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Investigation of Hepatoprotective Activity of Desmodium Gangeticum Leaf Extract against Paracetamol Induced-Liver Damage in Rats

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ABSTRACT

Desmodium gangeticum leaves (Family Fabaceae) commonly known as shalparni is a vigorously growing in India. The aim of the present study was carried out with the objective of phytochemical screening and to evaluate the hepatoprotective activity of aqueous extract of *Desmodium gangeticum* leaves. The rats were divided into five groups with six rats in each. Group I (Control) served as normal and received the vehicle alone (Sterile distilled water, 10 ml/kg, p.o.) for 5 days. Group II (Toxin control) animals on the 3rd and 4th day. Group III and IV were treated with DGL at a dose level of 400 mg/kg and 600 mg/kg body weight p.o. per day respectively for 5 days and on the 3rd and 4th day with hepatotoxic drugs was given 1 h after the treatment of the extract. Group V (Standard) was treated with standard drug silymarin (100 mg/kg p.o.) for 5 days and on the 3rd and 4th day hepatotoxic drugs was given 1h after the treatment of the drug. In hepatoprotective studies, the induced paracetamol toxicity elevated levels of serum marker enzymes ALT, AST, ALP and the level of BUN along with the decrease in total protein and albumin levels. It also increased the relative liver weight and decreased the level of liver total protein and GSH. The activity of catalase and GPx significantly decreased in paracetamol intoxicated animals. The pre-treatment of methanol extract of Desmodium gangeticum leaves at dose levels of 400 and 600 mg/kg had restored the ALT, AST, ALP and BUN levels towards normalization and the effects were comparable with standard drug (Silymarin 100 mg/kg). The data obtained from animal experiments are expressed as mean Â± SEM (standard error of mean). For statistical analysis data were subjected to analysis of variance (ANOVA) followed by Studentâ€™s t-test. Values are considered statistically significant at $p < 0.01$ for ANOVA and $P < 0.05$ for t-test.

Keywords: Desmodium gangeticum, hepatoprotective activity, Aspartate amino transferase, Alanine amino transferase.

1 Introduction

Liver diseases remain one of the serious health problems. They are mainly caused by toxic chemicals, excess consumption of alcohol, infections and autoimmune disorders. Most of the hepatotoxic chemicals damage liver cells by inducing lipid peroxidation and oxidative stress. These liver disorders remained unresolved because the therapies developed along the principles of modern system of medicine are often limited in efficacy, carry the risk of adverse effects and are very expensive, especially for the developing world. Therefore, the treatment of liver diseases with herbal medicines seems highly attractive and also is quite an old approach of various traditional systems of medicine[1].*Desmodium gangeticum* DC (Leguminosae, Subfamily-Papilionaceae) is an erect or suberect undershrub, distributed throughout central and eastern Himalayas, South India and Sri Lanka. The leaves are used as a substitute for tea by hill tribes in upper Assam. Preliminary phytochemical investigations on the *D. gangeticum* revealed the presence of flavonoids, glycosides, steroids, saponins, phenolic compounds, amino acids and fixed oils[4]. It was also reported that the whole plant is boiled and used against the treatment of liver parasite by Lao people[5]. Although the leaves were used traditionally for varied ailments, no scientific study has been reported. Hence a systematic study has been undertaken to evaluate the hepatoprotective and antioxidant activities of ethanol extract of leaves of *D. gangeticum* against Paracetamol induced hepatotoxicity in rats.

2. Methodology

1. Collection and Identification of Plant Material

The leaves of *Desmodium gangeticum* were collected.

2.Preparation of The Extract

The leaves were washed under tap water, shade dried, homogenized to fine powder and stored in airtight bottles. Ten grams of dried powder was first defatted with petroleum ether (to remove fat) and then extracted with methanol by using Soxhlet apparatus (Lin et al., 1999). The solvent was evaporated to dryness and the dried crude extract was stored in air tight bottle at 4°C. The percentage yield of methanol extract was 36%. The methanol extract of *Desmodium gangeticum Leaves* was used for the entire study.

Percentage yield = Actual yield/theoretical yield x 100

Actual yield = 3.6, Theoretical yield =10

=3.6/10x100 =36%

3. Phytochemical Analysis

Preliminary chemical tests were carried out for methanolic extract to identify different phyto-constituents.

4. Animals

Wistar albino rats of both sexes (180-220 g) were used for the study. All the rats were kept in standard plastic rat cages with stainless steel coverlids and paddy straw was used as bedding material. The animals were kept at the animal house of department of pharmacology. The animals were facilitated with standard environmental condition of photoperiod (12:12 h dark: light cycle) and temperature (25 ± 2°C). They were provided with commercial rat and mice feed (Pranav Agro Industries Ltd., Baroda. Amruth Brand rat & mice pellet feed) and water given ad libitum.

5.Selection of The Doses for Animal Study

The dose considered for the experiment on rats was obtained from conversion of human dose of *Desmodium gangeticum* (3-5 g/kg). The conversion factor of human dose (per 200 g body weight) is 0.018 for rats (Ghosh 1984). Hence the calculated dose for the rats (considering human dose 5 g/kg) is 450 mg/kg. Thus, hepatoprotective activity was done at two different doses 400 and 600 mg/kg body weight.

6. Hepatoprotective Studies

6.1. Paracetamol induced hepatotoxicity

The rats were divided into five groups with six rats in each. Experimental Design:

Group I	Group I (Control) served as normal and received the vehicle alone (Sterile distilled water, 10 ml/kg, p.o.) for 5 days.
Group II	Group II (Toxin control) animals received Paracetamol (3 g/kg, p.o.) on the 4th day.
Group III	Group III (DGL-400) were treated with DGL at a dose level of 400 mg/kg body weight p.o. per day respectively for 5 days and on the 4th day Paracetamol (3 g/kg, p.o.) was given 1 h after the treatment of the extract.
Group IV	Group IV (DGL-600) were treated with DGL at a dose level of 600 mg/kg body weight p.o. per day respectively for 5 days and on the 4th day Paracetamol (3 g/kg, p.o.) was given 1 h after the treatment of the extract.
Group V	Group V (Standard) was treated with standard drug silymarin (100 mg/kg p.o.) for 5 days and on the 4th day Paracetamol (3 g/kg, p.o.) was given 1 h after the treatment of the drug.

The animals were sacrificed 48 h after the dose of Paracetamol under mild ether anesthesia. The blood was collected and allowed to stand for 30 min at 37°C and then centrifuged to separate the serum to estimate various biochemical parameters. In above three hepatoprotective models, various in vivo antioxidant parameters were estimated from liver.
P.O (medication is taken by mouth "bid or twice a day")

6.2 Preparation of liver homogenate

The liver was quickly removed and perfused immediately with ice-cold saline (0.9% NaCl). A portion of the liver was homogenized in chilled Tris-HCl buffer (0.025 M, pH 7.4) using a homogenizer. The homogenate obtained was centrifuged at 5,000 rpm for 10 min, supernatant was collected and used for analysis.

6.3 Biochemical analysis from serum

The absorbance of all the biochemical parameters was measured in a UV–VIS Spectrophotometer - 1601.

6.3.1 Estimation of total protein content

The serum total protein was estimated by modified Biuret method using the total protein test kit.
Procedure
3.0 ml of Reagent I + Added to all the test tubes.
Thereafter, 0.03 ml serum was added for the test + 0.03 ml Reagent II was added for the standard, while in blank 0.03 ml of purified water was added. They were then mixed well and incubated at 37ºC for 5 minutes. The absorbance was read at 578 nm.

6.3.2 Estimation of albumin content

The serum albumin was estimated by the method given by Corcoran and Durnan (1977) using ready made albumin test kit.
Procedure
3.0 ml of albumin reagent (Reagent I) was added to all the test tubes. Thereafter, 0.03 ml serum was added for the test and 0.03 ml Reagent II was added for the standard, while in blank 0.03 ml of purified water was added. They were then mixed well and incubated at room temperature for 1 min. The absorbance was read at 630 nm.

6.3.3 Estimation of blood urea nitrogen (BUN) content

The serum blood urea nitrogen was estimated by Enzymatic Urease (Berthelot) method (Fawcett and Scott, 1960) using Urea Berthelot test kit .
Procedure
1.5 ml Solution I was added to clean test tubes. 0.01 ml serum was added for the test and
0.01 ml Reagent III was added for the standard. It was then mixed well and incubated at 37ºC for 3 min; then 1.5 ml of Solution II was added. It was then mixed well and incubated at 37ºC for 5 min. The absorbance was read at 578 nm against reagent blank.

6.3.4 Estimation of alkaline phosphatase (ALP) activity

Alkaline phosphatase activity was estimated by the method of Kind and King (1954) using ALP test kit.
Procedure
All the test tubes were marked properly as blank (B), standard (S), control (C), and test (T). 0.5 ml of working buffered substrate was added in clean tubes. 1.5 ml of purified water was added in all the tubes. They were mixed well and incubated at 37°C for 3 min.
0.05 ml of serum was added in test (T), 0.05 ml of reagent III (Phenol standard) was added in standard (S) and 0.05 ml of purified water was added in blank (B) tubes. All the tubes were mixed well and incubated at 37°C for 15 min. 1 ml of reagent II was added in all the tubes. 0.05 ml of serum was added in control (C). All the tubes were mixed well and absorbance was read at 510 nm. Serum alkaline
phosphatase activity is expressed as KA units.

6.3.5 Estimation of aspartate aminotransferase (AST) activity

The serum aspartate aminotransferase was estimated by the method of Reitman and Frankel (1957) using AST test kit .
Procedure
0.25 ml of Reagent I was added in clean test tubes and incubated at 37ºC for 5 minutes.
0.05 ml of serum was added in the test, 0.05 ml Reagent IV was added in standard and
0.05 ml distilled water was added in the blank. They were mixed well and incubated at 37ºC for 60 minutes. Thereafter, 0.25 ml of Reagent II was added to all the tubes, mixed well and allowed to stand at room temperature for 20 min. Then 2.5 ml of Solution I was added to all the tubes, mixed well and allowed to stand at room temperature for 10 min. The absorbance of blank, standard and test were read at505 nm.

6.3.6 Estimation of alanine aminotransferase (ALT) activity

The serum alanine aminotransferase was estimated by the method of Reitman and Frankel (1957) using ALT test kit (Span Diagnostics Ltd.).

Procedure

0.25 ml of Reagent I was added in clean test tubes and incubated at 37ºC for 5 minutes.

0.05 ml of serum was added in the test, 0.05 ml Reagent IV was added in the standard and 0.05 ml distilled water was added in the blank. They were mixed well and incubated at 37ºC for 30 minutes.

Thereafter, 0.25 ml of Reagent II was added to all the tubes, mixed well and allowed to stand at room temperature for 20 min. Then 2.5 ml of Solution

was added to all the tubes, mixed well and allowed it to stand at room temperature for 10 min. The absorbance of blank, standard and test were read at 505 nm.

7 Acute Toxicity Study

Acute oral toxicity (Ryu et al., 2004), study was performed as per OECD-423 guidelines (1987). 10 rats/group (5 males and 5 females) were used for the study. Group 1 was control group , Group III and Group IV were that of WFM at different doses (400, 600 mg/kg body weight). Single dose of the extract was administrated orally to each animal. Signs of toxicity, body weight, feed and water consumption of each animal was observed every day for 14 days.

8. Statistical Analysis

The data obtained from animal experiments are expressed as mean ± SEM (standard error of mean). For statistical analysis data were subjected to analysis of variance (ANOVA) followed by Student's t-test. Values are considered statistically significant at $p < 0.01$ for ANOVA and $P < 0.05$ for t-test.

RESULTS

Preliminary Phytochemical Analysis

The results of qualitative phytochemical analysis of the crude powder and the methanol extract of *Desmodium gangeticum* leaves is shown in Table.

Table 1: Preliminary qualitative phytochemical analysis of *Desmodium gangeticum leaves*

Phytochemical	Test	Methanolic extract
Alkaloids	Dragandroffs test Mayers test Wagners test	+ + +
Flavonoids	Shinoda test Alkaline reagent test	+ +
Cardiac glycosides	Keller-kilianni test	-
Phlobotannins	HCl test	+
Saponins	Frothing test	+
Steroids	Libbermann-Burchard test	-
Tannins	$FeCl_3$ test	+
Triterpenes	H_2SO_4 test	+

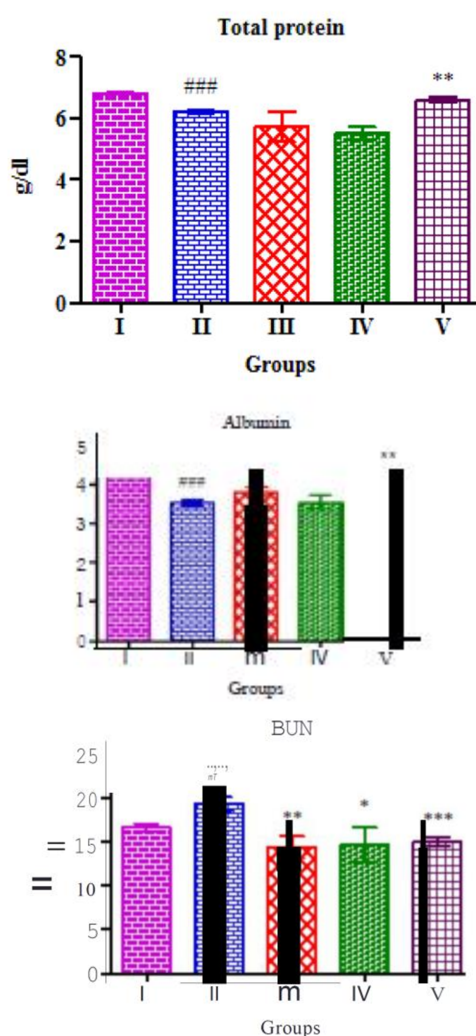
(-): absent, (+): present.

In methanol extract maximum amount of tannins, alkaloids, Flavonoids, phlobotannins, saponins and triterpenes were present. Cardiac glycosides and steroids were absent.

HEPATOPROTECTIVE STUDIES

Paracetamol induced hepatotoxicity

Oral administration of Paracetamol (PCT) caused significant liver damage as evidenced by altered biochemical parameters (Figure 6). PCT significantly ($P < 0.001$) decreased serum levels of total protein and albumin as compared to normal control group. APAP significantly ($P < 0.01$) enhanced BUN, ALP, AST and ALT levels in the blood circulation; about 3-fold increase was observed in AST and ALT levels in serum.Treatment with DGL did not exhibit potential effect on recovery of total protein and albumin levels; while in standard drug treated group, the level of total protein and albumin levels increased significantly ($P < 0.01$). The BUN and ALP levels also decreased significantly in lower as well as higher dose of DGL ($P < 0.01$, $P < 0.05$ respectively) as compared to toxin control group. 400 and 600 mg/kg of DGL treated group showed significant ($P < 0.001$, $P < 0.01$ respectively) decrease in AST level as compared to toxin control group. The result of AST was similar to that of the standard drug treated group ($P < 0.001$). ALT level decreased in DGL treated groups towards normalization though not significantly.Significant decreased level was observed in hepatic total protein ($P < 0.001$). The administration of PCT significantly decreased the hepatic non-enzymatic antioxidant GSH contents ($P < 0.05$). The treatment of DGL decreased liver weight significantly ($P < 0.05$) at both the dose levels as compared to toxin control group.



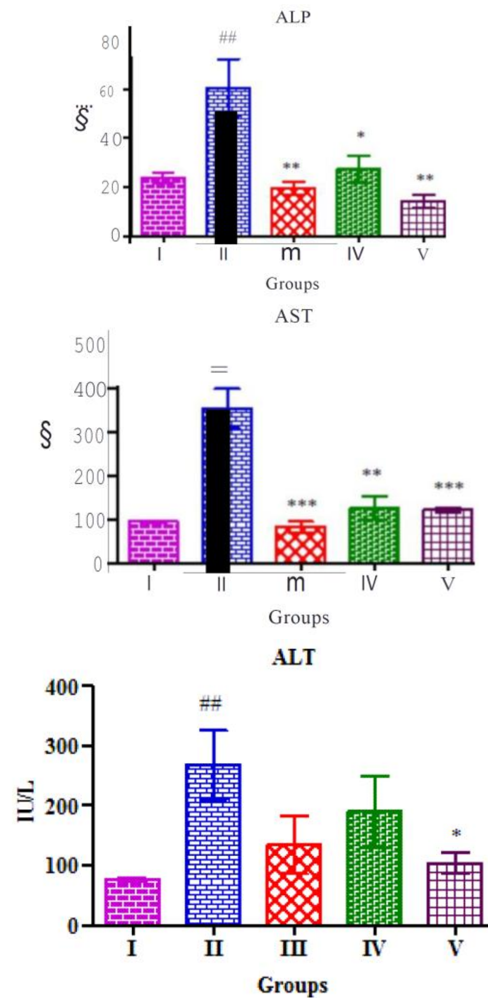
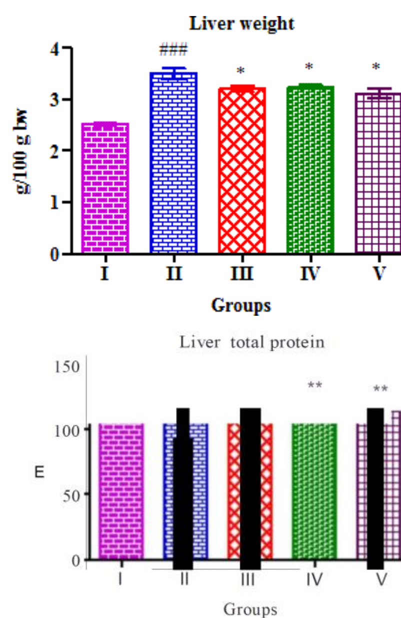


Figure 1: Effect of methanol extract of Desmodium gangeticum Leaves on different serum biochemical parameters in PCT (3 g/kg) induced hepatic damage in rats. Group I: Normal control, Group II: Toxin control PCT, Group III: DGL-400 mg/kg + PCT, Group IV: DGL-600 mg/kg + PCT, Group V: Silymarin-100 mg/kg + PCT. Results are expressed as mean ± SEM, (n = 6). #p < 0.05, ##p < 0.01, ###p < 0.001 as compared with normal control group; *p < 0.05, **p < 0.01, ***p < 0.01 as compared with toxin control group.



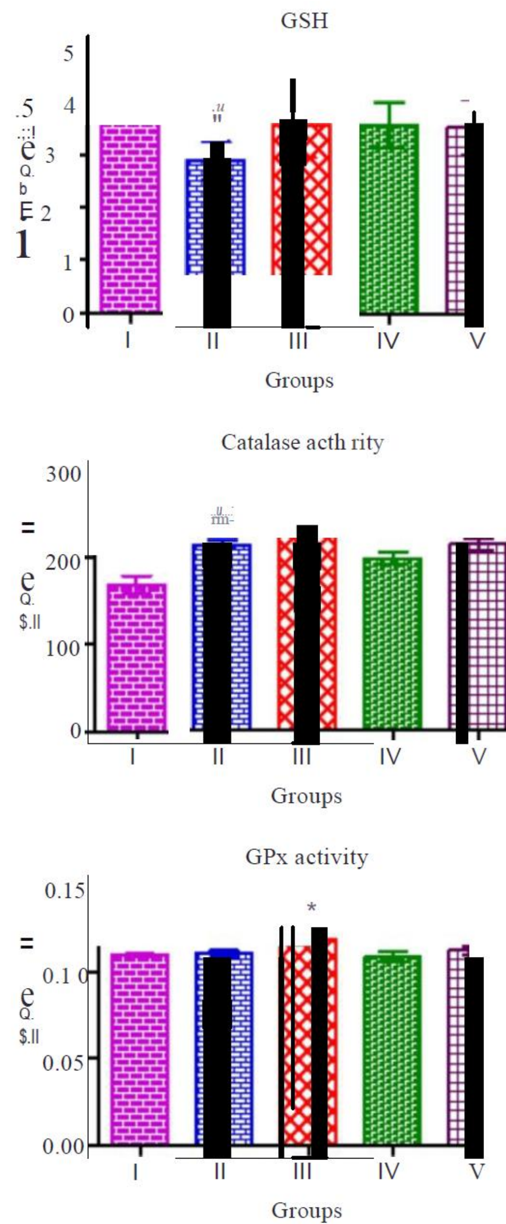


Figure 2: Effect of methanol extract of *Desmodium gangeticum* Leaves on relative liver weight, liver total protein and different liver antioxidants in APAP (3 g/kg) induced hepatic damage in rats. Group I: Normal control, Group II: Toxin control APAP, Group III: DGL- 400 mg/kg + APAP, Group IV: DGL-600 mg/kg + APAP, Group V: Silymarin-100 mg/kg + APAP. Results are expressed as mean ± SEM, (n = 6). #p < 0.05, ##p < 0.01, ###p < 0.001 as compared with normal control group; *p < 0.05, **p < 0.01,
***p < 0.01 as compared with control group.

In higher dose, the level of hepatic total protein increased significantly (P < 0.01). The hepatoprotective efficacy of the DGL-600 was comparable with that of standard drug silymarin. DGL treatment enhanced the production of GSH towards normal control, but not to a significant level. Administration of APAP did not diminish the antioxidative status of hepatic catalase and GPx activity.

Table 2: Groups Classification

S.NO	GROUPS	TREATMENT	DURATION	GSH-Px(µmol/mg protein)
1	Group- I	Normal Control	5 days	20 ± 0.6984
2	Group- II	Toxin control + PCT	2 days	64 ± 2.3652
3	Group- III	DGL of 400 mg/kg + PCT	5 days	32 ± 3.5238
4	Group- IV	DGL of 600 mg/kg + PCT	5 days	35 ± 5.6342
5	Group- V	Silmarin of 100mg/kg + PCT	5 days	42 ± 05648

Values are expressed as mean ± SEM, (n = 6). #p < 0.05, ##p < 0.01, ###p < 0.001 as compared with normal control group; *p < 0.05, **p < 0.01, ***p < 0.01 ascompared with control group.

4.7. ACUTE TOXICITY STUDY

In acute toxicity study, no adverse reactions or mortality were observed after administration of DGL (450, 1800, and 3600 mg/kg bw) and no behavioral changes were observed during the entire period of experimentation. Some alteration was noticed in daily feed and water intake in both male and female rats treated with single dose of extract as well as in control animals. As compared to the control group, drug treated groups had several consecutive days of reduced/increased feed and water consumption at different times in the study. These periods of reduced/increased feed and water intake were not significant to the overall feed and water consumption rates. Individual body weights were recorded daily during the experimental period. Mean body weight gains were calculated for each group. In control and DGL treated groups, body weight of animals slightly increased during experimental period, but the increase was not significant.

CONCLUSION

In physicochemical analysis, crude powder and methanol extract of Desmodium gangeticum leaves were free from heavy metals. In qualitative phytochemical analysis tannins and alkaloids were present in higher amount, while cardiac glycosides and steroids were totally absent. In quantitative analysis of phytoconstituents, total phenol content was higher than flavonoid content. Hence, the determination of pharmacognostical and phyto-physicochemical profile of Desmodium gangeticum leaves may be useful to supplement information in respect to its identification, authentication and standardization of herbal drugs. In other words, the pharmacognostic features examined in the present study may serve as tool for identification of the plant for validation of the raw material and for standardization of its formulations at herbal industrial level in the coming days.

In hepatoprotective studies, the induced diclofenac toxicity elevated levels of serum marker enzymes ALT, AST, ALP and the level of BUN along with the decrease in total protein and albumin levels. It also increased the relative liver weight and decreased the le vel of liver total protein and GSH. The activity of catalase and GPx significantly decreased in diclofenac intoxicated animals. The pre-treatment of methanol extract of *Desmodium gangeticum leaves* at dose levels of 400 and 600 mg/kg had restored the ALT, AST, ALP and BUN levels towards normalization and the effects were comparable with standard drug (Silymarin 100 mg/kg). The total protein, albumin, GSH levels and catalase, GPx activity increased significantly in the animals received pre-treatment of the DGL.

In Paracetamol induced hepatotoxicity models, the serum biochemical parameters and liver antioxidants were altered when animals were intoxicated with Paracetamol. The treatment with DGL restored the level of serum biochemical parameters as well as liverantio xidants in both the animal models. The administration of acetaminophen and DGL did not have any effect in serum total protein level, catalase and GPx activity.

In acute toxicity study, the methanol extract of Desmodium gangeticum leaves. had no mortality and observable acute toxic effect on the experimental animals and therefore can be considered as non-toxic. However, acute toxicity data sometimes is of limited clinical application since accumulative toxic effect may not be seen in short period with a single dose application. Hence, sub acute and chronic evaluation of the extract should be carried out in evaluating the safety profile of Desmodium gangeticum leaves.

These studies have shown that the methanol extract of Desmodium gangeticum leaves contain some active ingredients with the potential of being good hepatoprotective agents. For that, further study for detailed investigation of the mechanism of action of DGL is needed.

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