

# The Phytochemical Investigation and Pharmacological Evaluation of Hepatoprotective Activity of *Bougainvillea glabra* in Rats

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## Abstract:

An indispensable organ is the liver which is crucial to the body's removal of foreign substances and it plays an unexpected part in preserving, controlling, and restoring the body's homeostasis. It is involved in practically every biochemical route that leads to growth, the defense against illness, the supply of nutrients, the creation of energy, and reproduction. The metabolism of carbohydrates, proteins, and fats, as well as detoxification, bile secretion, and vitamin storage, are the liver's primary roles. Numerous hazardous substances, including ethanol, silymarin, chemotherapeutic drugs, thioacetamide, carbon tetrachloride, paracetamol, and microorganisms can cause hepatotoxicity. The present investigation goal is to assess the ethanolic hepatoprotective workings of *bougainvillea glabra* Leaf extract on Wister rats' liver damage brought on by paracetamol. The great *Bougainvillea glabra* plant is used in cooking and as an ornament that is additionally employed in conventional remedies to treat familiar illnesses. Numerous investigations have demonstrated the diverse pharmacological actions of certain extracts or components that were derived from the genus *Bougainvillea*. Certain kinds of *bougainvillea* have evolved into the origin of standard medical care for mortal wellness. The plant was collected and dried in the shade at room

temperature. The plant *Bougainvillea* was powdered and successively extracted using a Soxhlet apparatus with suitable solvents acetone, chloroform, aqueous, ethanol, and methanol. When different extracts were examined phytochemically, fixed oils, saponins, amino acids, glycosides, and alkaloids were discovered. The level of defense is determined by calculating biochemical parameters such as serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, total protein, total albumin, alkaline phosphatase, and the level of total serum direct and indirect bilirubin levels. Observing any changes occurring in rats before and after the experiment like the weight of the animals and liver weight of the rats. Additionally, a histologic evaluation was performed to rule out paracetamol-induced hepatotoxicity.

**Keywords:** Hepatoprotective activity, *Bougainvillea glabra*, Silymarin, and paracetamol-induced.

## Introduction

Herbal medicine was a leading health-care method in the early 20th century because antibiotics and analgesics were not readily available. Herbal medicine has become more popular recently, as seen by the fact that some of

its products reached peak performance levels comparable to those of synthetic medications (1). One important source of hepatoprotective medications is herbal medicine. Preparations made of single or several herbs have been used to treat liver diseases. Over 700 herbal formulations, both mono and poly, in the form of decoctions, tinctures, pills, and capsules derived from over 100 plants are reportedly in therapeutic use. Remarkably, several research addressing the hepatotoxic potential of herbal medications have been published in publications (2). Abuse of medicines and alcohol, along with certain medical disorders, are also linked to hepatotoxicity. For example, taking aspirin when younger increases the risk of liver damage (3,4). The susceptibilities of men and women to drug-induced hepatotoxicity differ. Drugs such as isoniazid, nitrofurantoin, and chlorpromazine tend to damage women more than men; azathioprine-induced hepatotoxicity primarily affects men (5). Numerous studies have revealed that another component, obesity, is also accountable for acute and chronic alcohol consumption-related hepatotoxicity (6). An over-the-counter (OTC) medication that is non-steroidal anti-inflammatory is paracetamol. The active metabolite damage that paracetamol causes to the liver should be taken with caution as it can result in liver damage. The suggested daily dose for the medication is 2000–4000 mg, with a maximum of 4000 mg (7).

Louis de Bougainville, a global traveler, discovered bougainvillea in Brazil in the 18th century and brought it to Europe, where it grew widely and gained popularity (8). The genus *Bougainvillea* is a globally distributed plant and it belongs to the member of Nyctaginaceae family (9). It treats a variety of illnesses, including looseness of the bowels, low blood pressure, and intestinal disorders (10). It is a small bloom with a paper-like texture and it is also known as a “PAPER FLOWER” (11). *Bougainvillea glabra* choice leaves have been demonstrated to exhibit antibacterial, antiulcer, antifertility, antiviral,

antioxidant, antidiabetic, and antimicrobial properties (12).

## Materials and Methods

### Plant material

The new plant of *Bougainvillea glabra* was gathered from Tirupati, Andhra Pradesh, India. The plant was authenticated by Dr. Madhava Chetty, Asst. Professor, SV University, Andhra Pradesh. After collecting immediately, the plant was washed and shade dried at 40°C and powdered coarsely, sieved with 40 mesh, and stored in air-tight containers.

### Chemicals

Paracetamol drug was purchased from Vijaya Entrepreneurs Pvt Ltd and All reagents purchased were of analytical quality.

### Preparation of extract

The dried fig leaves of *Bougainvillea glabra* were reduced to coarse powder and approximately 60 g of powder material was exposed to sequentially hot continuous extraction. A weighed quantity of each of the plant powdered materials was extracted by using a Soxhlet apparatus with ethanol of 300ml for 24 hrs with intermediate heating at 40°C. After that the residues were extracted. The Whatsmann filter paper was utilized, and the extract was subsequently evaporated using a rota evaporator. (Buchi, Flawil, Switzerland.) and further dried under vacuum. The obtained extract and percentage yield are shown in Table 1.0

### Phytochemical investigation

The extracted materials underwent phytochemical analysis and pharmacological assessment. The extracts underwent first phytochemical analysis to see whether different secondary metabolites including alkaloids, flavonoids, phenols, terpenoids, saponins, tannins, and glycosides reagent solvents of analytical quality<sup>13</sup>. The findings of the initial phytochemical analysis are displayed in Table .2.0

## **Pharmacological study**

### **Animals**

We bought a total of thirty Wistar Albino Rats (180-220g) from Sanzyme Pvt Limited, Hyderabad. Sawdust litter was employed in the metabolic cages (55×32.7×19cm), that the animals were kept in to allow no more than five animals of the same sex to reside in a single cage. From the time of arrival until the commencement of treatment, every animal was observed and allowed to acclimate for seven days. Rats were given a regular diet and access to food at will both before and throughout the trial. (Lipton India Ltd., Bangalore, India). Standard temperature, relative humidity, and a 12-hour light and dark cycle were maintained for each animal (14). Animal Ethics Committee laws and regulations governing the investigation of experimental plans in conscious animals were strictly adhered to during the experimental procedures. Being approved no CPCSEA REG NO: 1636/PO/RE/S/12/CPCSEA/2023/010.

### **Acute toxicity studies**

In acute toxicity investigations, male and female healthy rats were employed by OECD norms -425. After an overnight fast, the rats were split up into three groups, each consisting of five rats. The dosages of extracts were 100, 500, and 2000 mg/kg.p.o. body weight. For two hours, the rats' behavior and autonomic profiles were monitored constantly, and for up to 48 hours, any indications of toxicity or mortality were noted. (OECD-425,2001) (15). The drug under test was taken orally. The vehicle was administered to the control group's rats at the same dosage as the other treatment groups' animals. Oral administration used a 10 ml/kg dose volume. Based on each animal's body weight on the day of treatment, the amount of test material was provided to each one.

### **Hepatoprotective activity on *Bougainvillea glabra***

To cause liver injury in experimental animals, hazardous doses or repeated doses of hepatotoxins, such as rifampicin, carbon tetrachloride, paracetamol, thioacetamide, allyl alcohol, etc., are given (15). A test material is deemed an effective hepatoprotective agent if it prevents or reduces the hepatotoxicity caused by the toxin (16, 17). Currently this research, we are using paracetamol as a hepatotoxin inducer.

### **Pretreatment group**

For 14 days, every pretreated group of albino rats—apart from the control groups—received a daily dosage of silymarin (100 mg/kg), paracetamol (3 g/kg body weight), and an ethanolic extract of *bougainvillea glabra* (250–500 mg/kg).

### **Experimental design**

- Group 1: Received (Distilled water) as the control for 14 days.
- Group 2: For 14 days, a daily dosage of paracetamol (3g/kg of body weight p.o.) was administered.
- Group 3: Received a daily oral dosage of regular silymarin (100 mg/kg body weight) for 14 days, followed by a daily dosage of paracetamol (3g/kg body weight) after an hour.
- Group 4: Received a daily dosage of paracetamol (3g/kg body weight) and one hour later, a daily oral dosage of *B. glabra* (250 mg/kg body weight) for 14 days.
- Group 5: Received a daily dose of paracetamol (3g/kg of body weight) and after one hour a daily dosage of *B. glabra* ( 500mg/kg of body weight ) for 14 days (p.o).

### **Sample collection**

Following a 16-hour fast, blood samples were taken from the orbital sinus of the rats in

each group on the fourteenth day of therapy, while they were under light ether anesthesia. To separate the serum, the drawn blood samples were additionally centrifuged for ten minutes at 10,000 rpm. The biochemical parameters were assessed using the serum that had been isolated. like SGOT, SGPT, ALP, and BILIRUBIN (16).

### Histopathological study

Histopathology is the microscopic examination of tissues to look for abnormalities. This includes obtaining diseased tissues from a biopsy or necropsy, fixing them, making slices, staining them, and examining them under a microscope. The livers of the animals were removed and cleaned with normal saline (17). For 48 hours, the materials were fixed in 10% buffered neutral formalin. followed by 6 hours in bovine solution, and then paraffin-embedded. After cutting sections of 5 m thickness with an alcohol-xylene series, the sections were histopathologically inspected and stained with alum hematoxylin and eosin (18).

### Statistical analysis

The data is presented as the Mean± SEM of six creatures in every group. Dunnett's comparison tests were used to examine the data. \*p values <0.05 were considered statistically significant for liver enzymes (19).

### Results and Discussion

Table 1: The total yield of Ethanolic extract of *Bougainvillea glabra*:

Plant / Extract	Nature of the extract	Extraction Yield (% w/w)
Ethanolic extract of bracts of <i>Bougainvillea glabra</i>	Dark green semisolid	7.32 %

The preliminary qualitative phytochemical studies were performed to test the different chemical groups such as alkaloids, glycosides tannins, etc., present in ethanolic extract of *Bougainvillea glabra*.

Table 2: Qualitative phytochemical investigation of EEBG

S.no	Chemical test	Result
1.	Alkaloids	++
2.	Proteins	--
3.	Flavonoids	++
4.	Aminoacids	--
5.	Steroids	++
6.	Glycosides	++
7.	Tannins	+++

+ indicates present, and – is absent.

Table: 3 Acute toxicity studies of Ethanolic Extract of *Bougainvillea glabra*.

Route of administration	Groups	Dose mg/kg	No. of animals	No. of Survival	No. of Death
Oral	Control	10ml/kg	15	15	0
	Only para	3gm/kg	15	15	0
	Stand	100mg/kg	15	15	0
	Test (T <sub>1</sub> )	250mg/kg	15	15	0
	Test (T <sub>2</sub> )	500mg/kg	15	15	0

When taken orally up to 2000 mg/kg body weight, acute trials did not reveal any signs and symptoms of toxicity or fatality. Thus, by OECD rules 425, the extracts might be regarded as safe. For the first two weeks of the observation period, the rats showed no symptoms or indicators of poisoning. This shows that the extract is safe, and the data above imply that no toxicity has been observed in the extracts given at the prescribed levels.

Table 4: Body weight of the animal before and after the experiment and also liver weight of the animal.

Group	Initial body weight	Final body weight	Liver weight
GROUP 1	178.33±13.2	211.18±14.3	4.721±0.236
GROUP 2	185.43±16.3	171.38±17.7	6.522±0.524
GROUP 3	174.13±14.7	185.16±19.1*	3.829±0.463
GROUP 4	182.11±19.6	223.56±34.7**	3.254±0.385**
GROUP 5	195.33±13.9	200.85±56.9	4.187±0.398*

Rats given paracetamol develop liver damage. (6.522±0.524). A reduced body weight is one way this shows up (171.38±17.7). Standard sily-

marin rats have slightly increased body weight. In EEBG-treated rats at quantities of 250 and 500mg/kg treated rats. The ultimate body weight was (223.56±34.7) and (200.85±56.9) respectively. The liver weight is increased when *Bougainvillea glabra* (EEBG) on SGOT, SGPT ALP, TP, and TB serum enzymatic activity in paracetamol-induced liver damage in rats.

paracetamol is administered. (6.522±0.524). It means the liver is swollen or enlarged by inflammation and the liver is storing too much fat (steatosis).

Table 5: Liver function tests: Ethanolic extract of

GROUP	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/l)	TP (mg/dl)	TB (mg/dl)
GROUP 1	132.65±0.16	35.17±19.92	237.4±54.57	8.20±0.153	0.65±0.456
GROUP 2	217.5±89.96	99.93±19.61	345.1±27.89	5.60±0.404	2.25±0.299
GROUP 3	133.3±44.92*	38.91±4.313**	254.2±35.41	8.18±1.02	0.67±0.232
GROUP 4	151.5±60.03**	49.30±22.27	269.7±29.27*	8.42±1.81	0.62±0.207
GROUP 5	142.2±3.467**	34.65±2.00**	262.7±79.96**	9.03±0.463*	0.74±0.206*

Values are reported as the mean ±SD; statistical significance (p) is determined using Dunnet's one-way ANOVA. S-NS (no significant) \*p<0.001, \*\*p<0.01, \*p<0.05 computed by contrasting the treatment group with the groups under control.

In the group that simply took paracetamol, there was a rise in liver enzyme levels, which may indicate hepatotoxicity. The levels of 250mg/kg EEBG and 500mg/kg EEBG groups significantly decreased the levels compared to paracetamol. The ethanolic extract of *bougainvillea* bract has been demonstrated to have considerable hepatoprotective action. Increased levels of ALP are released into the bloodstream by injured liver cells. The level of Total protein is decreased in the paracetamol-only group (5.60±0.404) (20). It means Protein deficiency is associated with liver diseases.

The waste product bilirubin, which is orange-yellow, is mostly generated by heme breaking down normally. The pigment hemoglobin, which is present in red blood cells (RBCs), contains heme. In the end, the liver processes bilirubin to enable the body to excrete it. An increased blood level may result from any illness that hastens the breakdown of red blood cells or interferes with the processing and excretion of bilirubin. Elevated bilirubin levels signify injury to the liver or gallbladder. The bilirubin levels are high in the paracetamol-only group (2.25±0.299) compared to other standard silymarin groups and 250mg EEBG and 500mg/kg body weight of the animals.

**Histopathological changes**

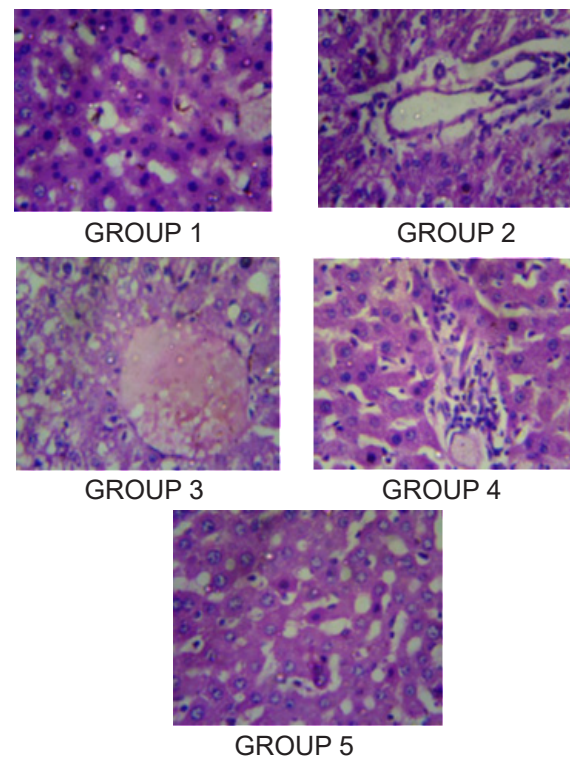


Figure 1: Histopathological changes showing the effect of EEBG on the rats with paracetamol-induced hepatotoxicity.

Hepatocytes in the control group exhibit no disease, and the liver has a normal lobular morphology. Both irritation and harmful alterations are absent. Hepatocytes exhibit cytoplasmic vacuolations and binucleation in group 2, and the section displays deformed lobular architecture (22). In groups 4 and 5 section shows distorted structure and hepatocytes don't exhibit any serious pathologies. Port tracts display infection around the porta. These visual assessments offer additional, unbiased proof that EEBG therapy successfully and dose-dependently shielded the liver from developing new cirrhosis.

### Conclusion

The goal of the current investigation was to assess any potential impacts of the ethanolic *Bougainvillea glabra* bract extract (EEBG) against paracetamol-induced hepatotoxicity in animals. In conclusion, we can say that the liver can be shielded by *bougainvillea* from the harmful effects of high dosages of paracetamol and *Bougainvillea glabra* revealed the hepatoprotective nature of the plant.

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### Conflicts of interests

The author declares no conflicts of interest.

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