PHARMACOLOGICAL AND TOXICOLOGICAL STUDIES OF *FICUS VIRENS* IN RATS

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CERTIFICATE

This is to certify that the thesis entitled "***Pharmacological and Toxicological Studies of Ficus Virens in Rats***" submitted by **Ms. JAYASHREE PATTAR, I.D. No. MVHK 803** in partial fulfillment of the requirements for the award of **Master of Veterinary Science in Veterinary Pharmacology and Toxicology** of the Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar is a record of bonafide research work carried out by her during the period of her study in this University under my guidance and supervision, and the thesis has not previously formed the basis for the award of any degree, diploma, associate ship, fellowship or other similar titles.

Bangalore,

July, 2010

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Dr. SHRIKRISHNA ISLOOR

Dedicated To,

My Beloved Brother Shankar

My loving Uncle Virupaxi and My Parents

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LIST OF ABBREVIATIONS

ALT	Alanine aminotransferase
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
BHT	Butylated hydroxytoluene
BUN	Blood urea nitrogen
n-BuOH	n-Butanol
CAT	Catalase
CCl_4	Carbon tetrachloride
$CHCl_3$	Chloroform
Cm	Centimeter
Cr	Creatinine
C	Degree(s) celsius
DPPH	1, 1-diphenyl-2-picrylhydrazyl
EC_{50}	Effective concentration
EDTA	Ethylene diamine tetra acetic acid
EtOAc	Ethanolic acid
Fig	Figure
ft	feet
g	Gram
g/dl	Gram per deciliter
GAE	Gallic acid equivalent
GPx	Glutathione peroxidase
Hb	Haemoglobin
H_20_2	Hydrogen peroxide
h	Hour
HPTLC	High performance thin layer chromatography
IAEC	Institutional Animal Ethics Committee
I.M.	Intra muscular
I.P.	Intra peritonial
IU/L	International units per liter

Kg	kilogram
LD_{50}	Median lethal dose
m	Meter
mg	Milligram
mg/dl	Milligram per deciliter
mg/kg	Milligram per kilogram
min	Minute
µl	Micro liter
g	Microgram
ml	Milliliter
NBF	Neutral buffered formalin
NOAEL	No Observable Adverse Effects Level
$O^{\cdot}_2$	Superoxide anion radical
OECD	Organization for Economic Cooperation and Development
OD	Optical density
OH^-	Hydroxyl ions
PCV	Packed cell volume
PUFA	Poly unsaturated fatty acid
RE	Rutin equivalent
%	Percentage
±	Plus or minus
SC_{50}	Scavenging concentration
SEM	Standard error mean
SOD	Superoxide dismutase
TEC	Total erythrocyte count
TLC	Total leucocyte count
TLC plates	Thin layer chromatography
UV	Ultraviolet

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Abstract

I INTRODUCTION

Indian traditional medicine is based on various systems including Ayurveda, Siddha, Unani and Homeopathy. The evaluation of these drugs is primarily based on phytochemical, pharmacological and other allied approaches such as chromatography, microscopy and others. With the emerging world wide interest in adopting and studying traditional systems and exploiting their potential based on different healthcare systems, the evaluation of the rich heritage of traditional medicine is essential.

Free radicals and reactive oxygen species play an important role in the pathogenesis of number of degenerative disease and other disease conditions like cancer, aging, liver diseases, respiratory diseases has been widely recognized. Many plants contain phytoconstituents like carotenoids, flavonoids, phenolics etc. which can be used for scavenging free radicals. Such plants can be the potential candidates for the use in stress related diseases (Munganthayaran, 2007).

Man and animals mostly depend on vegetable kingdom for their food. Plants by their metabolic activities besides being the source of feeds and fodder also elaborate other substances viz. alkaloids, glycosides, toxalbumins, essential oils, resins, bitter principles etc. which are important from medicinal and toxicological point of view. Many plants are categorized as poisonous plants. In India, there are more than 700 poisonous plant species belonging to more than 90 different families.

Most of the grazing livestock are indiscriminate eaters, during scarcity period they ingest wide variety of plants which may be poisonous causing serious harmful effects or even death. Many plants though do not cause fatal poisoning, may produce far reaching effects on the health and production of animals. Besides causing adverse effects in animals, the phytotoxins present in plants may enter the human food chain through animal products such as eggs, meat and milk.

Literally, a plant is a storehouse of hundreds of chemicals of diverse biological activities. Most of the chemicals present in the plants are harmless and are necessary for the survival of both the plant and animal kingdoms. The pathway of metabolism that is essential to life is known as primary metabolism and compounds (such as glucose, amino acids etc.) which are directly involved in these pathways are referred to as primary metabolites. Incidentally, a variety of compounds other than the primary metabolites (e.g. sugar) may occur secondarily (e.g. as a part of glycosides).However, the functions of these secondary metabolites (e.g. alkaloids, glycosides etc.) are not clearly understood and they do not appear to be essentially related to the sustenance of life. Plant toxins may be referred to as secondary plant metabolites which are toxic compounds. Most of these secondary toxic metabolites do not have any apparent function in the plant except for defense mechanism or survival adaptions (Garg, 2002).

Ficus virens (Syn.*Ficus infectoria, Ficus lacur*) a plant of the genus *Ficus* belonging to family Moraceae is found in India, Southeast Asia, Malaysia and Northern Australia. Its common name is white fig and is locally known as pilkhan and like many figs, its fruits are edible. It a medium sized tree which grows to a height of 24-27 m in Southeast Asia. It is a fig tree belonging to the group of trees known as strangler figs, because, its seeds can germinate on other trees and grow to strangle and eventually kill the host tree.

The genus *Ficus* constitutes an important group of trees with immense medicinal value. Among the many species, the most important are the 4 trees with milky latex, namely *Ficus racemosa* , *Ficus virens* , *Ficus religiosa*, and *Ficus benghalensis* that constitute the group "*Nalpamaram*" in Ayurveda. The barks of these species form an important ingredient in many Ayurvedic formulations, such as *Nalpamaradi tailam, Chandanasavam,* and *Saribadyasavam* (Sivarajan and Balachandran, 1994). They are used separately or in combination in different formulations.

Ficus virens has been reported to posses antioxidant property (Anandjiwala *et al.*, 2008: Abdel-Hameed ,2009), good corrosion inhibitor(Jain *et al.,* 2006) and leaves are found to have oestrogenic activity (Narayana *et al*., 2003).Stem /bark are used for therapeutic purpose in blood disease, apoplexy, vertigo and delirium and reported to contain α amyrin, β amyrin, lupeol, sitosterol, stigmasterol and campesterol (Amrit Pal Singh, 2006).

It is reported that all parts of plant are useful in diseases of blood, uterus, burning sensation, hallucinations and unconsciosness. Petroleum, ether, chloroform, methanol and water extracts of *Ficus virens* latex were found to be irritant to the mice ear (Narayana *et al*., 2003).

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 are toxic to cattle, rat and rabbit (Narayana and Shridhar, 2004).

Ficus is one of the genus which is rich in total phenolic, tannins and flavonoids, has been shown to posses various biological properties related to antioxidant or free radical scavenging properties (Larson , 1988). Most of the researches have been directed towards *in vitro* evaluation of antioxidant property of *Ficus virens.*However, there is no published literature available on *in vivo* antioxidant property of *Ficus virens* and also systematic toxicity studies are not available on *Ficus virens.* Therefore the present study was under taken to assess its *in vivo* antioxidant and evaluate toxic properties of plant *Ficus virens* in the rats.

Hence, the present study was aimed to conduct pharmacological and toxicological studies of methanol extract of *Ficus virens* leaves in a systematic way using rats as model with following objectives;

1. To study the phytochemical composition of *Ficus virens.*
2. To study the anti-oxidant effect of *Ficus virens.*
3. To study the acute and sub acute toxicity of *Ficus virens.*
4. To correlate the histopathological findings with biochemical studies.

II REVIEW OF LITERATURE

Globally, about 85 percent of the traditional medicines used for primary health care are derived from plants. Over 7500 plant species are used by 4635 communities for human and veterinary healthcare. India has an ancient heritage of traditional medicine. The Materia Medica of India provides a great deal of information on the folklore practices and traditional aspect of therapeutically important plants which are used in the treatment of liver disorders, their extracts, fractions and active constituents exhibit marked hepatoprotective action which has been related to their antioxidant properties (Handa,1997).

It is estimated that of 20,000 species of agricultural forms in India alone, about 9,500 species are of ethnobotanical importance.World Health Organization has listed over 21,000 plant species used around the world for medicinal purpose. In India about 2500 plant species belonging to more than 1000 genera are being used in indigenous systems of medicine (Tewari, 1997). India is tenth among the plant rich countries of the world and fourth among the Asian countries (Rajasekharan *et al.,* 2002).

India is the largest producer of medicinal plants and is called as World Botanical Garden of medicinal plants (Seth and Sharma, 2004). Many antioxidant-based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer. The antioxidative phytochemicals especially phenolic compounds found in vegetables, fruits and medicinal plants have received increasing attention for their potential role in prevention of human diseases (Cai *et al.,* 2004).

Plant of genus *Ficus* consisting of about 800 species of woody trees, shrubs and vines in the family Moraceae are collectively known as fig trees. Several members of the genus *Ficus* are being used traditionally in a wide variety of ethnomedical remedies all over the world (Kone *et al.*, 2004; Hansson, *et al.*, 2005). Phytochemical investigations of some *Ficus* species revealed that phenolic compounds constitute the major components of them (Tuyen *et al.*, 1999; Sandabe *et al.*, 2006). Also, some studies reported the presence of antioxidant activity in some *Ficus* species which was attributed to the phenolic content of them (Daniel *et al.*, 1998; Shukla *et al.*, 2004; Al-Fatimi *et al.*, 2007; Manian *et al.*, 2008).

2.1 Vernacular names of *Ficus virens* in various languages

Language	Vernacular names
English	Spotted fig, White Fig, Sour fig, Grey fig
Kannada	Basari, Basarigoli, Basarimara, Juvvi, Kabbasari, Kari basari, Matai ichchi, Plaksha, Ulabasari
Tulu	Biligoli
Hindi	Gasti, Kahimal, Kaim, Khabar, Keol, Pakar, Pilkhan
Malayalam	Bakri, Chakkila, Cherala, Cherla, Chuvannal, Itti, Jati.
Marathi	Bassari, Dhedumbara, Gandhaumbara, Lendwa, Pakari, Pepar.
Tamil	Jovi, Kallal, Kurugatti, Kurugu, Matai ichchi, Suvi.
Telugu	Badijuvi, Banda juvvi, Jati, Jatti, Juvvi.
Urdu	Pakharia
Gujarati	Pepri.
Punjabi	Bathar, Janglipipli, Pakhar, Palakh, Palkhi, Pilkhan, Pilkin.

Sanskrit	Ashvatthi, Charudarshani, Dridhapraroha, Gardabhanda, Jati, Kandaralu, Karpari, Parkati, Pimpari, Plaksha, Plavaka, Shringi, Suparshva, Varohashakhi, Vati.
Bengali	Pakar
Burmese	Hpak hi, Hyaung pan, Nyaung gyin, Nyaung shin
Chinese	Huang ge shu.
German	Java-Weide, Wurgefeige
Nepalese	Safedkabra
Malay	Ampulu (Indonesia), Ara nasi, Bulu bras (Java), Wunut baygu (Java).

2.2 Scientific classification of *Ficus virens*

Kingdom:	Plantae
Division:	Magnoliophyta
Class:	Magnoliopsida
Order:	Rosales
Family:	Moraceae
Genus:	*Ficus*
Species:	***Ficus virens***
Synonym(s):	***Ficus infectoria, Ficus lacur***

2.3 Description about *Ficus*

The genus has remarkable variation in the habitat of its species. It contains some of the giants of vegetable kingdom, e.g., banyan, peepal and Indian rubber and also small wiry climbers like *Ficus pumila*. Many of these species start their life as epiphytes often destroying their host by their vigorous growth, establish themselves by aerial roots and assume large dimensions by their spreading habits. All species of *Ficus* yield latex containing caoutchouc. Several species produce edible figs of varying palatability ranging from such well-known fruits as those of *Ficus carica* to those, which are hardly touched by birds. Many arborescent species are cultivated for shade and ornament to gardens, parks and road sides. The leaves of many of them are lopped for cattle and elephant as fodder (Anon, 1956).

Ficus a large genus of trees or shrubs, often climbers with milky juice, is widely distributed throughout the tropics of both hemispheres, but is particularly abundant in South East Asia. *Ficus* (Moraceae) a large, pantropical genus with over 750 species, represents an important component of tropical floras (Berg, 1989; Basset *et al.*, 2004). *Ficus* is an important component of rain forest vegetation, particularly in the lowlands. About 65 species occur in India. The more among them being *Ficus bengalensis* (banyan), *Ficus carica* (fig) and *Ficus elastic* (Indian rubber). It retained as a single, large genus because it is well defined by its unique reproductive system, involving syconia fig and specialized pollinator wasps (Novotny *et al.*, 2002).

2.4 Distribution and description about *Ficus virens*

Ficus virens is a plant of the genus *Ficus*. Its common name is white fig and like many figs, its fruits are edible. Ayurvedic synonyms are hasvaplaksa, susita, sitaviryaka, pundra, mahavroha, hasvaparna, pimpri, bhidura and manglachaya. It is a dominant species in the subtropical rainforest of northeastern India, Western Ghats of Karnataka, Kerala and Maharastra, China, Eastern Asia, Japan, Taiwan Bhutan, Nepal, Sri

Lanka,Cambodia, Laos, Myanmar,Thailand, Vietnam Indonesia, Malaysia, Papua New Guinea, Philippines, Australia pacific and Solomonlands.

Ficus virens is a medium sized tree which grows to a height of 24- 27m and up to 32m tall in wetter areas. This fig tree belongs to the group of trees known as strangler figs, because its seeds can germinate on other trees and grow to strangle and eventually kill the host tree. It has two marked growth periods in its Indian environment: in spring (February to early May), and in the time of the monsoon rains (June to early September).

2.4.1 Botany

Ficus virens is a large spreading evergreen low-crowned thick shady tree, 35 to 40 feet high with greenish-grey smooth bark, sending down aerial roots. Leaves are alternate, narrow, and abruptly acuminate. Fruits when ripe are white. The new leaves have a beautiful shade of reddish pink and are very pleasing to the eye. The leaves are 8-19 cm long and 3-6 cm wide, with a whitish midrib. Stipules are less than 1 cm long. The figs are in pairs and greenish-white to brown with spots, latex is present. Bark is flat to curve, measuring 2 to 3 mm in thickness, external surface is ash or grayish-brown in color and rough with numerous lenticels.

Internal surface of the bark is rough, fibrous, longitudinally striated, pale reddish, fracture and fibrous. Periderm is thin measuring 138µm and characteristic feature the periderm tubes, thin phellem, peel off as membranes of one cell thickness (Babu *et al.*, 2010).

2.4.2 Functional uses

2.4.2.1 Uses: Foliage buds are eaten as vegetable and pickle (Siwakoti *et al.* 1997). *Ficus virens* is good source of fuel wood (Ripu *et al.* 2006).

2.4.2.2 Actions: Pungent and astringent in taste and cold in potency.

2.4.2.3 Therapeutics

The barks are used for various purposes: as an astringent medicine, for cooling in action, as haemostatic, as laxative, in improving complexion and it useful in *pitta* and *kapha*. They are used in diabetes, diarrhoea, leucorrhoea, menorrhoea, nervous disorder, and vaginal diseases. It is also widely used in the treatment of skin diseases, ulcer and soreness in the mouth (Joshi and Upadhye, 2008; Gayathri and Kannabiran, 2008). The stem/bark is used in blood diseases, apoplexy, vertigo and delirium (Amrit Pal Singh, 2006).

Panchvalkala a reputed ayurvedic preparation, contains dry powder of stem bark of *Ficus virens* as one of the ingredient. It is astringent in taste, coolant, cures burning and quenches thirst. The decoction is extensively used as anti-inflammatory, to clear ulcers, dress wounds, as a douche in leucorrhoea and other vaginal diseases, and as a gargle in salivation (Chunekar *et al.*1999).

In scabies affected children, the decoction of panchvalkala is used externally and internally. Panchvalkala also forms a part of certain formulations for diarrhoea and leucorrhoea (Vidhyotini *et al.* 2002).

2.4.3 Phytochemical properties of *Ficus virens*

Anandjiwala *et al.* (2008) reported that preliminary phytochemical screening of panchvalkala and its individual components (stem bark of *Ficus benghalensis*, *F. glomerata*, *F. religiosa* , *F. virens* and *Thespesia populnea*) showed the presence of high amount of tannins and phenolics in all the samples. Subsequent quantification revealed that the total phenolic content of panchvalkala and *F.virens* is about 06.89 ± 0.21% w/w and 03.84± 0.03% w/w respectively. Total tannin content of panchvalkala and *F.virens* is about 03.45±0.15 % w/w and 01.64±0.07 % w/w respectively.

Abdel-Hameed (2009) reported that the phytochemical screening of certain Egyptian *Ficus* species leaf samples (*F. afzelli, F. decora, F. lyrata, F. nitida, F. sycomorus and F. virens*) revealed that phenolic compounds are major components of the most active fractions. He also measured the total phenolics and tannins of the EtOAc and n-BuOH fractions by using Folin–Ciocalteu's assay, while the total flavonoids and its subclass flavonols were estimated using aluminium chloride method for flavonoids and aluminium chloride/sodium acetate method for flavonols. The ethyl acetate and n-butanol fractions of *F. virens* showed the total phenols is about 93.50 ± 4.64 and 63.27 ± 4.32 mg/g GAE , total tannins is about 18.40 ± 1.47 and 26.28 ± 2.87 mg/g GAE , total flavonoids is about 48.85 ± 3.58 and 52.29 ± 2.94 mg/g RE and total flavonols is about 33.45 ± 3.11 and 27.34 ± 2.87 mg/g RE respectively.

Babu *et al.*(2010) conducted comparative pharmacognostic studies on the barks of four *Ficus* species (*Ficus racemosa, F. virens, F. religiosa* and *F. benghalensis*) and established the diagnostic keys of these important drugs based on the macroscopic, microscopic, and HPTLC profiles. Preliminary phytochemical screening of 4 *Ficus* spp. barks showed the presence of tannins, saponins, flavonoids, steroids, terpenoids, cardiac glycosides and absence of alkaloids and quinines .They also reported that the percentages of alcohol-soluble and water soluble extractives in *F. virens* are about 2.26 ± 0.78 and 4.39 ± 0.83, respectively and total ash and acid-insoluble ash in *F. virens* are about 11.97 ± 1.18, 2.59 ± 0.45 respectively.

2.4.4 Pharmacological properties of *Ficus virens*

Jain *et al.* (2006) reported that the acid extract of seeds, leaves and bark of the *Ficus.virens* plant has inhibitive action towards hydrochloric and sulfuric acid corrosion of aluminium when tested by using mass loss and thermometric technique.

Yadav *et al.* (2006) conducted study on folk medicine used in gynecological and other related problems in eight district of Haryana based on interviews, informal discussion and observations and reported that *Ficus virens* is one of the plants used for leucorrhoea .The leaves are boiled in water and the water is used as a wash for women genital.

Anandjiwala *et al.* (2008) evaluated the free radical scavenging activity of an ayurvedic preparation Panchvalkala and its individual components (stem bark of *Ficus benghalensis*, *F. glomerata* , *F. religiosa* , *F. virens* and *Thespesia populnea*) in three *in vitro* models *viz* . 1, 1-diphenyl-2-picrylhydrazyl radical scavenging activity, superoxide radical scavenging activity and reducing power assay. Panchvalkala and its individual components showed significant antiradical activity by bleaching 1, 1-diphenyl-2-picrylhydrazyl radical (EC_{50} ranging from 7.27 to 12.08 μg) which was comparable to pyrogallol (EC_{50} 4.85 μg). Thin layer chromatography of the methanol extracts when sprayed with 0.2% 1,1-diphenyl-2-picrylhydrazyl in methanol revealed several bands with antiradical activity as seen by bleaching of 1,1-diphenyl-2-picrylhydrazyl. All the samples showed good superoxide scavenging potential (EC_{50} ranging from 41.55 to 73.56 μg) comparable to ascorbic acid (EC_{50} 45.39 μg) in a dose-dependent manner. The reduction ability, Fe^{3+} to Fe^{2+} transformation was found to increase with increasing concentrations of all the sample extracts.

Abdel-Hameed (2009) reported that the methanol extracts of the leaves of eleven *Ficus* species growing in Egypt were subjected to free radical scavenging activity using 1,1-diphenyl picrylhydrazyl (DPPH) method. Six methanol extracts of six species showed high activity in order: *Ficus lyrata Warb.* > *Ficus afzelli G.* > *Ficus nitida L.* > *Ficus virens Ait.* > *Ficus sycomorus L.*> *Ficus decora Hort.* with SC_{50} 38.37, 60.22, 61.67, 74.00, 79.50 and 81.62 μg/ml, respectively. The free radical scavenging activity of different fractions obtained from successive fractionation of the six methanol extracts with organic solvents of different polarities; petroleum ether, $CHCl_3$, EtOAc and n-BuOH; showed that, the EtOAc and n-BuOH fractions have the high activity with $SC_{50} < 50$ μg/ml whereas petroleum ether and $CHCl_3$ fractions have weak activity at $SC_{50} > 200$ and 100 μg/ml, respectively. The EtOAc and n-BuOH fractions of *F. lyrata* showed the strongest free radical scavenging activity on DPPH with SC_{50} = 8.27 and 12.14 μg/ml, respectively and also the highest antioxidant capacity monitored by phosphomolybdenum method (928.48 and 728.53 mg equivalent to ascorbic acid/g extract).

Oudhia, (2010) reported that traditional healing of Indian state Chhattisgarh, the healers are adding *Parthenium hysterophorus* in complex herbal formulations having plus 100 herbs. These complex herbal formulations are used in treatment of different types of cancer to Type II Diabetes. They are practicing traditional allelopathic knowledge to enrich parthenium with medicinal properties. *Ficus virens* locally known as gasti one of selected herb added in herbal solutions used to enrich parthenium with medicinal properties and all parts of plant used in treatment.

2.4.5 Toxicological properties of *Ficus virens*

Petroleum, ether, chloroform, methanol and water extracts of *Ficus virens* latex were irritant to the mice ear (Narayana *et al*., 2003).

In the process of clinical investigation, 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 are toxic to cattle, rat and rabbit,(Narayana and Shridhar, 2005).

However, there are no systemic data available on toxicological studies of the plant *Ficus virens* in large, small and laboratory animals.

2.4.5.1 Toxicological features of other *Ficus* species are as follows:

It is reported that *Ficus carica* (Common fig) latex is toxic when administered parenterally to animals but has no toxic effects when administered orally. The leaves of *Ficus clavata* are reported to cause skin disease when fed to calves: those of *Ficus nemoralis* contain fair amount of saponins and cause haematuria in cattle (Anon, 1956).

Myburgh *et al.* (1994) reported two outbreaks of neurotoxicosis in cattle browsing on the leaves of *Ficus* species. In the first outbreak, three animals died and one became ill. A sheep developed severe nervous signs, including titanic spasms, when dosed with leaves of *Ficus ingens var.ingens* from the toxic camp where the cattle had died. The second outbreak resulted in death of 12 heifers within 48 hours of ingestion of the leaves of *F.cordata subsp. Salicifolia.* Clinical signs included hyperaesthesia, ataxia, muscle tremors and paddling motions while in lateral recumbency. Similar signs were reproduced by drenching the incriminated leaves to a steer. The sheep dosed with *F.ingens var.ingens* and two cattle, one of which had died during the second outbreak and the steer drenched with *F.cordata subsp. salicifolia*,were necropsied. Light microscopical examination consistently revealed oedema of the central nervous system. In the steer, focal demyelination was evident in localized areas of brain and spinal cord. Liver lesions ranged from mild degeneration to focal disseminated necrosis of hepatocytes.

Dechamp *et al.* (1995) reported an anaphylactic reaction which occurred shortly after ingestion of fresh figs. The IgE-dependent mechanism was demonstrated on the basis of positivity of the prick test performed with fresh figs (*Ficus carica)* extract. In addition, they were able to detect specific IgE to the same extract in the serum.

Krishna (1999) reported the toxicity studies of *Ficus.tsjahela* in cattle, feeding of *F.tsjahela* leaves 20 g/kg to Group I calves did not elicit any clinical signs. Feeding of *F.tsjahela* leaves 40 g/kg to Group II calves induced clinical signs of toxicity 48 h after feeding. Feeding of *F.tsjahela* leaves 60 g/kg to Group III calves induced clinical signs of toxicity at 24 h after feeding. This showed that 20 g/kg nontoxic dose and the maximum toxicity was with 60g/kg feeding of *F.tsjahela* leaves with a short latent period between feeding and the onset of clinical signs. Feeding of *F.tsjahela* leaves 40g/kg had a latent period of 48 h indicating this dose medium toxic dose. Clinical signs manifested during the study were clonic-tonic seizures, nystagmus, hyperaesthesia, ataxia, paddling movements and other neurological behaviours, significant ($p<0.05$) hyperglycemia was noticed

2.4.6 Oxidative stress and Antioxidants

2.4.6.1 Reactive oxygen species

Bandyopadhyay (1999) reviewed the toxicity of reactive oxygen species such as superoxide (O^{-}_2), hydrogen peroxide (H_20_2) and hydroxyl ions (OH^-). The toxicity changes included oxidative damage of cellular macromolecules such as lipids, protein and nucleic acid that lead to various pathological conditions.

Reactive oxygen species (ROS) include a number of chemically reactive molecules derived from oxygen. Some of these molecules are extremely reactive, while some are less reactive. Examples of oxygen free radicals are superoxide, hydroxyl, peroxyl (RO_2•), alkoxyl (RO•), and hydroperoxyl (HO_2•) radicals. Intracellular free radicals, *i.e.*, free, low molecular weight molecules with an unpaired electron, are often called as ROS. The ROS and free radicals are synonymously used. Free radicals and ROS are the oxidants that can readily react with most biomolecules, starting a chain reaction of free radical formation (Blokhina *et al.*, 2003).

2.4.6.2 Oxidative stress

An imbalance between oxidants and antioxidants in favor of the oxidants, potentially leading to damage was termed as "oxidative stress". Oxidative stress is the major factor involved in pathogenesis and progression of many diseases. The indispensable oxygen sometimes become toxic and results in the generation of reactive oxygen species. The ROS can trigger many disorders in biological systems. The oxidative damage to the cells and tissues occurs when the concentration of reactive oxygen species (ROS) exceeds the anti-oxidant capacity (Sies, 1993).

Several types of reactive species were generated in the body as a result of metabolic reactions in the form of free radicals or non radicals. These species were either oxygen derived or nitrogen derived species and called as pro-oxidants. A variety of stress conditions such as nutritional stress, environmental condition and internal stress were associated with free radical generation by decreasing the coupling of oxidation and phosphorylation in mitochondria that result in an increased electron leakage and over production of radicals (Dalton *et al.*, 1999).

Free radicals are reactive molecules involved in many physiological processes and human diseases, such as cancer, aging, arthritis, Parkinson's syndrome, chemia and liver injury. The elevation of free radical levels seen during the liver damage owing to enhanced production of free radicals and decreased scavenging potential of the cells. A variety of intrinsic antioxidants (reduced glutathione, superoxide dismutase, catalase and peroxidase) are present in the organism, which protect them from oxidative stress, thereby forming the first line of defence (Sandhir and Gill, 1999).

Oxidants were formed as a normal product of aerobic metabolism but can be produced at elevated rates under pathophysiological conditions. A balance between oxidant and antioxidant intracellular systems was hence vital for cell function, regulation and adaptation to diverse growth conditions (Fang *et al.*, 2002).

Lipid peroxides were formed by auto-oxidation of PUFA, primarily in cell membrane resulting in membrane damage. Free radical damage to protein has resulted in loss of enzyme activity. Mutagenesis and carcinogenesis were due to damage caused to DNA. Pro-oxidants attack macromolecules including protein, DNA and lipids causing cellular or tissue damage. Oxygen radicals have catalyzed the oxidative modification of lipids resulting in lipid peroxidation (Devasagayam *et al.*, 2004).

Oxidative stress an outcome of imbalance between ROS production and antioxidant defenses, which in turn evokes a series of events deregulating the cellular functions (Bandyopadhyay, 1999). The status of lipid peroxidation indicated by increased levels of TBARS and decreased levels of endogenous defense enzyme systems gives the direct evidence of oxidative stress (Khan, 1995).

2.4.6.3 Antioxidant substances

To counter the effect of pro-oxidants or ROS, the body equipped with antioxidants or free radical scavengers. Normal living cells develop multiple antioxidant defenses and DNA repair systems. The term antioxidant defined as a substance that delays or inhibits oxidative damage to target molecules (Halliwell and Gutteridge, 1990).

Antioxidants are substances present in lower concentration that significantly delays or prevent oxidation of substrates such as protein, lipids, carbohydrates and DNA

(Sen, 1995). These antioxidants are produced either endogenously or received from exogenous sources. The endogenous antioxidants enzymes are Superoxide dismutase (SOD), Catalase (CAT) and Glutathione peroxidase (GPx). Minerals like Selenium, Manganese, Zinc and vitamins like vitamin A, vitamin C and vitamin E also act as antioxidants. Other compounds with antioxidant activity include glutathione, flavonoids and uric acid (Irshad and Chaudhuri, 2002).

2.4.6.4 Enzymatic antioxidants

The superoxide dismutase catalyses conversion of $\dot{O}_2$ (Superoxide) to H_2O_2 (Hydrogen peroxide) and H_2O (Water). Subsequently, catalase converts H_2O_2 to H_2O and O^-_2 GPx reduces H_2O_2 to H_2O by oxidizing glutathione. Reduction of oxidized form of glutathione then catalyzed by glutathione reductase (Bulger *et al.*, 2001).

2.4.6.4a Superoxide Dismutase (SOD)

The endogenous superoxide dismutase may act as an important physiological enhancer of Endothelium Derived Relaxing Factor (EDRF) secreted from vascular endothelium that plays an important role in preventing atherosclerosis (Abrahamsson *et al.*, 1992).

The superoxide dismutase (SOD) a family of metallo-enzyme that converts O^-_2 to H_2O_2 by taking its electron. $O_2^{\dot{}}$ the only known substrate for SOD (Ray and Husain, 2002).

$$O^-_2 + O_2 \rightarrow H_2O_2 + O_2$$

2.4.6.4b Catalase (CAT)

CAT an enzyme present in most cells and found to act 10^4 times faster than peroxidases. It localized mainly in mitochondria and in subcellular respiratory organelles. It catalyses the decomposition of hydrogen peroxide to water and oxygen (Ray and Husain, 2002).

2.4.6.4c Glutathione Peroxidase (GPx)

GPx requires glutathione as a cofactor. It catalyses reaction of H_2O_2 with reduced glutathione to form glutathione disulphide and production of H_2O (Rotruck *et al.*, 1973).

2.4.6.5 Non enzymatic antioxidants

The non enzymatic antioxidants include the lipid soluble vitamins (vitamin E, beta-carotene), the water soluble vitamin (Vitamin C) and Glutathione. Vitamin E located in cell membrane which directly quenches reactive oxygen species including O_2^-, OH^- and O_2. Vitamin C capable of broadly scavenging reactive oxygen species (ROS) including major neutrophil oxidants like H_2O_2 and hypochlorous acid (Bulger *et al.*, 2001).

III MATERIALS AND METHODS

The present work was taken up to evaluate the pharmacological property, acute oral toxicity and repeated dose 28-day oral toxicity in Wistar albino rats. For pharmacological property, acute oral toxicity and repeated dose 28 day oral toxicity studies methanolic extract of *Ficus virens* leaves were used.

3.1 Collection of plant material

Ficus virens fresh leaves (Plate 1B) were collected from Talaguppa area in Shimoga District, of Karnataka State during the month of December 2009. The *Ficus virens* leaves were dried under shade for 10 days and were finely powdered and stored in air tight container until the preparation of extract.

3.2 Preparation of *Ficus virens* leaf extract

One hundred gram of whole leaf powder of *Ficus virens* was mixed in 1000 ml of methanol and kept for 5 days. The contents were periodically shaken using an electric shaker. After 5 days, contents were filtered through Buchner's funnel in a conical flask and it was further concentrated by rotary flash evaporator (Superfit India Ltd., Mumbai) at 39-40^{o}C till the solvent got completely evaporated and extract settled down to bottom. The residual methanol from the extract was evaporated after keeping the extract in a petri dish in a vacuum oven at 60^{o} C at the pressure of 25 psi. The residues were weighed after drying and their respective percentage yield was estimated.

PLATE 1

3.3 Phytochemical analysis

Phytochemical analysis of the *Ficus virens* leaf extract was carried out using HPTLC technique (Wagner *et al.*, 1984).

Analysis are performed for the following constituents:

1. Alkaloids
2. Anthracene derivatives
3. Flavonoids
4. Bitter principles
5. Coumarins
6. Saponins
7. Glycosides

3.3.1 Procedure of TLC

Pre-coated silica gel 60F 254 TLC aluminium 10x10 cm (Merck, Germany) type of plates were used for HPTLC. From each extract 5 µl of *Ficus virens* leaf extract were spotted using CAMAG Linomat 5 on to a TLC silica gel plate. Chromatography was performed using solvent systems. This procedure was followed for the analysis of alkaloids, anthracene derivatives, flavonoids, bitter principles, coumarins, saponins and glycosides.

3.3.2 Alkaloids

Most plant alkaloids are derivatives of tertiary amines, while others contain primary, secondary or quaternary nitrogen. The basicity of individual alkaloids varies greatly, depending on which of the four types represented. The pK_B values (dissociation constants) lie in the range pH 10-12 for very weak bases (e.g. Purines), pH 7 to 10 for weak bases (e.g. Cinchona alkaloids) and pH 3 to 7 for medium strength bases (e.g. Opium alkaloids).

3.3.2.1 Preparation of extracts for TLC

Ficus virens leaf extract (1g) was mixed with 10 ml of 0.5N HCl , contents were vortexed and pellets were discarded, to the supernatant, 30% Na_2CO_3 (pH 10) was added and centrifuged at 2000 rpm for 5 min, supernatant was discarded. The precipitate was washed with chloroform and chloroform extract was collected, again the residue washed with methanol and methanol extract was collected. The chloroform and methanol extract were concentrated to 1 ml and used for chromatography.

3.3.2.2 Chromatography solvent

a) Toluene: Ethyl acetate: Diethyl ammine :: 70:20:10

b) Ethyl acetate: Methanol: Water :: 100:13.5:10 were used as solvent systems for the detection of alkaloids in the *Ficus virens* leaf extract.

3.3.2.3 Detection

a) Without chemical treatment

TLC plates were observed under UV-254 nm and UV-366 nm.

b) Dragendorff reagent

The ready to use reagent (Sd fine-chern limited, Mumbai) was used. 1ml of Dragendorff reagent was diluted with 4 ml of acetic acid and 20 ml of water. The plate was immersed in the reagent for 1 second. The plate was examined under white light. This method was used to detect the presence of Alkaloids and heterocyclic nitrogen compounds.

3.3.3 Anthracene derivatives

The characteristic constituents of this drug group are anthraquinones and their reduced derivatives, oxanthrones, anthranols and anthrones. The anthraquinones possess phenolic group on C-1 and C-8, and keto group on C-9 and C-10. Most compounds in this group are present in the plant as O-glycosides. The glycoside linkage usually at C-1, C-8 or C-6.

3.3.3.1 Preparation of drug extracts for TLC

Ficus virens leaf extract (0.5g) was extracted by warming for 5 min on the water bath with 5 ml of methanol. The clear filtrate was used directly for HPTLC.

3.3.3.2 Chromatography solvent

Ethyl acetate: methanol: water:: 100:17:13, was used as solvent system for the detection of anthracene derivatives in the *Ficus virens* leaf extract.

3.3.3.3 Detection

a) Without chemical treatment

TLC plates were observed under UV-254 nm and UV-366 nm.

b) Natural products-polyethylene glycol

The plate was heated to 100°C for 3 min, then dipped in solution A (1 g diphenylboronic acid aminoether ester was dissolved in 200 ml ethyl acetate), dried in a stream of cold air, then dipped in solution B (10 g polyethylene glycol 400, dissolved in 200 ml dichloromethane).

c) Potassium hydroxide

Five percent ethanolic KOH was prepared and the plate was immersed in the reagent for 1second. The plate was observed in visible and UV-366 nm.

3.3.4 Bitter principles

Most of the bitter principles possess a terpenoid structure, representing derivatives of monoterpenes (secoiridoids), sesquiterpenes, diterpenes and triterpenes.

3.3.4.1 Preparation of drug extracts for TLC

Ficus virens leaf extract (1g) was extracted for 10 min with 10 ml methanol at 60°C on the water bath. The mixture was filtered and the filtrate was evaporated to a volume of about 2 ml.

3.3.4.2 Chromatography solvent

Ethyl acetate: methanol: water:: 77:15:08, was used as solvent system for the detection of bitter principles in the *Ficus virens* leaf extract.

3.3.4.3 Detection

a) Without chemical treatment

TLC plates were observed under UV-254 nm and UV-366 nm.

b) Vanillin-sulphuric acid

The reagent consisted of 5 % ethanolic sulphuric acid (Solution I) 1% ethanolic vanillin (Solution II). The plate was sprayed vigorously with 10 ml Solution I, followed immediately by 5 to 10 ml Solution II after heating the TLC plate at 100° C for 5 to10 min. The plate was examined under white light and UV 366 nm.

3.3.5 Flavonoids

The main constituents of flavonoids are 2-phenyl-γ-benzopyrones or structurally related mostly phenolic compounds. The various types of flavonoid structure differ in the degree of oxidation of ring C, and in the pattern of substitution in the A and/or B rings. Most of these compounds are present in the drug as mono or diglycosides.

3.3.5.1 Preparation of drug extracts for TLC

Ficus virens leaf extract (1g) was extracted with 10 ml methanol for 5 min on a water bath at about 60°C. The clear filtrate was used for chromatography. These rapid methods also extract both lipophilic and hydrophilic flavonoids.

3.3.5.2 Chromatography solvent

Ethyl acetate: formic acid: glacial acetic acid:water :: 100:11:11:27, the ethylacetate, formic acid and glacial acetic acid are mixed first and the water was added gradually with vigorous shaking . This was used as a solvent system for the detection of flavonoids in the *Ficus virens* leaf extract.

3.3.5.3 Detection

a) Without chemical treatment

TLC plates were observed under UV-254 nm and UV-366 nm.

b) Natural products-polyethylene glycol

The plate was heated to 100°C for 3 min, then dipped in solution A (1g diphenylboronic acid aminoether ester was dissolved in 200 ml ethyl acetate), dried in a stream of cold air, then dipped in solution B (10 g polyethylene glycol 400, dissolved in 200ml dichloromethane).

c) Fast blue salt B

The spray reagent was prepared by dissolving 0.5 g fast blue salt in 100 ml of distilled water. The plate was sprayed and was dried. The plate was examined under white light and UV-366 nm.

3.3.6 Coumarins

The active principles of coumarin drugs are benzo-α-pyrones. They can further classify as simple coumarins (herniarin, scopoletin), condensed coumarins (xanthothoxin, psoralene) and dimerin coumarins (daphnoretin).

3.3.6.1 Preparation of drug extracts for TLC

Ficus virens leaf extract (1g) was extracted by shaking with 10 ml methanol for 30 min on the water bath. The clear filtrate was evaporated to about one ml and 20 µl was applied to TLC plate.

3.3.6.2 Chromatography solvent

Toluene: ether (1:1, saturated with 10% acetic acid), was used as solvent system for the detection of coumarins in the *Ficus virens* leaf extract.

3.3.6.3 Detection

a) Without chemical treatment

TLC plates were observed under UV-254 nm and UV-366 nm.

b) Potassium hydroxide

Five percent ethanolic KOH was used as spray reagent. The plate was immersed in the reagent for 1s and was observed at UV-366 nm.

3.3.7 Saponins

The saponins are mainly triterpenes derivatives, with a smaller number of steroids. Sugar residues may be linked via a single OH-group (usually C-3-OH) of the aglycone (monodesmoside saponins) or more rarely via two OH– groups or a single OH-group and a carboxyl group (bis-desmoside saponins).

3.3.7.1 Preparation of drug extracts for TLC

Ficus virens leaf extract (2g) extracted by heating for 10 min under reflux with 10ml of 70% ethanol the clear filtrate evaporated to about 5ml, this was used for chromatography.

3.3.7.2 Chromatography solvent

Chloroform: methanol:water :: 64:50:10, was used as solvent system for the detection of saponins in the *Ficus virens* leaf extract.

3.3.7.3 Detection

a) Without chemical treatment

With the exception of glycyrrhetic acid, no saponins were detectable by exposure to UV-254 nm or UV-366 nm.

b) Blood reagent

Ten ml of 3.6% sodium citrate are added to 90 ml of fresh bovine blood.Two ml of this mixture are mixed with 30 ml phosphate buffer pH 7.4. The plate was sprayed in horizontal position and the plate was observed in visible light.

c) Vanillin-sulphuric acid

The reagent consisted of 5 % ethanolic sulphuric acid (Solution I) 1% ethanolic vanillin (Solution II). The plate was sprayed vigorously with 10 ml Solution I, followed immediately by 5 to 10 ml Solution II after heating the TLC plate at 100° C for 5 to10 min. The plate was examined under white light and UV 366 nm.

3.3.8 Glycosides

Glycosides are ether-like combinations of sugars with other organic structures (non-sugar aglycone or genin). Glycosides are relatively inactive when two parts of the molecules are connected; but when separated from sugar moiety, the genin or aglycone becomes more active *i.e.* toxic. During the digestion in animals, separation of aglycone takes place due to hydrolysis of the O_2 bond between the sugar and aglycone.

3.3.8.1 Preparation of drug extracts for TLC

Ficus virens leaf extract (1g) was extracted by shaking with 10 ml methanol for 30 min on the water bath. The clear filtrate was evaporated to about one ml and 20 µl was applied to TLC plate.

3.3.8.2 Chromatography solvent

Toluene: ether (1:1, saturated with 10% acetic acid) was used as a solvent system for the detection of coumarins in the *Ficus virens* leaf extract.

3.3.8.3 Detection

a) Without chemical treatment

TLC plates were observed under UV-254 nm and UV-366 nm

b) Aniline-diphenylamine phosphoric acid

The spray reagent was prepared using 4 g of diphenylamine and 4 ml aniline which were dissolved in 160 ml acetone. To this 30 ml of O-phosphoric acid was carefully added. The plate was immersed in the reagent for 1 second and then heated at

120°C. The plate was examined under white light. This method was used to detect the presence of sugars and glycosides.

3.4 Pharmacological study

3.4.1 Experimental Animals

Wistar albino rats procured from Indian Institute of Sciences, Bangalore, were used in the present work. The animals were kept separately in cages and were allowed to acclimatize to the experimental conditions for one week before the commencement of actual studies under standard hygienic conditions and provided with Amruth pellet feed supplied by Sai Durga Feeds and Foods, Kamraj Road, Bangalore – 560042 and water ad libitum. The animals were maintained as per the protocol outlined in publication of the Committee for the Purpose of Control and Supervision of Experiments on Animals standard guidelines (CPCSEA) and obtained approval from Institutional Animal Ethics Committee (IAEC) with reference No.32/LPM/IAEC/2009 for laboratory animals.

3.4 .2 Experimental Design

Wistar albino rats weighing 200±10 g were divided into six groups of six animals each of either sex. Group I animals served as normal control, administered with distilled water which was used for dissolving the *Ficus virens* extract. Group II animals served as hepatotoxic control. Group III animals served as standard control, treated with Vit-C (Ascorbic acid) in dose of 200 mg/kg for seven days. Group IV, V and VI animals were served as test groups, treated with daily doses of methanolic extract of *Ficus virens* leaves 50,100 and 150 mg/kg b.w.p.o respectively for 7 days (Mera and Rana, 2006). The animals of Groups II-VI were treated with a single dose of CCl_4 1 ml/kg b.wt, p.o in liquid paraffin (1:1) 6 h after the last treatment to produce acute hepatotoxicity (Sundaram and Mitra , 2007). On day 8 blood samples were collected and serum samples were separated for estimation of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and the rats were sacrificed by decapitation. To avoid gender difference in stress susceptibility hence antioxidant activity was conducted both in male and female rats (Chakraborti *et al.*, 2006). The group details and dose administered to male and female rats are as follows:

Group. no	**No. of female rats**	**No. of male rats**	**Treatment**	**Dose (mg/kg)**
I	6	6	Normal control (Dist.H_2O)	1 ml
II	6	6	CCl_4 control	1 ml
III	6	6	Standard control (Vit-C)	200
IV	6	6	Low dose	50
V	6	6	Medium dose	100
VI	6	6	High dose	150

3.4.2.1 Collection of organs

At the end of the study on day 8, rats were sacrificed humanely to collect liver tissue for assay of thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). The liver, spleen, kidney and heart tissues were collected in 10 % neutral buffered formalin for processing tissue for histopathology.

3.4.3 Estimation of Antioxidant enzymes

3.4.3.1 Tissue Preparation

Immediately after sacrificing the animals, tissues were processed for the estimation of activity of antioxidant enzymes as per the method of Bruce and Baudry (1995). Mainly liver sample was rapidly excised in ice cold normal saline and it was blotted dry and stored at -20 °C for further analysis. Liver was crushed in tissue homogenizer with 0.05 M phosphate buffer (pH 7.4) to make it 10 % liver homogenate w/v (1 g of liver tissue crushed in 10 ml of ice cold 0.05 M phosphate buffer (pH 7.4). This liver homogenate was centrifuged at 1,500 g for 1 h at 4°C and the supernatant obtained was used for the estimation of total proteins, superoxide dismutase, catalase and glutathione peroxidase levels.

For the estimation of thiobarbituric acid reactive substances (TBARS) in tissue was processed as per the modified method of Ramanarayan *et al*., (2000). Fresh liver tissue (0.5g) was collected immediately after the sacrifice and was placed in the tubes kept in ice bath, ice cold working extraction buffer (4ml of extraction buffer stock (20mM phosphate buffer pH 6.5 containing 0.002% (w/v) BHT) +0.6ml 76% TCA pH 2.2) was added and the liver tissue was finely ground and homogenate was used for the estimation of TBARS.

3.4.3.2 Protein estimation

Protein content of the liver tissue was determined by the method described by Lowry *et al.* (1951).

3.4.3.2.1 Principle

The phenolic group of tyrosine and tryptophan residues in protein will produce a blue purple color complex with maximum absorption in the region of 660 nm wavelength with Folin- Ciocalteau reagent which consists of sodium tungstate molybdate and phosphate. Thus, the intensity of color depends on the amount of these aromatic amino acids present and will thus vary for different proteins.

3.4.3.2.2 Reagents

1. **BSA stock solution** (1 mg/ml): Standard stock solution.
2. **Analytical reagents:**

   Solution A: Sodium carbonate 20 g in 1000 ml of 0.1 N Sodium hydroxide.

   Solution B: Copper sulphate (1 g in 100 ml distilled water)

   Solution C: Sodium potassium tartarate (2 g in 100 ml distilled water)

   Solution D: Mix 1 ml each of Solution B and Solution C.

   Later 50 ml of solution A was mixed with 1 ml of solution D.
3. **Folin - Ciocalteau reagent solution:** Dilute Folin-ciocalteau reagent with distilled water in the ratio of 1:2 just before use.

3.4.3.2.3 Procedure

1. Different dilutions of BSA solutions were prepared by mixing stock BSA solution (1 mg/ ml) and water in the test tube as given in the table. The final volume in each of the test tubes was 5 ml. The BSA range 0.05 to 1 mg/ ml

2. From these different dilutions, 0.2 ml protein solution was pipette out to different test tubes and 2 ml of alkaline copper sulphate reagent (analytical reagent) was added.
3. This solution was incubated at room temperature for 10 mins.
4. Then 0.2 ml of Folin - Ciocalteau reagent solution was added to each tube and incubated for 30 min.
5. The optical density reading was taken by measuring the absorbance at 660 nm.
6. A standard curve was plotted for the absorbance (OD values) against known protein concentration.
7. The absorbance (OD values) of unknown samples was measured and the protein concentration was determined using the standard curve plotted.

BSA (ml)	**Water (ml)**	**Sample concentration (mg/ml)**	**Sample volume (ml)**	**Analytical reagent (ml)**	**Folin - Ciocalteau reagent(ml)**
0.25	4.75	0.05	0.2	2	0.2
0.5	4.5	0.1	0.2	2	0.2
1	4	0.2	0.2	2	0.2
2	3	0.4	0.2	2	0.2
3	2	0.6	0.2	2	0.2
4	1	0.8	0.2	2	0.2
5	0	1.0	0.2	2	0.2

3.4.3.3 Estimation of thiobarbituric acid reactive substances (TBARS)

The level of TBARS which are the indicators of lipid peroxidation was measured in tissue homogenate by the method of Ramanarayan *et al.*, (2000).Which based on reaction of malonoldialdehyde (MDA), the breakdown product of membrane polyunsaturated fatty acids, with thiobarbituric acid (TBA) to give a brilliant pink color product. About 4 ml of homogenate was mixed with 16 ml of freshly prepared water: 1.5% Thiobarbituric acid (TBA) in 0.2M Tris Hcl at pH 7 (2:5), vortex the mixture and incubate in water bath at 80°C for 30 min. Then stop the reaction by placing the test tubes in ice bath and add 1 ml ice cold 91% trichloroacetic acid (TCA) followed by 4ml of chloroform, vortex the mixture. Collect the chloroform phase by centrifugation at 2000 g for 10 min at 4°C. The absorbance of chloroform phase having brilliant pink color was measured at 532 nm and the values were expressed as μmol L cm $^{-1}$ of MDA/g tissue.

3.4.3.4 Estimation of Superoxide Dismutase (SOD)

Superoxide dismutase (EC 1.15.1.1) was determined by the method described by Marklund and Marklund (1974).

3.4.3.4.1 Principle

Superoxide anion an intermediate in the auto-oxidation of pyrogallol which occurs at pH 8.2. The ability of SOD to inhibit the auto-oxidation of pyrogallol at pH 8.2 provides the basis for enzymatic activity.

3.4.3.4.2 Procedure

Preparation of homogenate

To 0.5 ml of tissue homogenate, 0.25 ml of ethanol and 0.15 ml of chloroform were added and mechanically shaken for 15 min .Then the contents were centrifuged at 13,000 g for 15 min at 4 °C. The supernatant was carefully separated and used for the test.

Standard pyrogallol autooxidation

To 2 ml of 0.1 M tris Hcl, 0.5 ml of homogenate was added. To this 1.5 ml of distilled water and 0.5 ml of pyrogallol were also added, mixed and the OD value was taken at 0, 1, 2 and 3 min intervals at 420 nm wave length (**X** value).

Assay mixture

Assay mixture consisted of 2 ml of 0.1m Tris Hcl, 0.5 ml of homogenate, 1.5 ml of distilled water and 0.5 ml of pyrogallol. OD value was taken for 3 min at 420 nm wave length.

Calculation: 1\ X value x OD x (Dilution factor) / Total protein (in mg/g tissue)

Unit of activity: The enzyme activity was expressed in terms of units per min per mg of protein. One unit of enzyme corresponds to the amount of enzyme that inhibits pyrogallol auto-oxidation reaction by 50 per cent.

3.4.3.5 Estimation of Catalase (CAT)

Catalase (EC 1.11.1.6) was estimated by the method of Caliborne (1985).

3.4.3.5.1 Principle

Catalase activity was determined by monitoring the decrease in absorbance spectrophotometrically at 240 nm due to decomposition of H_2O_2. The difference in extinction coefficient per unit time was a measure of the catalase activity.

3.4.3.5.2 Procedure

To 0.2 ml of homogenate, 1 ml of 30 mM H_2O_2 was added and the OD value was taken at 240 nm at an interval of 1 min for 3 min. Blank used contained 0.2 ml of distilled water plus 1 ml of 30 mM H_2O_2.

Calculation: OD value / Total protein

Unit of activity: Enzyme activity was expressed as μmol of H_2O_2 decomposed per min per mg of protein.

3.4.3.6 Estimation of Glutathione Peroxidase (GPx)

Glutathione peroxidase (EC 1.11.1.6) was determined by the method described by Rotruck *et al.* (1973).

3.4.3.6.1 Principle

GPx reacts with H_2O_2 and reduced glutathione giving rise to oxidoreductase will form a color complex with dinitrothiobenzoic Acid (DTNB). The intensity of color development directly proportional to amount of GPx present in the tissue.

3.4.3.6.2 Procedure

About 0.4 ml of 0.4 M phosphate buffer was taken and 0.1 ml of sodium azide, 0.2 ml of 4 mM GSH, 0.2 ml tissue homogenate, 0.1 ml of 30 mM H_2O_2 were added and the volume was made up to 2 ml with distilled water. It was incubated for 10 min in room temperature and then 3 ml of Na_2HPO_4 (0.3 M) and 1 ml of DTNB were added. The OD value was taken at 412 nm.

Calculation: OD value X Dilution factor / Total protein

Unit: µM of glutathione utilized / min / mg protein

3.4.4 Statistical analysis

The data obtained from the present study were subjected to statistical analysis. The data were analyzed by using one-way ANOVA.$P<0.05$ was considered significant. The post hoc analysis was carried out by Bonferroni's Multiple Comparison Test. Mean values and standard error of mean was calculated and all the values are expressed as Mean±SEM (GraphPad Prism, 2007).

3.5 Toxicological studies

3.5.1 Experimental Animals

Wistar albino rats procured from Indian Institute of Sciences, Bangalore, were used in the present work. The animals were kept separately in cages and were allowed to acclimatize to the experimental conditions for one week before the commencement of actual studies under standard hygienic conditions and provided with Amruth pellet feed supplied by Sai Durga Feeds and Foods, Kamraj Road, Bangalore – 560042 and water ad libitum. The animals were maintained as per the protocol outlined in publication of the Committee for the Purpose of Control and Supervision of Experiments on Animals standard guidelines (CPCSEA) and obtained approval from Institutional Animal Ethics Committee (IAEC) with reference No.32/LPM/IAEC/2009 for laboratory animals.

3.5.2 Pilot study

Pilot study for the methanol extract of *Ficus virens* leaves was conducted in both male and female Wistar albino rats as per the Organization for Economic Co-operation and Development (OECD) guideline for testing of chemicals.

The animals were fasted overnight prior to the administration of the substance. The methanolic leaf extract of *Ficus virens* in graded doses were administered as a single dose to animals by gavage using a gavaging tube. The volume of administration was maintained to 2 ml/200g through proper dilution of methanolic leaf extract of *Ficus virens*. The group details and dose administered to male and female rats are as follows:

Sl. No.	Group	No. of male rats	Dose (mg/kg)	Group	No. of female rats	Dose (mg/kg)
1	Group I	3	50	Group I	3	50
2	Group II	3	300	Group II	3	300
3	Group III	3	2000	Group III	3	2000
4	Group IV	3	3000	Group IV	3	3000
5	Group V	3	4000	Group V	3	4000
6	Group VI	3	5000	Group VI	3	5000

3.5.3 Acute oral toxicity study

Acute oral toxicity study for the methanolic extract of *Ficus virens* leaves was conducted in both male and female Wistar albino rats as per the Organization for Economic Co-operation and Development (OECD) guideline for testing of chemicals, Acute Oral Toxicity – Acute Toxic Class Method (OECD 423).

3.5.3.1 Study Procedure

3.5.3.1.1 Animal Preparation

Healthy Wistar albino rats aged 7 to 9 weeks weighing 200 ± 10g adult were acclimatized to the laboratory conditions for seven days prior to test before assigning the animals to treatment groups.

3.5.3.1.2 Animal groups and number of animals

Acute toxicity was conducted in both male and female rats separately. Ten groups of rats consisting 10 males in each group were used as one batch for determining LD_{50} value in male rats. Other ten groups consisting 10 female rats in each group were used as another batch for determining LD_{50} value in female rats.

3.5.3.1.3 Dose selection

The doses were selected based on Acute 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.

3.5.3.1.4 Administration of doses

The animals were fasted overnight prior to the administration of methanolic extract of *Ficus virens* leaves in graded doses were administered as a single dose to animals by gavage using a gavaging tube. The volume of administration was maintained to 2 ml/200 g through proper dilution of methanol extract of *Ficus virens* leaves. The group details and dose administered per kg are as follows:

Sl. No.	Group	No. of male rats	Dose (mg/kg)	Group	No. of female rats	Dose (mg/kg)
1	Control	10	Distilled water	Control	10	Distilled water
2	Group I	10	50	Group I	10	50
3	Group II	10	300	Group II	10	300
4	Group III	10	2000	Group III	10	2000
5	Group IV	10	2500	Group IV	10	2500
6	Group V	10	3000	Group V	10	3000
7	Group VI	10	3500	Group VI	10	3500
8	Group VII	10	4000	Group VII	10	4000
9	Group VIII	10	4500	Group VIII	10	4500
10	Group IX	10	5000	Group IX	10	5000

3.5.3.1.5 Observation of animals

General clinical observations were made at least once a day throughout the study period of 14 days considering the period of anticipated effects after dosing. All the animals were observed for health condition, morbidity and mortality at least twice daily.

3.5.4 Repeated dose 28-day oral toxicity study

Repeated dose 28-day oral toxicity study for the methanolic extract of *Ficus virens* leaves was conducted in both male and female Wistar albino rats as per the Organization For Economic Co-operation and Development (OECD 407) guideline for testing of chemicals.

3.5.4.1 Study Procedure

3.5.4.1.1 Animal Preparation

Healthy young adult Wistar albino male and female rats aged around 8-9 weeks weighing 200 ±10 g were acclimatized to the laboratory conditions for seven days prior to the study and before assigning the animals to different groups.

3.5.4.1.2 Selection of doses

The dose was 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, but if from the assessment of other data, no effects would be expected at a dose of 1000 mg/kg, a limit test might be performed. The limit test was primarily used in situations where there was information indicating that the test material was likely to be nontoxic, i.e., having toxicity below regulatory limit doses. Information about the toxicity of the test material can be gained from knowledge about similar tested compounds or similar tested mixtures or products, taking into consideration the identity and percentage of components known to be of toxicological significance. In those situations where there was little or no information about its toxicity, or in which the test material was expected to be toxic, the main test should be performed.

If there were no suitable data available, a range finding study may be performed to aid the determination of the doses to be used. Except for treatment with the test substance, animals in the control group should be handled in an identical manner to the test group subjects.

The dose was selected on the basis of preliminary study, acute oral toxicity study, range finding study for a period of 28 days, where the highest dose was chosen with the aim of inducing toxicity but not death or reverse suffering. Thereafter, a descending sequence of dose was selected with a view to demonstrate any dose related response and No Observable Adverse Effects Level (NOAEL) at the lowest dose. Two to four fold intervals were frequently optimal for setting the descending dose levels and addition of a fourth test group was often preferable to using very large intervals (e.g. more than a factor of 10) between dosages.

3.5.4.1.3 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. The group details and dose administered per kg are as follows:

3.5.4.1.3.1Animal groups and number of animals

Male and female rats acclimatized to laboratory conditions were assigned to control and treatment groups. Four groups consisting of 6 male rats and four groups consisting of 6 female rats in each group were used for the study.

Satellite group of control and high dose groups were maintained for both male and female rats separately as per the study protocol. These groups were maintained for further two weeks after the 28 day period without administering the *Ficus virens* leaf extract.

3.5.4.1.3.2 Administration of doses

The animals were administered with the *Ficus virens* leaf extract at a different concentration and distilled water separately, daily for a period of 28 days by oral gavage as a single dose. The volume of administration was maintained at 2 ml per animal through proper dilutions of *Ficus virens* leaf extract.

The group details and doses administered to male and female rats are as follows

Group Male rats	**Dose type**	**Concentration (mg/kg)**		**Group Female rats**	**Dose type**	**Concentration (mg/kg)**
Group I	Control	Distilled water		Group I	Control	Distilled water
Group II	Low dose	50		Group II	Low dose	50
Group III	Medium dose	200		Group III	Medium dose	200
Group IV	High dose	800		Group IV	High dose	800
Group V	Satellite	800		Group V	Satellite	800

3.5.4.1.4 Limit test

If a test at one dose level of at least 1000 mg/kg body weight/day or, 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 male and female rats are as follows:

Group Male rats	Dose type	Concentration (mg/kg)		Group Female rats	Dose type	Concentration (mg/kg)
Group I	Control	Distilled water		Group I	Control	Distilled water
Group II	Limit dose	1000		Group II	Limit dose	1000

3.5.4.1.5 Observations

General clinical observations were made at least once a day throughout the study period of 28 days considering the period of anticipated effects after dosing. All the animals were observed for health condition, morbidity and mortality at least twice daily.

3.5.4.1.6 Serum biochemical parameters

Serum biochemical parameters were estimated from the serum samples 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) anesthesia. The estimation of serum biochemistry was done by using clinical chemistry analyzer - Microlab 300 (Vitalab Scientific, The Netherlands). The following parameters were estimated using commercially available diagnostic kits from Merck (Ecoline®, Merck Specialties Limited, Kalyan Badlapur Road, M. I. D. C Area, Ambernath) by following the manufacturer instructions furnished in the leaflet supplied along with the diagnostic kit.

1. Aspartate aminotransferase (AST)
2. Alanine aminotransferase (ALT)
3. Blood urea nitrogen (BUN)
4. Creatinine (Cr)

3.5.4.1.7 Hematological parameters

Haematological parameters were estimated using blood samples collected from all the animals on day 0, 7, 14, 21 and 28 by retro-orbital plexus puncture technique using microhaematocrit capillary tubes under ketamine (40 mg/kg, I.P) and xylazine (10 mg/kg, I.M) anesthesia. Calcium Disodium EDTA was used as an anticoagulant. The following haematological parameters were estimated by using fully automatic blood cell counter (Model PCE-210, ERMA Inc., Tokyo, Japan).

1. Total Erythrocyte Count (TEC)
2. Total Leukocyte Count (TLC)
3. Packed Cell Volume (PCV)
4. Hemoglobin (Hb)

3.5.4.1.8 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.

3.5.4.1.9 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, and cranial, thoracic and abdominal cavities and their contents. The organs were collected for histopathological study.

3.5.4.1.10 Collection of organs

After overnight fasting the rats were weighed and sacrificed humanely on day 29 under anesthesia and necropsy was conducted on each carcass to observe any gross pathological changes.

The liver, spleen, kidney, heart, lung, stomach, intestine, brain, testis/ovary were separated from the adhering tissues using saline and collected by placing on the blotting paper and gently pressed to remove excess of saline.

The organs were weighed using electrical balance and the weight of each organ was recorded. Organ to body weight ratio i.e., organ weight/body weight was calculated and expressed in percentage.

The liver, spleen, kidney, heart, lung, stomach, intestine and brain were processed for histopathology by cutting sections of five microns thickness and stained with Haematoxylin and Eosin (Luna, 1968).

3.5.4.2 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 (GraphPad Prism, 2007).

IV RESULTS

Phytochemical analysis, antioxidant study, acute oral toxicity and repeated dose 28-day oral toxicity study of *Ficus virens* leaf extract in rats was carried out in the present investigation and the results obtained were statistically analysed and presented.

4.1 Phytochemical analysis

Phytochemical analysis of the *Ficus virens* leaf extract was carried out using High performance thin layer chromatography technique (Plates 2-3).

4.1.1 Alkaloids

a) Without chemical treatment

There was no pronounced quenching of fluorescence on TLC plates at UV-254 nm and no fluorescence blue or yellow on TLC plates at UV-366 nm (Plate 2A).

b) Dragendorff reagent

No brown or orange bands appeared on TLC plates (Plate 2B).

Inference

The *Ficus virens* leaf extract was negative for the presence of alkaloids.

4.1.2 Anthracene derivatives

a) Without chemical treatment

There was fluorescence quenching on TLC plates at UV-254 nm and development of red-brown bands on TLC plates at UV-366 nm (Plate 2C)

b) Natural products – polyethylene glycol

Red-brown bands appeared on TLC plates in visible light and UV - 366 nm (Plate 2D).

c) Potassium hydroxide

Development of red-brown bands on TLC plates in visible light and UV - 366 nm.

Inference

The *Ficus virens* leaf extract was positive for the presence of anthracene derivatives.

4.1.3 Bitter principles

a) Without chemical treatment

There was fluorescence quenching on TLC plates at UV-254 nm and UV- 366 nm (Plate 3A).

b) Vanillin-sulphuric acid

Brown-red fluorescence appeared on TLC plates at UV-366 nm (Plate 3B).

Inference

The *Ficus virens* leaf extract was positive for the presence of bitter principles.

PLATE 2

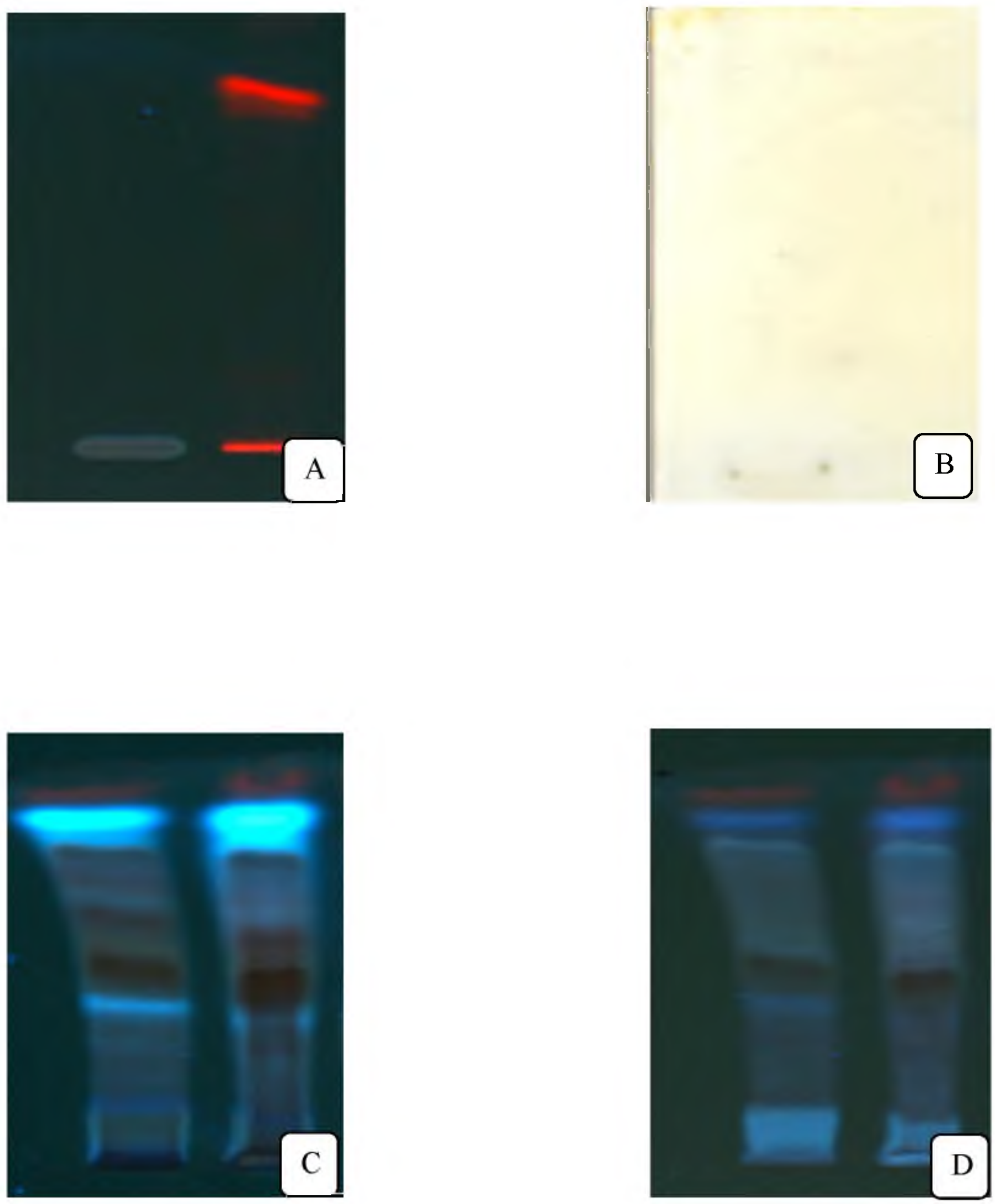
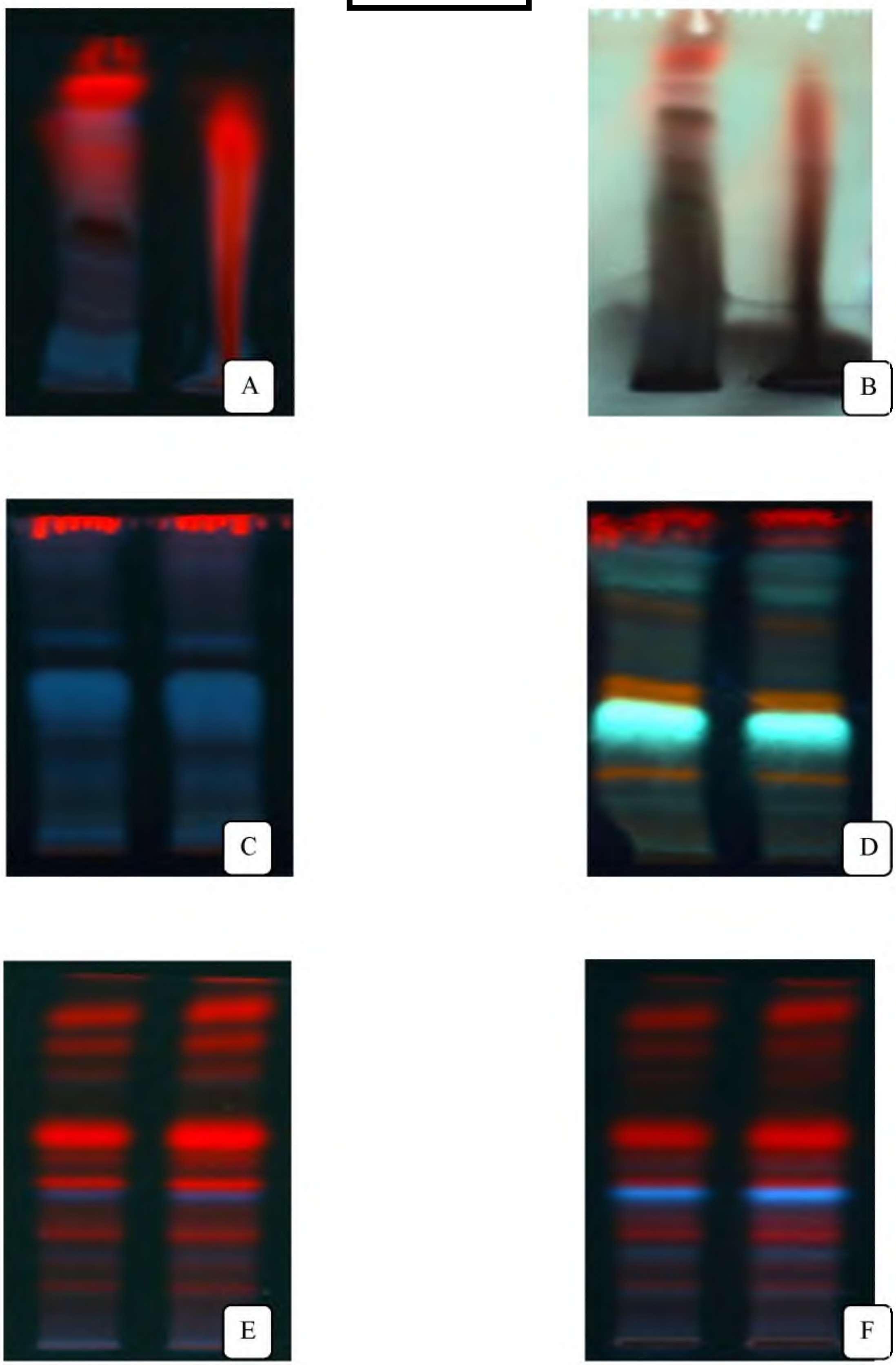


PLATE 3



4.1.4 Flavonoids

a) Without chemical treatment

Fluorescence quenching on TLC plates at UV-254 nm and light blue fluorescence appeared on TLC plates at UV-366 nm (Plate 3C).

b) Natural products – polyethylene glycol

Intense blue fluorescence appeared on TLC plate at UV-366 nm and orange bands appeared in visible light (Plate 3D).

c) Fast blue salt B

Blue fluorescence appeared on TLC plates at UV-366 nm.

Inference

The *Ficus virens* leaf extract was positive for the presence of flavonoids.

4.1.5 Coumarins

a) Without chemical treatment

There was a distinct fluorescence quenching on TLC plate at UV-254 nm and blue fluorescence appeared on TLC plate at UV-366 nm (Plate 3E).

b) Potassium hydroxide

Pronounced blue fluorescence appeared on TLC plates at UV- 366 nm (Plate 3F).

Inference

The *Ficus virens* leaf extract was positive for the presence of coumarins.

4.1.6 Saponins

a) Without chemical treatment

No saponins are detectable by exposure to UV-254 nm or UV-366 nm.

b) Blood reagent

No white zones were observed on TLC plate in the visible light.

c) Vanillin-sulphuric acid

No blue or yellowish zones were observed on TLC plate in the visible light.

Inference

The *Ficus virens* leaf extract was negative for presence of saponins.

4.1.7 Glycosides

a) Without chemical treatment

There was no fluorescence quenching on TLC plate at UV-254 nm.

b) Aniline-diphenylamine phosphoric acid

No orange yellow bands appeared on TLC plate.

4.2 Antioxidant study

4.2.1 Total protein in liver (mg/g of tissue)

The mean ± SE values of total protein in Wistar albino rats subjected to oxidative stress and treated with *Ficus virens* extract are presented in table.1 and fig 1.

4.2.1.1 Female groups

Group II (177.81±4.48) administered with CCl_4 showed significant ($P<0.001$) lower level of total protein concentration as compared to normal control group I (291.96±0.84). When treated groups *i.e* standard control Group III administered with Vit-C (200 mg/kg) and test groups, Group IV, V and VI administered with methanolic extract of *Ficus virens* leaves (50, 100 and 150 mg/kg respectively) compared to Group II, in this Group III, V, VI ($P< 0.001$) and Group IV ($P< 0.05$) showed significant higher level of total protein concentration as compared to CCl_4 control group II. When test groups IV, V and VI (199.35±1.34, 218.97±1.38 and 235.56±5.26) compared with standard control group III (246.94±6.16) there no significant ($P>0.05$) higher level of total protein concentration. Within test groups, Group VI showed significant ($P<0.001$) higher level of total protein concentration as compared to Group IV. Group V showed significant ($P<0.05$) higher level of total protein concentration as compared to Group IV. No significant ($P>0.05$) difference was noticed within other test groups.

4.2.1.2 Male groups

Group II (177.25±4.48) administered with CCl_4 showed significant ($P<0.001$) lower level of total protein concentration as compared to normal control group I

(296.67±3.88). When treated groups *i.e* standard control Group III administered with Vit-C (200 mg/kg) and test groups, Group IV, V and VI administered with methanolic extract of *Ficus virens* leaves (50, 100 and 150 mg/kg respectively) compared to Group II, in this Group III, IV, V and VI ($P< 0.001$) showed significant higher level of total protein concentration as compared to CCl_4 control group II. When test groups IV, V and VI (204.63±2.17, 225.59±6.93 and 252.17±3.00) compared with standard control group III (281.62±1.43) there no significant ($P< 0.05$) higher level of total protein concentration. Within test groups, Group VI showed significant ($P< 0.001$) higher level of total protein concentration as compared to Group IV. Group VI showed significant ($P<0.01$) higher level of total protein concentration as compared to Group V. Group V showed significant ($P<0.05$) higher level of total protein concentration as compared to Group IV.

4.2.2 Thiobarbituric acid reactive substances (TBARS) in Liver (μmoles L^{-1} cm^{-1} of MDA/g tissue)

The mean ± SE values of thiobarbituric acid reactive substances (TBARS) in Wistar albino rats subjected to oxidative stress and treated with *Ficus virens* extract are presented in table.2 and fig 2.

4.2.2.1 Female groups

Group II (24.77±0.13) administered with CCl_4 showed significant ($P<0.001$) higher level of TBARS as compared to normal control group I (7.31±0.34). When treated groups *i.e* standard control Group III administered with Vit-C (200 mg/kg) and test groups, Group IV, V and VI administered with methanolic extract of *Ficus virens* leaves (50, 100 and 150 mg/kg respectively) compared to Group II, in this Group III, IV, V and

VI (P< 0.001) showed significant reduction in TBARS as compared to CCl_4 control group II .Test groups IV, V and VI (22.07±0.04, 21.79±0.37 and 20.72±0.22) showed no significant (P>0.05) reduction in TBARS when compared with standard control group III (16.18±0.01).With in test groups, Group VI showed significant (P<0.01) reduction in TBARS as compared to Group IV. Group VI showed significant (P<0.05) reduction in TBARS as compared to Group V. No significant (P>0.05) difference was noticed within other test groups.

4.2.2.2 Male groups

Group II (24.88±0.02) administered with CCl_4 showed significant (P<0.001) higher level of TBARS as compared to normal control group I (7.96±0.34). When treated groups *i.e* standard control Group III administered with Vit-C (200 mg/kg) and test groups, Group IV, V and VI administered with methanol extract of *Ficus virens* leaves (50, 100 and 150 mg/kg respectively) compared to Group II, in this Group III, IV, V and VI (P< 0.001) showed significant reduction in TBARS as compared to CCl_4 control group II. Test groups IV, V and VI (22.21±0.01, 21.90±0.38 and 21.05±0.10) showed no significant (P>0.05) reduction in TBARS when compared with standard control group III (16.18±0.01).Within test groups, Group VI showed significant (P<0.05) reduction in TBARS as compared to Group IV. No significant (P>0.05) difference was noticed within other test groups.

4.2.3 Antioxidant enzyme profile

4.2.3.1 Superoxide dismutase (SOD) activity in Liver (units/mg protein)

The mean ± SE values of superoxide dismutase (SOD) activity in Wistar albino rats subjected to oxidative stress and treated with *Ficus virens* extract are presented in table.3 and fig 3.

4.2.3.1.1 Female groups

Group II (2.59±0.14) administered with CCl_4 showed significant ($P<0.001$) decrease in SOD activity as compared to normal control group I (6.15±0.57). When treated groups *i.e* standard control Group III administered with Vit-C (200 mg/kg) and test groups, Group IV, V and VI administered with methanol extract of *Ficus virens* leaves (50, 100 and 150 mg/kg respectively) compared to Group II, in this Group III ($P< 0.001$), Group VI ($P< 0.01$) showed significant increase in SOD activity as compared to CCl_4 control group II and no significant ($P>0.05$) difference was noticed in other test groups. When test groups IV, V and VI (2.52±0.16, 3.32±0.28 and 4.47±0.31) compared with standard control group III (5.76±0.39) there was no any significant ($P> 0.05$) increase in SOD activity. Within test groups, Group VI showed significant increase in SOD activity as compared to Group IV. No significant ($P>0.05$) difference was noticed within other test groups.

4.2.3.1.2 Male groups

Group II (2.75±0.15) administered with CCl_4 showed significant ($P<0.001$) decrease in SOD activity as compared to normal control group I (6.09±0.79). When treated groups *i.e* standard control Group III administered with Vit-C (200 mg/kg) and test groups, Group IV, V and VI administered with methanol extract of *Ficus virens* leaves (50, 100 and 150 mg/kg, respectively) compared to Group II, in this Group III ($P< 0.01$) showed significant increase in SOD activity as compared to CCl_4 control group II and no significant ($P>0.05$) difference was noticed in other test groups. When test groups IV, V and VI (2.33±0.14, 3.24±0.14, and 4.28±0.26) compared with standard control group III (5.00±0.35) there was no significant ($P>0.05$) increase in SOD activity. Within test groups, Group VI showed significant ($P< 0.05$) increase in SOD activity as compared to Group IV. No significant ($P>0.05$) difference was noticed within other test groups.

4.2.3.2 Catalase (CAT) activity in liver (µM of H_2O_2 utilized/min/mg protein)

The mean ± SE values of catalase (CAT) activity in Wistar albino rats subjected to oxidative stress and treated with *Ficus virens* extract are presented in table.4 and fig 4.

4.2.3.2.1 Female groups

Group II (44.01±3.64) administered with CCl_4 showed significant ($P<0.001$) decrease in catalase activity as compared to normal control group I (109.10±6.04). When treated groups *i.e* standard control Group III administered with Vit-C (200 mg/kg) and test groups, Group IV, V and VI administered with methanol extract of *Ficus virens* leaves (50, 100 and 150 mg/kg respectively) compared to Group II, in this Group III ($P< 0.001$), Group VI ($P< 0.05$) showed significant increase in catalase activity as compared to CCl_4 control group II and no significant ($P>0.05$) difference was noticed in other test groups. Test groups IV, V and VI (45.55±3.07, 62.39±5.27and 77.75±4.30) showed no significant ($P>0.05$) increase in catalase activity when compared with standard control group III (103.13±4.87).Within test groups, Group VI showed significant ($P<0.01$) increase in catalase activity as compared to Group IV. No significant ($P>0.05$) difference was noticed within other test groups.

4.2.3.2.2 Male groups

Group II (47.18±3.27) administered with CCl_4 showed significant ($P<0.001$) decrease in catalase activity as compared to normal control group I (107.91±6.05). When treated groups *i.e* standard control Group III administered with Vit-C (200 mg/kg) and test groups, Group IV, V and VI administered with methanol extract of *Ficus virens* leaves (50, 100 and 150 mg/kg respectively) compared to Group II, in this Group III ($P< 0.001$) showed significant increase in catalase activity as compared to CCl_4 control group II and no significant ($P>0.05$) difference was noticed in other test groups. Test groups IV, V and VI (42.37±2.89, 59.53±4.50, and 71.31±2.86) showed no significant ($P>0.05$) increase in catalase activity when compared with standard control group III (96.14±2.41).Within test groups, Group VI showed significant ($P<0.05$) increase in catalase activity as compared to Group IV. No significant ($P>0.05$) difference was noticed within other test groups.

4.2.3.3 Glutathione Peroxidase (GPx) activity in liver (units/mg protein)

The mean ± SE values of glutathione peroxidase (GPx) activity in wistar albino rats subjected to oxidative stress and treated with *Ficus virens* extract are presented in table 5 and fig 5.

4.2.3.3.1 Female groups

Group II (46.71±3.17) administered with CCl_4 showed significant ($P<0.001$) decrease in glutathione peroxidase activity as compared to normal control group I (91.05±3.96). When treated groups *i.e* standard control Group III administered with Vit-C (200 mg/kg) and test groups, Group IV, V and VI administered with methanolic extract of *Ficus virens* leaves (50, 100 and 150 mg/kg respectively) compared to Group II, in this Group III ($P< 0.001$), Group VI ($P< 0.05$) showed significant increase in glutathione peroxidase activity as compared to CCl_4 control group II and no significant ($P>0.05$) difference was noticed in other test groups. Test groups IV, V and VI (43.54±2.56, 50.80±2.36 and 64.93±3.97) showed no significant ($P>0.05$) increase in glutathione peroxidase activity when compared with standard control group III (88.52±4.55).Within test groups, Group VI showed significant ($P<0.01$) increase in glutathione peroxidase activity as compared to Group IV. No significant ($P>0.05$) difference was noticed within other test groups.

4.2.3.3.2 Male groups

Group II (47.18±3.27) administered with CCl_4 showed significant ($P<0.001$) decrease in glutathione peroxidase activity as compared to normal control group I (87.11±1.45). When treated groups *i.e* standard control Group III administered with Vit-C (200 mg/kg) and test groups, Group IV, V and VI administered with methanolic extract of *Ficus virens* leaves (50, 100 and 150 mg/kg respectively) compared to Group II, in this Group III ($P< 0.001$) showed significant increase in glutathione peroxidase activity as compared to CCl_4 control group II and no significant ($P>0.05$) difference was noticed in other test groups. Test groups IV, V and VI (40.11±1.79, 50.76±4.39, and 56.85±2.16) showed no significant (P> 0.05) increase in glutathione peroxidase activity when compared with standard control group III (82.56±2.45) Within test groups, Group VI showed significant (P< 0.01) increase in glutathione peroxidase activity as compared to Group IV. No significant (P>0.05) difference was noticed within other test groups.

4.2.4 Serum biochemistry

4.2.4.1 Aspertate aminotransferase(AST) activity (U/L)

The mean ± SE values of aspertate aminotransferase(AST) activity (U/L) in Wistar albino rats subjected to oxidative stress and treated with *Ficus virens* extract are presented in table.6 and fig 6.

4.2.4.1.1 Female groups

Group II (157.39±3.84) administered with CCl_4 showed significant (P<0.001) increase in AST value as compared to normal control group I (58.01±0.57). When treated groups *i.e* standard control Group III administered with Vit-C (200 mg/kg) and test groups, Group IV, V and VI administered with methanolic extract of *Ficus virens* leaves (50, 100 and 150 mg/kg respectively) compared to Group II, in this Group III, V and VI (P< 0.001) showed significant decrease in AST value as compared to CCl_4 control group II. When test groups IV, V and VI (154.29±2.85, 140.60±0.32 and 132.70±0.86) compared with standard control group III (114.62±1.29) there was no significant (P>0.05) decrease in AST value. Within test groups, Group V and VI showed significant (P<0.001) decrease in AST values as compared to Group IV. No significant (P>0.05) difference was noticed within other test groups.

4.2.4.1.2 Male groups

Group II (158.80±2.64) administered with CCl_4 showed significant ($P<0.001$) increase in AST value as compared to normal control group I (59.13±1.59). When treated groups *i.e* standard control Group III administered with Vit-C (200 mg/kg) and test groups, Group IV, V and VI administered with methanolic extract of *Ficus virens* leaves (50, 100 and 150 mg/kg, respectively) compared to Group II, in this Group III and VI ($P< 0.001$) showed significant decrease in AST value as compared to CCl_4 control group II. When test groups IV, V and VI (155.19±5.33, 147.21±4.36 and 135.13±0.66) compared with standard control group III (120.49±1.24) there was no significant ($P>0.05$) decrease in AST value. With in test groups, Group VI showed significant ($P<0.01$) decrease in AST values as compared to Group IV. No significant ($P>0.05$) difference was noticed within other test groups.

4.2.4.2 Alanine aminotransferase(ALT) activity (U/L)

The mean ± SE values of alanine aminotransferase (ALT) activity (U/L) in Wistar albino rats subjected to oxidative stress and treated with *Ficus virens* extract are presented (Table.7 and Fig.7).

4.2.4.2.1 Female groups

Group II (78.66±1.91) administered with CCl_4 showed significant ($P<0.001$) increase in ALT value as compared to normal control group I (24.18±0.71). When treated groups *i.e* standard control Group III administered with Vit-C (200 mg/kg) and test groups, Group IV, V and VI administered with methanolic extract of *Ficus virens* leaves (50, 100 and 150 mg/kg respectively) compared to Group II, in this Group III, V, VI (P<

Table 1: Mean± SE values of total protein (mg/g of liver tissue) in different groups of rats (n=6)

Groups	Female Group	Male Group
Group I	291.96±0.84	296.67±3.88
Group II	177.81±4.48[a]	177.25±4.48[A]
Group III	246.94±6.16[b]	281.62±1.43[B]
Group IV	199.35±1.34[d]	204.63±2.17[C]
Group V	218.97±1.38[cf]	225.59±6.93[CF]
Group VI	235.56±5.26[ce]	252.17±3.00[CDE]

Female groups: [a]P< 0.001 as compared to Group I, [b]P< 0.001, [c]P< 0.001 , [d]P< 0.05 as compared to Group II, [e]P< 0.001 as compared to Group IV, [f]P< 0.05 as compared to Group IV.

Male groups: [A]P< 0.001 as compared to Group I, [B]P< 0.001, [C]P< 0.001 as compared to Group II, [D]P< 0.001 as compared to Group IV, [E]P< 0.01 as compared to Group V, [F]P< 0.05 as compared to Group IV.

* P<0.05, ** P<0.01, ***P<0.001

Table 2: Mean ± SE values of thiobarbituric acid reactive substances TBARS (µM L^{-1} cm-1 MDA /g of tissue) in different groups of rats (n=6)

Groups	Female	Male
Group I	7.31±0.34	7.96±0.34
Group II	24.77±0.13^{a}	24.88±0.02^{A}
Group III	16.18±0.01^{b}	16.34±0.05^{B}
Group IV	22.07±0.04^{c}	22.21±0.01^{C}
Group V	21.79±0.37^{ce}	21.90±0.38^{C}
Group VI	20.72±0.22^{cd}	21.05±0.10^{CD}

Female groups: ^{a}P< 0.001 as compared to Group I, ^{b}P< 0.001, ^{c}P< 0.001 as compared to Group II, ^{d}P< 0.01 as compared to Group IV, ^{e}P< 0.05 as compared to Group VI.

Male groups: ^{A}P< 0.001 as compared to Group I, ^{B}P< 0.001, ^{C}P< 0.001 as compared to Group II, ^{D}P< 0.05 as compared to Group IV

* P<0.05, ** P<0.01, ***P<0.001

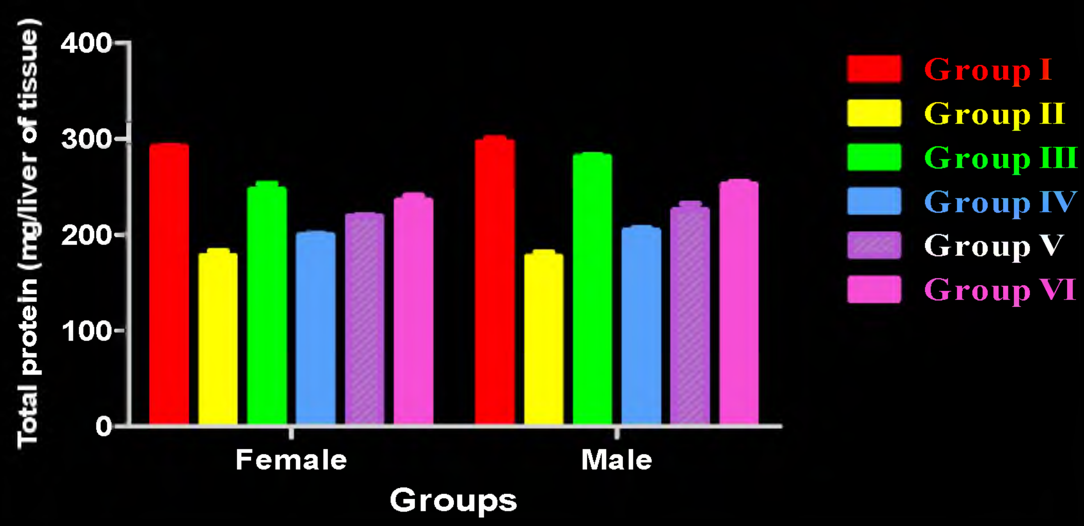


Fig.1 Mean ± SE of Total protein (mg/g of liver tissue)

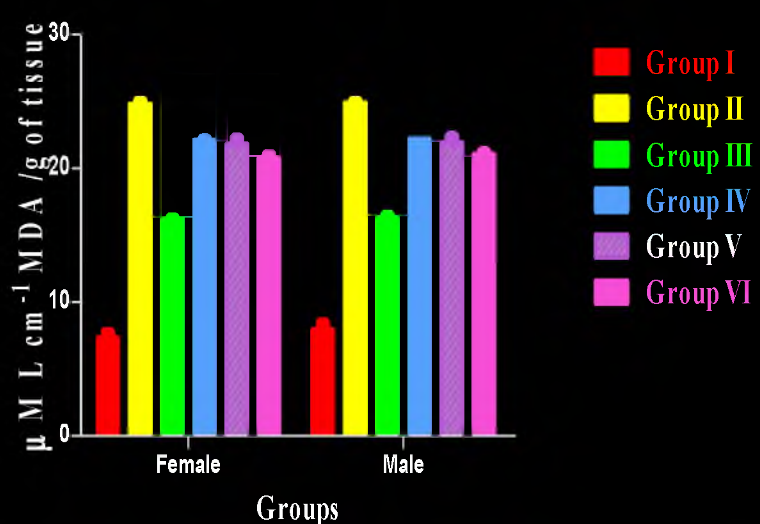


Fig.2 Mean ± SE values of TBARS (μ M L cm^{-1} MDA /g of tissue) in liver tissue of different groups of rats

Table 3. Mean ± SE values of superoxide dismutase (SOD) activity (units/mg protein) in different groups of rats (n=6)

Groups	Female	Male
Group I	6.15±0.57	6.09±0.79
Group II	2.59±0.14[a]	2.75±0.15[A]
Group III	5.76±0.39[b]	5.00±0.35[B]
Group IV	2.52±0.16	2.33±0.14
Group V	3.32±0.28	3.24±0.14
Group VI	4.47±0.31[cd]	4.28±0.26[C]

Female groups: [a]P< 0.001 as compared to Group I, [b]P< 0.001, [c]P< 0.05 as compared to Group II, [d]P< 0.01 as compared to Group IV

Male groups : [A]P< 0.001 as compared to Group I, [B]P< 0.01 as compared to Group II , [C]P< 0.05 as compared to Group IV

* P<0.05, ** P<0.01, ***P<0.001

Table 4: Mean ± SE values of catalase (CAT) activity (μmol of H_2O_2/min/mg protein) in different groups of rats (n=6)

Groups	Female	Male
Group I	109.10±6.04	107.91±6.05
Group II	44.01 ± 3.64^{a}	47.18 ± 3.27^{A}
Group III	103.13 ± 4.87^{b}	96.14 ± 2.41^{B}
Group IV	45.55±3.07	42.37±2.89
Group V	62.39±5.27	59.53±4.50
Group VI	77.75 ± 4.30^{cd}	71.31 ± 2.86^{C}

Female groups: ^{a}P< 0.001 as compared to Group I, ^{b}P< 0.001, ^{c}P< 0.05 as compared to Group II, ^{d}P< 0.01 as compared to Group IV

Male groups : ^{A}P< 0.001 as compared to Group I, ^{B}P< 0.001 as compared to Group II , ^{C}P< 0.01 as compared to Group IV

* P<0.05, ** P<0.01, ***P<0.001

Fig.3 Mean ± SE values of Superoxide dismutase (SOD) activity (units/mg protein) in liver tissue of different groups of rats

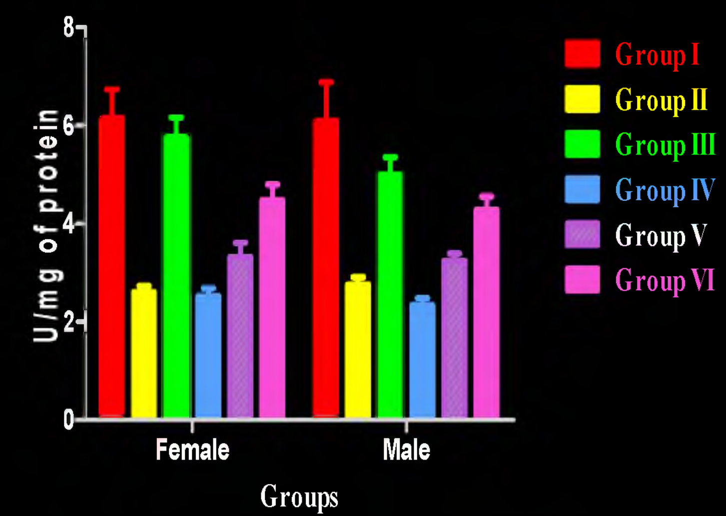


Fig.4 Mean ± SE values of Catalase (CAT) activity (μmol of H_2O_2/min/mg protein) in liver tissue of different groups of rats

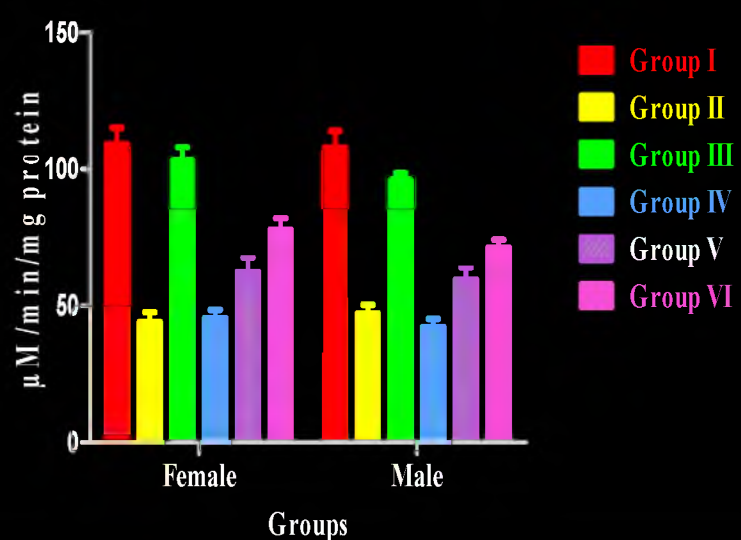


Table 5: Mean ± SE values of glutathione peroxidase (GPx) activity (units/mg protein) in different groups of rats (n=6)

Groups	Female	Male
Group I	91.05±3.96	87.11±1.45
Group II	46.71±3.17[a]	47.15±2.65[A]
Group III	88.52±4.55[b]	82.56±2.45[B]
Group IV	43.54±2.56	40.11±1.79
Group V	50.80±2.36	50.76±4.39
Group VI	64.93±3.97[cd]	56.85±2.16[C]

Female groups: [a]P< 0.001 as compared to Group I, [b]P< 0.001, [c]P< 0.05 as compared to Group II, [d]P< 0.01 as compared to Group IV

Male groups: [A]P< 0.001 as compared to Group I, [B]P< 0.001 as compared to Group II, [C]P< 0.01 as compared to Group IV

* P<0.05, ** P<0.01, ***P<0.001

Table 6: Mean ± SE values of serum AST activity (U/L) in different groups of rats (n=6)

Groups	Female	Male
Group I	58.01±0.57	59.13±1.59
Group II	157.39±3.84[a]	158.80±2.64[A]
Group III	114.62±1.29[b]	120.49±1.24[B]
Group IV	154.29±2.85	155.19±5.33
Group V	140.60±0.32[cd]	147.21±4.36
Group VI	132.70±0.86[cd]	135.13±0.66[CD]

Female groups: [a]P< 0.001 as compared to Group I, [b]P< 0.001, [c]P< 0.001 as compared to Group II, [d]P< 0.001 as compared to Group IV

Male groups: [A]P< 0.001 as compared to Group I, [B]P< 0.001, [C]P< 0.001 as compared to Group II, [D]P< 0.01 as compared to Group IV.

* P<0.05, ** P<0.01, ***P<0.001

Fig.5 Mean ± SE values of Glutathione Peroxidase (GPx) activity (units/mg protein) in liver tissue of different groups of rats

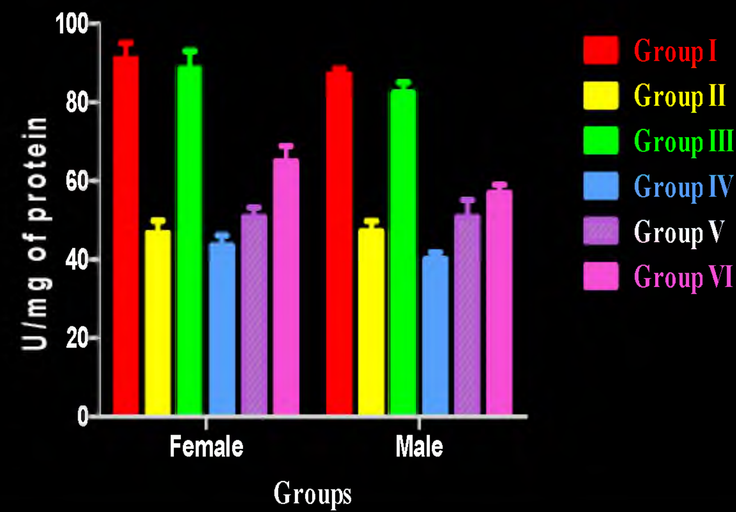


Fig.6 Mean ± SE values of Aspartate aminotransferase (AST) activity (U/L) in liver tissue of different groups of rats

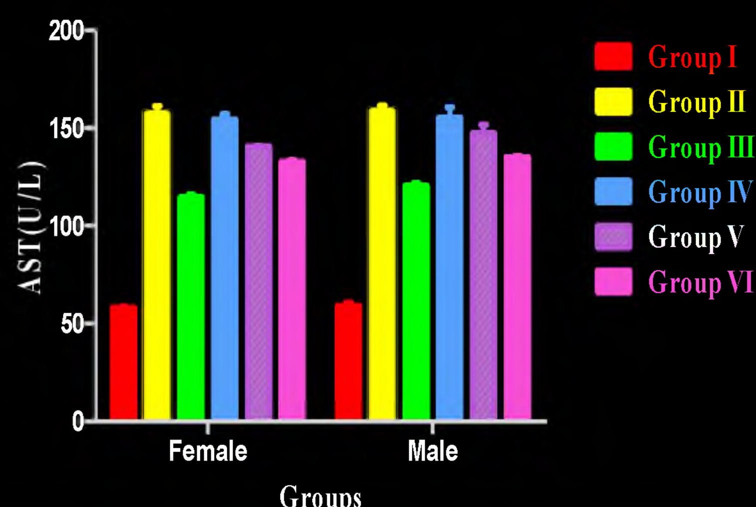


Table 7. Mean ± SE values of serum ALT activity (U/L) in different groups of rats (n=6)

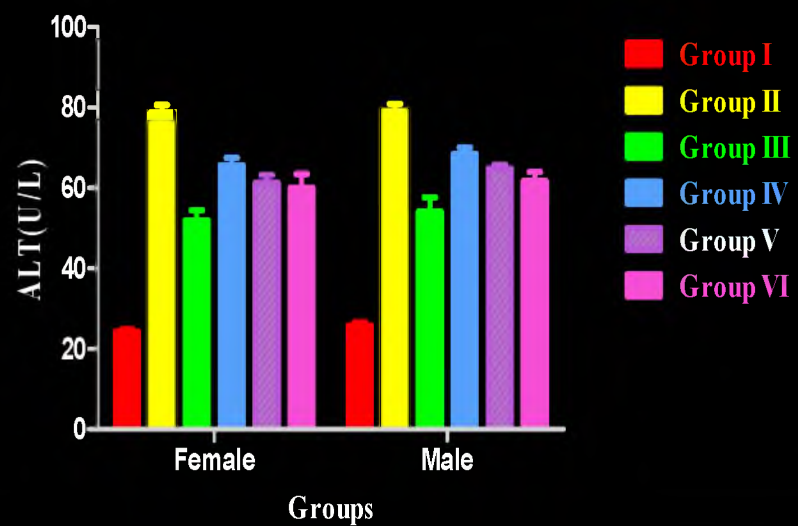
Groups	Female	Male
Group I	24.18±0.71	25.78±0.83
Group II	78.66±1.91[a]	79.12±1.71[A]
Group III	51.91±2.55[b]	54.03±3.55[B]
Group IV	65.63±1.83[d]	68.38±1.63[D]
Group V	61.22±1.94[c]	64.78±0.97[C]
Group VI	60.04±3.39[c]	61.73±2.21[C]

Female groups: [a]P< 0.001 as compared to Group I, [b]P< 0.001, [c]P< 0.001 , [d]P< 0.01 as compared to Group II .

Male groups: [A]P< 0.001 as compared to Group I, [B]P< 0.001, [C]P< 0.001, [D]P< 0.01as compared to Group II.

* P<0.05, ** P<0.01, ***P<0.001

Fig.7 Mean ± SE values of Alanine Aminotransferase (ALT) activity (U/L) in liver tissue of different groups of rats



0.001) and Group IV ($P< 0.01$) showed significant decrease in ALT value as compared to CCl_4 control group II. When test groups IV, V and VI (65.63±1.83, 61.22±1.94 and 60.04±3.39) compared with standard control group III (51.91±2.55) there was no significant ($P>0.05$) decrease in ALT value. With in test groups IV, V and VI no any significant ($P>0.05$) difference was noticed.

4.2.4.2.2 Male groups

Group II (79.12±1.71) administered with CCl_4 showed significant ($P<0.001$) increase in ALT value as compared to normal control group I (25.78±0.83). When treated groups *i.e* standard control Group III administered with Vit-C (200 mg/kg) and test groups, Group IV, V and VI administered with methanolic extract of *Ficus virens* leaves (50, 100 and 150 mg/kg respectively) compared to Group II, in this Group III, V, VI ($P< 0.001$) and Group IV ($P< 0.01$) showed significant decrease in ALT value as compared to CCl_4 control group II. When test groups IV, V and VI (65.63±1.83, 61.22±1.94 and 60.04±3.39) compared with standard control group III (51.91±2.55) there was no significant ($P>0.05$) decrease in ALT value. With in test groups IV, V and VI no significant ($P>0.05$) difference was noticed.

4.2.5 Histopathology

Histopathological studies were conducted in all the animals from treated group III, IV, V and VI were compared with that of the CCl_4 control group rats. Animals in model control group II (without any treatment but with hepatotoxicity by CCl_4) compared with normal group rats (Plate 4-5).

4.2.5.1Control group female and male rats

The histological features of the liver of the control groups of both male and female rats were apparently normal (Plate 4A and 5A).

4.2.5.2 Group II female and male rats

Animals in this model control group showed massive large scale necrotic area, inflammatory changes, vascular degeneration and haemorrhages with loss of architecture (Plate 4B and 5B).

4.2.5.3 Group III female and male rats

Animals treated with Vit-C showed significant restoration of hepatocytes with mild degree of congestion (Plate 4C and 5C).

4.2.5.4 Group IV female and male rats

This group treated with *Ficus virens* (50 mg/kg) showed non significant restoration of hepatocytes with massive degree of congestion, degenerative changes and necrosis as compared to Group III, but showed similar lesions, congestion and necrosis as compared to Group II (Plate 4D and 5D).

4.2.5.5 Group V female and male rats

This group treated with *Ficus virens* (100 mg/kg) showed non significant restoration of hepatocytes with congestion and necrosis as compared to Group III, but showed mild degree of congestion and necrosis as compared to Group II (Plate 4E and 5E).

PLATE 4

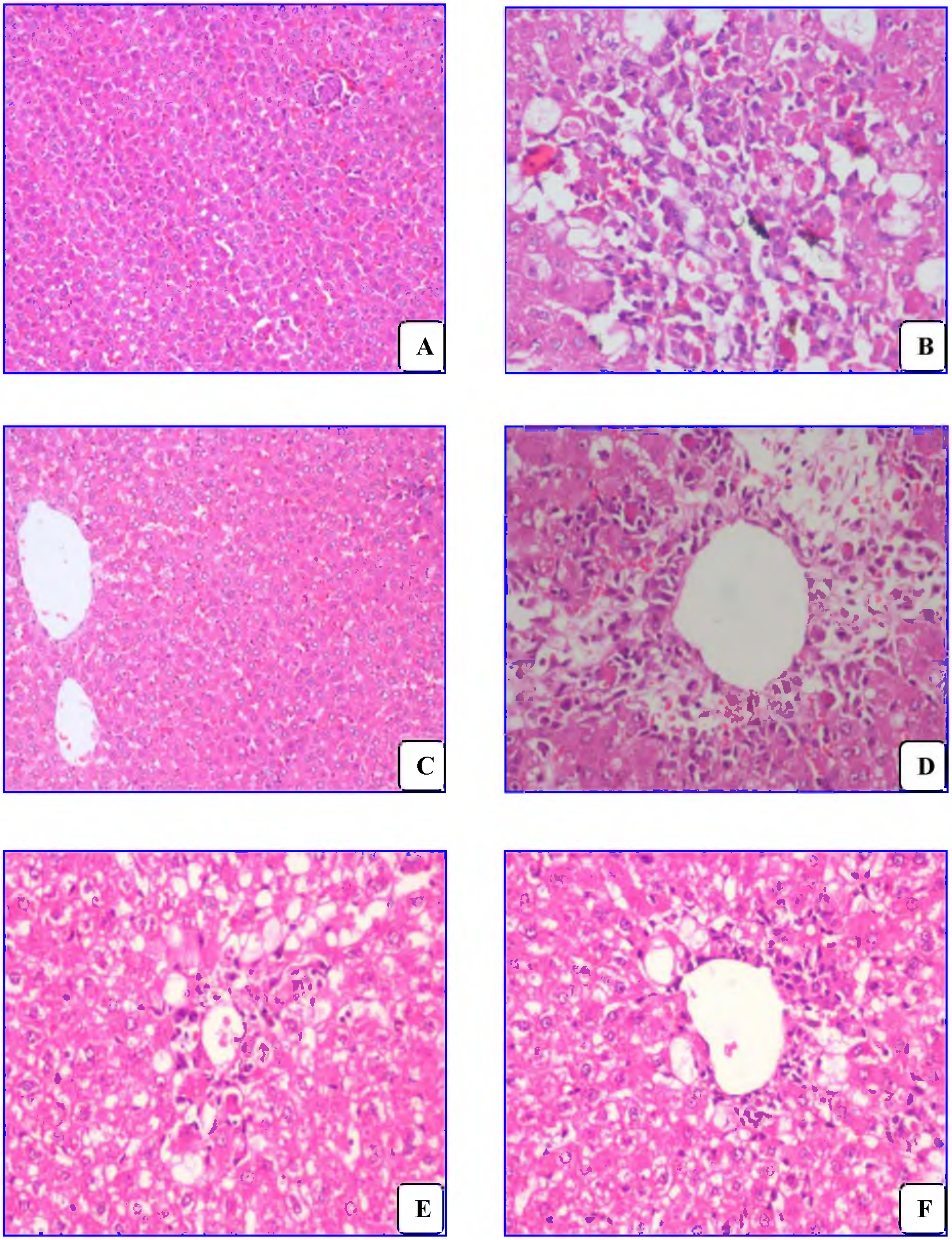
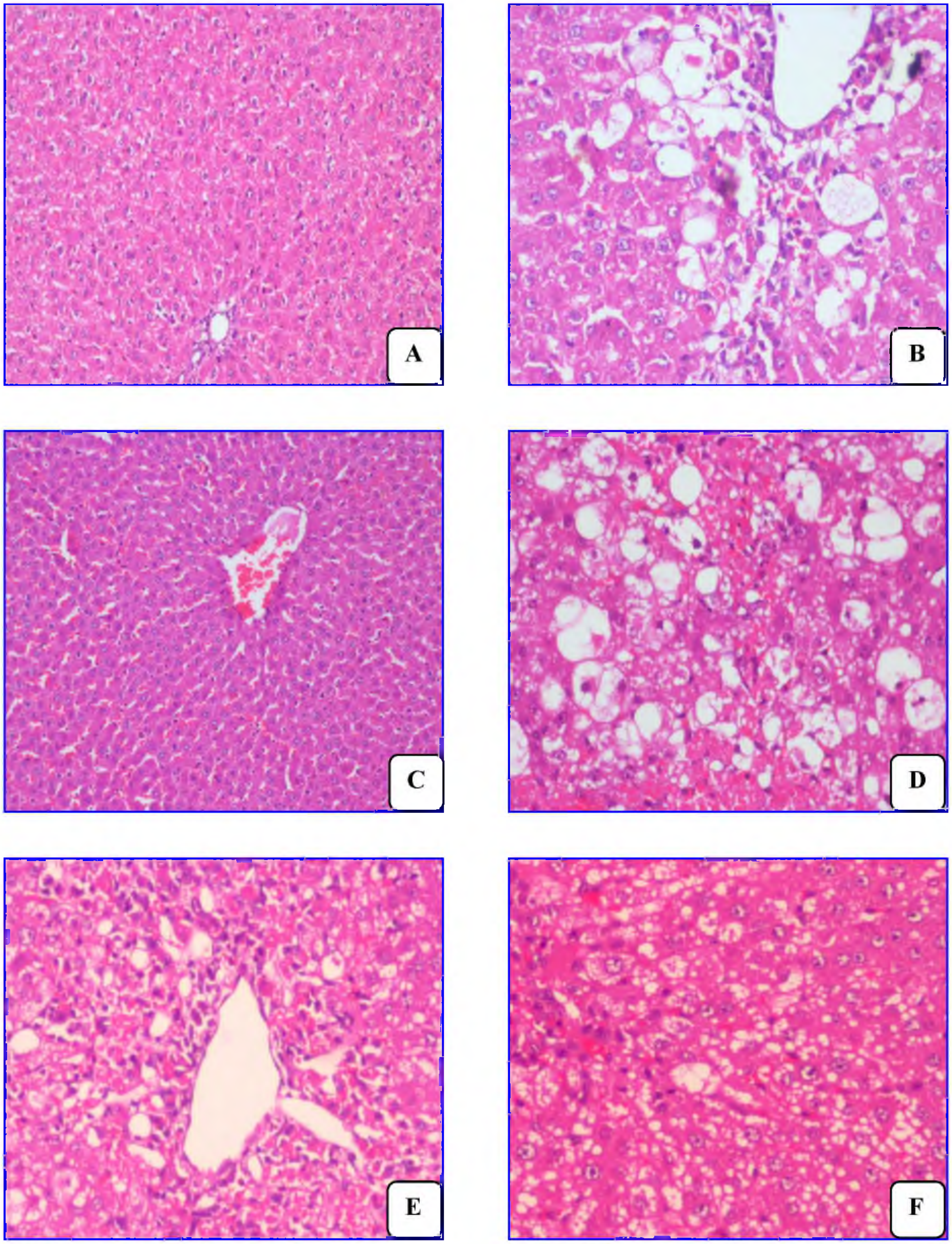


PLATE 5



4.2.5.6 Group VI female and male rats

This group treated with *Ficus virens* (150 mg/kg) showed non significant restoration of hepatocytes with mild degree of congestion and necrosis as compared to Group III , but showed mild degree of congestion and necrosis as compared to Group II (Plate 4F and 5F).

4.3 Pilot study

Six groups of rats consisting of 3 male in each group and another six groups consisting of 3 female rats were used for pilot study. No mortality observed in any of test groups in given dose and duration during the experiment period (Table.8).

4.4 Acute oral toxicity study

Ten groups of Wistar albino rats consisting of 10 male in each group and another ten groups consisting of 10 female Wistar albino rats in each group were used for estimating LD_{50} values in male and female rats separately. There were no deaths and clinical signs of toxicity in any of the test groups within 24 h 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. LD_{50} value of *Ficus virens* in rats was more than 5 g/kg (Table.9).

All the tested groups of animals were sacrificed on day 15. Detailed post mortem examination was carried out and all the experimental animals did not reveal any gross pathological changes. The histological examination of various organs of both male and female rats revealed normal appearance of all the organs.

Table 8. Mortality pattern in male and female rats in the pilot study

Group	No. of Male rats	Dose (mg/kg)	Mortality observed	Group	No. of Female rats	Dose (mg/kg)	Mortality observed
Group I	3	50	0	**Group I**	3	50	0
Group II	3	300	0	**Group II**	3	300	0
Group III	3	2000	0	**Group III**	3	2000	0
Group IV	3	3000	0	**Group IV**	3	3000	0
Group V	3	4000	0	**Group V**	3	4000	0
Group VI	3	5000	0	**Group VI**	3	5000	0

Table 9. Mortality pattern in male and female rats in the acute oral toxicity study

Groups	Dose (mg/kg)	No. of male animals	No. of female animals	Mortality observed	Mortality (%)
Control	Distilled. water	10	10	0	0
Group I	50	10	10	0	0
Group II	300	10	10	0	0
Group III	2000	10	10	0	0
Group IV	2500	10	10	0	0
Group V	3000	10	10	0	0
Group VI	3500	10	10	0	0
Group VII	4000	10	10	0	0
Group VIII	4500	10	10	0	0
Group IX	5000	10	10	0	0

4.5 Repeated dose 28-day oral toxicity study

Three groups of rats with 6 male and 6 female Wistar albino rats in each group were gavaged with the *Ficus virens* leaf extract 50,200 and 800 mg/kg daily for 28 days and the limit test group was gavaged with dose of 1000 mg/kg of *Ficus virens* leaf extract daily for 28 days and the results were recorded. The control group animals were gavaged with distilled water which was used for dissolving the *Ficus virens* leaf extract.

Satellite group consisting of 6 male and 6 female Wistar albino rats were gavaged with the *Ficus virens* leaf extract 800 mg/kg daily for 28 days and maintained for another 14 days without administration of *Ficus virens* leaf extract. There were no any apparent clinical signs of toxicity and mortality observed in any of the tested groups in range finding study and in limit test group.

4.5.1 Serum biochemical parameters

Serum collected on days 0, 7, 14, 21 and 28 of the experimental period were used for the estimation of AST, ALT, BUN and Cr for both male and female rats separately and the results are given in table 10-17 and fig 8-15.

4.5.1.1 Serum biochemical parameters of male and female rats

4.5.1.1.1 Aspartate aminotransferase (AST)

The values obtained for AST (U/L), for the control group (Group I) and treated group (Group II, III, IV and satellite) for both male and female rats are given in table 10-11 and fig 8-9.

Table 10. Effect of *Ficus virens* leaf extract on aspartate aminotransferase (U/L) on female rats in subacute toxicity study

Day	Control	Low dose	Medium dose	High dose	Limit dose	Satellite group
0	55.78±1.31	55.25±0.53	54.88±1.02	54.98±0.49	55.98±0.53	56.00±0.45
7	56.26±0.58	55.95±0.75	56.25±0.40	56.25±0.74	57.21±0.41	56.70±0.50
14	56.55±0.75	56.75±0.38	57.43±0.44	58.45±0.26	58.66±0.67	58.53±0.40
21	58.01±0.57	58.86±0.45	58.90±0.50	59.66±0.43	59.58±0.44	59.86±0.32
28	60.40±0.31	59.93±0.34	60.56±0.46	60.33±0.34	59.93±0.84	60.71±0.44
35	59.96±0.58	-	-	-	-	59.30±0.35
42	60.55±0.32	-	-	-	-	60.40±0.48

Table 11. Effect of *Ficus virens* leaf extract on aspartate aminotransferase (U/L) on male rats in subacute toxicity study

Day	Control	Low dose	Medium	High dose	Limit dose	Satellite group
0	54.10±0.99	53.23±0.92	53.38±1.33	53.31±0.27	53.35±0.24	53.50±0.30
7	56.05±0.52	56.00±0.55	55.75±0.39	56.08±0.39	57.30±0.38	57.70±0.25
14	57.88±0.36	58.63±0.29	58.20±0.46	59.18±0.26	58.76±0.26	58.60±0.20
21	59.30±0.31	59.36±0.35	59.41±0.46	59.18±0.26	60.00±0.28	59.68±0.52
28	60.30±0.23	60.03±0.28	60.03±0.39	60.03±0.31	60.45±0.39	60.51±0.28
35	60.11±0.81	-	-	-	-	60.31±0.31
42	59.86±0.41	-	-	-	-	60.70±0.52

Compared with the control group values of respective days,values are mean SEM P>0.05 n = 6

Fig. 8 Effect of *Ficus virens* leaf extract on Aspartate Aminotransferase(AST) in female rats in subacute toxicity study

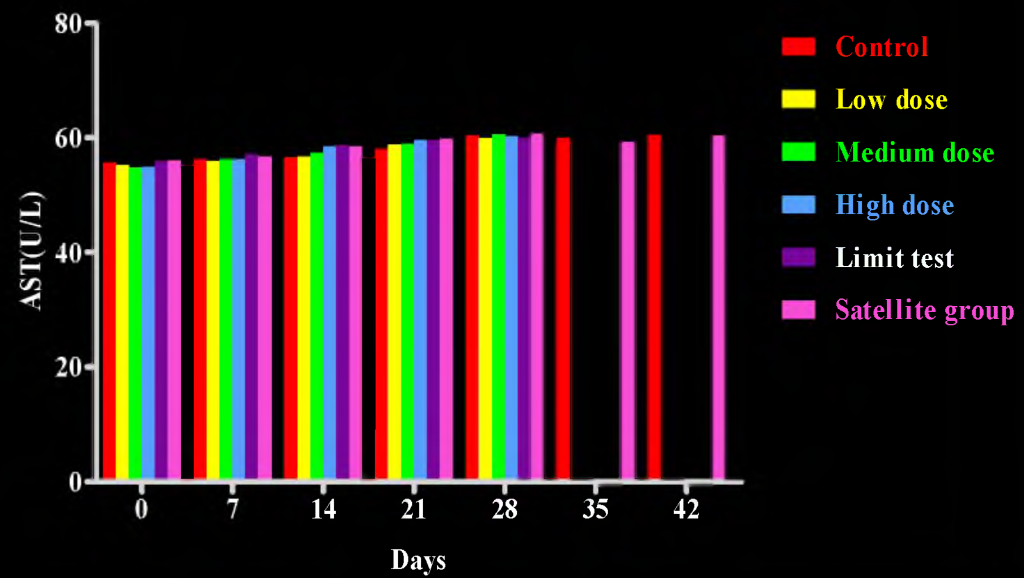


Fig.9 Effect of *Ficus virens* leaf extract on Aspartate Aminotransferase(AST) in male rats in subacute toxicity study

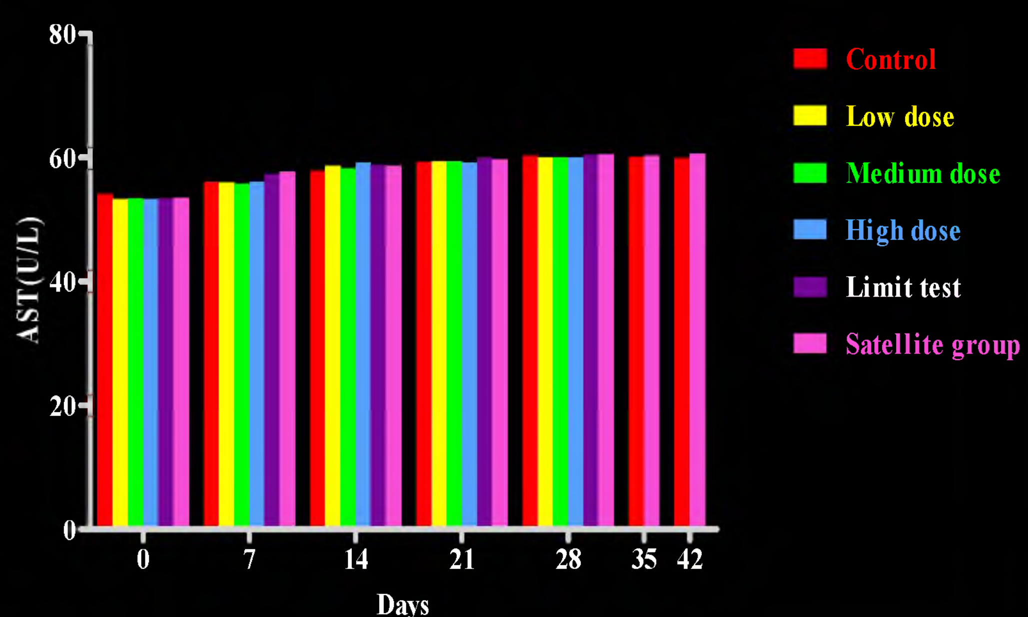


Table 12. Effect of *Ficus virens* leaf extract on alanine Aminotransferase (U/L) on female rats in subacute toxicity study

Day	Control	Low dose	Medium dose	High dose	Limit dose	Satellite group
0	23.60±0.75	23.36±0.47	23.20±0.39	22.93±0.72	23.41±0.23	23.36±0.33
7	24.36±0.57	23.80±0.35	23.90±0.43	24.60±0.77	25.30±0.33	25.25±0.45
14	24.98±1.02	23.78±0.36	24.60±0.50	25.75±0.73	25.93±0.34	25.65±0.46
21	25.86±0.80	25.43±0.33	25.26±0.73	25.76±0.97	26.23±0.41	25.96±0.28
28	26.00±0.52	25.50±0.73	25.16±0.63	26.21±0.50	26.75±0.40	26.36±0.22
35	26.25±1.34	-	-	-	-	25.92±0.34
42	23.60±0.99	-	-	-	-	25.78±0.36

Table 13. Effect of *Ficus virens* leaf extract on alanine Aminotransferase (U/L) on male rats in subacute toxicity study

Day	Control	Low dose	Medium dose	High dose	Limit dose	Satellite group
0	22.24±0.44	21.83±0.53	21.72±0.61	21.65±0.46	22.16±0.76	22.13±0.35
7	22.66±0.44	22.54±0.53	22.18±0.47	22.41±0.58	22.96±0.58	23.17±0.30
14	23.12±0.42	23.60±0.76	23.61±0.45	23.79±0.24	23.79±0.37	23.74±0.25
21	23.46±0.39	24.01±0.68	24.17±0.41	24.23±0.25	24.34±0.38	24.43±0.34
28	24.21±0.40	24.22±0.61	24.33±0.39	24.65±0.27	24.81±0.30	24.63±0.24
35	24.35±1.23	-	-	-	-	24.69±0.35
42	24.15±1.45	-	-	-	-	24.83±0.39

Compared with the control group values of respective days,values are mean SEM P>0.05 n = 6

Fig.10 Effect of *Ficus virens* leaf extract on serum Alanine Aminotransferase (ALT) in female rats in subacute toxicity study

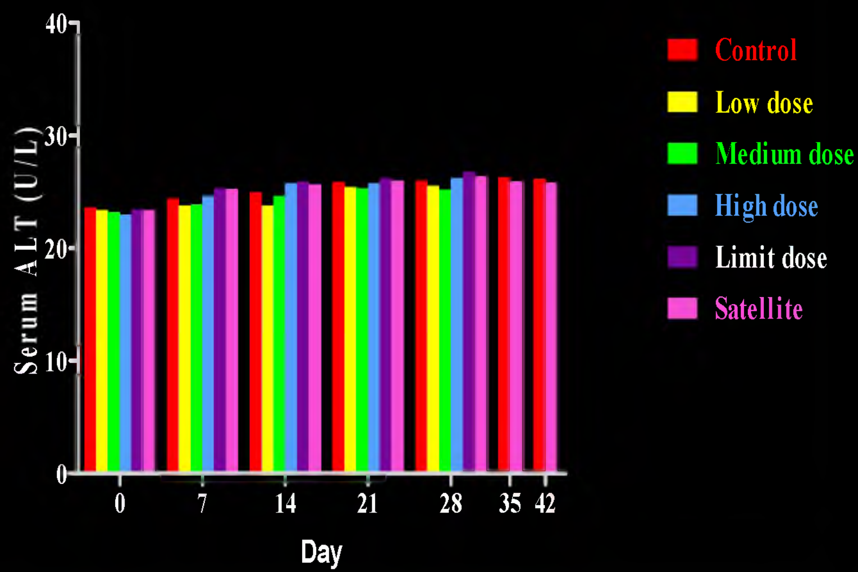


Fig.11 Effect of *Ficus virens* leaf extract on serum Alanine Aminotransferase (ALT) in male rats in subacute toxicity study

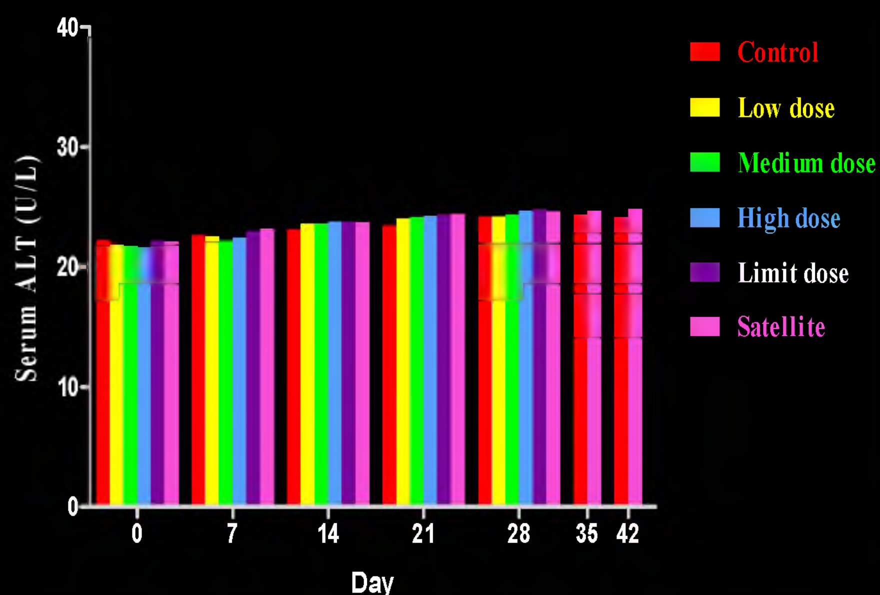


Table 14. Effect of *Ficus virens* leaf extract on BUN (mg/dl) on female rats in subacute toxicity study

Day	Control	Low dose	Medium dose	Highdose	Limit dose	Satellite group
0	19.69±0.48	18.95±0.51	18.98±0.53	19.36±0.91	19.91±0.81	20.06±0.31
7	19.49±0.70	19.32±0.28	19.61±0.44	20.00±0.87	20.37±0.81	20.64±0.20
14	20.03±0.51	19.58±0.28	20.00±1.26	20.72±0.87	21.11±0.69	21.03±0.19
21	20.48±0.52	19.97±0.27	20.44±0.96	21.11±0.88	21.45±0.72	21.75±0.25
28	20.53±0.43	20.23±0.47	20.87±0.97	21.53±0.80	21.95±0.67	22.13±0.29
35	20.50±0.52	-	-	-	-	21.60±0.45
42	20.40±0.33	-	-	-	-	21.62±0.58

Table 15. Effect of *Ficus virens* leaf extract on BUN (mg/dl) on male rats in subacute toxicity study

Day	Control	Low dose	Medium dose	High dose	Limit dose	Satellite group
0	18.86±0.96	18.45±0.69	18.64±0.30	18.73±0.46	18.83±0.91	18.94±0.19
7	18.91±0.90	18.66±0.62	18.78±0.39	19.34±0.55	19.89±0.84	19.81±0.23
14	18.90±0.77	18.73±0.72	19.19±0.39	19.85±0.55	20.51±0.77	20.43±0.16
21	19.55±0.69	19.18±0.45	19.64±0.29	20.40±0.52	21.10±0.80	21.02±0.13
28	19.46±0.74	19.47±0.42	20.19±0.57	20.98±0.62	21.64±0.72	21.68±0.15
35	20.15±0.63	-	-	-	-	21.45±0.34
42	20.17±0.60	-	-	-	-	20.95±0.31

Compared with the control group values of respective days, values are mean SEM $P>0.05$ $n = 6$

Fig. 12 Effect of *Ficus virens* leaf extract on Blood urea nitrogen (BUN) in female rats in subacute toxicity study

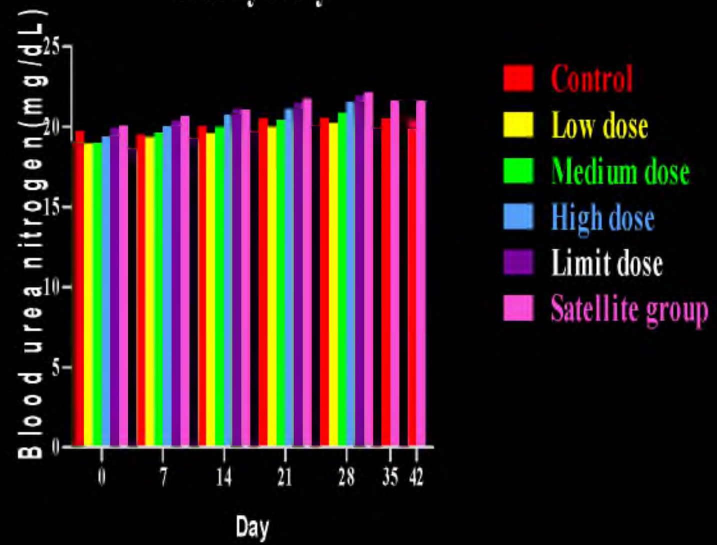


Fig. 13 Effect of *Ficus virens* leaf extract on Blood urea nitrogen (BUN) in male rats in subacute toxicity study

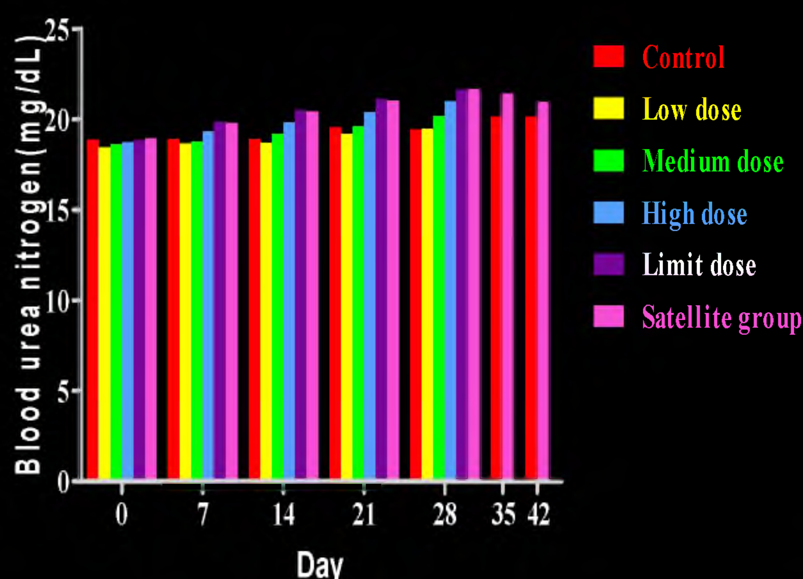


Table 16. Effect of *Ficus virens* leaf extract on creatinine (mg/dl) on female rats in subacute toxicity study

Day	Control	Low dose	Medium dose	High dose	Limit dose	Satellite group
0	0.61±0.02	0.61±0.01	0.61±0.00	0.60±0.01	0.62±0.01	0.61±0.01
7	0.61±0.01	0.61±0.01	0.62±0.00	0.62±0.01	0.63±0.01	0.62±0.01
14	0.61±0.01	0.62±0.00	0.63±0.00	0.63±0.01	0.64±0.01	0.63±0.01
21	0.62±0.01	0.63±0.00	0.63±0.00	0.64±0.01	0.65±0.01	0.64±0.01
28	0.62±0.01	0.62±0.01	0.64±0.00	0.65±0.01	0.65±0.01	0.65±0.01
35	0.62±0.01	-	-	-	-	0.65±0.00
42	0.62±0.01	-	-	-	-	0.65±0.00

Table 17. Effect of *Ficus virens* leaf extract on creatinine (mg/dl) on male rats in subacute toxicity study

Day	Control	Low dose	Medium dose	High dose	Limit dose	Satellite group
0	0.61±0.01	0.62±0.00	0.62±0.00	0.62±0.00	0.62±0.00	0.62±0.00
7	0.62±0.01	0.63±0.00	0.63±0.00	0.63±0.00	0.64±0.00	0.64±0.00
14	0.63±0.01	0.64±0.00	0.64±0.00	0.64±0.00	0.65±0.00	0.65±0.00
21	0.64±0.01	0.64±0.00	0.65±0.00	0.65±0.00	0.66±0.00	0.66±0.00
28	0.64±0.01	0.65±0.00	0.66±0.00	0.66±0.00	0.67±0.00	0.67±0.00
35	0.65±0.00	-	-	-	-	0.67±0.00
42	0.64±0.00	-	-	-	-	0.66±0.00

Compared with the control group values of respective days, values are mean SEM $P>0.05$ n = 6

Fig. 14 Effect of *Ficus virens* leaf extract on Creatinine (Cr) in female rats in subacute toxicity study

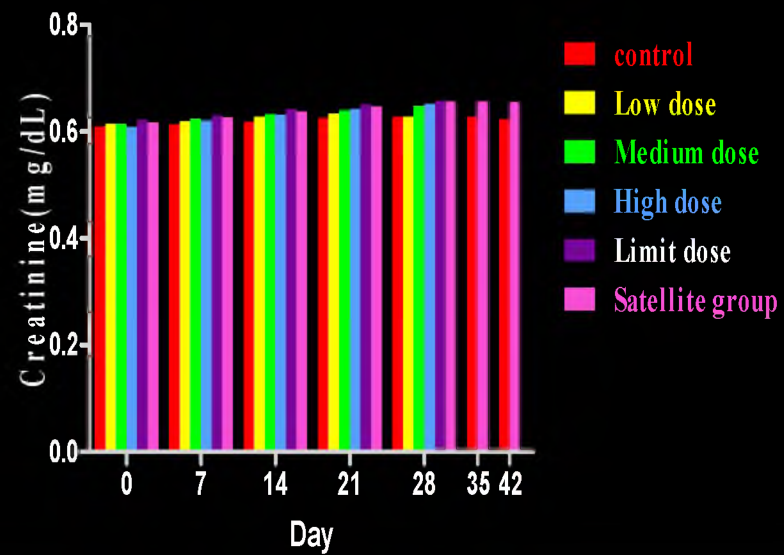
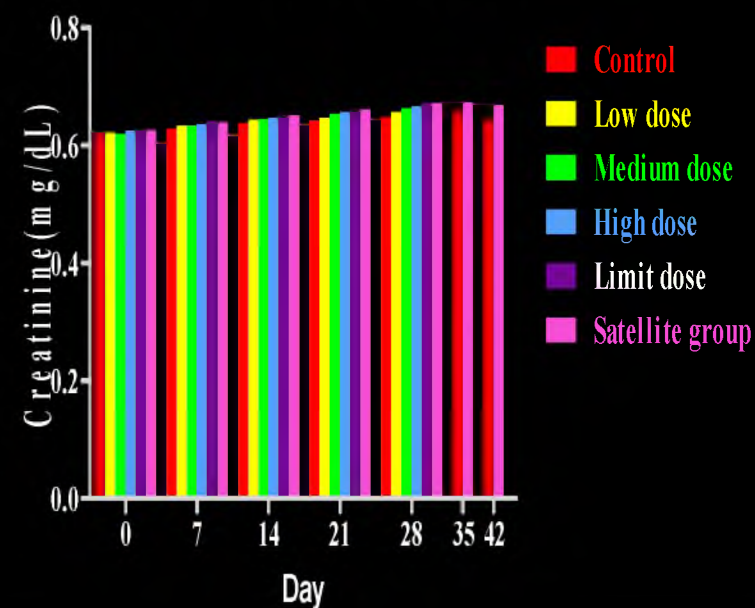


Fig. 15 Effect of *Ficus virens* leaf extract on Creatinine (Cr) in male rats in subacute toxicity study



There was no significant (P>0.05) change in AST (U/L), in treated groups compared to control group values.

4.5.1.1.2 Alanine aminotransferase (ALT)

The values obtained for ALT (U/L), for the control group (Group I) and treated group (Group II , III, IV , limit dose and satellite) for both male and female rats are given in the tables 12-13 and fig 10-11.

There was no significant (P>0.05) change in ALT (U/L), in treated groups compared to control group values.

4.5.1.1.3 Blood urea nitrogen (BUN)

The values obtained for BUN (mg/dl), for the control group (Group I) and treated group (Group II , III, IV , limit dose and satellite) for both male and female rats are given in the tables 14-15 and fig 12-13.

There was no significant (P>0.05) change in BUN (mg/dl) in treated groups compared to control group values.

4.5.1.1.4 Creatinine (Cr)

The values obtained for creatinine (mg/dl), for the control group (Group I) and treated group (Group II, III, IV, limit dose and satellite) for both male and female rats are given in the table16-17 and fig.14-15.

There was no significant (P>0.05) change in Cr (mg/dl) in treated groups compared to control group values.

4.5.2 Haematology

The blood samples were collected on day 0, 7, 14, 21 and 28 of the experimental period and were used for the estimation of TEC, TLC, PCV and Hb for both male and female rats separately.

The results obtained for TEC, TLC, PCV and Hb for both male and female rats in control group (Group I) and treated group (Group II, III, IV, limit dose and satellite) are given in table18-25 and fig 16-23.

There were no significant (P>0.05) changes in TEC, TLC, PCV and Hb in treated groups compared to control group values.

4.5.3 Body weight

There was no significant (P>0.05) change in body weight in tested groups in range finding study and in limit test group (Table 26-27 and Figure 24-25).

4.5.4 Feed intake

There was no significant (P>0.05) change in feed intake in tested groups in range finding study and in limit test group (Table 28-29 and Figure 26-27).

4.5.5 Pathology

4.5.5.1 Gross pathology

At necropsy none of the treated groups showed any gross pathological lesions in any of the organs in both male and female groups.

Table 18. Effect of *Ficus virens* leaf extract on TEC (10^6 cells/mm^3) on female rats in subacute toxicity study

Day	Control	Low dose	Medium dose	High dose	Limit dose	Satellite group
0	5.15±0.15	5.20±0.22	5.25±0.13	5.21±0.21	5.11±0.13	5.08±0.20
7	5.16±0.18	5.20±0.12	5.20±0.10	5.21±0.09	5.25±0.13	5.20±0.22
14	4.95±0.22	5.03±0.09	5.11±0.12	5.16±0.19	5.26±0.15	5.26±0.13
21	5.01±0.17	4.98±0.09	4.96±0.21	5.03±0.21	5.20±0.08	5.25±0.09
28	5.01±0.17	5.06±0.16	5.08±0.15	5.15±0.10	5.30±0.05	5.23±0.08
35	5.11±0.16	-	-	-	-	5.26±0.08
42	5.10±0.13	-	-	-	-	5.23±0.07

Table 19. Effect of *Ficus virens* leaf extract on TEC (10^6 cells/mm^3) on male rats in subacute toxicity study

Day	Control	Low dose	Medium dose	High dose	Limit dose	Satellite group
0	5.15±0.19	5.08±0.17	5.03±0.06	5.05±0.06	5.01±0.06	4.96±0.09
7	5.10±0.21	5.08±0.10	5.06±0.04	5.12±0.06	5.14±0.05	5.07±0.05
14	5.08±0.19	5.06±0.19	5.07±0.10	5.09±0.14	5.21±0.12	5.22±0.03
21	4.93±0.09	4.94±0.08	4.96±0.20	5.03±0.21	5.18±0.06	5.16±0.05
28	5.03±0.16	5.06±0.16	5.08±0.15	5.09±0.10	5.21±0.22	5.24±0.04
35	5.15±0.12	-	-	-	-	5.20±0.04
42	5.28±0.08	-	-	-	-	5.35±0.02

Compared with the control group values of respective days,values are mean SEM $P>0.05$ $n = 6$

Fig. 16 Effect of *Ficus virens* leaf extract on Total Erythrocyte Count (TEC) in female rats in subacute toxicity study

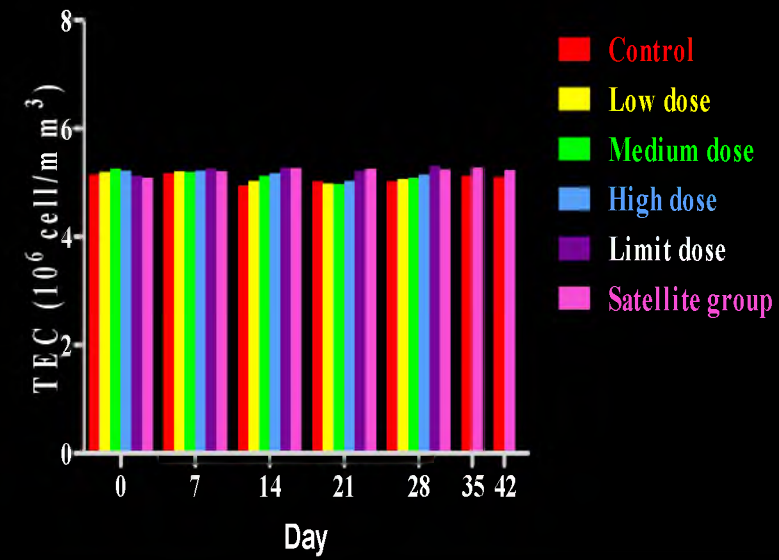


Fig. 17 Effect of *Ficus virens* leaf extract on Total Erythrocyte Count (TEC) in male rats in subacute toxicity study

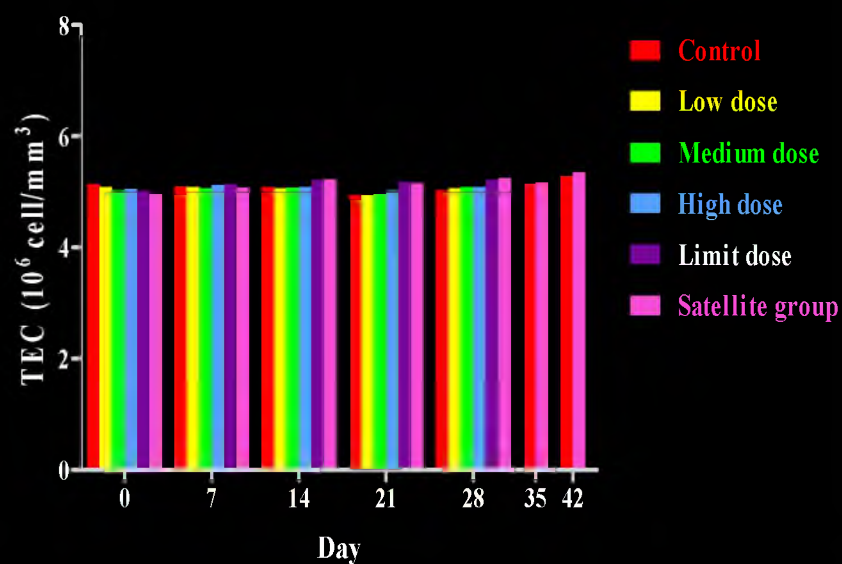


Table 20. Effect of *Ficus virens* leaf extract on TLC (10^6 cells/mm^3) on female rats in subacute toxicity study

Day	Control	Low dose	Medium dose	High dose	Limit dose	Satellite group
0	10.81±0.43	11.04±0.27	11.01±0.33	11.31±0.25	11.39±0.27	11.42±0.35
7	10.71±0.49	11.15±0.20	11.25±0.33	11.42±0.19	11.51±0.29	11.59±0.29
14	11.00±0.45	11.44±0.19	11.42±0.28	11.64±0.17	11.74±0.29	11.82±0.30
21	11.13±0.40	11.57±0.14	11.55±0.25	11.84±0.16	11.94±0.25	11.94±0.17
28	11.47±0.33	11.75±0.10	11.92±0.21	12.06±0.11	11.97±0.19	12.05±0.08
35	11.44±0.31	-	-	-	-	12.12±0.09
42	11.58±0.24	-	-	-	-	12.18±0.29

Table 21. Effect of *Ficus virens* leaf extract on TLC (10^6 cells/mm^3) on male rats in subacute toxicity study

Day	Control	Low dose	Medium dose	High dose	Limit dose	Satellite group
0	11.08±0.19	10.89±0.27	11.11±0.21	11.22±0.28	11.51±0.21	11.63±0.25
7	11.20±0.17	11.01±0.27	11.15±0.18	11.20±0.18	11.53±0.21	11.68±0.26
14	11.43±0.18	11.44±0.19	11.42±0.28	11.64±0.17	11.72±0.21	11.63±0.20
21	11.58±0.14	11.57±0.14	11.55±0.25	11.84±0.16	11.94±0.25	11.94±0.17
28	11.54±0.16	11.63±0.12	11.60±0.25	11.95±0.11	11.98±0.18	12.04±0.14
35	11.47±0.25	-	-	-	-	12.06±0.15
42	11.65±0.21	-	-	-	-	12.08±0.20

Compared with the control group values of respective days,values are mean   SEM $P>0.05$ $n = 6$

Fig. 18 Effect of *Ficus virens* leaf extract on Total Leukocyte Count (TLC) in female rats in subacute toxicity study

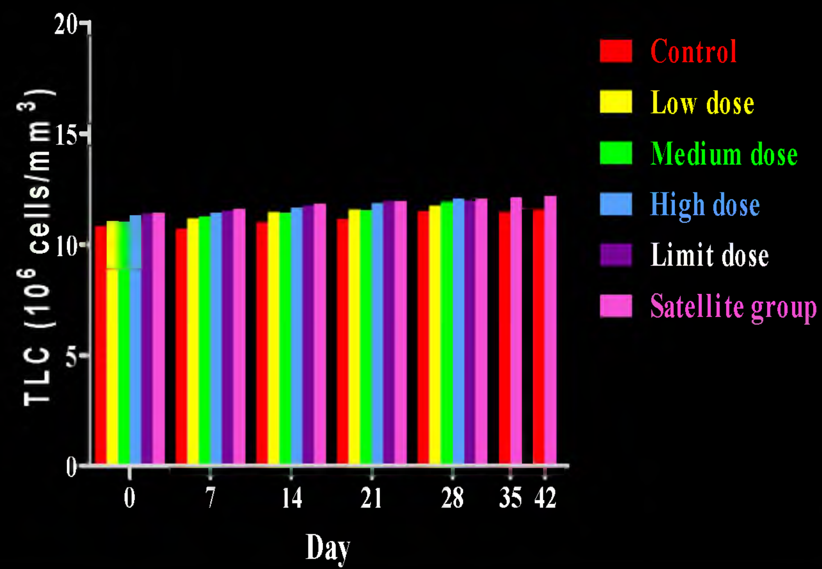


Fig. 19 Effect of *Ficus virens* leaf extract on Total Leukocyte Count (TLC) in male rats in subacute toxicity study

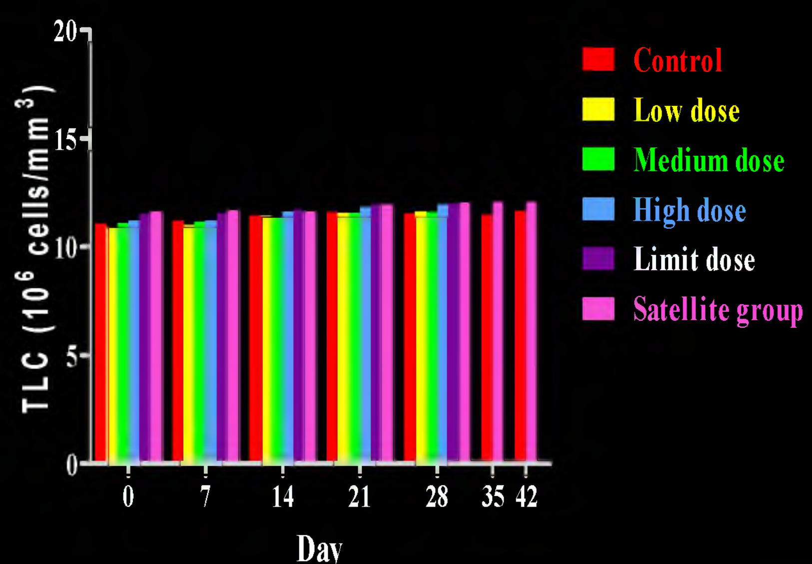


Table 22. Effect of *Ficus virens* leaf extract on Hb (g %) on female rats in subacute toxicity study

Day	Control	Low dose	Medium dose	High dose	Limit dose	Satellite group
0	12.68±0.30	12.50±0.12	12.80±0.07	12.75±0.21	13.03±0.33	13.15±0.23
7	12.70±0.60	12.71±0.23	12.61±0.16	12.70±0.55	12.95±0.13	12.88±0.29
14	13.00±0.52	12.88±0.21	12.83±0.09	13.05±0.21	13.03±0.12	13.08±0.29
21	12.63±0.31	12.86±0.21	12.86±0.28	13.15±0.23	13.01±0.11	12.98±0.17
28	12.73±0.40	12.81±0.25	12.88±0.32	13.18±0.42	13.55±0.26	13.36±0.17
35	12.60±0.38	-	-	-	-	13.55±0.15
42	12.86±0.30	-	-	-	-	13.55±0.25

Table 23. Effect of *Ficus virens* leaf extract on Hb (g %) on male rats in subacute toxicity study

Day	Control	Low dose	Medium dose	High dose	Limit dose	Satellite group
0	12.85±0.22	13.06±0.25	12.91±0.20	12.81±0.26	12.95±0.35	12.86±0.31
7	12.53±0.65	12.58±0.22	12.41±0.54	12.61±0.55	13.03±0.35	13.01±0.34
14	13.00±0.64	13.15±0.26	13.13±0.47	13.31±0.25	13.35±0.34	13.36±0.33
21	12.83±0.23	13.05±0.19	12.86±0.26	13.00±0.29	13.48±0.25	13.53±0.32
28	12.50±0.42	12.80±0.25	12.73±0.21	12.85±0.23	13.41±0.21	13.57±0.17
35	12.90±0.27	-	-	-	-	13.61±0.22
42	13.25±0.27	-	-	-	-	13.66±0.20

Compared with the control group values of respective days,values are mean SEM P>0.05 n = 6

Fig. 20 Effect of *Ficus virens* leaf extract on Haemoglobin(Hb) in female rats in subacute toxicity study

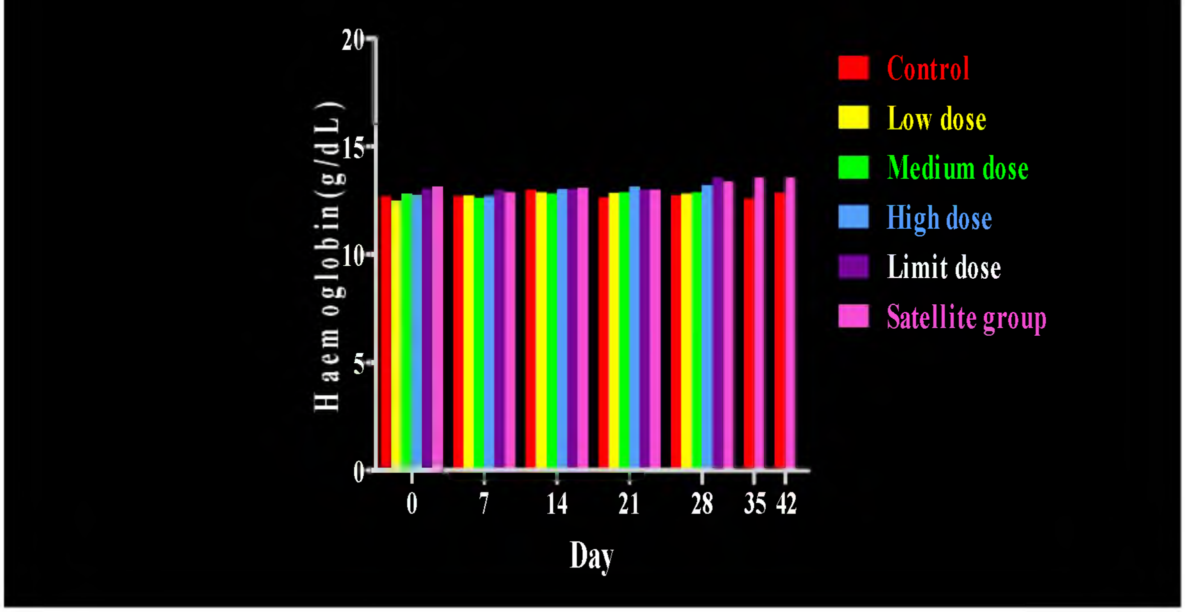


Fig. 21 Effect of *Ficus virens* leaf extract on Haemoglobin(Hb) in male rats in subacute toxicity study

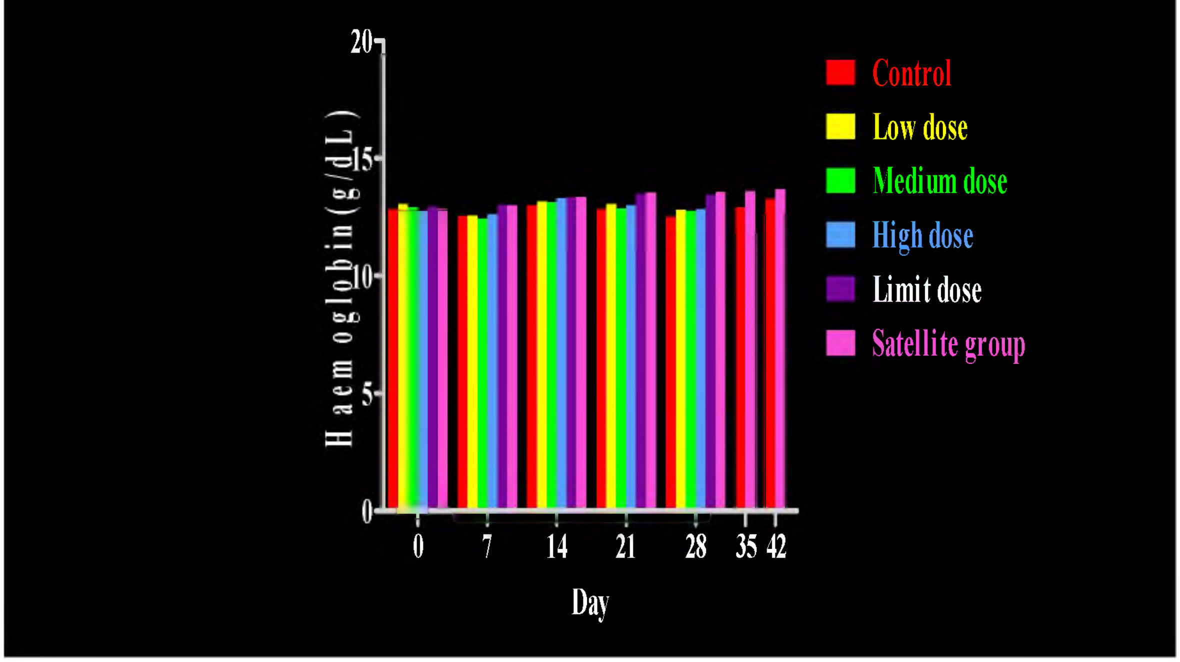


Table 24. Effect of *Ficus virens* leaf extract on PCV (%) on female rats in subacute toxicity study

Day	Control	Low dose	Medium dose	High dose	Limit dose	Satellite group
0	38.80±1.25	39.28±1.22	39.65±0.85	39.51±1.21	39.22±0.68	39.87±0.68
7	39.27±1.18	38.20±0.92	39.09±0.73	39.11±0.90	38.93±0.64	39.90±0.99
14	39.44±0.54	39.77±0.37	39.02±0.46	39.51±0.78	39.55±0.74	39.31±0.90
21	39.80±0.56	39.81±1.20	39.64±0.30	39.54±0.58	39.93±0.50	39.52±0.71
28	39.51±1.49	39.51±1.46	39.87±0.58	39.83±0.64	40.38±0.43	39.52±0.49
35	39.59±1.26	-	-	-	-	39.23±0.36
42	39.52±0.91	-	-	-	-	39.11±0.37

Table 25. Effect of *Ficus virens* leaf extract on PCV (%) on male rats in subacute toxicity study

Day	Control	Low dose	Medium dose	High dose	Limit dose	Satellite group
0	38.06±1.62	39.10±0.73	39.14±0.67	39.15±1.25	39.36±0.48	38.76±1.26
7	40.36±0.95	39.45±0.65	39.72±0.56	39.70±0.85	39.60±0.42	39.30±1.02
14	40.46±0.58	40.61±0.43	41.03±0.37	41.18±0.41	40.48±0.37	40.29±0.99
21	40.91±0.99	41.16±0.80	41.16±0.80	41.16±0.80	41.62±0.31	40.54±0.70
28	39.61±1.13	39.73±1.11	40.33±1.07	40.58±1.40	41.75±0.27	41.29±0.78
35	39.85±0.97	-	-	-	-	41.36±0.61
42	39.79±0.88	-	-	-	-	41.32±0.6

Compared with the control group values of respective days,values are mean SEM P>0.05 n = 6

Fig. 22 Effect of *Ficus virens* leaf extract on Packed Cell Volume (PCV) in female rats in subacute toxicity study

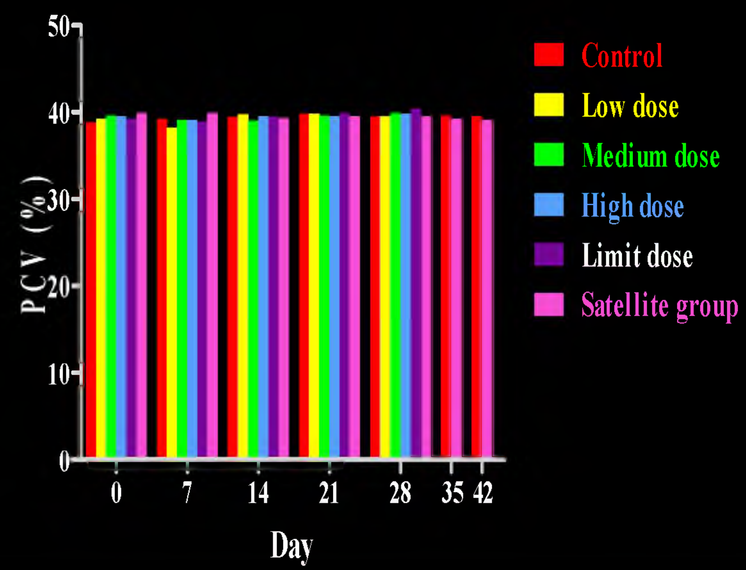


Fig. 23 Effect of *Ficus virens* leaf extract on Packed Cell Volume (PCV) in male rats in subacute toxicity study

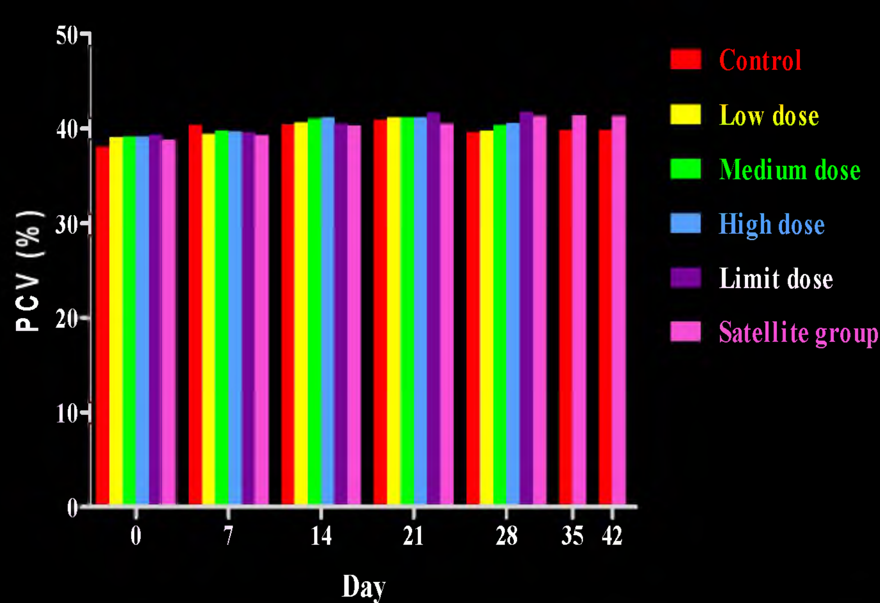


Table 26. Effect of *Ficus virens* leaf extract on body weight (g) on female rats in subacute toxicity study

Day	Control	Low dose	Medium dose	High dose	Limit dose	Satellite group
0	196.83±1.60	197.33±1.40	195.00±1.93	198.66±1.11	195.33±1.72	198.77±2.22
7	201.66±1.97	202.16±2.07	199.66±2.15	199.66±2.02	200.33±3.16	202.33±2.98
14	211.12±1.09	215.26±3.80	213.72±4.68	218.29±2.23	214.72±5.26	215.75±3.58
21	220.32±0.96	221.88±4.68	221.42±6.79	221.83±1.63	221.03±5.31	220.92±.93
28	235.69±1.50	234.22±3.12	236.30±4.27	234.33±1.96	234.25±2.21	233.16±4.60
35	244.36±1.36	-	-	-	-	244.16±4.24
42	251.43±1.40	-	-	-	-	253.96±2.98

Table 27. Effect of *Ficus virens* leaf extract on body weight (g) on male rats in subacute toxicity study

Day	Control	Low dose	Medium dose	High dose	Limit dose	Satellite group
0	195.83±1.86	193.50±1.70	196.00±2.00	192.92±1.69	194.50±1.96	195.16±2.31
7	203.50±2.33	201.61±1.32	205.02±1.96	201.25±1.41	204.00±1.97	203.75±2.27
14	224.50±2.23	226.35±2.96	231.00±2.41	228.50±1.52	229.50±2.04	231.27±2.02
21	235.00±2.82	233.90±2.95	240.05±0.50	240.55±1.91	241.83±1.66	242.23±2.04
28	246.16±2.77	250.50±1.98	250.16±2.72	251.33±0.61	250.50±1.17	251.66±2.33
35	255.80±2.20	-	-	-	-	260.86±0.40
42	263.82±1.35	-	-	-	-	267.10±0.60

Compared with the control group values of respective days,values are mean SEM P>0.05 n = 6

Fig. 24 Effect of *Ficus virens* leaf extract on Body weight(g) in female rats in subacute toxicity study

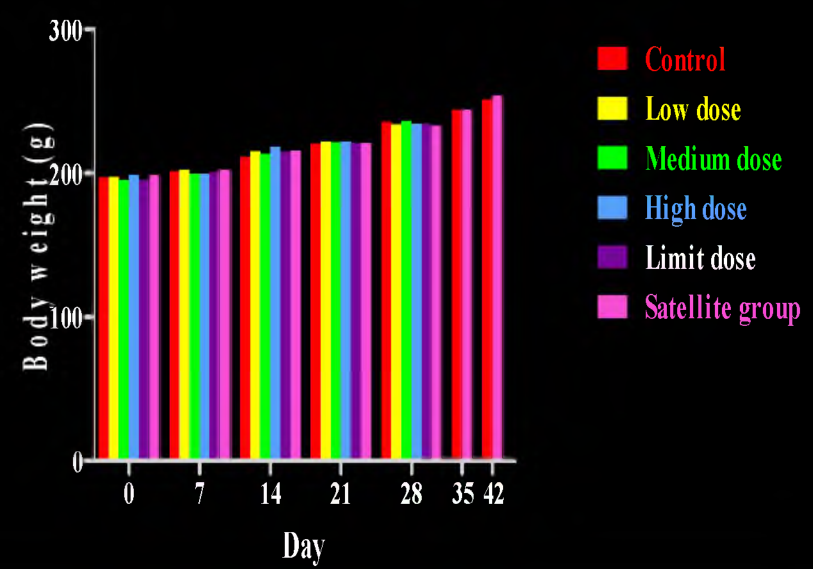


Fig. 25 Effect of *Ficus virens* leaf extract on Body weight(g) in male rats in subacute toxicity study

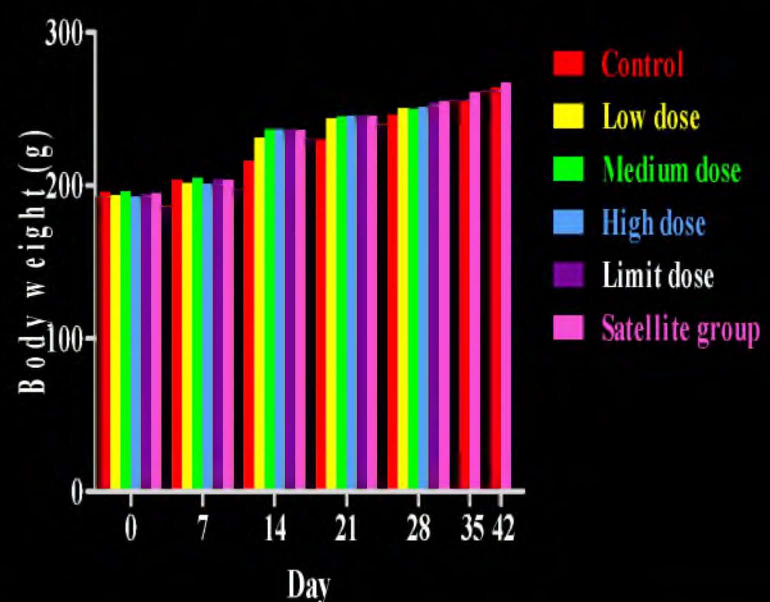


Table 28. Effect of *Ficus virens* leaf extract on feed intake (g/rat) on female rats in subacute toxicity study

Day	Control	Low dose	Medium dose	High dose	Limit dose	Satellite group
0	10.50±0.00	10.58±0.30	10.47±0.28	11.00±0.00	10.80±0.00	11.20±0.00
7	12.53±0.32	12.61±0.28	12.61±0.32	12.85±0.34	12.85±0.30	12.78±0.34
14	14.31±0.24	14.31±0.18	14.25±0.24	13.96±0.18	14.43±0.36	14.00±0.36
21	15.01±0.33	14.45±0.25	14.98±0.36	15.10±0.19	15.10±0.35	15.26±0.27
28	16.61±0.27	16.20±0.29	16.51±0.17	16.68±0.30	16.83±0.36	16.83±0.29
35	17.35±0.49	-	-	-	-	17.26±0.41
42	17.56±0.43	-	-	-	-	17.91±0.39

Table 29. Effect of *Ficus virens* leaf extract on feed intake (g/rat) on male rats in subacute toxicity study

Day	Control	Low dose	Medium dose	High dose	Limit dose	Satellite group
0	10.58±0.30	10.50±0.00	10.58±0.30	11.00±0.28	10.60±0.23	10.80±0.22
7	11.70±0.20	11.75±0.24	11.63±0.23	12.03±0.11	12.01±0.09	12.18±0.25
14	13.58±0.21	13.50±0.27	13.48±0.26	13.81±0.13	13.76±0.14	13.85±0.11
21	15.01±0.11	14.58±0.27	14.53±0.07	14.95±0.25	14.96±0.11	14.81±0.15
28	16.51±0.24	16.58±0.25	16.73±0.27	16.76±0.17	16.88±0.19	17.03±0.15
35	17.81±0.41	-	-	-	-	17.50±0.59
42	17.93±0.42	-	-	-	-	17.51±0.22

Compared with the control group values of respective days,**v**alues are mean SEM $P>0.05$ n = 6

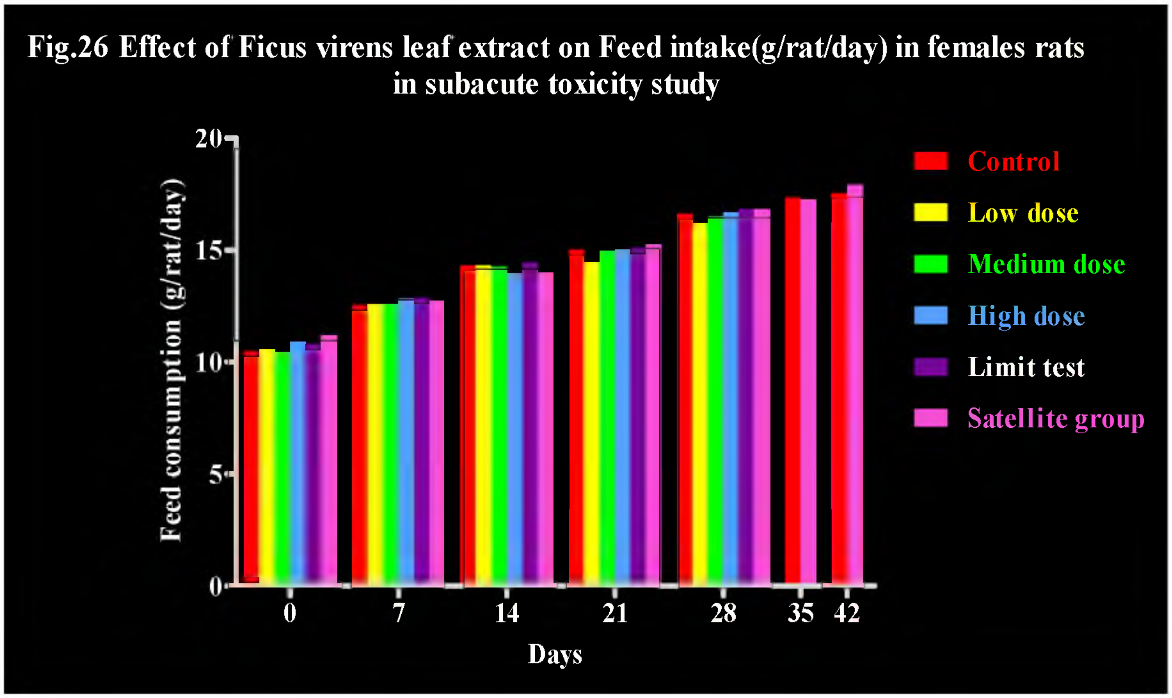


Fig.26 Effect of Ficus virens leaf extract on Feed intake(g/rat/day) in females rats in subacute toxicity study
Feed consumption (g/rat/day)
20
15
10
5
0
0
7
14
21
28
35
42
Days
Control
Low dose
Medium dose
High dose
Limit test
Satellite group

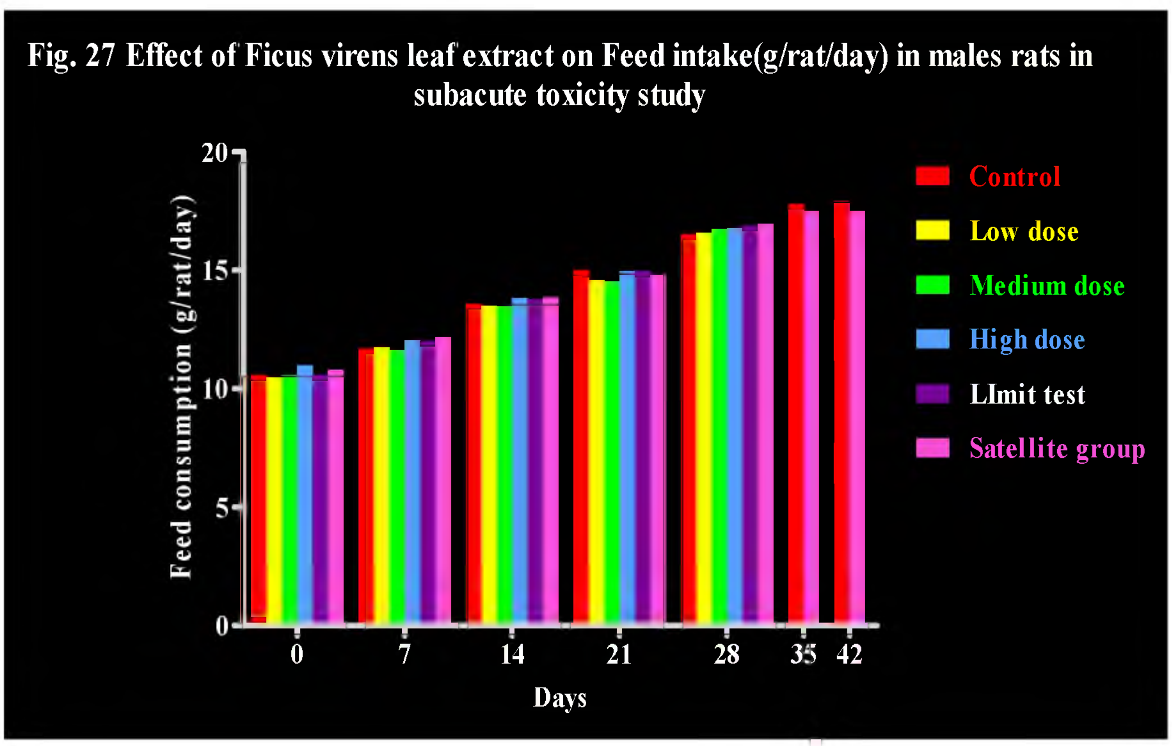


Fig. 27 Effect of Ficus virens leaf extract on Feed intake(g/rat/day) in males rats in subacute toxicity study
Feed consumption (g/rat/day)
20
15
10
5
0
0
7
14
21
28
35
42
Days
Control
Low dose
Medium dose
High dose
LImit test
Satellite group

4.5.5.2 Histopathology

Histopathological studies were conducted in all the animals from treated group II, III, IV and limit test were compared with that of the control group rats (Plate 6).

4.5.5.2.1 Control group male and female rats

The histological appearance of all the organs of the control group of both male and female rats found normal (Plate 6A and 6B).

4.5.5.2.2 Group II male and female rats

Liver, kidney, spleen, lung, heart, brain, stomach and intestine showed normal appearance of all these organs.

4.5.5.2.3 Group III male and female rats

Liver, kidney, spleen, lung, heart, brain, stomach and intestine showed normal appearance of all these organs.

4.5.5.2.4 Group IV male and female rats

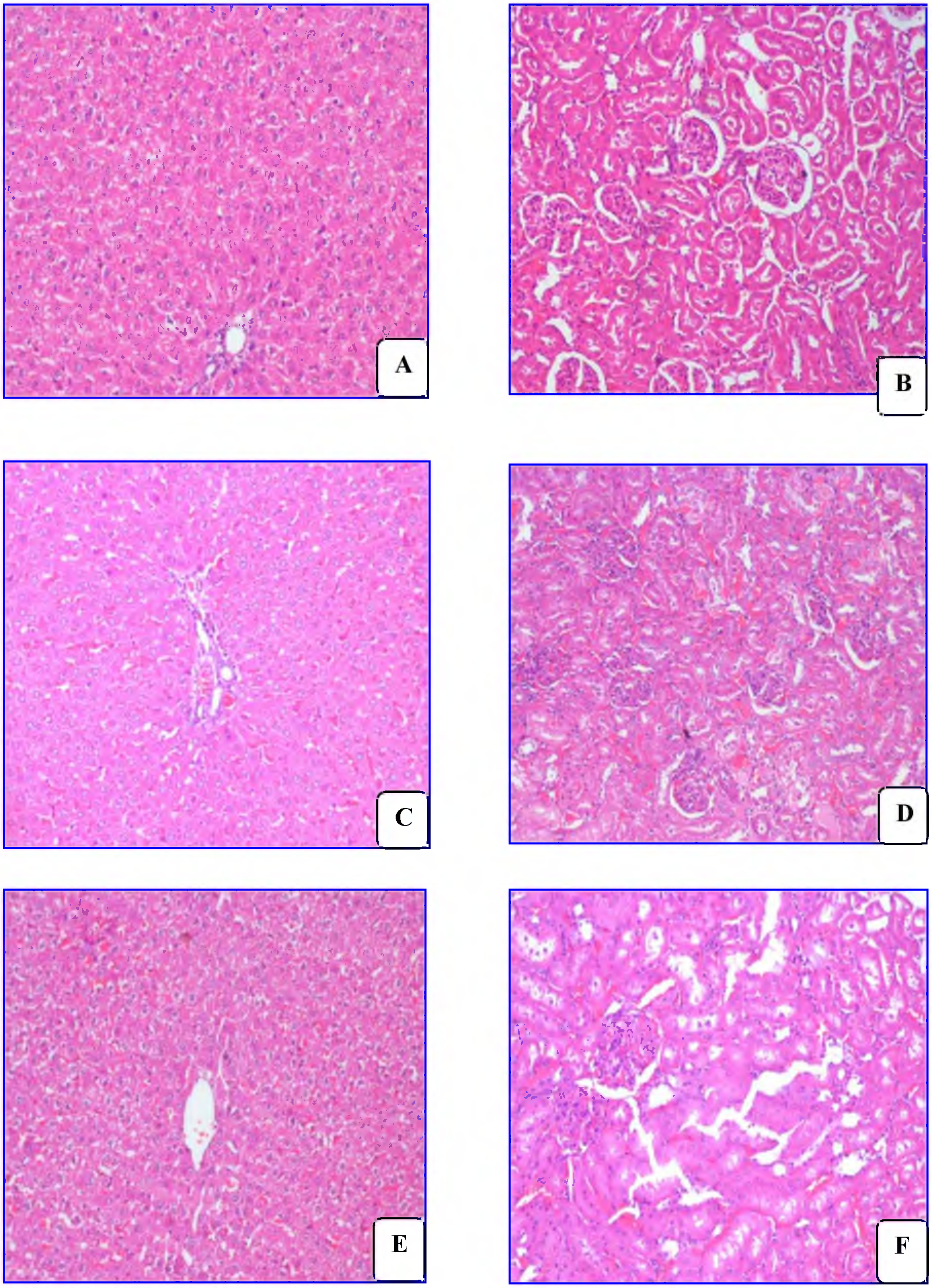
Liver: Slight granularity of hepatocytes (Plate 6C).

Kidney: Slight distension and desquamation of tubular epithelium, with normal appearance of glomeruli (Plate 6D).

4.5.5.2.5 Limit test male and female rats

Liver: Swollen granular hepatocytes with congestion of sinusoidal spaces and degenerative changes are evident (Plate 6E).

PLATE 6



Kidney: Tubular epithelium was swollen and distended, massive intertubular haemorrhages (Plate 6F).

4.5.5.2.6 Satellite group male and female rats

Liver, kidney, spleen, lung, heart, brain, stomach and intestine showed normal appearance of all these organs.

V DISCUSSION

The present study was carried out to evaluate the phytochemical properties, antioxidant property, and the toxic feature of the methanolic extract of *Ficus virens* leaves in rats. The results of the present study are discussed as here under.

5.1 Phytochemical analysis

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

In the present study, *Ficus virens* leaf extract was found positive for anthracene, flavonoids, bitter principle and coumarins and negative for alkaloids.

The *Ficus virens* leaf extract revealed positive for the presence of flavonoids. This finding is in accordance with the findings of Abdel-Hameed (2009) who stated that the phytochemical screening of certain Egyptian *Ficus* species leaf samples (*F. afzelli, F. decora, F. lyrata, F. nitida, F. sycomorus* and *F. virens*) revealed that phenolic compounds as major components of the most active fractions. Flavonoids are the constituents of phenolic comounds. They also measured total phenolics and tannins, flavonoids and its subclass flavonols. Further this finding supported by findings of Babu *et al.* (2010) who conducted the phytochemical analysis of the barks of four *Ficus* species including the *F. virens*. Preliminary phytochemical screening of barks showed the presence of flavonoids and absence of alkaloids, quinones.

5.2 Antioxidant activity

In the present study, the methanolic extract of *Ficus virens* leaves was evaluated for its anti-oxidant property in terms of increase in free radical scavenging activity in *in vivo* condition by using rat as model.

5.2.1 Total protein (mg/g of liver tissue)

In the present study, groups pre-treated with methanolic extract of *Ficus virens* leaves showed significantly increased ($P<0.001$) concentration in total protein when compared to CCl_4 induced oxidative stress group in both male and female rats. The present finding is in accordance with Francesco *et al.*(2009) who reported that liver is the major site of protein metabolism and membrane bound ATPase activity and protein thiols, which are indeed physiological free radical scavengers.

Similarly, Davies *et al.* (1987) who stated that proteins constitute one of the major targets of ROS and oxidation of proteins can lead to a loss of protein function as well as conversion of proteins to forms that are more susceptible to degradation by proteinases.

5.2.2 Thiobarbituric acid reactive substances (TBARS) in Liver (µmoles L^{-1} cm^{-1} of MDA/g tissue)

In the present study , groups pre-treated with methanolic extract of *Ficus virens* leaves showed significant reduction ($P<0.001$) in MDA levels as compared to CCl_4 induced oxidative stress group in both male and female rats. Kakali *et al.*(2001) reported that lipid peroxidation oxidative deterioration of polyunsaturated lipids and highly destructive process involving free radicals and alters the structure and function of cellular membrane .It leads to the generation of peroxides and hydroperoxides that can decompose to yield a wide range cytotoxic products most of which are aldehydes (Malondialdehyde and 4-hydroxynonenal).

The present finding is in accordance with the reports of Nalin and You (2004) who stated that the lipid peroxidation inhibitory effects of cactus pear fruit (*Opuntia ficus-indica*) extract reduced the MDA level. Meera *et al.*(2006) also documented similar observation with hydroalcoholic extract of *Taraxacum officinale* roots in rats which showed significant reduction in MDA levels as compared to CCl_4 control group.

5.2.3 Antioxidant enzyme profile

5.2.3.1 Superoxide dismutase (SOD) activity in Liver (units/mg protein)

In the present study, group VI pre treated with methanolic extract of *Ficus virens* leaves (150 mg/kg) showed significant increase ($P<0.05$) in superoxide dismutase activity as compared to CCl_4 induced oxidative stress group in female rats. But male group rats pre treated with methanolic extract of *Ficus virens* leaves showed no significant increase ($P>0.05$) in SOD activity was observed when compared to CCl_4 induced oxidative stress group.

Findings of the present investigation are supported by the findings of Patel *et al.* (2010) who reported that in colon homogenate of rats, aqueous extract of *Ficus bengalensis* bark markedly increased the superoxide dismutase (SOD) activity.

The above findings are in accordance with the findings of Sundaram *et al.* (2007) who reported superoxide dismutase, the enzyme responsible for dismutation of superoxide anion radicals, significantly depleted with CCl_4 induced oxidative stress group as compared to control group. Treatment with ethyl acetate soluble fraction of *Acacia arabica* bark in rats significantly increased superoxide dismutase levels.

In the present study, it was revealed that the male rats are more susceptible to oxidative stress compared to female rats. This might be attributed to the gender differences to the oxidative stress and indicative of the fact that the male are more susceptible to oxidative stress. This finding is in accordance with the observations of Chakraborti *et al*, (2006) who reported that there was gender difference in stress susceptibility in male and female rats and males were more prone to oxidative stress.

5.2.3.2 Catalase (CAT) activity in liver (µM of H_2O_2 utilized/min/mg protein)

In the present study, Group VI pre treated with methanolic extract of *Ficus virens* leaves (150 mg/kg) showed significant increase ($P<0.05$) in catalase activity as compared to CCl_4 induced oxidative stress group in female rats. But in male group rats pretreated with methanolic extract of *Ficus virens* leaves showed no significant increase ($P>0.05$) in catalase activity as compared to CCl_4 induced oxidative stress group.

However, the literature persuade did not reveal any reports on the role of catalase in the antioxidant activity of *Ficus virens*. Many reports are there about the antioxidant activity of different species of *Ficus* to support the finding of the present study. Smitha *et al*. (2009) reported that the catalase enzyme activity was markedly increased in *Ficus relegiosa* treated group. Similar observations were also made by Mansor and Mahmood (2009) who reported that the catalase activity was increased by various accessories of the plant *Ficus deltoidea*.

The above findings are in accordance with the findings of Meera *et al.* (2006) who stated that the H_2O_2 formed by superoxide dismutase and other processes scavenged by catalase that catalyzes the dismutation of H_2O_2 into water and molecular oxygen. Thus, the antioxidant enzyme catalase is responsible for detoxification of H_2O_2.Treatment of rats with hydroalcoholic extract of *Taraxacum officinale* roots showed significant increase in catalase activity as compared to CCl_4 control group.

5.2.3.3 Glutathione Peroxidase (GPx) activity in liver (units/mg protein)

In the present study, Group VI pre treated with methanolic extract of *Ficus virens* leaves (150 mg/kg) showed significant increase ($P<0.05$) in glutathione peroxidase activity as compared to CCl_4 induced oxidative stress group in female rats. But in male group rats pre treated with methanolic extract of *Ficus virens* leaves showed no significant increase ($P>0.05$) in glutathione peroxidase activity as compared to CCl_4 induced oxidative stress group.

The above findings are in accordance with the findings of Meera *et al.*(2006) who stated that the glutathione peroxidase is an enzyme that catalyzes the reduction of hydroperoxides, including H_2O_2 and functions to protect the cell from peroxidative damage, significant increase in glutathione peroxidase activity has direct free radical scavenging property and also strengthens the enzymatic antioxidant defense system.Treatment of rats with hydroalcoholic extract of *Taraxacum officinale* roots showed significant increase in glutathione peroxidase activity as compared to CCl_4 control group.

5.2.4 Serum biochemistry

5.2.4.1 Aspertate aminotransferase(AST) activity (U/L)

In the present study, Group VI pre-treated with methanolic extract of *Ficus virens* leaves (150 mg/kg) showed significant decrease ($P < 0.001$) in aspertate aminotransferase level as compared to CCl_4 induced oxidative stress group in both male and female rats.

The above findings are in accordance with the findings of Sundaram *et al.* (2007) who reported that the hepatic injury induced by CCl_4 results in an increase in serum AST level due to leakage of cellular enzymes into circulation. The decrease in the elevation of serum AST following treatment with ethyl acetate soluble fraction of *Acacia arabica* bark in rats is due to reduction in cell membrane disturbances.

5.2.4.2 Alanine aminotransferase (ALT) activity (U/L)

In the present study, Group VI pre-treated with methanolic extract of *Ficus virens* leaves (150 mg/kg) showed significant decrease ($P < 0.001$) in alanine aminotransferase level as compared to CCl_4 induced oxidative stress group in both male and female rats.

The above findings are in accordance with the findings of Sundaram *et al.* (2007) who reported that the hepatic injury induced by CCl_4 results in an increase in serum ALT level due to leakage of cellular enzymes into circulation. The decrease in the elevation of serum ALT following treatment with ethyl acetate soluble fraction of *Acacia arabica* bark in rats is due to reduction in cell membrane disturbances.

Rats administered with the plant extract in low and medium doses (50 and 100 mg/kg), did not show any increase in the antioxidant activity where as the rats administered with high dose (150 mg/kg) of methanolic extract of *Ficus virens* leaves showed significant increase ($P<0.05$) in free radical scavenging activity when compared to CCl_4 induced oxidative stress group.

In the present study also, the phytochemical analysis of the methanolic extract of *Ficus virens* leaf revealed the presence of flavonoids which might be responsible for the anti oxidant property observed in the high dose group.

The above findings are in accordance with the findings of Perrisoud *et al.*(1982) and Ratty *et al.*(1988) who reported that flavonoids are natural products , which have been shown to posses various biological properties related to antioxidant mechanisms.

The findings of the present study are in accordance with the findings of Anandjiwala *et al.* (2008) who evaluated the free radical scavenging activity of an Ayurvedic preparation panchvalkala and its individual components (stem bark of *Ficus benghalensis*, *F. glomerata*, *F. religiosa*, *F. virens* and *Thespesia populnea*) in three *in vitro* models *viz* . 1, 1-diphenyl-2-picrylhydrazyl radical scavenging activity, superoxide radical scavenging activity and reducing power assay. Panchvalkala and its individual components showed significant antiradical activity by bleaching 1, 1-diphenyl-2-picrylhydrazyl radical (EC_{50} ranging from 7.27 to 12.08 µg) which was comparable to pyrogallol (EC_{50} 4.85 µg).

The findings of the present study are in accordance with the findings of Abdel-Hameed (2009) who reported that the methanol extracts of the leaves of eleven *Ficus* species growing in Egypt were subjected to free radical scavenging activity using 1,1-

diphenyl picrylhydrazyl (DPPH) method. Six methanol extracts of six species showed high activity in the order of: *Ficus lyrata Warb.* > *Ficus afzelli G.* > *Ficus nitida L.* > *Ficus virens Ait.* > *Ficus sycomorus L.* > *Ficus decora Hort.* with SC_{50} 38.37, 60.22, 61.67, 74.00, 79.50 and 81.62 µg/ml, respectively.

5.2.5 Histopathology

In the present study, Group IV and V pre-treated with methanolic extract of *Ficus virens* leaves (50 and 100 mg/kg , respectively) showed congestion, necrosis in liver as similar to CCl_4 induced oxidative stress group in both male and female groups. Group VI pre-treated with methanolic extract of *Ficus virens* leaves (150 mg/kg) showed moderate ameolioration of CCl_4 induced oxidative stress where in moderate areas of necrosis and vascular degeneration were presented. In CCl_4 induced oxidative stress group, liver showed extensive areas of congestion, necrosis, vascular degeneration and inflammatory cell infiltration.

The above findings are in accordance with the findings of Sundaram *et al.* (2007) who reported the similar histopathological findings.

5.3 Acute oral toxicity study

In the assessment and evaluation of the toxic characteristics of a substance, determination of acute oral toxicity is usually an initial step. It provides information on health hazards likely to arise from short term exposure by the oral route. It is traditionally a step in establishing a dosage regimen in other studies by providing initial information on the mode of toxic action of a substance.

The methanol extract of *Ficus virens* even at the highest dose tested (5000 mg/kg) did not reveal any mortality in Wistar albino rats indicating that it is non toxic. Many plant extracts also did not reveal the mortality in the highest dose (5000 mg/kg).

Hence, the present findings are in accordance with the finding of the earlier findings of Devaraj *et al.* (2010) who reported that ethanolic extract of *Curcuma xanthorrhiza* plant did not reveal any toxic effects and mortality in mice at 5 g/kg in the acute oral toxicity study. Oduola *et al.* (2010) who found that *Morinda lucida* leaf extract was non-lethal at 6400 mg/kg in Wistar albino rats in acute oral toxicity study. This is further supported by Garg (2000) who mentioned that the chemicals with LD_{50} value >5 g/kg, could be considered as practically non toxic.

A conclusion can be drawn by the above finding that, many of the plant extracts are non toxic in the dose of 5000 mg/kg.

5.4 Repeated dose 28-day oral toxicity study

In the assessment and evaluation of the toxic characteristics of *Ficus virens* leaf extract, the determination of oral toxicity using repeated doses was carried out after initial information on toxicity was obtained by acute oral toxicity testing. This study provided information on the possible health effects and hazards likely to arise from repeated exposure over a relatively limited period of time.

Ficus virens leaf extract was administered orally by gavage daily for 28 days to male and female rats separately at 50, 200, 800 and 1000 mg/kg. The animals did not show any changes in general behavior or other physiological activities, no observable clinical signs of the toxicity and mortality during the entire period of the experiment in both the sexes.

Similar observations were also done by Jujun *et al.* (2008) who reported that *Garcinia mangostana* Linn.Rind plant extract did not reveal any toxicity in the dose of 1000 mg/kg in Sprague- Dwaley rats in repeated dose 28-day oral toxicity study.

5.4.1 Serum biochemical parameters

Serum obtained from blood samples collected on day 0, 7, 14, 21 and 28 of experiment period were used to estimate AST, ALT, BUN and Cr for both male and female rats separately.

5.4.1.1 AST and ALT

In the present study, there was no significant (P>0.05) increase in AST and ALT values in any of the tested groups (both male and female) group I, group II, group III and limit dose group administered with *Ficus virens* leaf extract 50, 200,800 and 1000 mg/kg respectively, compared with a respective control group values.

This finding is in accordance with the finding of Chivapat *et al.* (2001) who reported that there was no change in the above said parameters during the chronic toxicity of *Cassia siamea* in rats. Similarly, Pieme *et al.* (2006) reported that evaluation of acute and subacute toxicities of aqueous ethanolic extract of leaves of *Senna alata* did not show increase in ALT and AST values. ALT and AST are two liver enzymes that are associated to the hepatocellular damage. In general with liver disease, serum levels of AST and ALT will rise and fall at the same time (Sacher *et al.*, 1991). A mild elevation of AST level has been shown to be associated with liver injury or myocardial infarctions (Stroev, 1989).

This indicated that the methanolic leaf extract of the *Ficus virens* was not hepatotoxic at the dose and duration of the study. This was further strengthened by the histopathological findings of the present study, where in liver showed normal architecture of hepatocytes in all tested groups were evident.

This finding is in accordance with the findings of Kshirsagar *et al.* (2010) who reported that ethanolic extract of *Calotropis gigantea* R.BR in subacute toxicity study in rats did not reveal significant changes in AST and ALT values at the dose of 1000 mg/kg

5.4.1.2 BUN and creatinine

In the present study, there was no significant (P>0.05) increase in BUN and creatinine values in any of the tested groups (both male and female) group I, group II, group III and limit dose group administered with *Ficus virens* leaf extract 50, 200, 800 and 1000 mg/kg respectively, compared with a respective control group values. This was further strengthened by the histopathological findings of the present study, where in kidney showed normal architecture in all tested groups. This revealed the non toxic nature of the plant extract on kidney at the dose and duration tested in the rats of both sexes.

This finding is in accordance with the findings of Kshirsagar *et al.* (2010) who reported that ethanolic extract of *Calotropis gigantea* R.BR in subacute toxicity study in rats did not reveal significant changes in BUN and creatinine values in the dose of 1000 mg/kg.

5.4.2 Hematological parameters

In the present study, there were no significant (P>0.05) changes in the TEC, TLC, PCV and Hb concentration of all the treated groups compared to control group. This implied absence of any toxic effects on haemopoitic system in the present study.

This finding is in accordance with the findings of Kshirsagar *et al.*(2010) who reported that 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) at the dose of 1000 mg/kg.

5.4.3 Body weight

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

This finding is in accordance with the findings of Jujun *et al.* (2008) who reported that *Garcinia mangostana* Linn.Rind plant extract did not show significant decrease in body weight in the dose of 1000 mg/kg in Sprague- Dwaley rats in repeated dose 28-day oral toxicity study.

5.4.4 Feed consumption

In the present study, there was no significant (P>0.05) change in feed consumption in group I, group II, group III and limit dose groups administered with *Ficus virens* leaf extract 50, 200 , 800 and 1000 mg/kg respectively, compared with a respective control group feed consumption. This indicated the *Ficus virens* leaf extract was non toxic at the dose and duration of the study which was further supported by normal biochemical parameters and normal histological findings.

This finding is in accordance with the findings of Jujun *et al*. (2008) who reported that *Garcinia mangostana* Linn.Rind plant extract did not show significant decrease in feed intake in the dose of 1000 mg/kg in Sprague- Dwaley rats in repeated dose 28-day oral toxicity study.

5.4.5 Organ to body weight ratio

There was no change in the organ to body weight ratio of all the treated groups compared to control group.

Organ weight usually increases long term toxicity studies. Increase in organ weight is attributed to the pathological changes (Extoxnet, 1993). In the present study, the dose and duration of administration of the *Ficus virens* leaf extract to the rats might be less for causing any change in the organ weight.

This finding is in accordance with the findings of Jujun *et al*. (2008) who reported that *Garcinia mangostana* Linn.Rind plant extract did not show significant change in relative organ to body weight ratio at the dose of 1000 mg/kg in Sprague- Dwaley rats in repeated dose 28-day oral toxicity study.

5.4.6 Pathology

5.4.6.1 Gross pathology

At necropsy none of the treated and control rats showed any gross pathological lesions.

5.4.6.2 Histopathology

In the present study, 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.Satellite high dose group administered with 800 mg/kg of *Ficus virens* leaf extract showed normal architecture of the organs in histopathological study.

Similar observations were reported by Kshirsagar *et al.*(2010) in ethanolic extract of *Calotropis gigantea* R.BR in subacute toxicity study in rats at the dose of 1000 mg/kg.

The following conclusion were drawn from the present study

1. Phytochemical analysis of the *Ficus virens* leaf extract was revealed for the presence of flavonoids, bitter principles, coumarins and absence of alkaloids.

2. Methanolic extract of *Ficus virens* leaf was shown to have antioxidant activity on oral administration.

3. In acute oral toxicity study the methanolic extract of *Ficus virens* leaves produced no toxicity and mortality even in the highest dose (5000 mg/kg) and hence LD_{50} may be more than 5000 mg/kg.

4. In repeated dose 28-day oral toxicity study with *Ficus virens* leaf extract, no signs of toxicity, no mortality, no alterations in serum biochemistry or hematology and no histopathological changes were noticed in both male and female rats.

VI SUMMARY

The present study was conducted to evaluate the pharmacological property like antioxidant activity and toxicological properties like acute and sub-acute toxicity of methanolic extract of *Ficus virens* leaves. There are very few research reports on pharmacological and toxicological properties of *F.virens.* Hence, vigorous research efforts on pharmacological and toxicological properties of *F. virens* are required to use this plant in therapeutics.

Considering the need for systematic elucidation of pharmacological and toxicological properties of *Ficus virens*, this study was conducted to evaluate antioxidant activity and the acute, sub-acute toxicity of methanol extract of *Ficus virens* in Wistar albino rats.

The phytochemical analysis of the *Ficus virens* leaf extract revealed for the presence of flavonoids, bitter principles and coumarins.

The methanolic extract of *Ficus virens* leaves showed significant increase in antioxidant activity in both male and female, but male group showed less significant increase in antioxidant activity as compared to female group. When Group VI administered with high dose (150 mg/kg) of methanol extract of *F. virens* leaves showed significant increase in antioxidant activity as compared to other test groups, Group IV, V (50 and 150 mg/kg. b.wt.p.o) and positive control Group II (CCl_4, 1 ml/kg. in liquid paraffin, 1:1), though the values did not reach to normalcy and standard control. *In vivo* evalution of this methanol extract of *F. virens* in CCl_4 induced oxidative stress for 7 days at a dose 150 mg/kg showed marked protection as compared to other test groups and positive CCl_4 control, this was evident by significant changes in lipid peroxidation ($P<0.001$), superoxide dismutase ($P<0.05$), catalase ($P<0.05$), glutathione peroxidase ($P< 0.05$), AST($P<0.001$) and ALT ($P<0.001$) in female group and in male group lipid peroxidation ($P< 0.001$), AST($P<0.001$) and ALT ($P<0.001$). At lower doses, the protection was not consistent.

Acute oral toxicity and repeated dose 28-day oral toxicity were conducted in Wistar albino rats. The studies were conducted according to standard guidelines and methods.

Acute oral toxicity study was conducted in both male and female rats separately. There were no deaths and clinical signs in any of the tested groups within 24 h. The treated groups were kept under observation period of 14 days. No mortality and clinical signs of toxicity were observed in any of the tested group even at highest dose 5000 mg/kg. Hence, LD_{50} of methanolic extract of *Ficus virens* leaf in Wistar albino rats may be greater than 5000 mg/kg.

Repeated dose 28-day oral toxicity study was conducted in five groups each consisting of 6 male and another four groups each consisting of 6 female Wistar albino rats. The doses administered orally were distilled water (control), 50, 200, 800 and 1000 mg/kg of methanolic extract of *Ficus virens* leaves for a period of 28 days.

The biochemical parameters AST, ALT, BUN and creatinine did not show significant increase (P>0.05) indicating that *F. virens* leaf extract at the dose level selected did not exert any toxicity.

Hematological parameters such as TEC, TLC, PCV and Hb concentration did not show significant (P>0.05) change.

The body weight and feed consumption did not show significant change (P>0.05) indicating that *F. virens* leaf extract at the selected doses did not exert any toxicity.

The *Ficus virens* leaf extract did not produce any histopathological lesions in any of the organs.

The present study revealed that *Ficus virens* has antioxidant activity and did not exhibit any signs of toxicity in acute and sub-acute toxicity study in rats.

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VIII ABSTRACT

The methanolic extract of *Ficus virens* leaves was evaluated for its phytochemical analysis, antioxidant activity, acute oral toxicity and repeated dose 28-day oral toxicity in rats. The phytochemical analysis of the *F.virens* extract was carried out using High performance thin layer chromatography revealed for the presence of flavanoids, bitter principles, coumarins and absence of alkaloids. *In vivo* evalution of of *F. virens* in CCl_4 induced oxidative stress for 7 days at a high dose 150 mg/kg showed marked protection as compared to other test groups and positive CCl_4 control, this was evident by significant changes in lipid peroxidation ($P<0.001$), superoxide dismutase ($P<0.05$), catalase ($P<0.05$), glutathione peroxidase ($P< 0.05$), AST($P<0.001$) and ALT ($P<0.001$)in female group and in male group lipid peroxidation ($P< 0.001$), AST($P<0.001$) and ALT ($P<0.001$).At lower doses, the protection was not consistent.

In acute toxicity study, there were no deaths and clinical signs of toxicity in any of the tested groups. Hence, LD_{50} value may be greater than 5 g/kg . In repeated dose 28-day oral toxicity, at doses 50, 200 and 800 mg/kg and limit dose of 1000mg/kg of *Ficus virens* leaf extract showed no significant changes biochemical parameters (AST, ALT, BUN and creatinine) haematological parameters. No Significant ($P>0.05$) change in feed consumption and body weight gain. There were no gross and histopathological changes noticed. This indicates that *F. virens* non toxic at given dose and duration.

Keywords: *Ficus virens*; LD_{50} value; Antioxidant; Phytochemical analysis; High performance thin Layer Chromatogaphy.

Plate 1A: *Ficus virens* plant

Plate 1B: *Ficus virens* fresh leaves

Plate 1C: *Ficus virens* dried leaves

Plate 1D: *Ficus virens* leaves extract

Plate 2A: Blue fluorescence was not observed on TLC plate at UV-366 nm without chemical treatment for alkaloids.

Plate 2B: Yellowish brown bands were not observed on TLC plate on spraying with dragendorff reagent for alkaloids.

Plate 2C: TLC plate showing red brown bands at UV-366 nm without chemical treatment for anthracenes.

Plate 2D: TLC plate showing red brown bands at UV-366 nm on spraying with natural products – polyethylene glycol reagent for anthracenes

Plate 3A: TLC plate showing fluorescence quenching at UV-366nm without chemical treatment for bitter principles.

Plate 3B: TLC plate showing red, brown, blue fluorescence at UV-366 nm on spraying with vanillin-sulphuric acid reagent for bitter principles.

Plate 3C: TLC plate showing light blue fluorescence at UV-366 nm without chemical treatment for flavanoids.

Plate 3D: TLC plate showing blue, yellow, orange fluorescence at UV-366 nm on spraying with natural products – polyethylene glycol reagent for flavanoids.

Plate 3E: TLC plate showing blue fluorescence at UV-366 nm without chemical treatment for coumarins.

Plate 3F: TLC plate showing pronounced blue fluorescence at UV-366nm on spraying with potassium hydroxide reagent for coumarins.

Plate 4A: Section of the liver showing normal architecture of hepatocytes in control group I female rats. (H and E X 100)

Plate 4B: Section of the liver showing massive large scale of necrotic area, inflammatory changes, vascular degeneration and haemorrhages with loss of architecture in the CCl_4 control group II female rats. (H and E X 200)

Plate 4C: Section of the liver showing significant restoration of hepatocytes with milder degree of congestion in Vit-C control group III female rats. (H and E X 100)

Plate 4D: Section of the liver showing non significant restoration of hepatocytes with massive degree of congestion and necrosis in Group IV female rats.

(H and E X 200)

Plate 4E: Section of the liver showing non significant restoration of hepatocytes with milder degree of congestion and necrosis in Group V female rats.

(H and E X 200)

Plate 4F: Section of the liver showing slight significant restoration of hepatocytes with milder degree of congestion and necrosis Group V female rats.

(H and E X 200)

Plate 5A: Section of the liver showing normal architecture of hepatocytes in control Group I male rats. (H and E X 100)

Plate 5B: Section of the liver showing massive large scale of necrotic area, inflammatory changes, vascular degeneration and haemorrhages with loss of architecture in the CCl_4 control Group II male rats. (H and E X 200)

Plate 5C: Section of the liver showing significant restoration of hepatocytes with milder degree of congestion in Vit-C control Group III male rats. (H and E X 100)

Plate 5D: Section of the liver showing non significant restoration of hepatocytes with massive degree of congestion and necrosis in Group IV male rats. (H and E X 200)

Plate 5E: Section of the liver showing non significant restoration of hepatocytes with milder degree of congestion and necrosis in Group V male rats. (H and E X 200)

Plate 5F: Section of the liver showing slight significant restoration of hepatocytes with milder degree of congestion and necrosis Group V male rats. (H and E X 200)

Plate 6A: Section of liver showing normal architecture in control Group I in sub acute toxicity study. (H and E X 100)

Plate 6B: Section of kidney showing normal architecture in control Group I in sub acute toxicity study. (H and E X 100)

Plate 6C: Section of liver showing slight granularity in group IV (High dose) in sub acute toxicity study. (H and E X 100)

Plate 6D: Section of kidney showing slight distension and desquamation of tubular epithelium with normal appearance of glumeruli in group IV (High dose) in sub acute toxicity study. (H and E X 100)

Plate 6E: Section of liver showing swollen granular hepatocytes with congestion of sinusoidal spaces and degenerative changes are evident in group IV (Limit dose) in sub acute toxicity study (H and E X 100)

Plate 6F: Section of kidney showing tubular epithelium is swollen and distended, massive intertubular haemorrhages in group IV (Limit dose) in sub acute toxicity study. (H and E X 100)