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Research Article

# TOXICOLOGICAL STUDIES OF *FICUS VIRENS* IN WISTAR ALBINO RATS

Pattar Jayashree*, N.B Shridhar, Matham Vijaykumar[1], K Suhasini, Jayakumar and M.L Satyanarayana[2]
Department of Pharmacology and Toxicology, Veterinary College, KVAFSU, Hebbal, Bangalore, India
[1]Department of Pharmacology and Toxicology, Veterinary College, KVAFSU, Nandinagar, Bidar, India
[2]Department of Veterinary Pathology, Veterinary College, KVAFSU, Hebbal, Bangalore, India

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*Email: jayashri.vet@gmail.com

**ABSTRACT**
The present study was conducted to investigate the toxic effect of oral administration of methanolic leaf extract of *Ficus virens* in Wistar albino rats.The phytochemical analysis of the *F.virens* extract was carried out using high performance thin layer chromatography revealed for the presence of flavonoids, bitter principles, coumarins and absence of alkaloids, anthracene derivatives. Acute oral toxicity study (OECD 423) showed no clinical signs of toxicity and mortality even at dose of 2000-5000 mg/kg in 24 h and 14 day observation period. For repeated dose 28-day oral toxicity study (OECD 407) extract was given at dose of 50, 200, and 800 mg/kg and limit dose of 1000mg/kg for 28 days and compared with the control group given with the distilled water. The result showed no significant increase (P>0.05) in biochemical parameters (AST, ALT, BUN and creatinine) and haematological parameters. No significant decrease in feed consumption and body weight gain, also there were no treatment related gross and histopathological changes. In conclusion, methanolic leaf extract of *F. virens* did not revealed any clinical signs of toxicity and mortality in both acute and repeated dose 28-day oral toxicity study at given dose and duration in Wistar albino rats. $LD_{50}$ value may be more than 2000 mg/kg, can be classified as category 5 and indicating non-toxic nature of the methanolic leaf extract of *Ficus virens*.
**Keywords**: *Ficus virens*. OECD; $LD_{50}$ value; Phytochemical analysis; Repeated dose

## INTRODUCTION

*Ficus virens* (Syn. *Ficus infectoria, Ficus lacur*) is a plant belonging to the genus *Ficus* and family Moraceae is found in India, Southeast Asia, Malaysia and Northern Australia. Its common name is white fig and is locally in Hindi language known as pilkhan. It has been reported that all parts of plant are useful in diseases of blood, uterus, burning sensation, hallucinations and unconsciousness, also posses *in vitro* antioxidant property[1,2]. Petroleum, ether, chloroform, methanol and water extracts of *Ficus virens* latex were found to be irritant to the mice ear[3]. In the process of clinical investigation of toxicity cases, a number of naturally occurring plants have been implicated in Western ghat regions of Karnataka and reported that the fresh leaves of *Ficus virens* and its extract were toxic to cattle, rat and rabbit[4]. Systematic toxicity studies were not conducted on *Ficus virens.* Therefore the present study was aimed to evaluate the toxic properties of plant *Ficus virens* in Wistar albino rats.

## MATERIALS AND METHODS

### Plant extract

*Ficus virens* fresh leaves were collected from Talaguppa area in Shimoga District, of Karnataka state during the month of December 2009 and were dried under shade (10 days), finely powdered and stored in air tight container until the preparation of extract.

The powder (100 g) was mixed in 1000 ml of methanol, kept for 5 days and were periodically shaken using an electrical shaker. After 5 days, contents were filtered and it was further concentrated by rotary flask evaporator (Superfit India Ltd., Mumbai) at 39-40°C. The residual methanol from the extract was evaporated after keeping the extract in a petri dish in a vacuum oven at 60° C at the pressure of 25 psi. The extract was then weighed to calculate the yield and stored in air tight container.

### Preliminary phytochemical analysis

Phytochemical analysis of the methanolic leaf extract of *Ficus virens* was carried out using high performance thin layer chromatography (HPTLC) technique[5].

### Acute and repeated dose 28-day oral toxicity study

Toxicity study of methanolic extract of *Ficus virens* leaves was conducted in Wistar albino rats as per the Organization for Economic Co-operation and Development (OECD) guidelines for testing of chemicals, Acute Oral Toxicity – Acute Toxic Class Method [6] (OECD 423) and repeated dose 28-day oral toxicity study[7] (OECD, TG 407).

### Experimental protocol

Healthy young adult Wistar albino rats aged around 6-8 weeks weighing 200 ±10 g were acclimatized to the laboratory conditions for seven days prior to the study and maintained on normal diet (Amrut Laboratory Animal Feeds, Bangalore) and water *ad libitum*. The animal experiment protocols have been approved by the Institutional Animal Ethics Committee (IAEC) with reference No.32/ LPM/IAEC/ 2009 for laboratory animals.

### Acute toxicity study

Three animals were used for each step for determining $LD_{50}$ value. The doses were selected based on Acute Toxic Class method OECD 423. The dose level to be used as the starting dose was selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight. Nine doses were selected for determining $LD_{50}$ value. All the animals were observed for health condition, morbidity and mortality at least twice daily.

### Repeated dose 28-day oral toxicity study

Animals were divided into four groups containing six animals each, the doses for repeated dose 28 day oral toxicity were selected based on initial information on toxicity obtained by acute oral toxicity testing which should not produce mortality but should cause toxicity. If no any information available or no any $LD_{50}$ value determined or no any toxicity data obtained in acute oral toxicity study, then doses were selected on the basis of Draft updated test guideline 407 for "repeated dose 28-day oral toxicity study in rats". For selecting the doses three test groups and a control group were used.

**Range finding study**

The methanolic leaf extract of *Ficus virens* was administered in a constant volume over the range of doses to be tested by varying the concentration of the dosing preparation for 28 days. All the animals were observed for health condition, morbidity and mortality at least twice daily**.** The group details and dose administered per kg are as follows:

| Group rats | Dose type | Concentration (mg/kg) |
|---|---|---|
| Group I | Control | Distilled water |
| Group II | Low dose | 50 |
| Group III | Medium dose | 200 |
| Group IV | High dose | 800 |

**Limit test**

If a test at one dose level of at least 1000 mg/kg body weight/day, for dietary or drinking water administration, an equivalent percentage in the diet, or drinking water (based upon body weight determinations), using the procedures described for this study, produces no observable toxic effects and if toxicity would not be expected based upon data from structurally related compounds, then a full study using three dose levels may not be considered necessary. The limit test applies except when human exposure indicates the need for a higher dose level to be used. The group details and doses administered to rats are as follows:

| Group | Dose type | Concentration (mg/kg) |
|---|---|---|
| Group I | Control | Distilled water |
| Group II | Limit dose | 1000 |

**Hematological and serum biochemical parameters**

Blood samples were collected on day 0, 7, 14, 21 and 28 during the study period by retro-orbital plexus puncture technique using microhaematocrit capillary tubes under ketamine (40 mg/kg, I.P) and xylazine (10 mg/kg, I.M) anaesthesia. The haematological parameters TEC, TLC, Hb and PCV were estimated by using fully automatic blood cell counter (Model PCE-210, ERMA Inc., Tokyo, Japan) and serum biochemistry (AST, ALT, BUN and creatinine) was done by using clinical chemistry analyzer - Microlab 300 (Vitalab Scientific, The Netherlands) following the use of commercially available diagnostic kits from Merck (Ecoline®, Merck Specialties Limited, Kalyan Badlapur Road, M. I. D. C Area, Ambernath)

**Body weight and feed consumption**

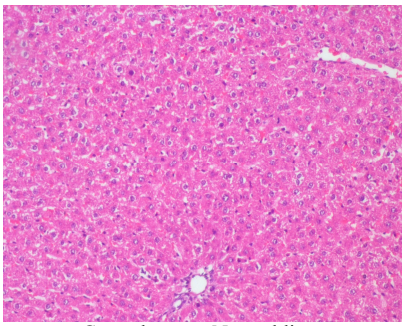
The animals were weighed individually at the beginning of the study and at weekly intervals till the end of study. Feed consumption measurements were made once in a week throughout the study period.

**Pathology**

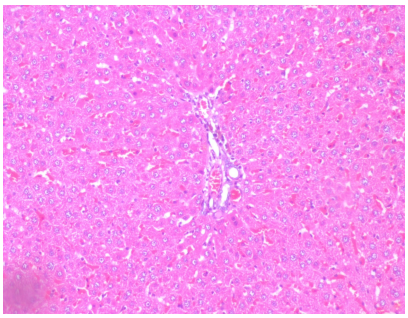
At the end of study period, all the animals in control and treated groups were humanely sacrificed and subjected to detailed gross necropsy including examination of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents. The organs were collected for histopathological study.

**Statistical analysis**

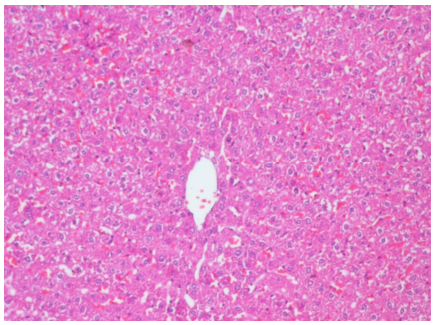
The data obtained from the present study were subjected to statistical analysis. The data were analyzed by using two-way ANOVA, Bonferroni post-test. Mean values and standard error of mean were calculated and all the values are expressed as Mean±SEM. Significance was judged at $P<0.05$.



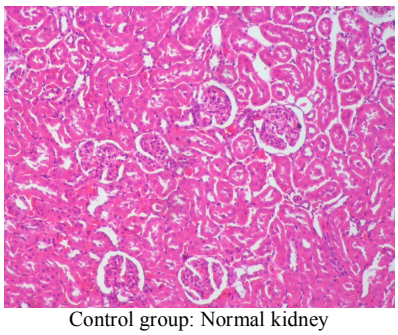
Control group: Normal liver



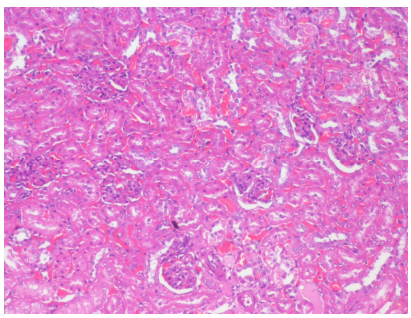
High dose (800 mg/kg): Normal architecture of hepatocytes



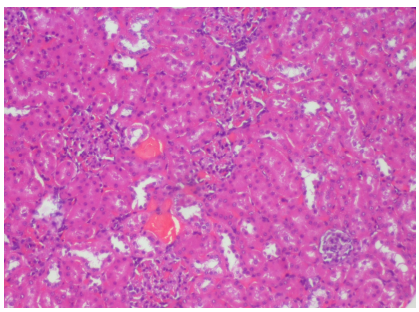
Limit dose (1000 mg/kg): Normal architecture of hepatocytes



Control group: Normal kidney



High dose (800 mg/kg): Normal architecture of tubular epithelium



Limit dose (1000 mg/kg): Slight distension of tubular epithelium

**Effect of methanolic leaf extract of *Ficus virens* in repeated dose 28 day oral toxicity study in Wistar albino rats**

## RESULTS AND DISCUSSION

### Phytochemical analysis

In the present study, methanolic leaf extract of *Ficus virens* was found positive for flavonoids, bitter principle, coumarins and negative for alkaloids, anthracene derivatives.These findings were supported by preliminary phytochemical analysis of the barks of four *Ficus* species including the *F. virens* and this plant showed for the presence of flavonoids and absence of alkaloids [8].

### Toxicity study

### Acute oral toxicity study

In the present study, there were no mortality and clinical signs of toxicity in any of the test groups within 24h after the administration of *Ficus virens* extract. The treated groups were kept for observation for a period of 14 days. No mortality and clinical signs of toxicity were observed in any of the tested groups in a given dose and duration.

It has been supported by *Morinda lucida* leaf extract was non-lethal at 6400 mg/kg in Wistar albino rats in acute oral toxicity study [9] and this was further supported by the chemicals with $LD_{50}$ value >5 g/kg, could be considered as practically non toxic[10].

### Repeated dose 28-day oral toxicity study

The animals did not show any changes in general behavior or other physiological activities, no observable clinical signs of the toxicity, morbidity and mortality during the entire period of the experiment.

Similar observations were supported by *Garcinia mangostana* Linn.Rind plant extract did not reveal any toxicity at the dose of 1000 mg/kg in Sprague- Dwaley rats in repeated dose 28-day oral toxicity study [11]

### Hematological and serum biochemical parameters

In the present study, there were no significant (P>0.05) increase in haematological (TEC, TLC, PCV and Hb) and serum biochemical parameters (AST, ALT, BUN and creatinine) in any of the tested groups as compared to control group in given dose and duration of the study.

These findings were in accordance with the findings of ethanolic extract of *Calotropis gigantea* R.BR in subacute toxicity study in rats did not reveal significant changes in hematological parameters (TEC, TLC, PCV and Hb) and serum biochemical parameters(AST, ALT , BUN and creatinine) at the dose of 1000 mg/kg[12].

### Body weight and feed consumption

In the present study, there were no significant (P>0.05) decrease in feed consumption and body weight in treated groups II, III, IV and limit dose group administered with *Ficus virens* methanolic leaf extract 50, 200,800 and 1000 mg/kg respectively, compared with control group I. This indicated that the *Ficus virens* methanolic leaf extract was non toxic at the given dose and duration of the study.

Similar findings were reported for *Garcinia mangostana* Linn.Rind plant extract did not show significant decrease in body weight and feed consumption at dose of 1000 mg/kg in Sprague- Dwaley rats in repeated dose 28-day oral toxicity study [11].

### Gross and histopathological examination

At necropsy none of the treated groups showed any gross pathological lesions, also liver revealed normal architecture of hepatocytes, kidney showed normal architecture of tubular epithelium and histopathology of all other organs were found normal in both treated groups as compared to control group.

Similar observations were reported for ethanolic extract of *Calotropis gigantea* R.BR in subacute toxicity study in rats at the dose of 1000 mg/kg [12].

## CONCLUSION

There are very few infancy research reports on toxicological property of *Ficus virens*. Even at highest dose *Ficus.virens* did not reveal any clinical signs of toxicity, morbidity and mortality in any of the treated groups in both acute and repeated dose 28 day oral toxicity study, hence $LD_{50}$ value of methanolic leaf extract of *Ficus virens* in Wistar albino rats was more than 2000 mg/kg body weight and no any observed adverse effects noticed. As per globally harmonised system (GHS) of classification and labelling of chemicals, methanolic leaf extract of *Ficus virens* can be classified as category 5 and NOAEL is greater than 800 mg/kg body weight/day. As the extract was negative for alkaloids, hence it may be contributing to non-toxic nature of plant *Ficus virens*.

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**Table 1: Effect of methanolic leaf extract of *Ficus virens* on serum biochemical parameters in repeated dose 28 day oral toxicity study**

| | AST ( U/L) | | | ALT ( U/L) | | | BUN (mg/dL) | | | Creatinine (mg/dL) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Days** | **0** | **14** | **28** | **0** | **14** | **28** | **0** | **14** | **28** | **0** | **14** | **28** |
| **Group I** | 55.78±1.31 | 56.55±0.75 | 60.40±0.31 | 23.60±0.75 | 24.98±1.02 | 26.00±0.52 | 19.69±0.48 | 20.03±0.51 | 20.53±0.43 | 0.61±0.02 | 0.61±0.01 | 0.62±0.01 |
| **Group II** | 55.25±0.53 | 56.75±0.38 | 59.93±0.34 | 23.36±0.47 | 23.78±0.36 | 25.50±0.73 | 18.95±0.51 | 19.58±0.28 | 20.23±0.47 | 0.61±0.01 | 0.62±0.00 | 0.62±0.01 |
| **Group III** | 54.88±1.02 | 57.43±0.44 | 60.56±0.46 | 23.20±0.39 | 24.60±0.50 | 25.16±0.63 | 18.98±0.53 | 20.00±1.26 | 20.87±0.97 | 0.61±0.00 | 0.63±0.00 | 0.64±0.00 |
| **Group IV** | 54.98±0.49 | 58.45±0.26 | 60.33±0.34 | 22.93±0.72 | 25.75±0.73 | 26.21±0.50 | 19.36±0.91 | 20.72±0.87 | 21.53±0.80 | 0.60±0.01 | 0.63±0.01 | 0.65±0.01 |
| **Limit Dose** | 55.98±0.53 | 58.66±0.67 | 59.93±0.84 | 23.41±0.23 | 25.93±0.34 | 26.75±0.40 | 19.91±0.81 | 21.11±0.69 | 21.95±0.67 | 0.62±0.01 | 0.64±0.01 | 0.65±0.01 |

AST: Aspartate aminotransaminase, ALT: Alanine aminotransaminase, BUN: Blood urea nitrogen

**Table 2. Effect of methanolic leaf extract of *Ficus virens* on haematological parameters in repeated dose 28 day oral toxicity study**

| | TEC ($10^6$ cells/$mm^3$) | | | TLC ($10^6$ cells/$mm^3$) | | | Hb (g %) | | | PCV (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Days** | **0** | **14** | **28** | **0** | **14** | **28** | **0** | **14** | **28** | **0** | **14** | **28** |
| **Group I** | 5.15±0.15 | 4.95±0.22 | 5.01±0.17 | 10.81±0.43 | 11.00±0.45 | 11.47±0.33 | 12.68±0.30 | 13.00±0.52 | 12.73±0.40 | 38.80±1.25 | 39.44±0.54 | 39.51±1.49 |
| **Group II** | 5.20±0.22 | 5.03±0.09 | 5.06±0.16 | 11.04±0.27 | 11.44±0.19 | 11.75±0.10 | 12.50±0.12 | 12.88±0.21 | 12.81±0.25 | 39.28±1.22 | 39.77±0.37 | 39.51±1.46 |
| **Group III** | 5.25±0.13 | 5.11±0.12 | 5.08±0.15 | 11.01±0.33 | 11.42±0.28 | 11.92±0.21 | 12.80±0.07 | 12.83±0.09 | 12.88±0.32 | 39.65±0.85 | 39.02±0.46 | 39.87±0.58 |
| **Group IV** | 5.21±0.21 | 5.16±0.19 | 5.15±0.10 | 11.31±0.25 | 11.64±0.17 | 12.06±0.11 | 12.75±0.21 | 13.05±0.21 | 13.18±0.42 | 39.51±1.21 | 39.51±0.78 | 39.83±0.64 |
| **Limit Dose** | 5.11±0.13 | 5.26±0.15 | 5.30±0.05 | 11.39±0.27 | 11.74±0.29 | 11.97±0.19 | 13.03±0.33 | 13.03±0.12 | 13.55±0.26 | 39.22±0.68 | 39.55±0.74 | 40.38±0.43 |

TEC: Total erythrocyte count, TLC: Total leukocyte count, Hb: Haemoglobin, PCV: Packed cell volume

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