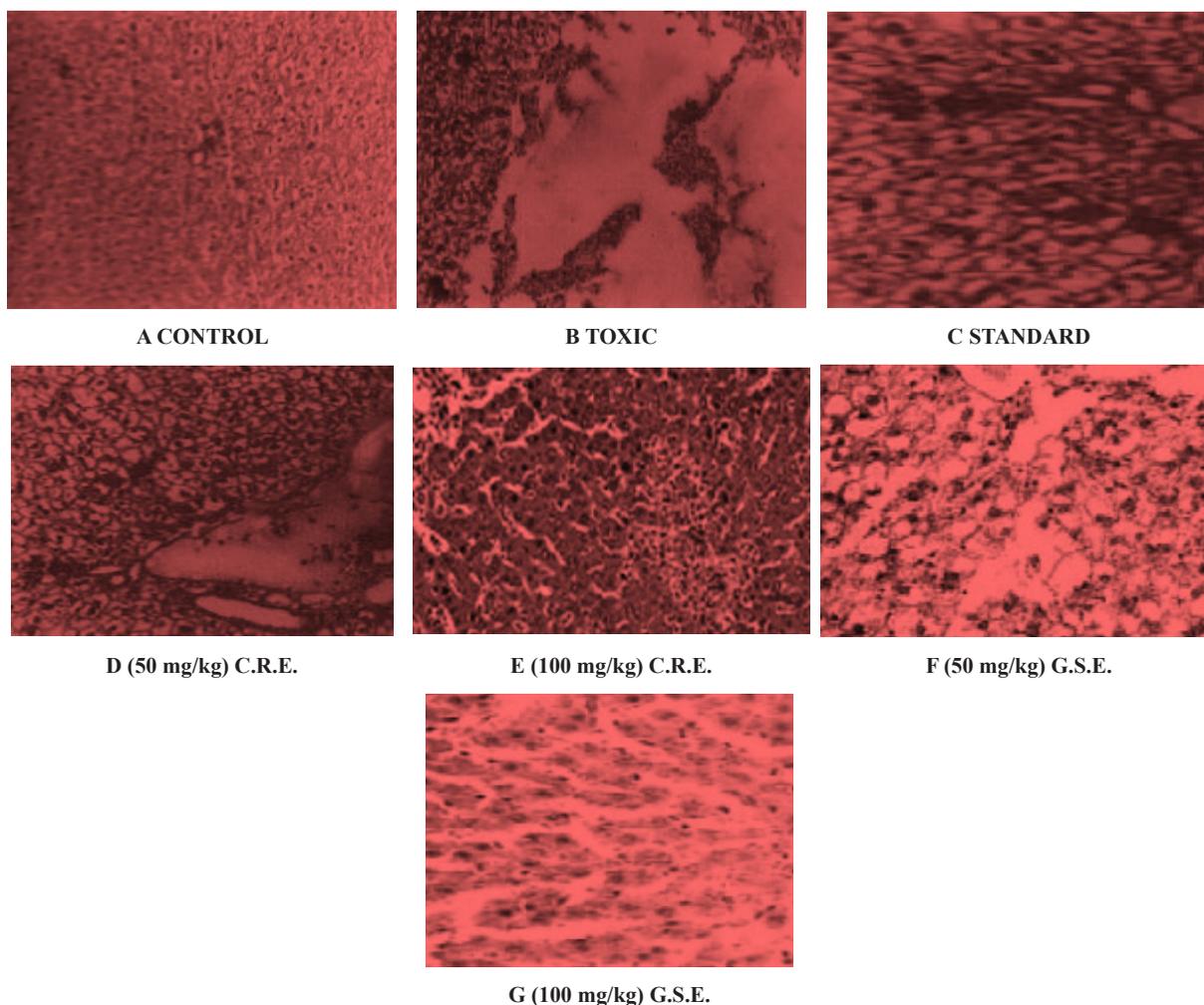
**Table 2:** Fluorescence analysis of powdered *Gymnema Sylvestre* (Retz.) R. Br. ex. Sm.

SL. No.	Experiments	Visible/daylight	UV light	
			254 nm	365 nm
1.	Drug powder as such	Green	Dark green	Light green
2.	Drug + 1N NaOH	Light green	Dark green	Greenish brown
3.	Drug + 1M HCl	Brown	Brown	Dark brown
4.	Drug + picric acid	Green	Light green	Brown
5.	Drug + 1M $\text{H}_2\text{SO}_4$	Light brown	Green	Greenish brown
6.	Drug + acetic acid	Yellowish-brown	Light brown	Brown
7.	Drug + ammonia soln. + $\text{HNO}_3$	Green	Dark green	Blackish green
8.	Drug + dil. $\text{HNO}_3$	Dark green	Green	Blackish green
9.	Drug + methanol	Dark brown	Dark brown	Black

**Table 3:** Hepatoprotective effect of methanolic extracts on serum biological levels in CCl<sub>4</sub> induced liver damaged rats.

SL.No	Groups	SGOT IU/L (mean ± SEM)	SGPT IU/L (mean ± SEM)	Alkaline phosphatase (ALKP) IU/L (mean ± SEM)	Total bilirubin (TB) (mg/dl) (mean ± SEM)
1	Control group-I	116.0 ± 1.9	101.4 ± 2.0	184.7 ± 1.6	1.4 ± 0.2
2	Toxic control group-II	340.5 ± 5.4**	382.2 ± 4.8**	426.7 ± 4.8**	4.3 ± 0.0**
3	Standard group-III (25mg/kg)	118.6 ± 3.1*	117.8 ± 2.1*	177.2 ± 5.0*	1.7 ± 0.0*
4	CRE group-IV (50 mg/kg)	291.9 ± 6.0*	298.0 ± 6.0*	377.6 ± 6.4*	2.8 ± 0.0*
5	CRE group-V (100 mg/kg)	282.7 ± 5.0*	251.5 ± 4.4*	274.8 ± 5.8*	2.5 ± 0.0*
6	GSE group-VI (50 mg/kg)	194.6 ± 6.0*	190.0 ± 5.4*	198.2 ± 5.6*	2.2 ± 0.6*
7	GSE group-VII (100 mg/kg)	140.8 ± 4.5*	127.3 ± 5.3*	191.1 ± 4.3	2.1 ± 0.0*

Values are mean ± SEM, n = 6. \*\*p < 0.01, when compared with a control group, \*p < 0.01 when compared with toxic group.

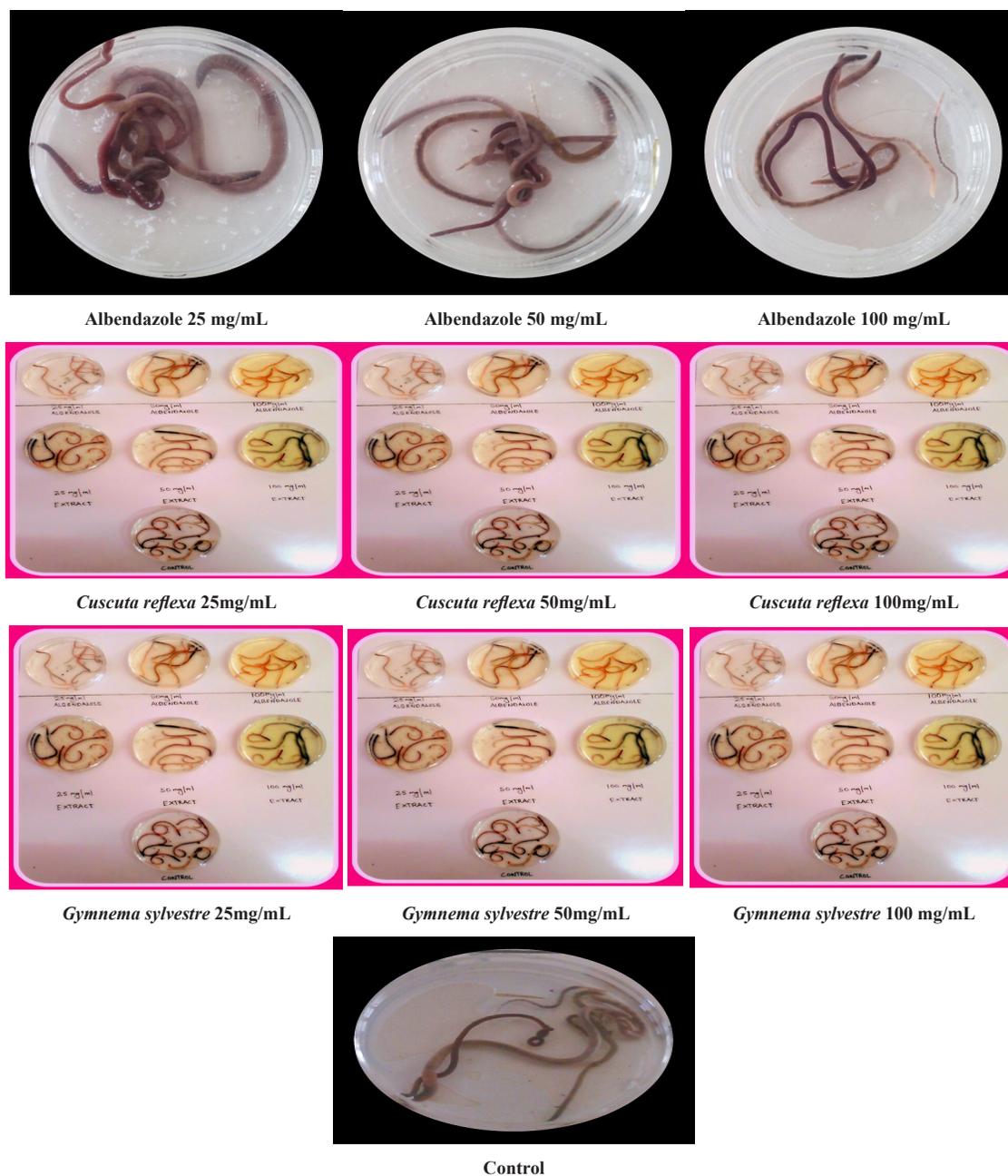


**Figure 1:** Response of *Cuscuta reflexa* and *Gymnema Sylvestre* on histopathological modification of rat liver in carbon tetrachloride (CCl<sub>4</sub>) induced hepatotoxicity (100x magnification).

3.1,  $117.8 \pm 2.1$ ,  $177.2 \pm 5.0$  and  $1.7 \pm 0.0$ , respectively. A high dose (100 mg/kg) of CRE treatment group (group V) showed serum levels of SGOT, SGPT, ALKP, and total bilirubin were  $282.7 \pm 5.0$ ,  $251.5 \pm 4.4$ ,  $274.8 \pm 5.8$ ,  $2.5 \pm 0.0$ , respectively. But high dose (100 mg/kg) of the GSE treatment group (group VII) showed serum levels of SGOT, SGPT, ALKP, and total bilirubin were  $140.8 \pm 4.5$ ,  $127.3 \pm 5.3$ ,  $191.1 \pm 4.3$ ,  $2.1 \pm 0.0$ , respectively. *C. reflexa* and *G. Sylvestre*, both the plants showed

hepatoprotective activity, but *G. Sylvestre* showed very good liver protection at the dose of 100 mg/kg.

The histopathological study of a control group of animals showed normal nucleus, nucleolus, cytoplasm, and central vein (Figure 1 A); in CCl<sub>4</sub> toxic group of animals showed total loss of liver architecture (Figure 1 B). But *C. reflexa*-treated group showed moderately improved liver architecture (Figures 1 D and E). While *G. Sylvestre* treated group showed significant



**Figure 2:** Effect of methanolic extract of *Cuscuta reflexa*, *Gymnema Sylvestre*, and Albendazole on Indian earthworm *Pheritima Posthuma*.

improvement in liver architecture (Figure 1 F & 1 G), which was almost similar to silymarin treated group of animals (Figure 1C).

### Anthelmintic Activity

Albendazole, *C. reflexa*, and *G. Sylvestre* showed death time of earthworm *Pheritima Posthuma* at  $109.83 \pm 2.82$  minutes,  $131 \pm 6.99$  minutes and  $111.83 \pm 4.16$  min, respectively for 25 mg/mL concentration. Albendazole, *C. reflexa*, and *G. Sylvestre* were showed death time of earthworm at  $89.5 \pm 5.23$  minutes,  $119 \pm 5.87$  minutes and  $94.33 \pm 9.75$  minutes, respectively for 50 mg/mL concentration. Also, for 100 mg/mL of Albendazole, *C. reflexa* and *G. Sylvestre* showed death time of earthworm at  $63.83 \pm 1.75$  minutes,  $79.6 \pm 4.84$

minutes, and  $71.2 \pm 3.26$  minutes, respectively. At the same time, the paralysis time of worms occurs at  $36.66 \pm 2.59$  minutes,  $42 \pm 3.65$  minutes, and  $38.86 \pm 4.38$  for 100 mg/kg concentration of Albendazole, *C. reflexa*, and *G. Sylvestre*, respectively. Both the methanolic extracts showed significant results against the earthworms, but *G. Sylvestre* has shown a better effect than *C. reflexa* which was compared with the standard drug Albendazole (Figure 2).

### CONCLUSION

*C. reflexa* and *G. Sylvestre* showed significant hepatoprotective and anthelmintic activity. In this study, authors were observed that various phytoconstituents were present,

different pharmacognostic parameters such as macroscopic, microscopic, and fluorescence parameters, acute toxicity study were regarded safe for further use, and the hepatoprotective and anthelmintic activity of methanolic extracts of *C. reflexa* and *G. Sylvestre* proved to be useful in herbal medicine for the treatment of liver disease and helminthiasis disease.

The hepatoprotective activity may be confirmed to the presence of phenols and flavonoids, and anthelmintic activity may be attributed to the presence of tannins and saponins in methanolic extracts of *C. reflexa* Roxb. and *G. Sylvestre* (Retz.) R. Br. ex. Sm.

#### ACKNOWLEDGEMENT

The authors express their gratitude to the Management, Bharat Technology, Uluberia, Howrah, and West Bengal for providing their continuous support throughout the work. The authors are also grateful to Prof. (Dr.) Lakshmi Kanta Kanthal and Mrs. Anima Jena for their continuous encouragement and valuable input and co-operation while carrying out this study.

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