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Quantitative Phytochemical Analysis and Acute Toxicity Study of Ficus racemosa

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ABSTRACT

Background
Ficus racemosa is a medicinal plant traditionally used for its therapeutic benefits. This study aims to quantify key phytochemicals and evaluate the acute toxicity of Ficus racemosa to establish its safety profile.

Methods
A quantitative analysis of Ficus racemosa extract was conducted to measure the concentrations of flavonoids, phenols, and alkaloids. Wistar rats were utilized in acute toxicity research. They received a single oral dosage of 2000 milligrams per kilogram of body weight of Ficus racemosa extract for a duration of fourteen days, the animals were monitored for indications of toxicity, such as alterations in their behavior, body mass, or deaths. Gross histopathological examinations of vital organs (liver, kidney, and heart) were performed to assess potential toxic effects.

Results
The quantitative analysis indicated significant concentrations of flavonoids and phenols, suggesting antioxidant and anti-inflammatory potential. The acute toxicity study showed no toxicity indicators,without any alterations in behavior, body weight, or at dosages as high as 2,000 mg per kilogram body weight.Gross histopathological examination revealed no significant tissue damage in the vital organs like liver, kidney, or heart.

Conclusion
Ficus racemosa is rich in phytochemicals especially flavonoids and phenols and has no lethal dose up to 2000mg/kg of the body weight. These results justify the traditional usage of Ficus racemosa and provide a basis for its secure therapeutic use. More pharmacological investigations are suggested in order to investigate its potential.

Ficus racemosa (syn. *Ficus glomerata*), being from the Moraceae family, has been widely employed in Asian traditional medicine, particularly in Ayurvedic, Unani, and Siddha systems of medicine. Known as 'Udumbara' in Ayurveda, this plant has a history of use for conditions including diabetes, dysentery, inflammation and illnesses related to the gastrointestinal system [1]. The richness of bioactive compounds allows for the utilization of the plant's bark, leaves, fruits and latex in health care [2].

Flavonoids, phenols, alkaloids, and terpenoids are the phytochemicals that are the essential cause of the beneficial pharmacological attributes of Ficus racemosa [3]. The anti-inflammatory and antioxidant properties of flavonoids and phenolic compounds regularly play a role in the treatment of chronic illnesses linked to diabetes and heart diseases [4, 5]. The benefit for both antimicrobials and anti-inflammation is boosted by alkaloids and terpenoids [6].

The Ficus racemosa has a historical application, but scientific investigations about its safety at high doses are still rather few. The evaluation of potential harmful effects and the safety of plant extracts for therapeutic uses is in urgent need of toxicity studies [7]. Research into other Ficus genus plants has shown important pharmacological activities that feature low toxicity [8]. A detailed evaluation of the higher dose acute toxicity of Ficus racemosa is critically dependent on available research.

This research seeks to carry out a quantitative phytochemical investigation of Ficus racemosa and to assess its acute toxicity in Wistar rats. The present study will examine significant bioactive substances such as alkaloids, phenols and flavonoids, while evaluating the effects of a high dose (2000 milligrams per kilogram of body weight) of the plant extract, thereby contributing important insights into the safety of Ficus racemosa and supporting its historical medical applications in modern medicine.

Materials and Methods

Plant Authentication and Voucher Number

The *Ficus racemosa* fruits were sourced from the local market and in Tirupati, Andhra Pradesh. An assistant professor in the Department of Botany at S.V. University, Tirupati, who is a botanist, identified and verified the plant. Under the voucher number, 0811 a voucher specimen has been placed in the department's herbarium for future use.

Plant Extraction

The collected fruits of *Ficus racemosa* were thoroughly cleaned, shade-dried, and then ground into a rough powder. The powdered material has been filtered by sieve number forty to achieve uniform particle size and stored in an airtight container to protect it from moisture and contamination, preserving its phytochemical properties for subsequent analysis.

To prepare the plant extract, 100g of the powdered material was macerated in a 70% v/v hydroalcoholic solvent for seven days. The mixture was occasionally stirred to enhance the extraction of substances that are bioactive. After the maceration process, the mixture was filtered, as well as the filtrate was subjected to evaporation via a rotary evaporator under reduced pressure, resulting in a semi-solid extract. Which was stored for further studies [9].

Preliminary Phytochemical Screening

The hydroalcoholic extract of fruits of Ficus racemosa underwent preliminary phytochemical screening to detect the existence of bioactive compounds like carbohydrates, proteins, terpenoids, polyphenols, sterols, alkaloids, flavonoids, tannins, saponins, and glycosides. The methodology described by Jaiswal Bhagat Singh et al. [10] was employed for this screening.

Acute Toxicity Studies

Acute toxicity studies followed the OECD (Organization for Economic Co-operation and Development) rules on animal experimentation (Guideline No. 423). This protocol is specifically for 'Acute Oral Toxicity Studies,' which involves single-dose administration of the extract. The study was approved by the IAEC (Institutional Animal Ethical Committee), with the trial conducted under approval number [KAMSRC/Pharm/IAEC/2020/1] [11].

Experimental Animals, Housing, and Feeding Conditions

Eight to twelve-week-old, healthy female Wistar rats weighing 150 to 180grams, were selected for the study. The animals were housed in polypropylene cages (370mm×210mm×150mm) with bedding made of corn cobs (three rats per cage). The setting they were kept in was temperature-controlled at 22±3°C, with relative humidity ranging from 30% to 70% and a 12-hour light/dark cycle. Water and rodent food were provided to the animals without restriction.

Prior to the investigation, the rats were given 21 days to become used to the lab environment. They have been examined for health status and fasted overnight before dosing.

Exclusion Criteria

Pregnant female Wistar rats, those weighing over 180 g, & study participation have been restricted to rats older than 12weeks.

Quantitative Phytochemical Analysis

Determination of Total Flavonoid Content

Using "the aluminum chloride colorimetric method, the overall flavonoid content in the hydroalcoholic extract of *Ficus racemosa* was "ascertained [12].

Preparation of Standard Solution

Quercetin was used as a reference compound. Varying concentrations (five,ten, twenty, thirty, forty and Fifty µg/mL) of quercetin have been prepared in double-distilled water for calibration.

Methodology

150 µL of five percent $NaNO_2$ was added to a sample solution one mg/mL of the extract and left to stand for five minutes. 150 µL of ten percent aluminum chloride was then added, and the mixture underwent incubation for sixty minutes. Lastly, a two-milliliter of 1M NaOH was included, as well as 5 mL of distilled water was added to dilute the mixture. With a spectrophotometer set at 510 nm, the absorbance was determined. Quercetin equivalents (QE) in milligrams were used to express the results.

Determination of Total Alkaloid Content

A colorimetric technique was used to determine the total alkaloid content, which forms a yellow alkaloid-bromocresol green complex [13].

Reagent and Standard Preparation

Bromocresol Green Solution: 69.8 milligrams of bromocresol green was dissolved in 3 mL of 2N sodium hydroxide and five mL of distilled water, and the mixture was then diluted to 1000 milliliters.Phosphate Buffer(pH 4.7): Made by mixing 0.2M citric acid and 2M sodium phosphate solutions.Atropine Standard Solution: To create a standard curve, "one milligram of atropine was dissolved in ten milliliters of distilled water.

Determination of Total Phenolic Content

The Folin-Ciocalteuprocedure have been utilized to determine the total phenolic" content [14].

Preparation of Standard Solution

Through dissolving ten milligrams of gallic acid in methanol, a stock solution of gallic acid one milligram/mL) was created.

Test Sample Preparation

A methanol stock solution containing one milligram per milliliter of Ficus racemosa extract was made.

Procedure

A 1 mL aliquot of the plant extract, along with different doses of gallic acid, was transferred into a 25 mL volumetric flask. "Subsequently, 1 mL of Folin-Ciocalteu reagent was introduced, followed by the addition of 10 mL of 7% Na_2CO_3 solution. Following a 2-hour incubation, the absorbance was measured at 760 nm with a spectrophotometer. Results were presented as milligrams of gallic acid equivalents" (GAE).

Results

Quantitative Phytochemical Analysis of Ficus racemosa

Total Flavonoid Content (TFC):

The total flavonoid content of the hydro-alcoholic extract of Ficus racemosa was determined to be 2.81 mg/100 mg (Table 1 , Figure 1 and Figure 2).

Total Alkaloid Content:

The alkaloid content of Hyptissuaveolens was found to be 1.32 mg/100 mg (Table 1 and Figure 1and Figure 3).

Total Phenolic Content (TPC):

(The total phenolic content of Hyptissuaveolens was calculated as 2.14 mg/100 mg, using the gallic acid

(Tabel 1 and Figure 1 and Figure 4) .

Acute toxicity studies

The acute toxicity study of Ficus racemosa, commonly known as the cluster fig, demonstrated that the extract exhibits a significant safety profile at various dosage levels (Tabel 2). In experiments involving animal models, no lethality or severe adverse effects were observed at doses up to a certain threshold, indicating a relatively high tolerance. Behavioral assessments and physiological observations showed no major changes in the treated subjects compared to the control group (Tabel 3 and Table 4). These findings suggest that Ficus racemosa possesses a favorable safety margin, although further investigation is warranted to fully understand its potential therapeutic benefits and mechanisms of action.

Table 3: Clinical Signs & Symptoms Recorded During Acute Toxicity Study

Observation	**Ficus racemosa** **Step I and Step II**
Temperature	Normal
General physique	"Normal
Change in skin	No effect
Diarrhea	Not present
Drowsiness	Not present
Breathing difficulty	Not present"

Food intake	Normal
Sedation	Not present
Coma	Not present"

Table 2: Summary of Necropsy Findings

	Dose (mg/kg bw)	**Necropsy Findings**
Ficus racemosa	2000	The animal was sacrificed 15days after the dose, and no significant findings were found.

Table 4: Body weights of animals given FR fruit hydroalcoholic extract. The mean ± S.E.M. is used to express all values.

	Dose mg/kg b.w	**Before Treatment**	**7th Day**	**14th Day**	**B.W Diff (g) (Day 0-7)W7-W0**	**B.W Diff (g) (Day 0-7)W7-W0**
F.R Step I	2000mg/kgbw	162.01± 0.57	164.33± 1.20	165± 1.73	2.33	3
F.R Step II	2000mg/kgbw	166.66± 1.20	170.33± 0.88	172.33± 0.66	3.67	5.67

Table 1 Quantitative estimation of Phytochemicals Present in *Ficus racemosa* Fruit Extract by Colorimetry.

Phytochemical	Unit	Conc
Flavonoid content	mg of quercetin/gm of *Ficus racemosa*	2.81
Alkaloid content	mg of atropine/gm of *Ficus racemosa*	1.32
Phenolics content	mg of GAE/gm of *Ficus racemosa*	2.14

Figure 1: Overlaid bar graph depicted the Quantitative estimation of Phytochemicals present in *Ficus racemose* extract by colorimetry.

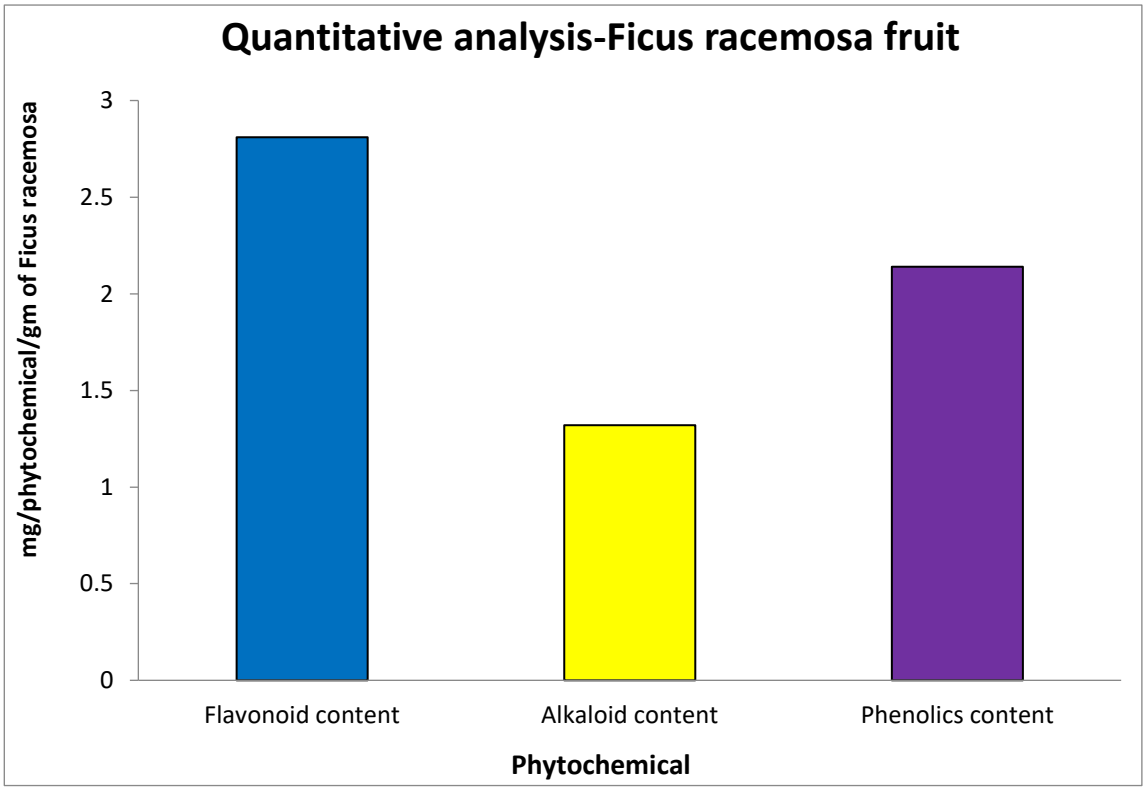


Figure 2: Standard Curve Quercetin

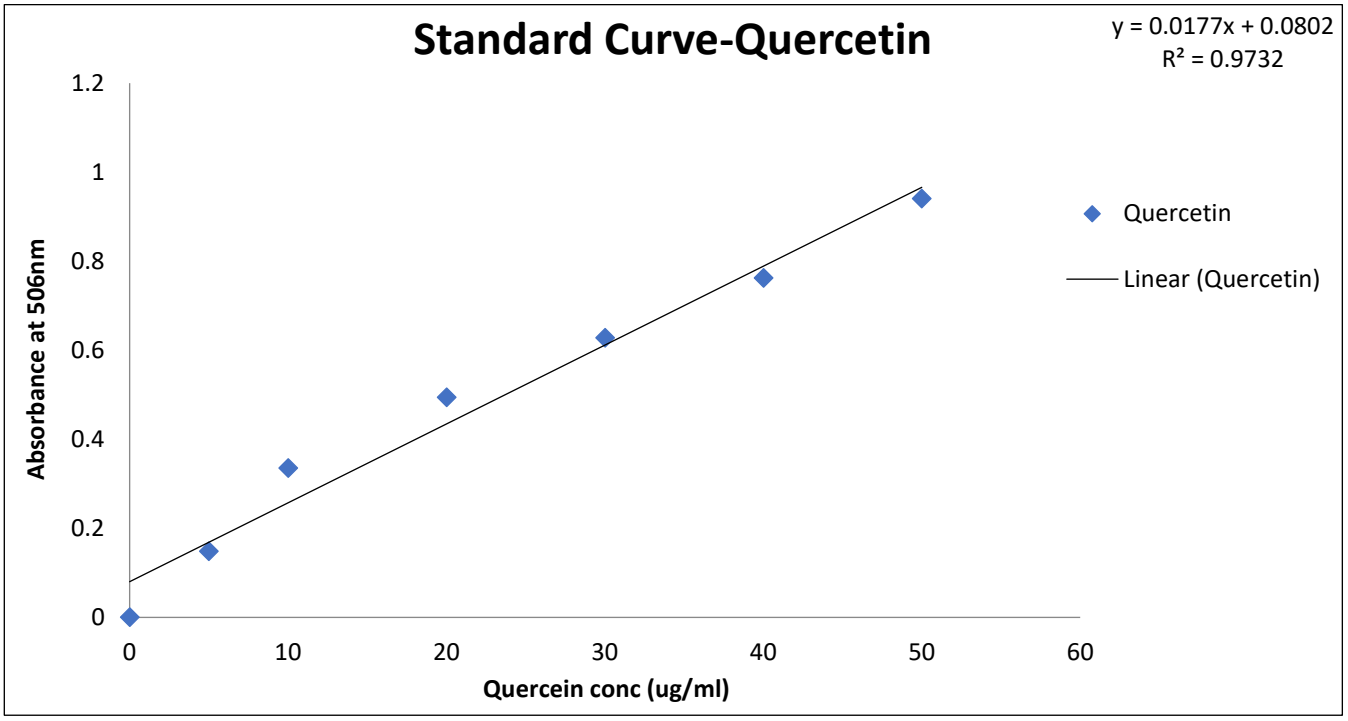


Figure 3: Standard Curve Atropine

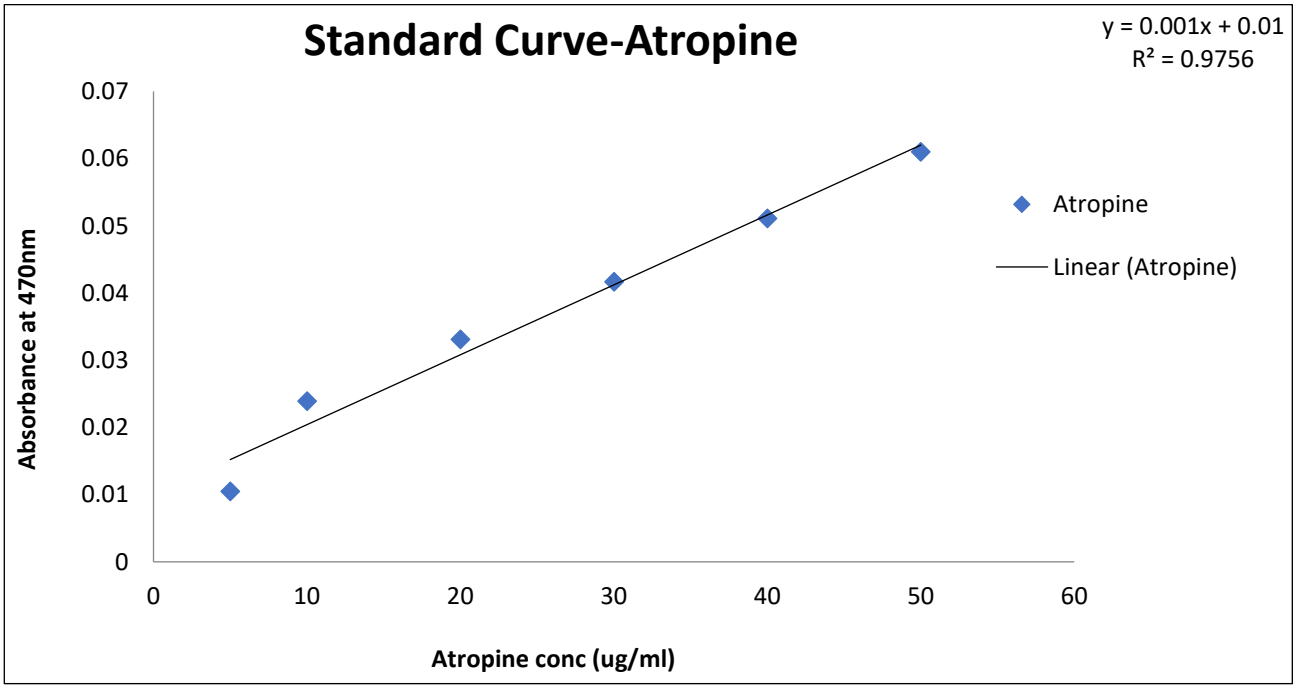
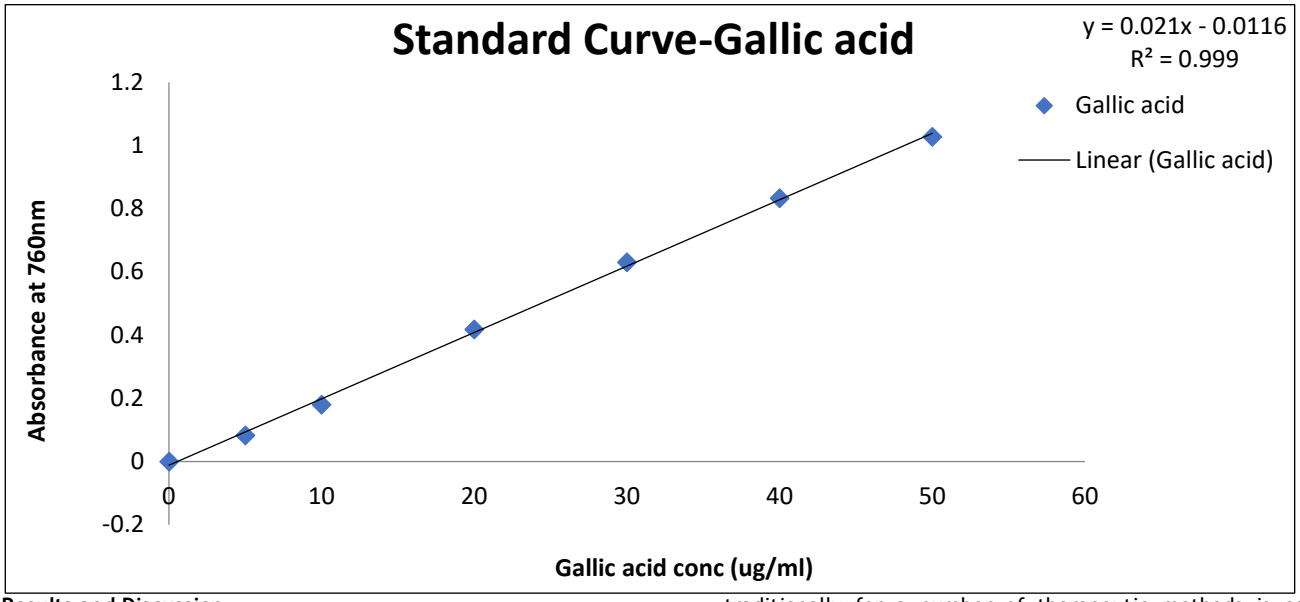


Figure 4: Standard Curve Gallic acid



Results and Discussion

DISCUSSIONINTRODUCTION

The present study concentrated on phytochemical screening, quantitative analysis, as well as acute toxicity assessment, of *Ficus racemosa*, it was extensively utilized in traditional medicine for its medicinal attributes. Our results reveal valuable insights into the biologically active compounds present in the hydroalcoholic extract of *Ficus racemosa* and its safety profile, confirming its potential for future pharmacological applications.

The "preliminary Phytochemical analysis of the *Ficus racemosa* hydroalcoholic extract demonstrated the existence of several important classes of biologically active compounds such as flavonoids, alkaloids, phenols, tannins, and terpenoids. These compounds are known to exhibit significant pharmacological effects, including antioxidant, anti-inflammatory, antibacterial,and antidiabetic activities. Of these compounds, flavonoids are the most effective antioxidants, which could be why *Ficus racemosa* is used in wound healing and to treat" inflammation [15,16].

The quantitative findings demonstrated that the extract had a rich abundance of phenolic compounds as well as flavonoids. The aluminum chloride colorimetric method serves to quantify flavonoids, and the results are presented as quercetin equivalents.The research findings are in line with those of Zhishen et al. [17], who discovered antioxidant properties in extracts abundant in flavonoids. Investigations are currently going into the effects of flavonoids on oxidative stress, which may help clarify why Ficus racemosa is employed in the treatment of diabetes, cancer, and cardiovascular diseases [18].

The Folin-Ciocalteu reagent measures phenolic chemicals related "with the antioxidant properties of medicinal plants by means of hydrogen atom transfer and free radical scavenging [19]. The high amounts of larger phenolic compounds present in Ficus racemosa are in line with" the traditiona lusage of this plant for both gastrointestinal problems as well as wound healing. The findings showed that the phenolic content was in line with prior studies, among them Sharma et al. [20], who found phenolic compounds in Ficus racemosa extracts at a similar concentration.

The extract served the significant basis for measuring its alkaloid content, which includes recognized compounds for their wide range of pharmacological effects, including analgesic, antibacterial, as well as anti-inflammatory properties. Our results are in line with those from Patel et al. [21], who found a significantquantity of alkaloids in Ficus racemosa and its uses in various therapeutic methods.As per the study, Ficus racemosa may have anibacterial and ani-inflammatory properties, which supports the traditional usages of plant for the treatment of infections and inflammatory diseases. The results show that Ficus racemosa is safe at high concentrations and using it traditionally for a number of therapeutic methods is correct [22].

Investigations at this high dose revealed no evidence of acute toxicity, consistent with the outcomes from previous studies on similar medicinal plants. Our findings about the absence of negative effects are in line with those of Jaiswal et al. [23], who found that Ficus' racemosa extract shows a positive safety profile in animal studies. The finding demonstrated that Ficus racemosa'bioactive compounds are probably the reason for its therapeutic uses, far less likely to be toxic.

Ficus racemosa phenolic,flavonoid, as well as alkaloid content in points to its potential therapeutic uses, particularly for diseases that results from stress related to oxidative stress and inflammation. Treatment of conditions like inflammation,infections, and diabetes with Ficus racemosa relies significantlyon the presence of these bioactive compounds. Regardless the fact that this study emphasized on acute toxicity, it is essential to do furtherresearch in investigating chronic toxicity as well as long-term safety.

Furthermore, a comprehensive understanding of the exact mechanisms through which the phytoconstituents of Ficus racemosa produce their therapeutic effects is essential. Future research should analyze pharmacokinetics and bioavailability in addition to the potential synergistic effect of the plants's chemical constituents.Significant clinical trials should be completed before Ficus racemosa extracts can be used to effectively treat human diseases.

CONCLUSION

This investigation focuses on the therapeutic potential of Ficus racemosa, because of its high phytochemical content. Quantitative phytochemical analysis results showed that this fruit, which has phenols, flavonoids, and alkaloids, exhibits effects that function as anti-antioxidants, antiinflammatories, and medicinally these biologically active chemical compounds might help the plant perform its traditional applications in the disease prevention of several types. The experimental animals had no adverse effects at a dose of 2000 mg/kg body weight of the hydroalcoholic extract of Ficus racemosa, demonstrating its acute toxicity safety. A histopathological analysis revealed that the plant was safe as there was no significant damage to the vital organs.

Furthermore, this study discovered that for future pharmacological investigations, Ficus racemosa is the ideal option. A combination of a low toxicity and high phytochemical content provides the opportunity for a more in-depth investigation of its medicinal effects. Research in the future ought to concentrate on making clear the specific mechanisms of action of the active chemicals and carrying out long-term toxicity assessments to establish their safety for ongoing application. Findings from these studies could demonstrate the

way forward for the development of new pharmaceuticals from this medicinal plant species.

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