



## Assessment of Hepatoprotective activity of Hydroethanolic Extract of *Luffa Acutangula* Leaves Extract in Experimental Animals

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### ABSTRACT :

*Luffa Acutangula* plant is well known for its medicinal and therapeutics values in Indian folk medicine. However, to be clinically useful, more scientific rational are essential. In the present study, hydroethanolic extract of *Luffa Acutangula* was evaluated for hepatoprotective activity in CCL4 rat model. To evaluate the hepatoprotective activity of different concentrations of the HEELA (200 and 400 mg/kg orally) was evaluated for CCL4 rat model. HEELA showed a dose-dependent protection against gross damaging action of CCL4 on animals. The treatment with HEELA shown significant protection of biochemical parameters in rats. Thus, our present study results clearly demonstrate that HEELA is in possession of good preventive and therapeutic action.

**Keywords:** Liver, Hepatoprotective, CCL<sub>4</sub>, *Luffa Acutangula*, Hydroethanolic, Antioxidant

### INTRODUCTION

One of the body's most important organs, the liver controls a number of internal physiological functions. Structure or function alterations in the liver are liver diseases (1). In maturity, the liver—the largest organ—makes up 2% of body weight at 1.5 kg. Metabolic processing takes place mostly in the liver, the biggest organ in vertebrates. It performs an impressive range of essential activities in the maintenance and operation of the body. Key activities include glucose, protein, and lipid metabolism, detoxification, and bile production. The liver is often compromised by environmental pollutants, unhealthy dietary practices, alcohol, medications, and over-the-counter remedies. Impaired liver function disrupts many homeostatic systems, leading to potentially severe effects (2).

Internal homeostasis is mostly regulated by the liver. Almost every metabolic activity related to development, immunity, nutrition, energy production, and reproduction involves it (3). Extrinsic factors may impair the structure and function of the liver, leading to liver damage. By controlling the metabolism of glucose, lipids, and proteins, the liver ensures that the body's energy levels remain stable and that all of its processes run smoothly.

Neurotransmitter levels in both central and peripheral neurons are primarily impacted by the oxidative stress-inflammation axis and the insulin signaling pathway, which connect the liver to the brain (4). Exposure to elevated amounts of environmental pollutants has led to an increase in liver toxicity. Centrilobular hepatic necrosis, kidney and liver damage, and cancer may all be outcomes of carbon tetrachloride (CCl<sub>4</sub>), a powerful hepatotoxin. When it comes to liver diseases, herbal formulations are the way to go (5).

The liver weighs around 1.5 kilograms (3.5 pounds). A horizontal measurement of around 8 inches (20 cm) is necessary, 6.5 inches (17 cm) in vertical height, and 4.5 inches (12 cm) in thickness. Medicinal plants have significantly contributed to the creation of novel pharmaceuticals and remain essential in the drug discovery process. Non-alcoholic fatty liver disease (NAFLD) is a rapidly proliferating hepatic condition of the twenty-first century, impacting roughly two billion individuals globally. There will likely be an increase in the number of people impacted by NAFLD in the next years, which is a worry for economies and world health (6).

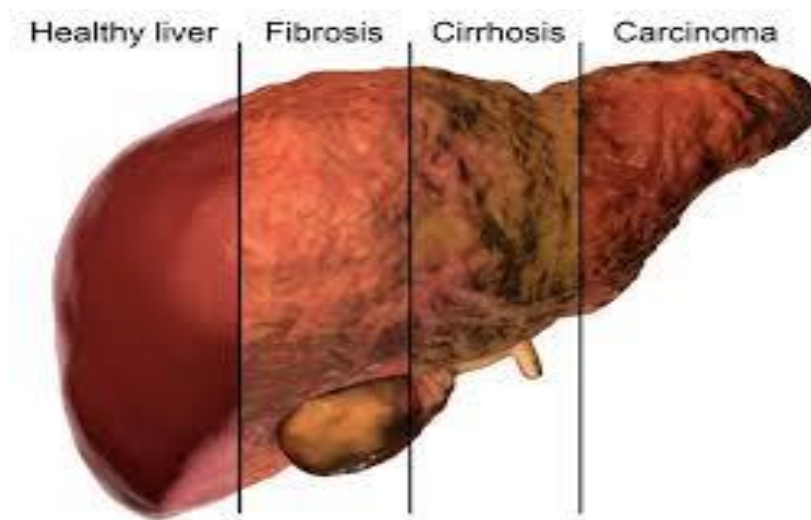


Figure 1.1 - Liver

The liver, biliary tree, and gallbladder are considered together owing to their anatomical proximity, interdependent functions, and shared characteristics of many diseases affecting these organs. It plays a far more prominent role in normal physiology and is the locus of a diverse range of diseases. The liver is located directly behind the stomach. Nonetheless, it also extends over the center of the upper abdomen and partially into the left upper quadrant of the abdomen (7).

#### **Free Radicals:**

Molecules that contain an unpaired electron are called free radicals. These compounds are very reactive because one electron is free in them. Natural processes related to cytotoxicity, regulation of vascular tone, and neurotransmission rely on them as crucial mediators. When testing the reactivity of free radicals, radiolysis is an effective approach for producing target free radicals.

#### **Protectors Against Free Radicals (Antioxidants)**

**Antioxidant:** Even trace quantities of substances existing in proximity to an oxidizable substrate will substantially impede or block the oxidation of the substrate (8).

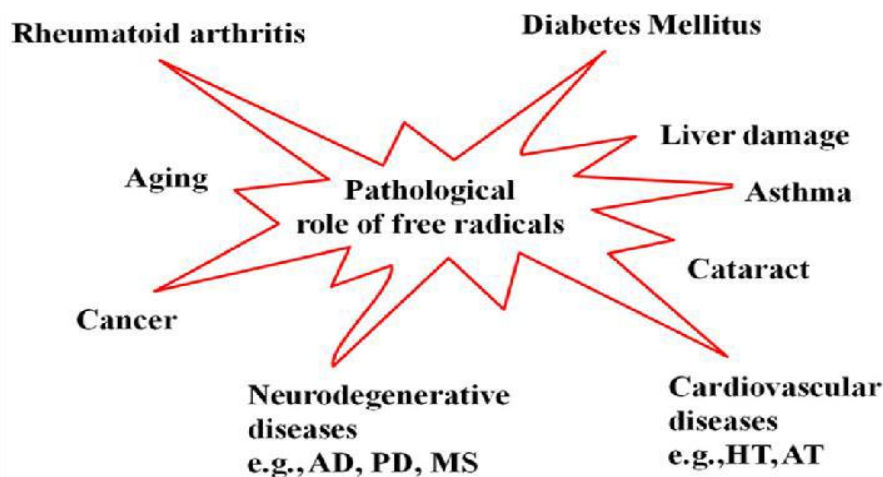


Figure: Role of free radical in various diseases

#### **Hepatotoxicity**

Since antiquity, mankind have used medicinal herbs to mitigate ailments. Contemporary analytical technology and understanding of bioactive chemicals in plants have facilitated enhanced insights into medicinal flora. Pharmaceutical, pharmacological, nutritional, and herbal hepatotoxins damage the liver. Hepatotoxicity costs about 50 million people worldwide. and isomerization are methods the liver, the main digestive organ, metabolizes drugs. P450 enzymes convert drugs into conjugated, water-soluble molecules that are excreted in urine or bile (9).

## HEPATOTOXICITY CAUSES

Hepatotoxicity is the result of

- Contamination with toxic compounds
- Dangerous individuals
- Dangerous substances themselves
- biological risks, and so on throughout the patient's lifetime.

It can likewise be achieved by

- Previous liver issues
- Abdominal sicknesses and stomach related infections
- Adverse responses of drugs
- Toxicity
- Poisoning
- Overdose from drugs

## SYMPTOMS OF HEPATOTOXICITY

The signs and side effects of Hepatotoxicity are the accompanying

- Feeling sick to your stomach and
- vomiting up food
- Experiencing stomach aches
- Not wanting to eat
- Constipation
- Lethargy
- Weakness
- Yellowing of the skin or jaundice
- Yellow sclera (white piece of the eyes' eyeball)
- Liver extension
- Edema in the feet
- Increase of weight
- Bleeding time takes additional time
- Water maintenance (10).

## HEPATOTOXICANT

Hepatotoxins and hepatotoxicants are terms used to describe substances that cause harm to the liver. Overdosing on some medicinal medications, synthetic chemicals, natural toxins like microcystins, herbal treatments, nutritional supplements, and other exogenous molecules may all be hepatotoxicants. Interactions between these compounds and macromolecules found in cells, lipid peroxidation, DNA damage, and oxidative stress. The liver metabolizes these compounds into reactive metabolites. 3 Liver damage occurs when defensive and aggressive forces are out of whack; a complex system is associated with hepatotoxicity, which may be caused by a wide variety of chemical and environmental factors (11).

## PLANT PROFILE

*Luffa acutangula* is more often known as ridge gourd. One species belonging to the genus *Luffa* is *Luffa acutangula*. Its immature fruits are the main reason it is sold as a vegetable. You may make cleaning sponges out of ripe fruits. A ridged cucumber is the closest comparison to the fruit. Ridge gourd is another name for the medicinal herb *Luffa acutangula* (12). It is prevalent throughout the Asian subtropics. People often look to India as a major starting point. Many parts of Africa, as well as India, Southeast Asia, China, and Japan, as well as Egypt, cultivate this plant widely. The Cucurbitaceae family, of which *Luffa acutangula* (L.) Roxb. is a member, has more than 975 species of flowering plants and 98 genera. There are a lot of ornamental or fruit-bearing annual or perennial species native to tropical and temperate zones (13).

**Synonyms:** Types of Cucumis include *acutangulus*, *lineatus*, and *longus* var. *indicus*. *Megacarpus cucumis* and *operculatus cucumis* are two species of cucumbers. The *acutangula* citrus fruit *Momordica tubiflora*, *Luffa acutangula* variations: *amara*, *drastic*, *fluminensis*, *foetida*, *forskalii*, *gosa*, and var. All *amara* are related (14).

### Plant Classification

- Class: Magnoliopsida
- Kingdom: Plantae

- Division: Magnoliophyta
- Phylum: Cucurbitales
- Classification: Cucurbitaceae
- Subclassification: Cucurbitoidea
- Classification: acutangula, Luffa. (15).

**1.5.3 Habit and Habitat** - Custom and environment It may flourish in many soil types, in naturalized tropical and subtropical regions, and can be cultivated in summer or during the monsoon season. *L. acutangula* is a pantropical climbing plant grown across India. It is disseminated by seeds, which can be sown in either June or July or in February or March (16).



**Figure: Leaves of Luffa acutangula**

**Scientific Classification:**

- Assamese : Jeeka
- Bengali : Jhingge or Jhinga
- Hindi : Turai, Tori
- Gujarati : Turiya
- Kannada : Eere kay
- Lao : mark noy
- Vietnamese : mướp khía.
- Tamil : Pirkanga
- Telugu : beera kaaya
- Thai : Buap liyam
- Marathi : dodaki
- Konkani : Gossale
- Indonesian : gambas, oyong
- Javanese : oyong
- Malayalam : Peechinga
- Malay : Petola segi
- Sinhalese : Watakolu (17).

**Phytochemistry**

More than fifty substances, including proteins, fatty acids, volatile components, saponin triterpenes, flavonoids, and anthraquinones, have been discovered and characterized via research into phytochemistry. Hydrogen connection we isolated many ribosome inactivating proteins (RIPs) from *Luffa acutangula*. There is a lot of interest in the possible medicinal uses of RIP because to its diverse pharmacological activities, which include those of an abortifacient, antifungal, anti-tumor, antiviral, and HIV-1 integrase inhibitor. In 2002, Junkai et al. extracted luffaculin and another RIP from seeds using SDS-PAGE. Luffaculin 1 and 2 have 28 kD molecular masses. According to Junkai et al. (2002), Both RIP limit growth of human leukemia K562 cells ( $IC_{50} = 1.1 \times 10^{-6}$  and  $2.0 \times 10^{-7}$  mol/L, respectively), indicating anticancer potential. Flufangulin (3), another seed-isolated RIP, reduced cell-free

translation ( $IC_{50} = 3.5 \text{ nM}$ ) but not HIV-1 reverse transcriptase. Schilling and Heiser found 10 Luffa flavonoids in 1981. Leaves and flowers contained just apigenin-7-glucoside (4) and luteolin (18).

### **Medicinal Uses**

You may use the leaves of the LA plant both internally and topically for their medicinal advantages. The pharmacological properties of the leaves are broad and varied, ranging from hemostatic to anti-inflammatory to emollient to mucilaginous to vulnerary to depurative to tonic. Injuries such as cuts, wounds, hemorrhoids, menorrhagia, skin discolouration, boils, necrotic ulcers, ophthalmic problems, burns, scalds, corns, diarrhea, dysentery, headaches, vomiting, and acute inflammations, and bronchitis are some of the more common pathological conditions that call for their usage. Nephrolithiasis, stomach ulcers, skin disorders, and edema of the lower extremities are some of its other uses. When applied topically to serious wounds, the leaf pulp or juice stops bleeding by narrowing tiny arterioles and speeds up the healing process. Wounds, ulcers, boils, burns, earaches, toothaches, eye infections, and insect stings are some of the conditions that it helps alleviate. Pulverized, heated leaves are applied to painful wounds. It speeds up the healing process of wounds and reduces swelling without leaving scars. When dysentery is present internally, the patient is given cumin seeds and leaf juice mixed with twice as much ghee as usual. In cases of menorrhagia, hemorrhoids, or excessive bleeding, the herb is highly advised (19).

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## **MATERIALS AND METHOD**

The planning of methodology entails a sequence of procedures executed systematically to attain the established objectives in accordance with the authorized rules and recommendations. It encompasses all processes from the field trip to observation, including the selection and harvesting of the medicinal plant, the choice of particular solvents for extraction, the formulation of protocols, and the final implementation of the standardized procedure. This needs a well-developed intellect and a proficient, delicate technical skill to manage the materials and processes in a really scientific way.

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## **ANIMALS AND MAINTENANCE**

### **Procurement of experimental animals**

The study used male Wistar rats weighing 150–220 grams. Animals came from Meerut's Translam Institute of Pharmaceutical Education and Research animal housing facility.

### **Animal housing**

Typical animal housing featured a 12-hour light/dark cycle,  $55\% \pm 10\%$  humidity, and  $25^{\circ}\text{C} \pm 5^{\circ}\text{C}$  temperature. Only four species fit in each cage. Food and water were continuous. The Institutional Animal Ethics Committee (IAEC) of T.I.P.E.R. Meerut, established by the Committee for the Control and Supervision of Animal Experiments, authorized the animal experiments (CCSEA), (IAEC/PH-25/TIPER/223, Date: 26/04/2025).

### **SELECTION OF PLANT**

A wide literature review was conducted to rule out similar in the past, and plants were chosen based on their historic applications. A literature review was conducted using both print and digital resources.

### **IDENTIFICATION, COLLECTION AND AUTHENTICATION**

We went to Rajpura on Mawana Road in Meerut in search of Luffa actutangula leaves, which we later collected. Preliminary studies were predicated on Professor Vijay Sharma's verification of the plant's genuineness at C.C.S. University in Meerut.

### **DRYING AND SIZE REDUCTION OF PLANT MATERIAL**

For 30 days at a temperature of  $25 \pm 2^{\circ}\text{C}$ , the plant's leaves were left to dry in a shaded area. Drying the leaves was followed by hand-grinding them into a powder and passing them through a 40-mesh filter.

### **EXTRACTION OF PLANT MATERIAL**

The process of hydroethanolic extraction included the use of a specified ratio of ethanol to distilled water, usually 70:30. The Soxhlet extractor was used with 500 g of leaf powder, which was placed into a thimble constructed of filter paper. Pour the hydroethanolic solvent into a flask with a round base. Join the flask and condenser using the Soxhlet apparatus. To heat the system, use a heating mantle or a water bath set to a temperature between 60 and  $80^{\circ}\text{C}$ , which is ideal for ethanol. Give the solvent a chance to boil, then condense before returning to the extraction chamber to seep into the plant matter. Once the solvent has been removed, it is sucked back into the flask. Gather the liquid from the flask with the circular bottom after the process is finished. To eliminate the solvent, concentrate the extract by subjecting it to lower pressure in a rotary evaporator. To get the extract, dry the leftovers in a desiccator. If you want to do further phytochemical screenings or pharmacological research, keep the extract in a sealed container at  $40^{\circ}\text{C}$ .

### **MACROSCOPIC CHARACTERS**

(a) **Size** – A graduated ruler in millimetre was used for the measurement of the length and thickness.

(b) **Colour** – Observed colour.

(c) **Surface characteristics** – The Material was observed for the surface characteristics and consistency.

(d) **Odour** – The material was checked for the odour.

(e) **Taste** – The taste of leaves was observed.

#### **Phytochemical Screening of Leaves Extract**

##### **FOR DETECTIONS OF CARBOHYDRATES**

Separately, 5 milliliters of alcohol was used to dissolve the extract, and the mixture was filtered. The presence of carbohydrates was determined by analyzing the filtrates. Two milliliters of a concentrated solution was added to the filtrate in a test tube after two drops of an alcoholic  $\alpha$ -naphthol solution had been put to it. The presence of carbs caused sulfuric acid and the test tube to retain their violet color.

1. **Benedict's test** – To prepare Benedict's reagent, which is an alkaline solution with a cupric citrate complex, the filtrate was cooked in warm water with it. When reducing sugars were present, a precipitate that was orange-red in color became visible.

2. **Fehling's test**

Following Fehling's solutions A and B, copper sulfate in distilled water, potassium tartrate, and sodium hydroxide in distilled water were applied to the filtrate, followed by alkali neutralization. The reddish precipitate showed fewer carbohydrates.

3. **Tollen's test**

The presence of aldose sugar was shown when a silver mirror formed on the inside of the test tube after 100 mg of extract was added to 2 ml of Tollen's reagent.

##### **FOR DETECTION OF ALKALOIDS**

After filtering, the water-based extract was subjected to an alkaloids test after being stirred with a few drops of diluted hydrochloric acid.

1. **Dragendorff's test (Potassium bismuth iodide solution)**

Alkaloids were found in the orange-brown precipitate that was formed using Dragendorff's reagent with a little quantity of extract.

2. **Mayer's test using a solution of potassium bismuth iodide)**

By adding Mayer's reagent to the extract cream and seeing a precipitate, the presence of alkaloids was verified.

3. **Hager's test (Saturated solution of picric acid)**

a little amount of extract, and Hager's reagent. Using a yellow precipitate to isolate alkaloids.

4. **Wagner's test (Solution of iodine in potassium iodide)**

Wagner's reagent treated with some amount of extract. Reddish brown precipitate presence of alkaloids (20).

##### **FOR DETECTION OF GLYCOSIDE**

###### **Test I**

200 mg of drug was extracts heating with test tube with 5 ml dil. sulfuric acid 1000°C for 2 minutes on the water bath, filtered, pipette out supernatant of filtrate added 5% solution of NaOH in acid extract. Then added 0.1 ml of fehling solution A and B and heating with ph paper for 2 minutes. Red precipitate was obtained.

###### **Test II**

Take 200 mg of drug extract and added 5ml of dil.sulfuric acid on boiling water bath. After adding 0.1 ml Fehling's A and B solutions, the test was supplemented with equal water and NaOH. The solution was heated in a water bath for 2 minutes till blue, using pH paper as proof. indicating alkanization. We noted the amount of red precipitate. If the second test's precipitate is larger than the first test's, glycosides could be present. If the first test's precipitate is smaller, the two tests should be compared.

### **FOR DETECTION OF SAPONINS**

#### **1. Froth test**

The extract was steeped for fifteen minutes in a graduated cylinder containing twenty milliliters of distilled water and sake. A 1-centimeter layer of saponin-containing foam was produced.

#### **2. Foam test**

A solution of the extract was prepared by diluting 1 milliliter with 20 milliliters of distilled water and stirring it in a graduated cylinder to ensure a constant concentration of saponins (21).

### **FOR DETECTION OF STEROIDS**

#### **1. Salkowski's test**

Some amount of taking chloroform and extract then filter. The filtered in adding some drop of concentrate sulphuric acid, sake and shaken were allowed to stand. Golden yellow colour appearance of obtained present of steroids.

#### **2. Liebermann Burchard's test**

Some amount of chloroform and extract was taken in a flask then filter in filtered mix with some drops of acetic anhydride. Heated and cool. The cooled with added concentrate sulphuric acid on the test tube side wall brown ring was not obtained indicate absence of phytosteroids.

### **FOR DETECTION OF TANNINS**

#### **1. Ferric chloride test**

The presence of tannins caused a greenish-black or dark blue hue to be created by adding a few drops of ferric chloride solution to 3 ml of extract solution.

#### **2. Gelatin test**

A 1% gelatin solution was combined with sodium chloride in the extract solution. A white precipitate is produced in the presence of tannins.

### **FOR DETECTION OF FLAVONOIDS**

#### **1. Alkaline reagent test**

Some amount of sodium hydroxide solution adding with alkaline extract reagent intense yellow colour in adding some amount of dilute acid become the colourless. It indicates that presence of flavonoids (22).

### **FOR DETECTION OF PROTEINS AND AMINO ACIDS**

#### **1. Ninhydrin test**

0.25 ninhydrine reagent (indene 1,2,3 triode hydrate) adding the extract solution and warmed some minutes. Blue colour was not observed indicate the absence of amino acid.

#### **2. Biuret test**

1ml of 10% sodium hydroxide solution adding with extract solution then warmed. In this solution few drop of 0.7% copper sulphate solution add. Violet presence of proteins (23).

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## **ACUTE TOXICITY STUDY**

The acute toxicity evaluation followed the guidelines laid forth by OECD-423. Finding a safe dose range for future pharmacological investigations was the goal of the acute toxicity study; the study also tracked any changes in behavior or physiological parameters brought on by the extract's administration. Toxicology evaluations were conducted on the selected experimental rats. Before animals are fasted overnight, do trials. The dosage of the extract is adjusted orally according to the patient's weight. In the first four hours after treatment, the animals' behavior was closely observed for any changes. After 24 hours, and every day for 14 days, evaluations were carried out to identify any changes in behavior or deaths related to humane concerns. No fatalities were recorded even after 14 days. The extract remains safe up to doses of 2000 mg/kg. To further investigate pharmacological effects, the test extracts were given at 200 and 400 mg/kg body weight, or one-fifth and one-tenth of the maximum acceptable dose.

### Screening for in Vitro Antioxidant Activity

#### BY PHOSPHOMOLYBDATE METHOD

In this adjustment the antioxidant action is abridgement based to mo six to mo 5 by the consecutive alertness of blooming phosphate /mo circuitous at acerb pH 12 and analysis sample. The band aid reagent contains 0.1 ml of Abacus in 0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. After being heated to 95 degrees Celsius, Half an hour was given for the combination to come down to room temperature. The whitish fluid's absorption at 695 nm was unnoticed. Samples were tested for their antioxidant activity using milligrammes per gramme of ascorbic acid.

#### PHARMACOLOGICAL STUDIES

**Selection of doses** – Doses was selected on the basis of *E. Prabhu Daniel et al.* and maximum tolerated safe doses was calculated from toxicity studies for hydroethanolic extract. The doses were selected which is 1/10<sup>th</sup> and 1/5<sup>th</sup> of the maximum toletared safe dose 1000 mg/kg then medium dose was 200 mg/kg and 400mg/kg as it is the just double dose of the above i.e. 200 mg/kg and all the doses were administered by oroul route.

#### CCl<sub>4</sub> Induced Chronic Hepatotoxicity

Each group of rats was assigned an identifying mark using picric acid as a dye in accordance with regular operating procedures. Each rat in the group was tagged at a specific location: Head, Tail, Back, while one remained unmarked. Each rat was weighed, and the dosage was determined appropriately (24).

Male Wistar rats weighing between 150 and 220 grams were chosen and randomly assigned to five groups, each including six rats.

Group I - For eight weeks, the control group received 1 milliliter per kilogram of distilled water orally.

Group II - For 8 weeks, animals that were not subjected to hepatotoxicity were given distilled water at a dosage of 1ml/kg orally and 1.5 ml/kg of CCl<sub>4</sub>, mixed 1:1 with olive oil, administered orally twice weekly.

Group III – The control group received silymarin (100 mg/kg/day, PO for 8 weeks) and CCl<sub>4</sub> (1.5 ml/kg, 1:1 in olive oil, PO twice weekly, PO for 8 weeks).

Group IV – HEELA received hydroethanolic *L. actangula* extract (200 mg/kg/day, p.o. for 8 weeks) and CCl<sub>4</sub>, 1.5 ml/kg, 1:1 in olive oil, weekly twice for 8 weeks.

Group V - HEELA received hydroethanolic *L. actangula* extract 400 mg/kg/day, p.o. for 8 weeks and CCl<sub>4</sub>, 1.5 ml/kg, 1:1 in olive oil, p.o. weekly twice for 8 weeks.

Following a 24-hour period of administering CCl<sub>4</sub>, blood was drawn from the retro-orbital plexus while the subjects were under moderate ether anesthesia. In order to assess different biochemical parameters, the blood was centrifuged at 350 g for 10 minutes after standing for 30 minutes at 37°C.

#### Statistical Analysis

Mean ± SEM is the way the results from animal research are expressed. The data were submitted to an ANOVA test for the mathematical investigation.

## RESULTS & DISCUSSION

#### Certifying Plants

The authentication of the plant was done by done by Prof. Vijay Sharma, (Professor) Department of Botany, C.C.S. University, Meerut. The voucher specimen Ref. No.- Bot/PB/132.

#### MACROSCOPICAL CHARACTERS

The leaves of *L. acutangula* were determined for macroscopical characteristics like colour, size, surface, texture, odour and taste as shown in table

#### Macroscopic characters of *L. acutangula* leaves

Sr. No.	Orgnoleptic Parameters	Observation
01.	Size	Uneven
02.	Color	Green
03.	Taste	Bitter
04.	Odour	Characteristics



05.	Qualities	Flexible and soft
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### EXTRACTION

The extraction was done by soxhlet extraction technique by using the solvents ethanol (70% and Water 30% v/v). Extract were the air dried and stored in yellowish-brown colored air tight bottle at 10-15 °C temperature. The extracts were used to carry out the qualitative tests and to evaluate pharmacological activities.

### Yield of *L. acutangula* leaf extract

Sr. No.	<i>Luffa acutangula</i> leaves extract	Yield of Extract (gm)	% Yield
1.	Hydroethanolic Extract of <i>Luffa acutangula</i> leave	3.9	4%

### CHEMISTRY OF THE INGREDIENTS

The phytochemical examination of hydroethanolic *L. acutangula* leaf extract revealed several secondary metabolites, including alkaloids, tannins, saponins, terpenoids, and flavonoids. However, as can be seen from the table, the extract had negative results for phenols, carbs, and sterols 3.3

### Extract from *Luffa acutangula* leaves: preliminary qualitative testing

Photochemical	Test	Extract
Alkaloids	Dragendroff's test	+
	Mayer's test	+
	Hager's test	+
	Wagner's test	+
Carbohydrates	Benedict's test	+
	Fehling test	+
	Tollen's test	+
Glycosides	Test I	+
	Test II	+
Saponin test	Froth test	-
	Foam test	+
Steroids	Salkewski test	+
	Liebermann Burchard test	-
Fat and Tannins	Ferric Chloride test	+
	Gelatin test	+
Flavonoids	Alkaline reagent test	+
Proteins and Amino Acids	Ninhydrin test	-
	Biuret test	+

**Note:** Sign indicates, (+) presence;(-) absence-

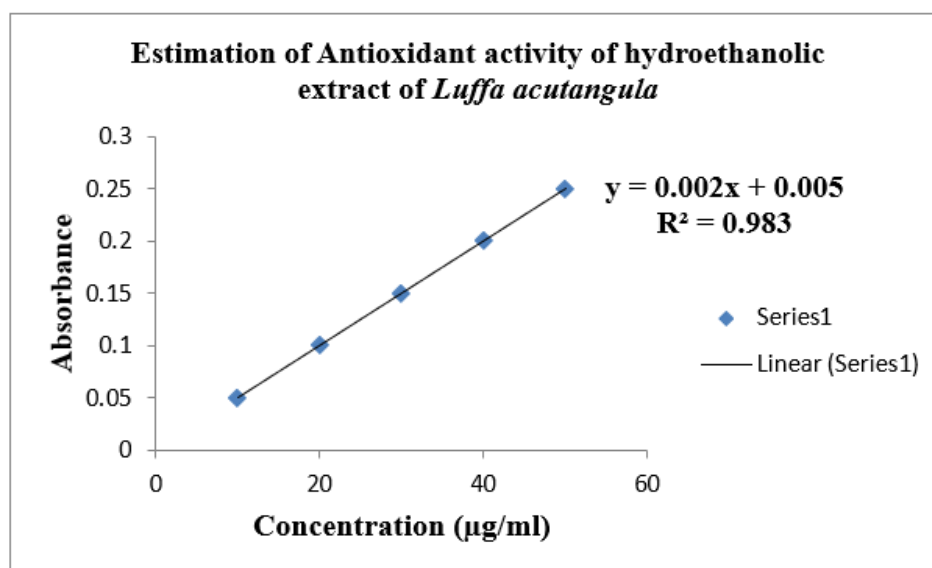
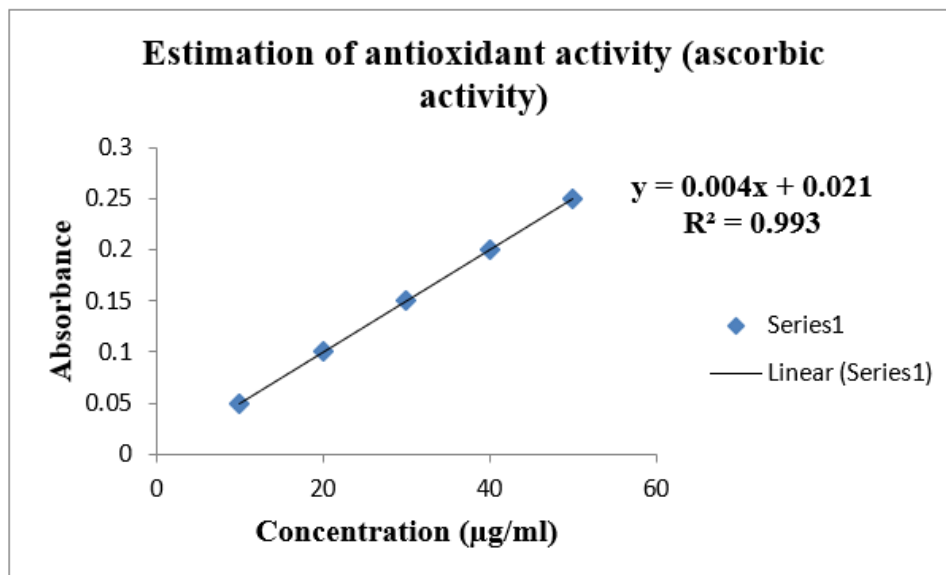
Phytochemical analysis revealed glycosides, tannins, and flavonoids to be present. There were a lot of extracts in steroids back then, but no fatty materials or volatile oil components. Amino acids and carbohydrates were detected in the Aq. extract.

### Study on Acute Toxicology:

Up to 2000 mg/kg, the *L. acutangula* extracts were safe and did not cause death. Half and a tenth of the extracts' dosages were sufficient to demonstrate pharmacological activity.

**IN-VITRO ANTIOXIDANT ACTIVITY**

In present investigation, the antioxidant property of *L. acutangula* was assayed in hydroethanolic extracts by using phosphomolybdate method. The data shows that the hydroethanolic extract is of much antioxidant nature.

**Antioxidant activity of HELA compared with standard (Ascorbic acid)**

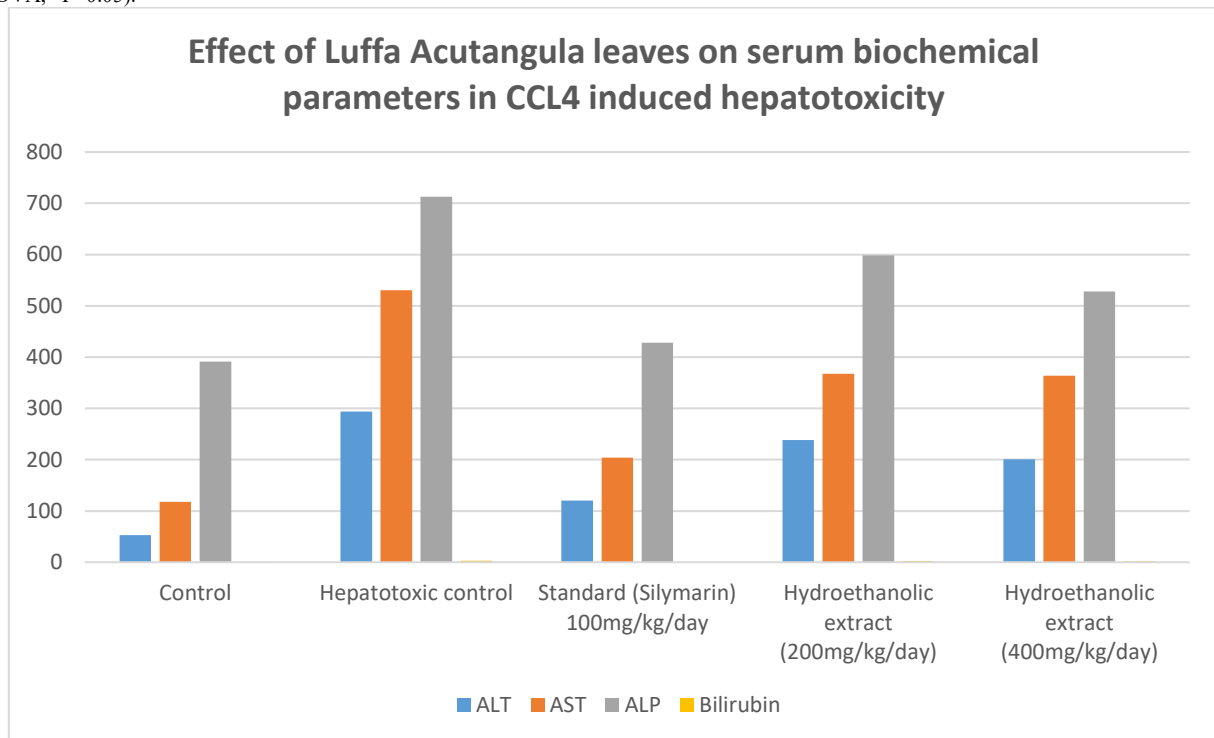
The antioxidant potential of *Luffa acutangula* leaf extracts is quantified in terms of ascorbic acid equivalents (µg/mL of extract). The total antioxidant activity of *Luffa acutangula* leaf extract was determined to be 14.00 µg/ml.

**HEPATOTOXICITY STUDY****Research on the hepatoprotective effects of *Luffa acutangula* hydroethanolic extract in a chronic hepatotoxicity model**

Group	Treatment	ALT	AST	ALP	Bilirubin
I	Control	52.99±1.33	118.01±1.28	390.98±1.43	0.43±0.04
II	Hepatotoxic control	294.01±1.73	530.69±1.98	712.89±2.32	3.11±0.03

III	Standard (Silymarin) 100mg/kg/day	120.6±1.05**	203.97±2.00**	428.16±2.63**	0.66±0.02**
IV	hydroethanolic extract 200mg/kg/day	238.12±1.93*	367.34±1.68*	598.34±1.92*	2.32±0.03*
V	hydroethanolic extract 400mg/kg/day	201.04±2.43*	364.02±1.64*	527.98±1.23*	1.97±0.03*

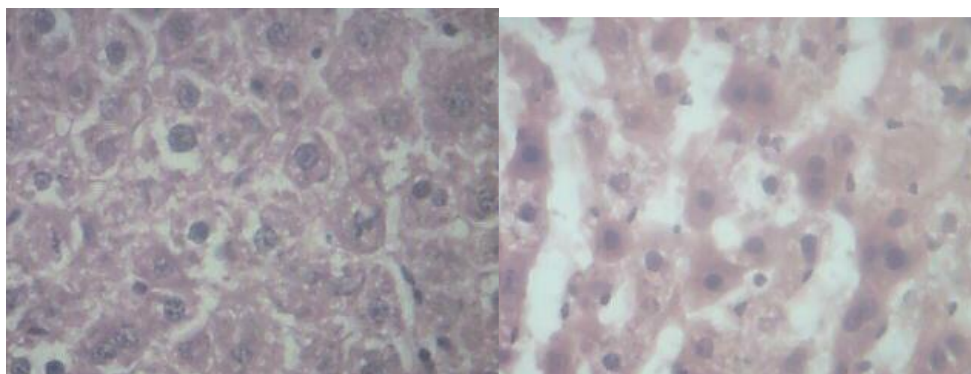
The mean plus or minus the standard error of the mean (n=3) is reported. P We compared GP II to GP I and GP III-V to GP II (\*\*P<0.01, one-way ANOVA, \*P<0.05).



**Impact of hydroethanolic extract of *Luffa acutangula* on serum biochemical markers in a study of chronic hepatotoxicity produced by CCl<sub>4</sub>.**

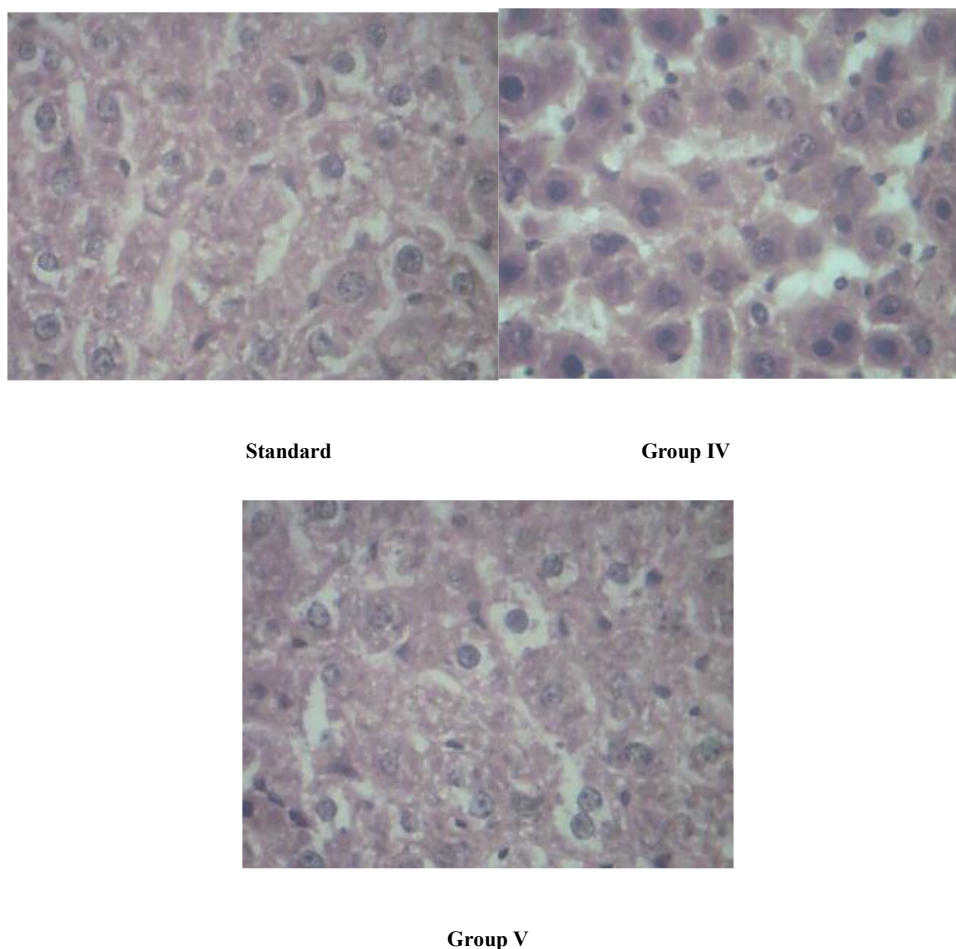
CCl<sub>4</sub> induced hepatic injury, as shown by modified serum biochemical markers. CCl<sub>4</sub> markedly elevated serum enzyme concentrations. Enzyme levels considerably lowered (P < 0.001, P < 0.01) following pre-treatment with 200 mg/kg or 400 mg/kg *L. acutangula* extract compared to the toxin control group.

**Histopathology**



**Normal**

**Control**



**Figure 3.3: Histopathology of liver Sections**

## CONCLUSION

The researchers set out to compare the outcomes of several *Luffa actangula* extracts and identify the most effective hydroethanolic extract in terms of its hepatoprotective properties. Plant materials were found to include glycosides, tannins, phenolic compounds, and flavonoids during hydrographic analysis. There found a lot of steroids in the hydroethanolic extracts, but no fatty substances or volatile oil components. Through hydroethanolic extraction, carbohydrates and amino acids were isolated. The phosphomolybdate technique evaluated the antioxidant properties of hydroethanolic extracts at 14.00  $\mu\text{g/ml}$  and 3.33  $\mu\text{g/ml}$  concentrations.

The potential pharmacological hepatoprotective effects of a hydroethanolic *L. actangula* extract were studied in this study. The hepatoprotective effectiveness of hydroethanolic extracts was tested against  $\text{CCl}_4$ -induced chronic hepatotoxicity, and the results showed that these extracts really work. *Luffa actangula* leaves contain cardiac glycosides and bufadienolides, which may explain the plant's hepatoprotective effects.

The results indicated that the hydroethanolic extract's better antioxidant capabilities could be responsible for its increased hepatoprotective potential; nevertheless, more investigation is needed to clarify the molecular mechanism. Protective methods against  $\text{CCl}_4$ -induced liver damage are commonly utilized to assess hepatoprotective medicines (Clauson, 1989). James and Pickering (1976) found that experimental liver damage caused by carbon tetrachloride histologically resembles viral hepatitis. According to Mahendala et al. (1985), the gold standard for detecting hepatic diseases is a panel of highly sensitive tests that includes ALT, ALP, AST, and blood bilirubin. A major biomarker of pathological abnormalities in biliary flow, alkaline phosphatase (ALP) showed a large rise in Wistar rats with extended dosages of  $\text{CCl}_4$  (Ploa and Hewitt, 1989), indicating serious hepatic damage. The use of ALP in chemically induced liver failure has been the focus of our research. Serum bilirubin levels are inversely proportional to the increase in this enzyme activity that  $\text{CCl}_4$  causes. Due to its dual effects of lowering high ALP activity and increasing bilirubin levels, this extract may stabilize biliary dysfunction in rats after prolonged  $\text{CCl}_4$ -induced hepatic damage.

*Luffa acutangula* hydroethanolic extract and Silymarin, the gold standard hepatoprotective medication, protected rats' livers from experimental liver injury. These markers dropped significantly after liver tonic and *Luffa acutangula* leaf extract. This supports emerging evidence that the extract may preserve the liver.

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