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PHYTOCHEMICAL EVALUATION AND *IN VIVO* HEPATOPROTECTIVE ACTIVITY OF *STERCULIA LYCHNOPHORA* (FRUIT)

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ABSTRACT

The ninth most prevalent cause of mortality in both developed and developing nations, cirrhosis is the most drug-stimulated liver injury, placing liver disease among the world's major health concerns. Since ancient times, medicinal herbs have been utilized to cure liver disorders as it is devoid of any side effects. This study deals with phytochemical evaluation and *in vivo* hepatoprotective activity of *Sterculia lychnophora* (fruit). The plant material was collected & subjected to extraction by hydroalcoholic solvent. Further, procedures for checking hepatoprotective activity in animals were performed by standard procedures. Results showed that percentage yield was found to be 8.65%. The phytochemical screening presence of alkaloids, glycosides, carbohydrates, tannins, resins, flavonoids, steroids, proteins and amino acids. Total phenol & flavonoid content was found to be 0.758 mg/100mg & 0.822 mg/100mg. The level of AST in rats treated with standard drug was found to be 36.95±0.63 while in rats treated with Hydroalcoholic extract of *Sterculia lychnophora* it was estimated to be 65±0.35. Further the level of ALP & ALT in Hydroalcoholic extract of *Sterculia lychnophora* treated rat was noted as 60.50±1.07 & 134.68±1.20. The level of triglyceride in hydroalcoholic extract of *Sterculia lychnophora* treated rats was seen to be 124.85±0.75. The total cholesterol & bilirubin level was observed to be 158.87±0.50 & 132.36 ± 0.35 respectively in rats treated with plant extract. The findings of this investigation confirm the presence of physiologically active chemicals with hepatoprotective potential in *Sterculia lychnophora* extracts.

Keywords: Liver disease, Medicinal plants, Phytochemicals, *Sterculia lychnophora*, Hepatoprotective

INTRODUCTION

The liver is a vital organ. Its role in the metabolism and excretion of xenobiotics from the body is crucial. Additionally, it manages the metabolism and excretion of drugs and other xenobiotics from the body by using detoxifying agents to protect against foreign chemicals and warding them off. The liver's major functions include detoxification, bile secretion, glucose, protein, and lipid metabolism, as well as vitamin storage (Desai *et al*., 2012; Bogdanos *et al*., 2013).

According to the ninth most prevalent cause of mortality in both developed and developing nations, cirrhosis is the most drug-stimulated liver injury, placing liver disease among the world's major health concerns. However, it is

brought on by viral diseases or by consuming toxic foods, chemicals, or drug overdoses.

These substances are known as hepatotoxins. Microcystins and other synthetic chemicals, such as antibiotics, tetrachloride, chemotherapeutic agents, dimethyl nitrosamine, aflatoxin, carbon tetrachloride (CCl4), pyrrolizidine alkaloids, allyl alcohol, Thioacetamide (C2 H5 NS), and biomobenzen, may also contribute to it. Chronic medications may also potentially have side effects (Knolle *et al*., 2016; Asrani *et al*., 2019).

The liver's vulnerability to chemical attacks from numerous hazardous compounds, contaminants from the environment, xenobiotics, and chemotherapeutic drugs may be suppressed. Maintaining a healthy liver is a problem for overall health and well-being, as is the treatment of such diseases with artificial medicines, key substances that have been isolated, or important components of native medicinal plants that are used in conventional medicine. Despite this, only a small number of medications having potential negative effects on humans are utilized to treat liver problems (Jutiviboonsuk, 2012).

Since ancient times, medicinal herbs have been utilized to cure liver disorders. With a variety of chemical architectures, several leads derived from plant sources have been identified as possible hepatoprotective drugs. Only a small number of the hepatoprotective phytomolecules reported in the scientific literature were effective against different kinds of liver injury. The scientific community has been most interested in the compounds silymarin, andrographolide, neoandrographolide, curcumin, picroside, kutkoside, phyllanthin, hypophyllanthin, and glycyrrhizin. Numerous herbal medications have had their capacity to protect the liver clinically assessed. Hepatitis, alcoholic liver disease, and liver cirrhosis have all been treated effectively with silymarin, glycyrrhizin, and Liv-52. Considering these facts, this study deals with phytochemical evaluation and in vivo hepatoprotective activity of *Sterculia lychnophora* (fruit) (Govind, 2021; Hong *et al*., 2015). In several Asian nations, *Sterculia lychnophora*, a type of traditional Chinese medicine (TCM), has been utilised as a food and a traditional medicinal. Tussis, a sore throat, toothaches, constipation, laryngitis, cough, menorrhagia, and discomfort have all been treated with decoctions in the past. *Sterculia lychnophora* demonstrates a variety of pharmacological effects, including analgesic, anti-pyretic, antibacterial, anti-hypertensive, anti-inflammatory, weight-loss, laxative, and calcium oxalate inhibitory impact, according to research conducted in vitro and in vivo.

MATERIALS AND METHODS

Collection of plant materials

Organoleptic characters, morphological characters, and microscopical examination would help in identifying crude drug. For identification of unknown drugs herbariums and leading botanical gardens are of great help. The fruits of selected plant namely *Sterculia lychnophora* were identified and collected from local area of Bhopal on the basis of geographical availability. All collected plant drug were cleaned, shade dried, pulverized into moderately coarse powder and stored in airtight container for further use.

Defatting

Plant materials was defatted with petroleum ether (40-60°C) for about 12 hrs separately & complete defatting was censured by placing a drop from the thimble on a filter paper which did not exhibited any oily spot.

Extraction

The defatted plant materials were subjected to extraction by hydro alcohol (methanol: water) in the ratio of (70: 30) as solvents. The process was carried out for 48 hrs. The liquid extract was collected in a tarred conical flask. The solvent removed by distillation. Last traces of solvent being removed under vacuum. The extracts obtained with each solvent were weighed to a constant weight and percentage w/w basis was calculated.

Preliminary phytochemical screening

Preliminary phytochemical screening means to investigate the plant material in terms of its active constituents. In order to detect the various constituents present in the different extracts of fruits of *Sterculia lychnophora*, were subjected to the phytochemical tests as per standard methods (Pandey and Tripathi, 2014).

Estimation of total phenolic content

The total phenolic content was estimated according to the method. The aliquots of the extract (10-50 µg/ml) was taken in a test tube and made up to the volume of 2 ml with distilled water. Then 1ml of folin-ciocalteu reagent (1:10 with water) and 1ml of sodium carbonate solution (7.5g/l) were added. After mixing, solution was incubated at room temperature for one minute and the absorbance was recorded at 765 nm against the reagent blank. Using Gallic acid, a standard curve was prepared. Using the standard curve, the total phenolic content was calculated and expressed as Gallic acid equivalent in mg/100mg of extract (Parkhe and Bharti, 2019).

Estimation of total flavonoids content

Total flavonoid contents of extract were determined and expressed as Quercetin equivalent in mg/100mg of extract. An aliquot (3ml) of extracts or standard solution of Quercetin (5, 10, 15, 20 and 25µg/ml) was added with 1 ml of 2% $AlCl_3$ solution. The mixture was incubated for 5 min at room temperature. The solution was mixed well and the absorbance was measured at 4200 nm. Using the standard curve, the total flavonoid content was calculated (Parkhe and Bharti, 2019).

In vivo hepatoprotective activity

Animals:

Randomly bred 30 male wistar rats weighing 200±20g grams were obtained. All animals were examined good health at the time of receipt Age of the animals at the start of treatment was approximately 2 months. Animals were maintained in an environment-controlled room (Centrally Air-Conditioned with 100% fresh air replacement). All the animals were housed in polypropylene cages having bedding of steam autoclaved paddy husk and each cage was fitted with a stainless steel top grill having provision for keeping pellet feed and a glass water bottle with stainless steel drinking nozzle. Animals were kept in experimental animal room under 12:12 hours Light and Dark cycle at 23-28^{o}C and

relative humidity of 45-65%, with standard as food and drinking water *ad libitum*.

The present study had the approval by Institutional Ethical Committee. The care and maintenance of all the animals were as per the approved guidelines of CPCSEA.

Acute oral toxicity study

Acute oral toxicity was conducted according to the method of Organisation for Economic Co-operation and Development (OECD, 2002). Hydroalcoholic extract of *Sterculia lychnophora* (5, 50, 300, and 2000 mg/kg) was administered orally for 4 days of six groups of rats (n=6) and the animals were kept under observation for mortality as well as any behavioral changes for evaluation of a possible anti-pyretic activity.

Grouping of animals

Group I: 0.9% saline solution was administered by per oral route.

Group II: 6 g/kg ethanol was administered by per oral route with the help of oral feeding canula for 21 days than self recovery for 7 days.

Group III: 6 g/kg ethanol was administered by oral route with the help of oral feeding canula for 21 days than silymerin (100 mg/kg, p.o.) was administered for 7 consecutive days.

Group IV and V: 6 g/Kg ethanol was administered by oral route with the help of oral feeding canula for 21 days than hydroalcoholic extract of *Sterculia lychnophora* (100 and 200 mg/kg, p.o.) respectively, and were administered respectively for 7 days consecutively.

Biochemical Parameters:

Aspartate Aminotransferase (AST)

0.5 ml asparate substrate and added 0.1 ml of serum sample. Then incubated the tube at 37°C for 30 min after removed added 0.5 ml DNPH solution then kept for 20 min at room temperature then added 5 ml of 0.4N NaOH solution and obtained the optical density for compared the colours by using photoelectric calorimeter with green filter at 520 mμ.

Alanine Aminotransferase (ALT)

0.5 ml alanine substrate and added 0.1 ml of serum sample. Then incubated the tube at 37°C for 30 min. after removed added 0.5 ml DNPH solution then kept for 20 min at room temperature then added 5 ml of 0.4N NaOH solution and obtained the optical density for compared the colours by using photoelectric calorimeter with green filter at 520 mμ.

Alkaline Phosphate (ALP)

1 ml of substrate and added 1ml of alkaline buffer then mixed 0.1 ml of serum and stand for 15 min on water bath at temperature 37°C, after that added 1ml sodium bicarbonate solution then added 1ml amino pyrine solution and 1 ml of potassium fericynide solution, mixed well and obtained optical density reddish brown colour developed and compared by photoelectric calorimeter at 520 mμ.

Total Cholesterol

0.2 ml serum in test tube then added 5 ml freshly prepared colour reagent mixed well and stand for 10 min in dark place and obtained optical density by using photoelectric calorimeter at 660 mμ.

Triglycerides (TGL)

Test sample 0.2 ml, in which added 4 ml of 10% trichloroacetic acid. Then centrifuge for 5 to 10 min for complete precipitation of triglycerides. After centrifuge poured the supernatant out and dissolved each precipitated in 8 ml of 1N NaOH. Then taken 2 ml and diluted upto 10 ml then used 0.25 and 0.50 ml solution for measurement of trigiycerides

Table 1: Extractive values obtained from *Sterculia lychnophora* fruits

S. No.	Solvent	Time of extraction	Color of extract	% Yield
1.	Methanol + water (70:30)	48 Hours	Brown colour	8.65%

Table 2: Preliminary phytochemical screening of *Sterculia lychnophora* fruits

S. No.	Phytoconstituents	Test Name	Hydroacoholic Extract
1.	Alkaloids	Hanger's Test	+
2.	Glycosides	Leagel's test	-
3.	Saponins	Froth test	+
4.	Terpenoids	Salwaski's test	-
5.	Phenols	Ferric chloride test	+
6.	Carbohydrates	Gelatin Test	+
7.	Flavonoids	Lead acetate	+
8.	Proteins & Amino acids	Precipitation test	+

(+) Positive, (-) Negative

Table No. 3: Estimation of total Phenol and flavonoids content

S. No.	Extract	Total phenol content (mg/ 100 mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
1	Hydroalcoholic	0.758	0.822

Table 4: Effect of hydroalcoholic extract of *Sterculia lychnophora* on the AST ALT ALP in ethanol-induced hepatic damage in rats

Groups	AST	ALT	ALP
Normal	35.3± 0.45	55.35± 0.78	110.1± 1.15
Control	67.85±0.35	84.68±1.85	170.0±0.84
Standard	36.95±0.63**	57.9±1.02**	120.0±1.18**
Hydroalcoholic extract of *Sterculia lychnophora* -100	39.50±0.40*	63.15±1.15*	145.2±1.10*
Hydroalcoholic extract of *Sterculia lychnophora* -200	37.65±0.35**	60.50±1.07**	134.68±1.20**

Values are expressed as mean±S.E.M. (n = 6); Values are statistically significant $^{*}P < 0.05$ vs. control group respectively (One-way ANOVA followed by Dunnett's test) by graph pad prism.

Table 5: Effect of hydroalcoholic extract of *Sterculia lychnophora* on the TG, TC and bilirubin in ethanol induced hepatic damage in rats

Groups	Triglyceride	Total cholesterol	Serum Bilirubin
Normal	100.05±1.29	120.10±1.25	104.0±0.40
Control	220.80±1.65	240.60±0.95	190.0±0.30
Standard	109.08±1.25***	145.85±0.75***	120.0 ± 0.35***
Hydroalcoholic extract of *Sterculia lychnophora* -100	128.82±0.35*	180.10±0.65**	143.0 ± 0.58***
Hydroalcoholic extract of *Sterculia lychnophora* -200	124.85±0.75**	158.87±0.50**	132.36 ± 0.35***

Values are expressed as mean±S.E.M. (n = 6); Values are statistically significant $^{*}P < 0.05$ vs. control group respectively (One-way ANOVA followed by Dunnett's test).

RESULTS AND DISCUSSION

The percentage yield was found to be 8.65%. The phytochemical screening presence of alkaloids, glycosides, carbohydrates, tannins, resins, flavonoids, steroids, proteins and amino acids. Also, earlier studies confirm that Flavonoids, triterpenoids, saponins, alkaloids, and other phytoconstituents have been shown to have hepatoprotective effect. Total phenol & flavonoid content was found to be 0.758 mg/100mg & 0.822 mg/100mg.

It is known that in case of liver injury Cytosolic and endoplasmic enzymes are released from liver cell as a indicating the presence of damage to liver structure and function. These show up as an increase in AST, ALT, and ALP levels. The hepatoprotective effect of the plant extract and solvent fractions can therefore be determined by monitoring the levels of these biomarkers of liver injury.

The level of AST in rats treated with standard drug was found to be 36.95±0.63 while in rats treated with Hydroalcoholic extract of *Sterculia lychnophora* it was estimated to be 65±0.35. Further the level of ALP & ALT in Hydroalcoholic extract of *Sterculia lychnophora* treated rat was noted as 60.50±1.07 & 134.68±1.20.

Also, the liver serves as the primary location for both bilirubin detoxification and protein synthesis, particularly albumin synthesis. The ability of the liver to synthesise and detoxify substances was evaluated in this study using the levels of total protein, albumin, and bilirubin. The level of triglyceride in hydroalcoholic extract of *Sterculia lychnophora* treated rats was seen to be 124.85±0.75. The total cholesterol & bilirubin level was observed to be 158.87±0.50 & 132.36 ± 0.35 respectively in rats treated with plant extract.

The findings of this investigation confirm the presence of physiologically active chemicals with hepatoprotective potential in *Sterculia lychnophora* extracts. The compounds in them may be powerful antioxidants, preventing oxidative stress from damaging cell membranes and aiding in the regeneration of the hepatocellular membrane. They also contain substances that aid in the breakdown of lipids, preventing fat from forming in ethanol-induced liver damage (fatty liver). As a result, the plant may be helpful as a food supplement in protecting against free radicals and chemical-induced liver impairments, according to the findings of the current study, which found evidence for *Sterculia lychnophora* hepatoprotective potential.

CONCLUSION

In conclusion, this study added to the body of evidence showing that the hydroalcohlic extract had hepatoprotective effects similar to those of the usual medication. The pre- and post-treatment of the hydroalcoholic fraction with the solvent fractions showed a dose-dependent decrease in all liver damage indicators. The hepatoprotective activity of the plant is therefore thought to be distributed to semi-polar bioactive components found in the n-butanol fraction, according to this study. Although the plant extract's hepatoprotective mechanism is still not fully understood, one of the anticipated mechanisms is the found antioxidant activity.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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