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Hepatoprotective Activity of Ficus Pumila Leaves Intoxification against Aspirin in Experimental Rats

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ABSTRACT: Ficus pumila L. has been used as a functional plant for a long time in East Asia, especially its fruits, as a dietary component in Japan and parts of China. A series of bioactive compounds, including Phenolic acids, flavonoids, terpenoids, alcohols, and steroids, have been extracted from the stems, leaves, flowers, and fruits of Ficus pumila L. Accumulated studies have demonstrated that Ficus pumila L. has multiple therapeutic activities, including antioxidant, anti-inflammatory, antibacterial, antitumor, hypoglycemic, and cardiovascular protective effects. The present study was designed to investigate the hepato protective effect of ethanolic extract of Ficus pumila L., Moraceae, on aspirin induced hepatotoxicity in rats. Experimental rats were divided into five groups; group 1 served as a control received vehicle (Distilled water), group 2 served as a toxic control, received aspirin (100 mg/ kg, i.p.) in sterile water, groups 3 and 4 received 200, 400mg/kg bw, p.o. ethanolic extract of F. pumila along with and group 5 received as reference standard drug Silymarin 200 mg/kg. All the treatment protocols followed 21 days for Aspirin induced hepatotoxicity model, after treatment rats were sacrificed and blood was used for biochemical and liver was used for histological studies. Administration of aspirin caused a significant elevation in the levels of liver marker enzymes ($P < 0.05$ and $P < 0.01$in experimental rats. Administration of ethanolic leaves extracts of F. pumila L. significantly prevented aspirin induced hepatotoxicity elevation in the levels of serum diagnostic liver marker enzymes in experimental groups of rats. The effect of extract was compared with a standard drug. The changes in biochemical parameters were supported by histological profile. The ethanolic extract of F. pumila L. protects against aspirin induced hepatotoxicity in liver injury in experimental rats.

Keywords: Hepatoprotective Activity, Anti-inflammatory, Antibacterial, Antitumor, hypoglycemic, and Cardiovascular Protective effects.

I. INTRODUCTION:

Oxidative stress and free radicals enhance the severity of hepatic damage, which can be overcome by the antioxidant mechanism. Plant extracts can be the best source of such antioxidants and mediate Hepatoprotective activity. In this chapter, high-dose paracetamol-induced hepatotoxicity in rat model is discussed with explanations of biochemical and Histopathological studies. These Hepatoprotective plants contain the Phytoconstituents such as phenyl compounds, coumarins, essential oils, Monoterpenoids, triterpenoids, triterpenoids, steroids, alkaloids and other nitrogenous compounds. Coumarins and naturally occurring benzopyrene derivatives are hepatoprotectively active compounds, flavonoids, triterpenoids and tannins are well known for their Hepatoprotective activities. Gallic acid (GA), a trihydroxybenzoic acid, possesses promising Hepatoprotective effects. The Hepatoprotective activity of betulinic acid, 3-methoxyquercetin, catechin, epicatechin, quercetin and quercetin was tested in vitro against CCl_4 -induced damage in rat hepatoma cells. Ursolic acid (UA), one of the pentacyclic triterpene acids, is ubiquitous in the plant kingdom and is found in fruits, vegetables and medicinal plants. It is well known for its Hepatoprotective effects for both acute chemically induced liver injury and chronic liver fibrosis and cirrhosis. Another pentacyclic triterpene acid, oleanolic acid, also reported for the same activity [1, 2].

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II. MATERIALS AND METHODOLOGY:

Instruments and Chemicals:

A wide range of instruments and chemicals were used during the course of this Study as follow:

Instruments:

- ✓ Soxhelt Apparatus Quick FIT, EX5/83. ENGLAND.
- ✓ Water bath BUCHI 461 SWITZERLAND
- ✓ Sensitive balance HF-300G A&D COMPANY, Limited
- ✓ Haemacytometer Hawksley, ENGLAND.
- ✓ Capillary Tubes.
- ✓ Condenser.
- ✓ Rota vapor apparatus.

Chemicals:

- ✓ aspirin
- ✓ Absolute Petroleum Ether
- ✓ Silymarin SGOT enzyme Kit (Giri diagnostic kit pvt ltd)
- ✓ SGPT enzyme kit (Giri diagnostic kit pvt ltd)
- ✓ Chloroform (Central drug house pvt ltd) New Delhi

Collection and Identification of the Tested Plants *Ficus Pumila L.:*

Ficus pumila L. leaves were procured on Febuary 2022 from plants growing in Pot N Plant Ghaziabad, Uttar Pradeshand dried at room temperature. The leaves were identified and authenticated by Dr. Ashok, Head of Department of Botany, CCS University Meerut.

Preparation of Extracts of *Ficus Pumila L.:*

Extraction involves the separation of bioactive portion of the plant tissues from the inactive components by using selective solvents in standard extraction procedure. The powdered leaves (500g) were extracted by using acetone by sequentially extracted using petroleum ether, chloroform, acetone and aspirin in Soxhlet apparatus. Where as a known weight of the coarsely powdered part of each plant was extracted successively with chloroform (60 -80 c°) and aspirin (98%) using soxhlet extractor. The chloroform extract was kept a side and the ethanolic extracts were filtered, evaporated, dryness using Rota vapor apparatus (under reduce pressure), and the yields were calculated. The residue obtained was kept in dry clean bottles till used [3].

Preliminary Phytochemical Studies:

The various extracts *Ficus pumila L.* of obtained were subjected to qualitative analysis to test the presence of various phytochemical constituents like alkaloids, carbohydrates, glycosides, flavonoids, steroids, terpenoids, phenols, proteins, tannins etc [4, 5].

Tests for Alkaloids:

Mayer‟s Test:

A pinch of dried extracts was taken and 2ml of dilute hydrochloric acid was added, mixed filtered. To the filtrate, one or two drops of Mayer‟s reagent were added. Formation of pale yellow precipitate indicates the presence of alkaloids.

Dragendroff's Test:

A pinch of dried extracts was taken treated with 2ml of 2% Acetic acid, mixed thoroughly and filtered. To the filtrate 2 drops of Dragendorff‟s reagent was added. Formation of orange-brown precipitate indicates the presence of alkaloids.

Hager's Test:

A pinch of dried extracts was taken and treated with drops of Hager‟s reagent. Formation of yellow precipitate indicates the presence of alkaloids.

Wagner's Test:

A pinch of dried extracts was taken and treated with drops of Wagner‟s reagent. Formation of brown precipitate indicates the presence of alkaloids

Tests for Sugar and Carbohydrates:

Molish's Test:

A small quantity of extracts was dissolved separately in 4ml of distilled water and filtered. Filtrate was treated with 2-3 drops of 1% Alcoholic Sulphuric acid was added from the sides of the test tube. Brown ring at the junction of two liquids indicates the presence of carbohydrate.

Tests for Glycosides:

Anthrone Test:

A pinch of extracts was taken in a watch glass and 2 drops of alcohol was added to extract. An equal quantity of anthrone was added and mixed thoroughly and dried. Then one drop of concentrated sulphuric acid was added, separated in a thin film with a glass rod in a watch glass. and heated over the water bath. Formation of dark green color indicates the presence of glycosides.

Test for Anthraquinone Glycosides:

Borntrager's Test:

A pinch of the extracts was boiled with dilute sulphuric acid, filtered while hot and filtrate was extracted with solvent like benzene. It was shaken well and the organic layer was separated and to this equal volume of dilute ammonia was added. Rose pink colour in ammonia layer indicates the presence of anthraquinone glycoside.

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Test for Cardiac Glycosides:

a) Legal's Test:

The extracts were hydrolyzed for few hours in a water bath. The hydrolysate was added with 2ml of pyridine, sodium nitropruside solution and was made alkaline with sodium hydroxide solution. Orange colour shows the presence of cardiac glycoside.

Tests for Proteins:

Small quantity of extracts was dissolved in a few ml of water and subjected to the following test:

Biuret Test

To the extract solution few drops of biuret reagent (1%CuSO4 and 10% NaOH), 1 drop of Copper sulphate solution and 10 drops of sodium hydroxide solution were added. Purple or violet color shows the presence of proteins.

Million's Test:

Few drops of Million‟s reagent were added to the extract solution. Reddish brown color shows the presence of proteins.

Tests for Amino Acid:

a) Ninhydrin Test:

To the extract few drops of Ninhydrin reagent were added. Purple color shows the presence of amino acids.

Tests for Saponins:

Foam Test:

1ml of the aspirin extract solutions were taken in a measuring cylinder. To this, 20 ml of distilled water were added and shake well.

Haemolysis Test:

The aspirin extracts of the plant were spread over a glass slide to form a thin film layer on which a drop of human blood was placed and spread over the extract layer. After 30 minutes, the slide was examined under microscope for change in the structure and shape of red blood cells. Control was always maintained to see the change in red blood cells structure for haemolysis.

Test for Glycosides:

Small amount of extracts is treated with distilled water and subjected to molish's test. To the extracts is hydrolyzed with dilute hydrochloric acid and subjected to Lieberman –burchara‟s, legal's and Borntrager's test to detect presence of different glycosides.

Test for Reducing Sugar:

A small portion of extracts was dissolved in water and treated with Fehling‟s and Benedict‟s reagent to detect presence of sugars.

Test for Phytosterols:

The extracts were heated with solution of alcoholic potassium hydroxide until complete Saponification. Then it was diluted with distilled water and extracted with ether. The ethereal extract is evaporated and the residue (unsaponifiable matter) was subjected to Liebermann's and Lieberman Burchara‟s test.

Tests for Phenolic Compounds:

Ferric Chloride Solution Test:

The extracts were taken in water and warmed; to this 2ml of Ferric Chloride solution was added. Formation of green colour is due to the presence of phenolic compounds.

Lead Acetate Solution Test:

To the extracts (2ml) lead acetate solution was added separately. Formation of precipitate indicates the presence of Phenolic compounds.

Tests for Tannins:

A pinch of the dried extracts was dissolved in aspirin, mixed thoroughly and filtered. The filtrate is tested for the presence of tannins by the following test;

Ferric Chloride:

To the filtrate dilute Ferric chloride solution was added. Formation of greenish blue precipitate is due to presence of tannins.

Lead Acetate:

To the filtrate lead acetate solution was added (10%). Formation of white colour precipitate is due to the presence of tannins.

Gelatin Solution:

To the filtrate 1% solution of gelatin solution containing 10% Sodium Chloride was added. Formation of white colour precipitate is due to the presence of tannins.

Tests for Terpenoids:

Noller's Test or Salkowshi Test:

A pinch of dried extract in a test tube was taken and a bit of Tinfoil and 0.5 ml of thionyl chloride was added. It was heated gently. Formation of the pink colour is due to the presence of terpenoids.

Determination of Fixed Oils and Fats:

Spot Test:

A small quantity of various extracts was pressed separately between two filter papers. Oil stain in the filter paper indicates the presence of the fixed oils.

Tests for Steroids:

Liebermann's Burchard Test:

The extract was dissolved in 2ml of chloroform and 10 drops of acetic anhydride, 2

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drops of concentrated sulphuric acid were added. Formation of green colures due to the presence of Phytosterols.

Salkowski Test:

A extract was treated with chloroform and a few drops of concentrated sulphuric acid was added along the sides of the test tube. The lower layer of the chloroform showed red colour due to the presence of steroids

Laboratory Animals:

Wistar albino rats (150-200g) used in studies was procured from IFTM University Muradabad. The animals were fed with standard pellet diet (Hindustan lever Ltd. Gwalior Road Agra) and water ad libitium. All the animals were acclimatized for a week before use. The experimental protocols were approved by Institutional Animal ethics Committee after scrutinization. Animals were received the drug by oral gavage tube. All the animals were care of under ethical consideration as per the CPCSEA guidelines (CPCSEA, 2003) with regular inspections of rats [6].

Induction of Liver Damage by Aspirin and CCl4:

The concentration of aspirin was determined by using the following formula: Wt/Vol (mg/mL). Each tablet of aspirin was dissolved in 3ml of distilled h2o. A total of 33-tablets of aspirin (each 300mg, 33-tab X 300mg = 9900mg) were dissolved in 99mL of distilled water, making 100mg/ml. 1ml/kg was administered orally to the experimental groups. 16mL of CCl4 from the stock was dissolved up to 100ml of olive oil. 2mL/kg was administered subcutaneously to the experimental groups [7].

Acute toxicity studies:

Healthy Wistar albino female rats of sex weighing 100-170 g maintained under standard laboratory conditions were used for acute oral toxicity test according to Organization for Economic Co-operation and Development guidelines 423. Animals were observed individually at least once during first 30 min after dosing, periodically during first 24 h. Observations were done daily for changes in skin and fur, eyes, mucus membrane (nasal), respiratory rate, circulatory signs (heart rate), autonomic effect (salivation, lacrimation, perspiration, urinary incontinence and defecation) and central nervous system (drowsiness, gait, tremors and convulsion) changes.

Determination of acute toxicity (LD50):

14 days single dose oral acute toxicity and gross behavioral study Number of animals required: 6 rats (male)

Number of groups: 2 groups (3 animals each group)

Dose levels: 4000mg/kg body weight of the animals

Study duration: 14 days

Preparation of dose:

Ethanolic extract of *Ficus pumila L.* leaves was suspended in 3% CMC, to prepare a dose of 4000 mg/kg body weight of animal, and administered 1ml/100gm body weight of the animal.

Experimental Design:

The procedure was divided into two phases, Phase I (observation made on day one), and Phase II (observed the animals since next 14 days). Two set of healthy female animal (each set of 3 rats) were used for the experiment. First set animals were divided and fasted for 18 hours deprived from food, water withdrawn before 4 hours of the dosing, body weights were noted before and after dosing with ethanolic extract of *Ficus pumila* L. (4000mg/kg) orally. Individually animals were observed for 4 hours to see any clinical symptoms, any change in behavior or mortality. 6 hours post dosing again body weights recorded. Form the next day onwards, each day for 1 hour the behavioral change, clinical symptoms or mortality was observed in the same animals for next 14 days and animal body weights were recorded on 8th and 14th day. The same procedure was repeated with another set of animals to nullify the errors [8].

Flow Chart OECD Guidelines:

Test Procedure Starting Dose of 300mg/kg B.W.
↓
300mg/kg / 3 animals
↓
0 - 1 (None of animals died)
↓
2000mg/ kg
↓
2 – 3 (None of animals died)
↓
3000mg/kg (none of animals died)
↓
4000mg/ kg / 3 animals
↓
None of animals died
↓
LD50 cut off 4000mg/ kg B.W.

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Chemicals:

All the chemicals and solvents were of analytical grade. Standard kits for SGOT, SGPT and ALP etc. were obtained from Span Diagnostics Ltd., India. Male Albino rats weighing between 150-200gm used in the experiment were kept in animal house under standard environmental conditions and had free access to feed and water and libitum. The animals were fasted for 16 hours before experiment but allowed free access to water.

Table.1 The Rats were divided into Five Groups each Containing Four Rats

Group 1	Control	Received water (5ml/kg. p.o) for 21 days once daily, and served as normal control
Group 2	Negative control	Received water (5ml/kg. p.o) for 21 days once daily and 40% aspirin v/v (2.0ml/100g body wt, p.o.) for 21 days.
Group 3	Standard	Received 40% aspirin v/v (2.0ml/100g body wt, p.o.) for 21 days and standard drug silymarin (25 mg/kg. p.o.) for 21 days once daily
Group 4	High dose	Received 40% aspirin v/v (2.0ml/100g body wt, p.o.) for 21 days and Received ethanolic extract of *Ficus pumila*(400mg/kg) 21 days once daily
Group 5	Low dose	Received 40% aspirin v/v (2.0ml/100g body wt, p.o.) for 21 days and Received ethanolic extract of *Ficus pumila* (200mg/kg) 21 days once daily

Experimental Procedure:

The rats were weighed after the adaptation period and marked with serial numbers and divided randomly into 5 groups, 5 rats each, and then the doses were calculated according to individual body weights.

Blood Samples:

Blood was obtained by puncturing retro orbital plexus (Poole, 1989), under anesthesia using Halothane and heparin zed capillary tubes. Blood drops were collected, gently, serum was separated by centrifugation (2500 rpm for 15 min), and EDTA was used as an anticoagulant for hematological parameters. Samples were collected before and after dosing with the tested plants extracts at day 0, 5 and at day 10.

Assessment of Liver Function:

Blood sample were collected into dry clean bottles and allowed to clot for 30 min at room temperature. Serum separated by centrifugation at 2500 rpm for 15 min and stored at -20c° until analyzed. Biochemical parameters, i.e. Alanine amino Transeferase (ALT), Aspartate Amino Transferase (AST), alkaline phosphatase (ALP), Total Bilirubin (TBIL) were analyzed according to the reported methods.

Total Bilirubin:

Total Bilirubin in serum is determined using the method of Jendrassik and Grof, (1938).

Principle: Bilirubin in the presence of a sluphanlic acid diazonium salt from a red colored azo compound in alkaline solution. Bilrubin is coupled with diazotized sulfanilic acid in the presence of caffeine to give an azo dye. The optical density was measured by spectrophotometer at wave length 546nm.

Calculation:

Total Bilirubin is calculated as follow:

Absorbance tube Total × 17.5 = Total Bilirubin (mg/dl).

Alkaline phosphatase: It is an optimized method according to recommendation of Chemie (1972).

Principle: In Alkaline medium serum alkaline phosphates splits pnitrophenyl phosphate, in the presence of Mg+2 ions, into p-nitro phenyl and Phosphate. At the PH of reaction, p-nitro phenyl was colored yellow, the optical density measured in a spectrophotometer at wavelength 405nm.

Reaction: P-nitrophenylphosphate + H2o ALP phosphate + p-nitro phenol

Calculation:

ALP is calculated as follows: U/1=2760 × A 405nm/min (A = the mean of sample absorbance reading)

Alanine Amino Transeferase (ALT):

It is an enzymatic method, which measure Gultamic Pyruive Transamine in serum and Schmidt and Schmidt, (1963).

Principle: Alanine Amino Transfers is measured by monitoring the concentration of pyruive hydrazone formed with 2-4 Dinitrophenylhydrazine.

Reaction: α - Oxoglutarate+L-alanine ALT L-glutrate+pyruivate

The absorbance of samples was read against the reagent blank after 5min at wavelength 546 nm in spectrophotometer.

Aspartate Amino Transferase (AST):

It is an enzymatic method that measure Gultamic Oxaloacetic Transaminase in serum and Schmidt and Schmidt, (1963).

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Principle: Aspartate amino transferase is measured by monitoring the concentration of oxaloacetate hydrozone formed with 2-4 dintrophenyl hydrazine.

Reaction:

α-oxoglutarate +L-aspartate AST L-glutarate + oxaloacetate. The absorbance of samples was read against the reagent blank after 5min at wavelength 546 nm in spectrophotometer and cuvette of 1cm light path.

Haematological Studies:

Haemoglobin Concentration (Hb), packed cell volume (PCV), red blood cells count (RBC), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC), were measured. Blood samples were collected into dry clean bottles; the anticoagulant was ethylene diamine tetra acetic acid (EDTA).

Red Blood Cell Count (RBCs):

Total Erythrocytes were counted by using Neubauer Haemocytometer and Hayem's solution.

Calculation: 200×50×R cells = 10.000×Rμl.

Hemoglobin Concentration:

The concentration of Hb was measured by the Cyanmethaemoglobin method. The procedure consists of adding 20μl of blood to 5 ml of a modified Drabkin's solution. After 10min the solution of Cyanmethaemoglobin is compared against a standard in either spectrophotometer (wavelength 540nm).

Assessment of Hepatoprotective Activity:

After 24h of aspirin administration, on 22nd day, blood was obtained from animals by puncturing retro orbital plexus. Blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500rpm at 30°C for 15 min and utilized for the estimation of various biochemical parameters including SGOT & SGPT serum Bilirubin and serum protein.

After collection of blood samples, the animals were sacrificed under deep ether anesthesia. Morphological parameters like weight of animals, weight of liver have also been used to evaluate the protective effect of the drug. Hepatoprotective chemical causes loss in liver weight/100 gm body weight of rats. Histopathological studies of the liver in aspirin induced hepatotoxicity. The Histopathological evaluation of aspirin toxicity in all the groups was examined and shown in figures. The description is as follows, Section of rat liver treated with vehicle control group shows liver parenchyma with intact architecture which is the normal appearance. Section of liver in toxicant control group shows partially effaced architecture. Some of the hepatocytes show apoptotic changes, perivenular mononuclear inflammatory infiltration, scattered inflammatory infiltration within the parenchyma which is due to toxicity. Section of liver in silymarin treated group shows liver parenchyma with intact architecture. Some of the central veins show congestion with diffuse congestion of sinusoids. Section of liver in test drug aspirinqueous treated group's shows intact architecture, few regenerative hepatocytes, sinusoidal congestion and scattered mononuclear inflammatory cells which is similar to silymarin treated group [9, 10, 11, 12].

III. RESULTS AND DISCUSSION:

There are many factors which are responsible for the liver damage or injuries such as chemicals and drugs. In the present study aspirin was used to induce Hepatotoxicity, since it is clinically relevant. Aspirin produces a constellation of dose related deleterious effects in the liver (Leo *etal.*, 1982). The majority of aspirin is metabolized in the liver and individuals who abuse alcohol by routinely drinking 50-60 g (about 4 to 5 drinks) of aspirin per day are at risk for developing alcoholic liver disease (Zakhari *et al.*, 2007). In addition, both acute and `chronic aspirin administration cause enhanced formation of cytokines, especially TNF-alpha by hepatic Kupffer cells, which have a significant role in liver injury (Zhou *et al.*, 2003; Thurman *et al.*, 1998;Tsukamoto*et al.*, 2001). Besides the development of fatty liver (steatosis), another early sign of excessive aspirin consumption is liver enlargement and protein accumulation, both of which are common findings in alcoholics and heavy drinkers (Baraona *et al*., 1977).

Ficus pumila L. are commonly used in the native system of medicine. Various parts of the plantlike leaves and roots are medicinally important. In order to investigate the medicinal use of *Ficus pumila L.*in Hepatoprotective, we evaluated crude extract for its Hepatoprotective activity using different *in vitro* assays and *in vivo* rat model of Hepatoprotective activity. Preliminary Phytochemical analysis of ethanolic extract of *Ficus pumila L.* had showed the presence of Phytoconstituents like alkaloids, flavonoids, tannins, saponins and Cardiac glycosides. Flavonoids and alkaloids are widely distributed in the plant which has the property to cure

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Hepatoprotective activity. Due to this reason the plant has chosen to this study. This shows that the *ethanolic leaves extract of Ficus pumila L.* may contain substances that inhibit liver damage and thus preventing a critical step in hepatotoxicity. Elevated levels of serum glutamic oxaloacetic transaminase (SGOT) and serum glutamicpyruvic transaminase (SGPT) are indications of hepatocellular injury (Yue *et al.*, 2006).

In the present study *AEAC* and *AQEAC* at a dose of 500 mg/kg, p.o caused a significant inhibition in the levels of SGOT and SGPT towards the respective normal range and this is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by aspirin. On the other hand suppression of elevated ALP activities with concur-rent depletion of raised bilirubin level and an increase in the total plasma protein content suggests the stability of biliary dysfunction in rat liver during hepatic injuries with toxicants (Mukherjee *et al.*, 2002). These results indicate that *AEAC* and *AQEAC* preserved the structural integrity of the hepatocelluar membrane and liver cell architecture damaged by aspirin which was confirmed by Histopathological examination. On examining the liver function tests of aspirin induced animals, the SGOT, SGPT, ALP, Total bilirubin has significantly increased After treatment with the ethanolic extract of *Ficus pumila* L. (200 mg/kg and 400 mg/kg) the excretion of has SGOT, SGPT, ALP, Total bilirubin significantly decreased Although the low dose was more potent than the high dose when compared with silymarin treated group, which is a standard. Ethanolic extract *Ficus pumila L.* of has shown promising in vitro efficacy on Hepatoprotective activity, we have observed increase in the absorbance indicating the inhibition of Nucleation and Aggregation of calcium oxalate in in vitro studies. For the *in vivo* Hepatoprotective activity, of *ethanolic leaves extract of Ficus pumila L.*, aspirin-induced hepatotoxicity rat model of was used. Since the liver damage inducing treatment, aspirin, was given orally, therefore, the extract was given p.o. in order to prevent any potential interaction of aspirin with plant constituents inside gut, interfering with absorption of either of the two. Administration of aspirin resulted in the increased toxicity, which might be due to the Hepatotoxicity, as evident by increase in SGOT, SGPT, ALP as compared to normal.

Table.2 Nature of Phytoconstituents present in the *Ficus pumila L.* ethanolic leaves crudeextract

S.No.	Phytochemical Tests	Inference
1.	Test for carbohydrates	+
2.	Test for alkaloids	-
3.	Test for saponins	+
4.	Test for tannins	-
5.	Test for starch	+
6.	Test for proteins and amino acids	+
7.	Test for Phytosterols	-
8.	Test for phenols	+
9.	Test for glycosides	-
10.	Test for reducing sugars	+

(+) = indicates the presence of constituents, and (-) = indicates the absence of constituents

Table.3 Effect of extracts of ethanolic extract of *Ficus pumila L.* leaves on SGOT

Groups	SGOT level mean ± SEM
Control	1760±1.02
Negative Control	2097.90± 2.468**a
Standard	1759.53±2.33**b
ethanolic leaf extract of *Ficus pumila L.* 200mg/kg	1735.64±1.73*b
ethanolic leaf extract of *Ficus pumila L.*400mg/kg	1750.26±1.99***b

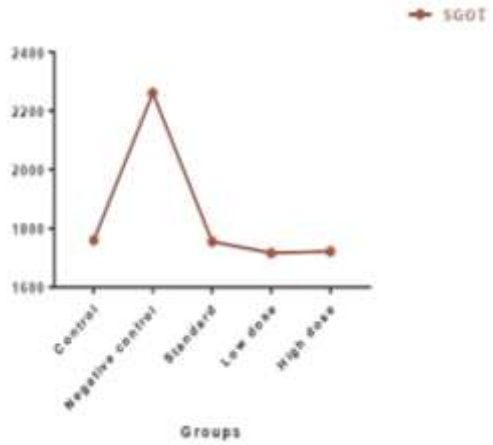

Fig.1: Effect of ethanolic leave extract of *Ficus pumila L.* on SGOT levels. Graphical representation of Effect of ethanolic leave extract of *Ficus pumila L.* on SGOT.

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Values are expressed as Mean ± SEM, n=6

Comparison:
a -Group I vs. Group II
b- Group II vs. Group III, IV & V; NS Non significant;
*P<0.05, **P<0.01;***P<0.001
One way ANOVA followed by Dennett's "t" Test

Effect of ethanolic leave extract of *Ficus pumila L.* on SGOT:

There was significant (p<0.001) increase in serum SGOT in aspirin induced group when compared to control group. There was significant (p<0.001) decrease in serum SGOT in Silymarin treated group when compared to control group. There was significant (p<0.001) decrease in serum SGOT in ethanolic leave extract of *Ficus pumila L.* treated group at a dose of 200mg/kg/p.0 when compared to control group. There was significant (p<0.001) decrease in serum SGOT in ethanolic leave extract of *Ficus pumila L.* treated group at a dose of 400mg/kg/p.o when compared to control group. There was a significant (p<0.01) decrease in serum SGOT level in Silymarin treated rats when compared to aspirin induced. The ethanolic leaves extract of *Ficus pumila L.* at a dose of 200mg/kg/p.o showed a significant (p<0.010) decrease in serum SGOT level when compared to aspirin induced group. The *ethanolic leaves extract of Ficus pumila L.*at a dose of 400mg/kg/p.o showed a significant (p<0.001) decrease in serum SGOT level when compared to aspirin induced group.

Table.4 Effect of extracts of ethanolic leave extract of *Ficus pumila L.* on SGPT

Group	SGPT Level Mean ± SEM
Control	1875±2.11
Negative Control	2263.36± 1.46**a
Standard	1870.98± 2.65*b
ethanolic leave extract of *Ficus pumila L.* 200mg/kg	1883.34±2.22**b
ethanolic leave extract of *Ficus pumila L.* 400mg/kg	2090.66± 2.32**b

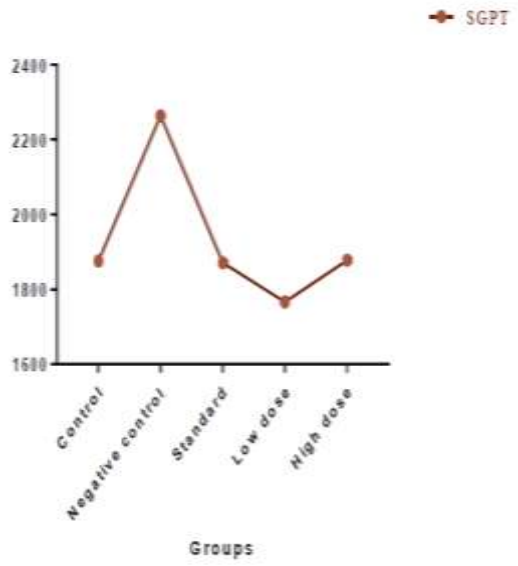

Fig.2: Graphical representation of Effect of *ethanolic leaves extract of Ficus pumila L.* on SGPT
Values are expressed as Mean ± SEM, n=6

Comparison:
a -Group I vs. Group II
b- Group II vs. Group III, IV &V;NS Non significant;
*P<0.05, **P<0.01;***P<0.001
One way ANOVA followed by Dunnet"s "t" Test

Effect of *ethanolic leaves extract of Ficus pumila L.* on SGPT:

There was significant (p<0.01) increase in serum glutamic pyruvate transaminase level in aspirin induced rats when compared to control group. There was significant (p<0.05) decrease in SGPT in aspirin treated group when compared to control group. There was significant (p<0.01) decrease in serum SGPT in ethanolic leave extract of *Ficus pumila L.* treated group at a dose of200mg/kg/p.0 when compared to control group. There was significant (p<0.01) decrease in serum SGPT in ethanolic leave extract of *Ficus pumila L.* treated group at a dose of 400mg/kg/p.o when compared to control group. There was a significant (p<0.01) decrease in serum SGPT level in Silymarin treated rats when compared aspirin induced group. The ethanolic leave extract of *Ficus pumila L.* at a dose of 200mg/kg/p.o showed a significant (p<0.01) decrease in serum SGOT level when compared to aspirin induced group. The ethanolic leave extract of *Ficus pumila L.* at a dose of 400 mg/kg/p.o showed a significant (p<0.001)

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decrease in serum SGOT level when compared to aspirin induced group.

Table5: Effect of Ethanolic Leave extract of *Ficus pumila L.* on Bilirubin

Group	Total Bilirubin Level Mean ± SEM
Control	1.48±0.01
Negative Control	2.61 ± 0.16***a
Standard	1.47 ± 0.01*b
ethanolic leave extract of *Ficus pumila L.* 200mg/kg	1.82 ± 0.03**b
ethanolic leave extract of *Ficus pumila L.* 400mg/kg	1.53 ± 0.01**b

Effect of ethanolic leave extract of *Ficus pumila L.* on Total Bilirubin

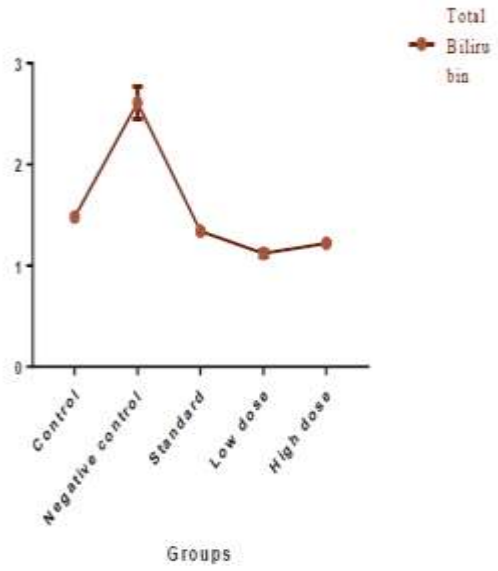

Fig.3: Graphical representation of Effect of *ethanolic leaves extract of Ficus pumila L.* on Bilirubin

Values are expressed as Mean ± SEM, n=6

Comparison:

a -Group I vs Group II

b- Group II vs Group III, IV & V; NS Non significant;

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

One way ANOVA followed by Dunnet"s "t" Test

Effect of ethanolic leave extract of *Ficus pumila L.* on Total Bilirubin:

There was significant (p<0.01) increase in Bilirubin level in aspirin induced group when compared to control group. There was significant (p<0.01) decrease in Bilirubin in Silymarin treated group when compared to control group. There was significant (p<0.05) decrease in Bilirubin in ethanolic leave extract of *Ficus pumila L.* treated group at a dose of 200mg/kg/po when compared to control group. There was significant (p<0.001) decrease in Bilirubin in ethanolic leave extract of *Ficus pumila L.* treated group at a dose of 400mg/kg/p.o when compared to control group. There was a significant (p<0.01) decrease in Bilirubin in Silymarin treated rats when Compared aspirin treated. The ethanolic leave extract of *Ficus pumila L.* at a dose of 200mg/kg/p.o showed a significant (p<0.05) decrease in serum bilirubin when compared to aspirin induced group. The ethanolic leave extract of *Ficus pumila L.* at a dose of400 mg/kg/p.o showed a significant (p<0.001) decrease in Bilirubin when compared to aspirin induced group. The results were shown in the Table and Graph.

Table.6: Effect of ethanolic leaves extract of *Ficus pumila L.* on ALP

GROUP	ALP Level Mean ± SEM
Control	35.06±0.12
Negative Control	53.47±0.01*a
Standard	37.8±0.03**b
ethanolic leave extract of *Ficus pumila L.* 200mg/kg	35±0.72***b
ethanolic leave extract of *Ficus pumila L.* 400mg/kg	39.98±0.11*b

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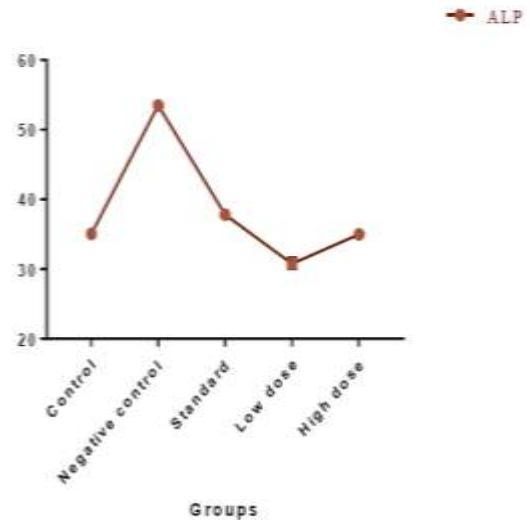

Fig.4: Graphical representation of Effect of ethanolic leaves extract of *Ficus pumila L.* on ALP
Values are expressed as Mean ± SEM, n=6

Comparison:
a -Group I vs Group II
b- Group II vs Group III, IV &V;NS Non significant;
*P<0.05, **P<0.01;***P<0.001
One way ANOVA followed by Dunnet"s "t" Test.

Effect of ethanolic leaves extract of *Ficus pumila L.* on ALP:

There was significant (p<0.01) increase in ALP in aspirin induced group when compared to control group. There was significant (p<0.01) decrease in ALP in Silymarin treated group when compared to control group. There was significant (p<0.05) decrease in ALP in ethanolic leaves extract of *Ficus pumila L.* treated group at a dose of 200mg/kg/p.0 when compared to control group. There was significant (p<0.001) decrease in ALP in ethanolic leaves extract of *Ficus pumila L.* treated group at a dose of 400mg/kg/p.o when compared to control group. There was a significant (p<0.05) decrease in ALP in Silymarin treated rats when compared aspirin treated. The ethanolic leaves extract of *Ficus pumila L.* at a dose of 200mg/kg/p.o showed a significant (p<0.001) decrease in ALP when compared to aspirin induced group. The ethanolic leaves extract of *Ficus pumila L.* at a dose of 400mg/kg/p.o showed a significant (p<0.05) decrease in ALP when compared to aspirin induced group.

Histopathological Examination of Hepatocytes:

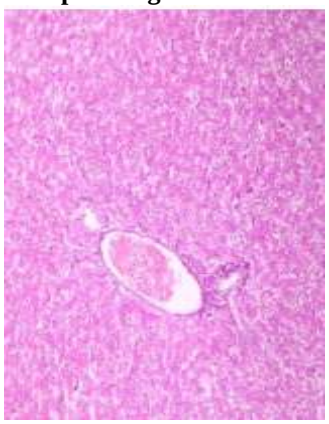

Fig.5: Normal Contol group, showing normal hepatocytes

Fig.6: Aspirin treated animal group shows that hepatic cells damage and congestion of the liver

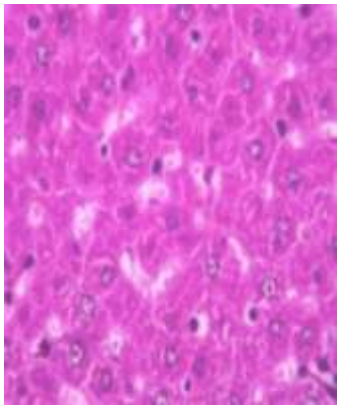

Fig.7: Hepatocytes in group treated with Standard (Silymarin 200 mg/kg)

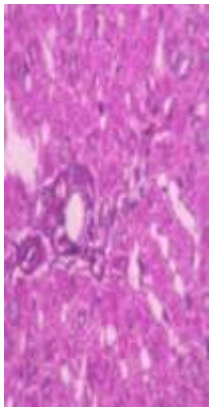

Fig.8: *Ficus pumila*ethanolic leave extract of 400 mg/kg shows that few regenerative hepatocytes, sinusoidal congestion and scattered mononuclear inflammatory cells

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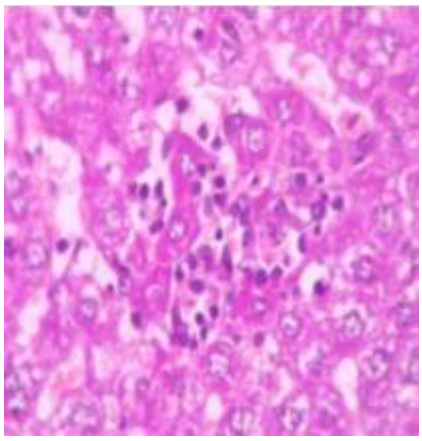

Fig.9: *Ficus pumila L.* ethanolic leave extract of 200 mg/kg shows that few regenerative hepatocytes, sinusoidal congestion and scattered mononuclear inflammatory cells

IV. CONCLUSION:

The Hepatoprotective effect of ethanolic extract of *Ficus pumila L.* leaves was confirmed by the following measures: The isolated livers from the toxicant (aspirin) treated animals exhibited increase in wet liver weight. Indeed, extract treated animals exhibited decrease in the values of above physical parameters as an indication of hepatoprotection. Serum marker enzymes such as SGPT, SGOT and total bilirubin, showed marked increase. The same is observed in liver diseases in clinical practice and hence are having diagnostic importance in the assessment of liver function. In the present study, the ethanolic extract of *Ficus pumila L* .leaves significantly reduced the elevated levels of above mentioned serum marker enzymes.

Hence, at this point it is concluded that the ethanolic and aqueous extract of *Ficus pumila L.* leaves possess Hepatoprotective activity. In support to this study, Histopathological results also show significant activity of the plant. In toxicant treated animals there will be severe disturbances in thecytoarchitecture of the liver. The same is observed in case of humans who are suffering from major liver disorders. In addition to this there is regeneration of hepatocytes also observed, which indicating Hepatoprotective activity. Finally based on improvement in serum marker enzyme levels, physical parameters, functional parameters and Histopathological studies, it is concluded that the ethanolic extract of *Ficus pumila L.* leaves possesses Hepatoprotective activity and thus supports the traditional application of the same under the light of modern science.

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