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INVESTIGATION ON HEPATOPROTECTIVE ACTIVITY OF PSIDIUM GUAJAVA AGAINST RIFAMPICIN AND ISONIZID INDUCED HEPATOTOXICITY IN RAT

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ABSTRACT

Background: The main aim of this project was to evaluate the hepatoprotective property of aqueous extract of Psidium guajava leaf against rifampicin and isoniazid induced hepatotoxicity. The combination of Anti-tuberculosis drugs isoniazid and rifampicin have property to treat tuberculosis, these drugs are used are associated with toxic reaction in tissues, mainly in the hepatic region, leading to liver disease (hepatitis). In regenerating and protecting the liver, Guava, an aqueous plant Leaf extract derived from the Guava plant, is effective. **Methods:** Wistar albino rats weighing 150–200 g were used to form 5 study groups, each group consisting of 6 rats. Isoniazid and Rifampicin (50 mg/kg each) body weight; was used to induce hepatotoxicity and Guava Leaf as a standard. Hepatoprotective activity of toxicity was evaluated by monitoring the serum enzyme levels and histopathology. **Results:** As evidenced by biochemical measurements, Isoniazid and Rifampicin treatment induced hepatotoxicity in rats: AST (aspartate aminotransferase), ALP (alkaline phosphatase) and ALT (serum alanine aminotransferase) activities and when drugs were administered to animals, their total bilirubin levels increased, and their albumin and total protein levels decreased. The livers of animals treated with drugs also showed changes in histopathology. Drugs induced significant biochemical and histological changes when AAM was administered simultaneously. **Conclusion:** AAM exhibited hepatoprotective action against antitubercular drugs induced hepatotoxicity.

KEYWORDS: Hepatoprotective, Psidium guajava, Rifampicin, Isoniazid.

INTRODUCTIONS

General: It is known that medicine was conceived in sympathy and born out of necessity. Man has been afflicted with illness since the beginning of time; the search for remedies to combat it is equally old, so drugs are ancient as disease itself.

If we study the history of medicine from antiquity to modern times, we will analyze that the medical knowledge has been derived, to a very great degree from intuitive and observational prepositions, tempered by evaluating interpretations. From the newsiest times, tribal priests and the medicine men used various plant, minerals, animal organelles, usually in associated with strange rituals and in causations, to drive out the evil spirits which they believed to be the main reason of the diseases.

Intimately connected to the history of botany is the history of medicinal plants. From the crude beginning of the earlier physical botanists, the study of drug plants had developed into modern Pharmacognosy.

On many fronts, traditional systems of medicine are still widely practiced.

- Lack of drug supply
- Population rise
- cost of treatments is prohibitive
- several allopathic drugs have side effect
- Eliminates the root cause of the disease.
- Freedom from approaching various specialists.
- Cure for many obstinate diseases.
- Natural sources of drugs are easily accessible.

- Resistant infections to allopathic drugs currently used.
- Changing lifestyles and social pathologies can be used to treat diseases.

Drugs and other exogenous substances are mainly metabolized in the liver since most drugs taken orally are absorbed through the gastrointestinal tract (GIT), the liver is the entry point to the tissues for such a compound. It is vulnerable both to the patent drugs, which is caressed from the gastro intestinal tracts to the hepatic veins, as well as to any metabolites that enter through the hepatic vein into various organ systems. Distortion of metabolic functions is related to liver injury.[1]

Because of their strategic placements within the body, metabolism and elimination are continuously exposed to xenobiotics in addition to their essential functions. A variety of liver disorders can result from the absorption of toxins from the gastrointestinal tract. As a result, liver diseases continue to pose a danger to health. Some conventional or synthetic drugs may cause harm or even death when used to treat hepatic diseases. Hepatitis is ineffectively treated with conventional or synthetic drugs, which can sometimes have harmful side effects. People in all countries, even these in developing country, gravitate towards CAM (complement arise and alternatives medicines) over the past decade. But there are not more drugs available for the cure of liver disorder.[2]

Hepatotoxicity

Hepatotoxicity implies (chemical driven liver damage), An enormous array of pharmaceuticals and environmental chemicals can potentially harm the liver, as the liver metabolizes and detoxifies drugs in the body.[3]

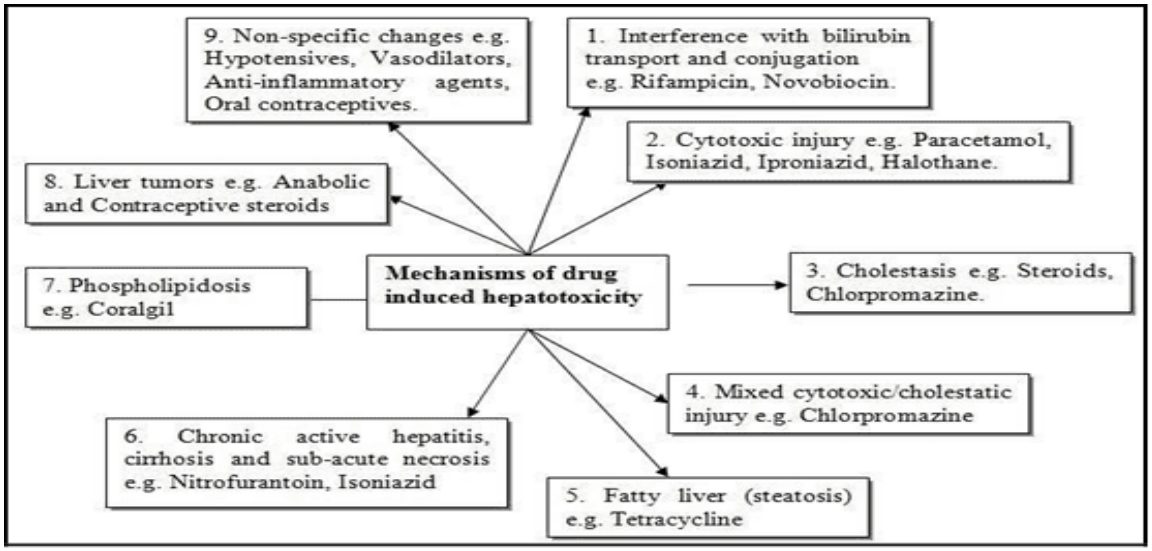

Fig: Mechanism of Hepatotoxicity.

Drugs and Toxins Responsible for Hepatotoxicity

Hepatocellular damage	Examples
Micro-vesicular (fatty change)	ethanol, Tetracycline, salicylates,yellow, phosphorous
Macro- vesicular (fatty change)	Amiodarone, Ethanol, Methotrexate
Centrilobular necrosis	Bromo-benzene, CCL4, Rifampicin
Hepatitis, chronic and acute	isoniazid, methyldopa, nitrofurantoin,Phenytoin, oxiphenisatin
Fibrosis-cirrhosis	amiodarone, ethanol, Methotrexate.
Benign (Neoplastic diseases)	Oral contraceptives, other steroids

Metabolism and mechanisms of toxicity

In current anti-tuberculosis drugs containing INH (isoniazid), pyrazinamide, and rifampicin,hepatic toxicity has a serious adverse effect. Multiple drugs are generally more potent at increasing the side effects of anti-tuberculosis drugs, so although INH, Rifampicin, and Pyrazinamide are each potentially hepatotoxic, when taken together they have a higher toxiclevel.

Isoniazid

It is hepatotoxic to take isoniazid because it converts into Isonicotinic acid and hydrazine, and transformation pathway is more significant in slows acetylator than rapid acetylator.[4]

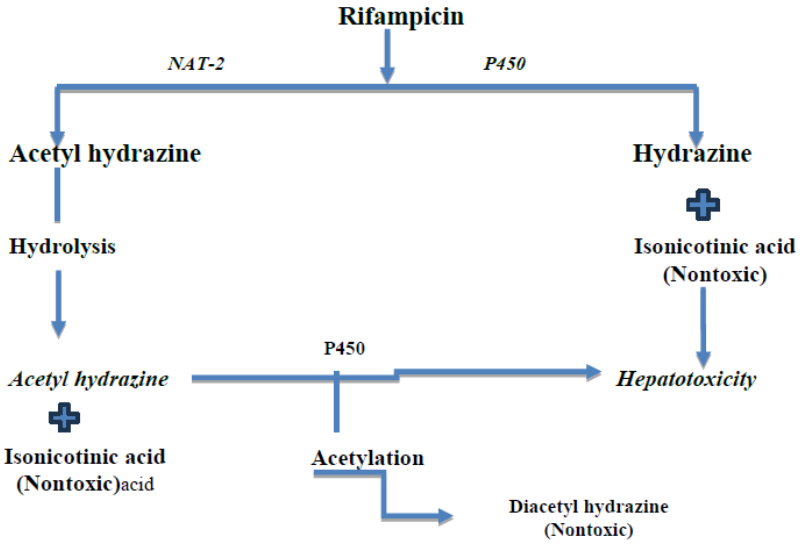

Figure: INH Metabolism and Hepatotoxicity.

RIFAMPICIN

In addition, a separate hydrolysis of 3-formyl rifampicin produces deacetyl rifampicin via deacetylation.[5] Further in the treatment course, rifampicin may cause hepatocellular dysfunction, though this will resolve without discontinuing the drug.[6] It is unknown how rifampicin induces hepatotoxicity. There is no evidence that there is a toxic metabolite present.[7] Rifampicin activates the hepatic CYP450 system in the liver and intestine, causing many other compounds to be metabolized more rapidly.[8] Hepatotoxicity has been associated with the combined use of rifampicin and isoniazid. Combining rifampicin and isoniazid facilitates the formation of hydrazine, which might explain why this combination is more toxic.[9]

Ant tubercular drugs are implicated in the pathogenesis of hepatitis C by oxidative stress. Deficiencies in intracellular antioxidant defenses, as well as excessive production of oxidants, lead to as imbalanced in to reduction- oxidation status of the hepaticicell.[10]

Liver: The largest gland is the human body is livers. Besides its role in metabolism, glycogen also play a major role. in plasma proteins synthesis and detoxification. By emulsifyinglipids, it produces bile, an alkaline compound that aid digestions. Bile, an alkalizes component that aids digestions, is produced when lipids are emulsified. A wide variety of high volumes biochemicals reaction are also performed or regulated by its.[11]

Anatomy

"A thorough understanding of liver anatomy is essential for modern liver surgery." There is the largest gland in the body located beneath the diaphragm in the right lower rib cage, theliver. The peritoneum, as well as the visceral peritoneum, It is almost entirely covered by a layer of dense, irregular tissue. Two lobes make up the liver: the larger right lobe and the Falciform ligament separates the smaller left lobe. In addition to an inferior quadrate's lobe, the right lobe includes a posterior quadrates lobe, according to anatomy Lobe.[12]

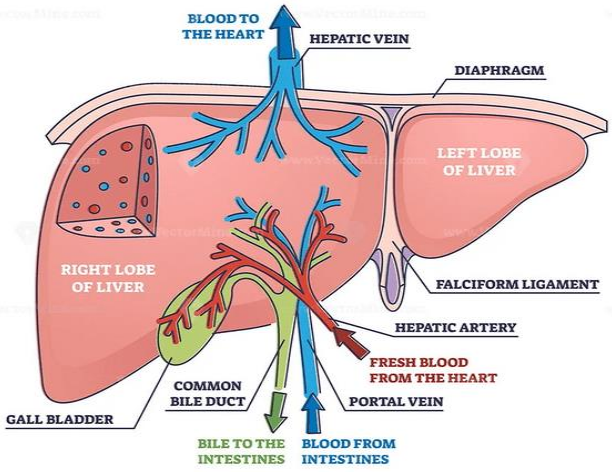

Figure: Anatomy of liver.

- **Circulation function**

Transfers of blood from portals to synthetic circulatory. Activities of its reticulo-endothelial system. (kuffer cell) in immune mechanism. Blood storages. (Regulations of blood volumes).

- **protective and detoxification functions**

By conjugation, methylation, oxidation, and reduction, Kocher cells eliminate foreign bodies from blood (phagocytosis).Ammonia is removed from blood, particularly through the portal vein, after it has been absorbedfrom the intestines.

Metabolism of Drug: Drugs are bio transformed in the liver. By converting non-polar drugs into polar compounds, it can be made water soluble for urinary excretion.[13]

Acute liver failure: It is most commonly caused by a viral infection, toxic drugs and chemicals as well as acute fatty liver changes that cause acute liver failure.

- **The following characteristics characterize acute liver failure**

Jaundices blood sugar decrease (hypoglycemia) acid base and Electrolyte disturbance (hypokalemia) Hepatic encephalopathy Elevation of serum enzyme (LDH, AST,)

Chronic liver failure: Cirrhosis leads to fibrosis and regrowth of nodular fibrosis.

The effects of chronic liver failure are

high blood pressure(hypertension) liver encephalopathy Hepatol renal syndrome.

Cirrhosis: Cirrhosis is premalignant lesion. As a progressive and irreversible disease, itultimately leads to death. Hepatic cirrhosis is characterized by: Hepatic necrosis, leading to hepatic failure and death. A fibrosis that involves both the central vein and the portal vein. Hepatic cells surviving regenerative nodules hyperplasia as a result. Hepatic lobular architecture is distorted. Hepatic involvement across the board.

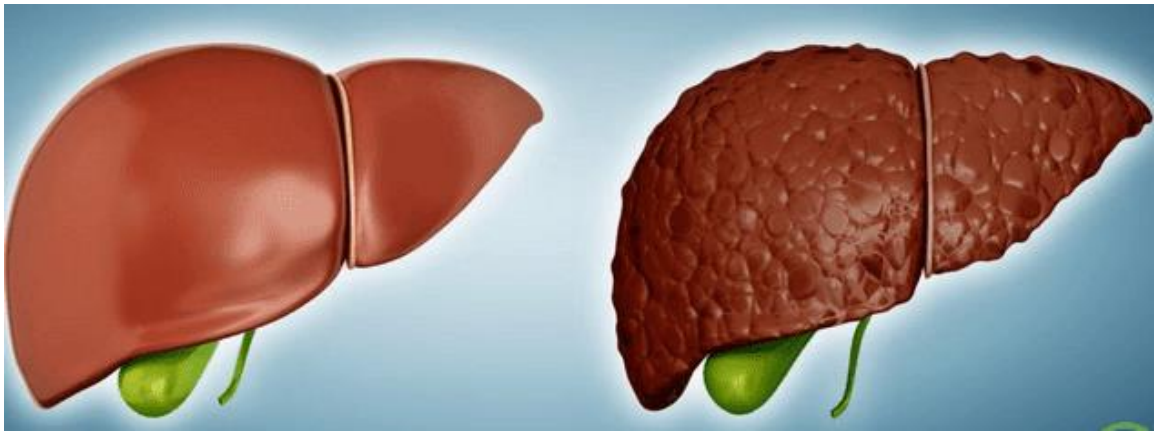

Figure: Liver Cirrhosis

- Mesenchymal as hematoma
- Hepatoblastomas

Congenital Liver Diseases

Liver cystic disease

In polycystic kidney disease, the bile duct is malformed.

Durbin – Conjugated bilirubin excretion defect.

Crislers (Najjar syndrome) – In homologous patients, it is characterized by the absence of the enzyme (Type A disease).The lesser form of Crigler-Najjar syndromes (Type B diseases) with partial enzymes deficiency and more prolong symptoms

Circulatory disorders

Impair blood flow into the hepatic system (Bani syndrome)- Ascites and variceal bleeding are the clinical signs of extrahepatic portal vein obstruction.

impaired blood flow from liver- Centrilobular necrosis and passive congestion.

Liver vein outflow obstruction-Budd- Chiari syndrome- The syndrome is characterized by liver enlargement, pain, and ascites, caused by obstruction of two or more hepatic veins. Eno-occlusion disease - In hepatic vein neoplasms, subendothelial swelling and finely reticulated collagen are common features.

Viral hepatitis

Hepatitis A, B, C, and E are the most common viruses that cause hepatic diseases. Viral hepatitis is not classified as other viral infections that may cause liver disease. These agents include Epstein-Barr virus, Cytomegalovirus, Vercelli-Zoster virus, Herpes virus and Yellow Fever virus.

Bacterial hepatitis

Bacteria that cause bacterial hepatitis include Escherichia coli, other gram-negative bacteria, an aerobic bacterium,

Staphylococcus aureus, and streptococci. A case of systemic bacterial hepatitis may cause bacteria to reach the liver. A systemic bacteremia may reach the liver through the hepatic artery or through the bile duct or portal vein from the intestine.

Protozoal hepatitis

(Hepatic amoebiasis) - Amoebic protozoa invading portal veins in colonic mucosa cause focal enzymatic necrosis of the hepatocytes caused by the amoeba. There is a moderate increase in serum bilirubin and SGPT.

Liver Function Tests

One or more, but not often all, of the liver's functions are compromised when it is sick. The whole functioning of the liver cannot be tested. The several "Liver Function Tests" (LFTs) measure disruptions in certain liver functions. It could be feasible to extrapolate a conclusion from a single test since several tests yield aberrant results that are quite similar in a given liver illness. Since many functional changes are not reflected by clear structural changes in the liver cells, the results of the liver biopsy may not be comparable with LFTs. The following tests are carried out in order to comprehend the different liver functions. Serum total, indirect, and direct bilirubin are tests for the excretion of bile pigments and salts. Bile pigment, urobilinogen, and urine bile salts.

Tests for plasma proteins

Turbidity test (thymol turbidity)

- Determination of albumin, globulin and A/G ratio (all plasma protein).
- Determination of fibrinogen present in plasma

Investigative method

- Ultrasonography, computerized sonography and magnetic resonance imaging - disclosesmass lesions in the liver or dilation of the biliary system.
- Arteriography and isotope scans-hepatic blood flow can be detected by this.
- Gallium Scanning-useful in detecting neoplasm and abscesses in the liver.

Plant profile

Guava leaf

Description

The guava tree (Psidium guajava L.), which is a member of the Myrtaceous family, is a highly special and traditional plant that is planted because of its many nutritional and therapeutic qualities. In tropical regions like India, Indonesia, Bangladesh, Pakistan, and the Americas, guava has been cultivated and used extensively. In many nations, the roots, bark, and stem of guava trees are used to cure diarrhea, diabetes, stomachaches, and other illnesses. The dark green-colored, elliptical, oval guava (Pisidia guajavae) folium; GL) is distinguished by its obtuse-type apex. Guava pulp and seeds are utilized for treating gastrointestinal and respiratory conditions, as well as to boost platelets in dengue fever patients. Because of its antiseptic, cough sedative, antimicrobial, antidiarrheic, antihypertensive, anti-obesity, and antidiabetic qualities, GLs are also often employed. The effectiveness of GL isolate as powerful tumor fighting, cancer fighting, and cytotoxic agents has also been shown by studies conducted on animal models.[14]

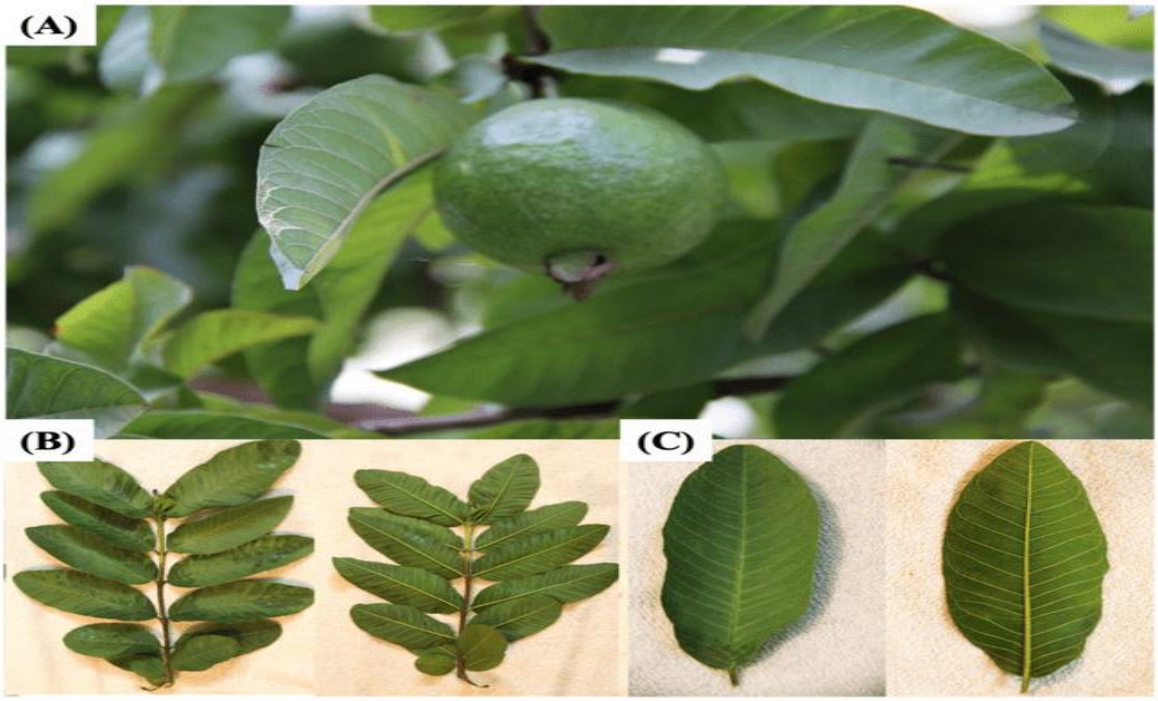

Figure: Guava A, B, C Types.

TAXONOMY

Earlier P. pomiform as well as P. pyriform were regarded as two distinct genera. Despite their little differences, they are now acknowledged as belonging to the same species. Globally, more than 150 guava species are grown.[15]

Guavas can have either a sweet or sour flavor. Fresh sweet guavas are consumed when they are fully ripe. In the wild, little, sour guavas are typically used for processing. Additionally, the varieties Redland, Supreme, Ruby Indian, Ruby, Blitch, Hart, and Webber are guava cultivars that may be found all over the world. Safed, Prayagraj, Karala is seedless, red-fleshed, and apple-colored are commercial types from India. Depending on the color, guavas are also categorized as either red or white.

History

There is a tree attributed to Shiva called Seadrome, which has been used in Hindu prayers by ancient people in Psidium guajava It is own place in indigenous systemsof medicines or drug.

Morphology

Morphology Features the guava tree is a tiny tree or shrub that grows to a height of two to seven meters and has several branches. The tree's smooth, thin, copper-colored bark sheds off. When the top surface is removed, the greenish skin beneath it becomes visible. When the tree reaches full maturity, its trunk reaches a diameter of 25 cm. The tree's twigs are bent downward and have a quadrangular shape. They have short petioles, are leathery, and are positioned in opposition to one another. They are 7–15 cm in length and 3–5 cm broad, with an uneven form that ranges from oval to elliptical. They are made up of parallel lines, or veins, that radiate outward from the central axis. Clusters of flowers with four to five white petals as well as 250 stamens, measuring two to five centimeters in diameter, are carried on the leaf axils. (Manica et al., 2000). According to botany, guava belongs to the genus Psidium and family Myrtle. Depending on the kind, they are round, ovoid, or pear-shaped, measuring 5–10 cm in diameter and weighing 50–200 g. The exocarp has a thin, pale yellow surface with a tinge of pink. The mesocarp, which is located directly adjacent to the exocarp, is granular, thick, white to whitish or shaded pink in color, and can reach a length of 3–12 mm. The mesocarp is delicious, acidic, and juicy. The endocarp, or center pulp, lies behind the granular flesh. A reservoir of rocky yellowish seeds, its endocarp is juicy and somewhat deeper in color. The pulp contains 112–535 seeds and has a diameter of 6 mm. Stone cells and cells of parenchyma are the two types of cell walls and tissues found in the pulpy pulp of berries. The distinctive gritty feel of the stone is caused by its cells, which are made of lignified tree material and resistant to digestion via enzymatic means. Guavas have a short shelf life of three to five days at room temperatures because of their rapid respiration and fast metabolism.[16]

Chemical Constituents

Polysaccharides

Macromolecules known as polysaccharides are found throughout nature. They consist of lengthy polymeric chains made up of simple sugar molecules. The polysaccharides in question exhibit a range of biological, pharmacological, and physicochemical characteristics, including antioxidant, anti-inflammatory, antidiabetic, immunomodulatory, and antitumor activities. Guava leaf polysaccharides (GLPs) can be isolated using ultrasound-assisted extraction (UAE) (time: 20 min, power: 404 W, temperature: 62 °C). These GLPs contain about 9.13% uranic acid.

Psidium guajava

- **Kingdoms:** Plantaee
- **Orders:** Myrtaless
- **Famialy:** Myrtaceaee
- **Sub-familyi:** Myrtoideaee
- **Genuss:** Psidiume
- **Speciese:** P. guajavae
- **Binomial Names:** Psidium guajavai

Medicinal Uses

Several medicinal properties of Psidium guajava are found in ethanol medicine: antipyretic, Astringent, antidysentery, antiscorbutic, antidiarrheal, haemostatic, aphrodisiac demulcent, and as an antidote of snake poison,[17] In various parts of the body, A variety of disorders can be treated with extracts from the Guava leaf plant.

Traditions of India. On hyperglycemic states, Blood sugar levels, body weight, and insulin levels were restored to normal.[18] Plants are used as astringents, appetizers, laxatives, tonics, restoratives, and febrifuges.

Pharmacological Activities

Contractile Activity

Study of the effects of alcoholic extract from Guava leaf .It has traditionally been used to treat asthma and related illnesses in guinea pig isolated ileum and tracheal chain. The isolated organ bath method was used to study these effects. An extract of thisplant The ileums and bronchial chain of isolated guinea pigs showed improvement at dosages of 1 mg/ml or 2 mg/ml. Additionally, the contractions carried on by histamine were inhibited. Alcoholic extracts prevented histamine-induced contraction in the tracheal chain and ileum of guinea pigs. Guava leaf was found to induce relaxations via depressing H1-receptors. As a result of investigating the anti-histamine effect of the extract, it was observed that the ileum and tracheal chain of the guinea pigs relaxed completely. In its alcoholic extract, Guava leaf contains one or more antihistaminic substances which have traditionally been used to treat asthmatic symptoms.

Anti-microfilaria Activity, Antifungal Activity, Analgesic Activity, Analgesics, antipyretic, and anti-inflammatory agents, Hypoglycemic Effects, Anti-dyslipidemic Activity, Immuno-modulatory Activity, Anti-proliferative Activity, Anti-fertility Effect, Anti-genotoxic Activity, Anti-oxidant Properties, Cardiac Activity.

LITERATURE REVIEW

The hepato-protectives effects of Ginkgo select Phyto some in rifampicin was reported by **Naik SR et al., 2008** (For 30 days, 500 mg/kg, p. o daily.) The antioxidant activity of induced liver injury in rats. The scavenging of ROS and the protection against GSH depletion are the two major mechanisms of GBP's hepatoprotective effects. In the form of Ginkgo Select Phyto some, G biloba flavonoids react with ROS, stabilizing them and oxidizing them to less reactive, more stable radicals Free radical scavenging activity is probably attributed to OH group flavonoids due to their high reactivity.

Orhan DD et al., 2007 In rats exposed to carbon tetrachloride (1 ml/kg, S.C.) induced acute liver damage, Vitis vinifera L showed hepatoprotective effects. In rats, ethanol extracts from Vitis vinifera may protect livers from CCl4-induced liver injury by inhibiting cytochrome P450-dependent oxygenase, preventing lipid peroxidation, and stabilizing hepatocyte membranes.

Pramyothin P et al., 2005 In rats treated with ethanol, Thunbergia laurifolia Linn extract was investigated for its hepatoprotective effects (4 g/ (kg, po for 14 days) Studies in vitro and in vivo. These results suggest that Thunbergia laurifolia and the compound silymarin exhibits hepatoprotective effects both in primary rat hepatocyte cultures and in rats**.**

Pramyothin P et al., 2006 Reported Phyllanthus emblicanin Linn extract was found to be protective against ethanol (Taking ethanol 5 g/kg p. o. for seven days is toxic) induced rat hepatic injury. Restoring normal levels of AST, ALT, and IL-1beta, 75mg/kg of Phyllanthus embolic extract enhanced liver cell recovery.

Pushpavalli G et al., 2010 studied Chrysin's influence on D-galactosamine-induced liver enzymes and lipid profiles (a single intraperitoneal injection 400 mg/kg BW) induced lever D-Ga l N hepatotoxicity rats had higher HDL-C levels in plasma and tissues and decreased levels of total, triglycerides, free fatty acids, phospholipids, cholesterol, very low-density lipoprotein-C, and low-density lipoprotein-C, demonstrating the hepatoprotective and antihyperlipidemic properties of chrysin.

Ravikumar V et al., 2005 reported It has hepatoprotective effects against D-galactosamine/lipopolysaccharides (30 mg/kg body weight/300 mg/kg body weight) induced liver disease (hepatitis) in rats. Using Triad procumbens, we found hepatocellular damage induced by d-Ga l N/LPS could be significantly alleviated.

Ravinder Pal et al., 2006 reported Garlic reduces hepatic injury induced by isoniazid and rifampicin in rats. Result: When INH+RIF co-treated animals were given garlic at 0.25g/kg per day, histopathological injuries were not induced (50mg/kg/day).

Seth Madhavan Santhosh et al., 2007 Experimental rats were shown to be protected against liver toxicity caused by isoniazid and rifampicin by chitosan**.**

OBJECTIVES AND PLAN OF THE STUDY

Literature survey report revealed that Guava leaf has potent antioxidant properties, and used as a hepatoprotective agent. Further it was observed from the literature that, no scientific evaluation for hepatoprotective action of A. Guava leaf extract has been carried in anti-tubercular drug induced hepatotoxicity.[19]

To confirm the folk medicinal claim of Guava leaf extract, this study examined its hepatoprotective properties against rifampicin-isoniazid-induced hepatotoxicity in albino rats.

PLAN OF WORK

- ❖ Plants and plant parts are collected and authenticated.
- ❖ Cold maceration with distilled water is the method of extracting dried s.
- ❖ Assessing phytochemical constituents of extracts through preliminary screening.
- ❖ Researching the hepatoprotective effects of selected plant extracts by extensive pharmacological studies.

Detailed biochemical studies and statistical analysis.

- ✓ Serum glutamate oxalo- Acetic transaminase (SGOT) and serum pyruvic transaminase (SGPT).
- ✓ ALP stands for an enzyme called alkaline phosphatase.
- ✓ Bilirubin concentration.
- ✓ Every Protein
- ✓ The albumin

MATERIAL AND METHOD

Selections of plant

The selections of medicinal plants for hepatoprotective activity were compiled from list of plants. Literature survey report revealed that the guava plant has not been previously investigated for Hepatic protective action against liver damage caused by isoniazid and rifampicin. Hence the present work involves pharmacological evaluation hepatoprotective activity.

Plant material collection

Fresh s of Guava leaf (Family- Myrtaceous) was collect from Botanical Garden of NGI (Naraina Group of Institution), Kanpur, India.

Animal used

Albino Wistar rats weighing 150-200g of either sex was used for pharmacological experiments.

Figure: Albino Wistar rats.

Housing of animals

It is recommended that no more than six animals per cage be housed in polyacrylic cages (38x28x10cm); temperature and relative humidity are maintained at 26°-2°C, with a 12-1-hour light/dark cycles. Standard dry pellet diet and water were freely available to them. The institutionally committees for ethical used of animal reviewed and approved all procedures described[20] Animals were cared for before experiments began.

Chemicals and Reagents

List of Chemicals/Reagents

S.N.	Names
1.	Acetic acid glacial
2.	Acetone
3.	Ammonia
4	Benzene
5.	Bismuth carbonate
6	Bismuth nitrate
7	Benzene
8	Chloroform
9.	Cupric acetate
10	Cupric sulphate
11.	Qualigens Acids
12	Ferric chloride
13.	Formaldehyde
14	Hydrochloride acid
15.	Iodine
16.	Lead acetate
17	Magnesium metal
18.	Picric acid
19.	Potassium iodide
20	Pyridine
21.	Resorcinol
22.	Sodium carbonate
23.	Sodium chloride
24.	Sodium citrate
25.	Sodium hydroxide
26.	Sodium nitroprusside
27	Sulphuric acid
28.	Rifampicin-Isoniazid
29.	SGOT, SGPT, SALP, Kit

Table: List Instruments.

Sr. No	Instruments
1	Autoanalyzer
2	Rota vapor
3	UV spectrophotometer
4	Homogenizer
5	Digital Balance
6	Deep Freezer
7	Centrifugation
8	Microscope
9	Autoanalyzer

METHODOLOGY

Drying and communities of plant material

The was air dried in pharmacy lab of NGI Institution Kanpur. The dried leaf' were manually powdered with iron hammer and was passed through sieve no. 40.

Preparation of crude extract

The powdered drug (500gm) was macerated with chloroform water for 48 h with occasionally stirring. It was subsequently condensed and filtered with a rotary evaporators.

Table: Percentage yield, Color and consistency of AAM.

Solvent	Colour and consistency	% yield
Chloroform water	Buff coloured thick mass	18.2%

Preliminary Phytochemical Analysis of Guava leaf' extracts

In this work, chemical tests for identification of various phytoconstituents present were conducted in various samples of Guava leaf extracts.[21]

Tests for carbohydrates

Molish test

Molish reagent (α-Naphthol) was added to the test tube to extract solutions, along with concentrated H2SO4. A purple color ring forms at the junction when carbohydrates are present.

Fehling solution tests

3 ml of each extracts was addends to each Fehling A and Fehling B solution. For 5-10 minutes, the mixtures were boiled in a water bath. As a result of the formation of cuprous oxide, a reddish-brown color was obtained.

Tests for alkaloid

Dragendorff's test

A reddish-brown precipitate was formed after adding 1 ml of Dragendorff's reagent to different extract solutions.

Mayer's test

Mayer reagents were addends to several extracts solutions, forming cream-colored precipitates that indicated alkaloids.

Wagner tests

The addition of 1 ml of Wagner's reagent to different extract solutions confirmed the presence ofalkaloids by a reddish-brown precipitate.

Hager's test

After adding 1ml of Hager reagents and extract solution, yellow precipitate was observed, confirming the presence of alkaloids.

Fats and fixed oils tests

The copper sulphate solutions was addeds to 6 drops of the different extract samples, a solution of 10% sodium hydroxide was then added. Fats and fixed oils appear as clear blue solution inthis test.

Tests for anthraquinones glycoside

Borntrager test

In test tubes, each extract sample was boiled for 5 minutes with 1 ml of Sulphuric acid, and then filtered while still hot. Chloroforms equal parts were added to a cooled filtrate. In order to separate the lowers layer of chloroform, it was shaken with half of its volume of dilute ammonia. Anthraquinones glycoside produce rose-pink to red colouration in the ammoniacal layer.

Cardiac Glycosides test

Keller - Killiani test (test for de oxy sugars)

In 0.5 ml chloroform extract, glacials acetics acid containing ferric chloride was added after the extract was evaporated to dryness. Sulphuric acid concentrated was add to the side of a small tube after transfer. presence of cardiac glycosides was indicated by the blue color of acetic acid layer.

Legal's test

The pyridine treatment of the extract samples was follow by the additions of an alkaline sodium nitroprusside solution. This indicates the presence of cardiac glycosides, asevidenced by the blood red color.

Baljet's test

Sodium picrate was used to treat different extract Elly sample. An orange color indicates cardiac glycoside presence**.**

Flavonoids test

Various extract samples were treated with sodium hydroxide solution. After adding a few drops of dilute hydrochloric acid, intense yellow colour turned colorless, indicating flavonoids.

Shinoda test

We added a few magnesium turns and several drop of conventions hydrochloric acid to the different extract samples and after a few minutes' observation, either a pink scarlet, crimson redor sometimes even a green hue appeared to indicate flavonoids were present.

Tests for tannins

Ferric chloride test

The ferric chloride solution colored different extract samples blue or green depending on whether they contained hydrolysable tannins or condensed tannins. Gelatin test.

An extract solution containing 1% Gelatin solution and 10% sodium chloride was treated with different extract samples. Precipitate formation indicated tannin presence.

Proteins test

Heat test

When heated on a boiling water bath, the extract samples coagulated, indicating protein presence.

Hydrolysis test

Hydrolyzed samples of different extract were boiled with ninhydrin solution and then hydrolyzed again with hydrochloric acid.

Steroids and triterpenoids test

Salko weskit test

Sulphuric acid concentrations were added to different extract samples. At the lower layer, red and yellow colours indicated steroids and triterpenoids respectively.[22]

Acute toxicity studies (OECD 423)

For the acute toxicity study, albino mice of both sexes were selected. Pilot studies were conducted on mice to decide on the dosage levels for the nextstudy prior to the

LD_{50} determination. In 1% CMC, aqueous extracts of Guava leaf were taken at various dose levels (The dosages are 200 mg/kg, 500 mg/kg, 1000 mg/kg, 1500 mg/kg, and 2000 mg/kg). Each dose level was administered to mice in pairs. Control animals received 10ml/kg of 1% CMC dissolved in distilled water orally. In order to determine the actual LD50, the extract of Guava leaf was administered once oral to groups of mice after fasting for approximately 18 hours. Control animals received 10ml/kg carboxymethyl cellulose in distilled water (1%).

The animals were maintained under surveillance for 14 days after being observed continuously for two hours and sporadically for a further four hours. After 72 hours, the animals' behavior and any other hazardous signs were noted.

Pharmacological studies Preparation of solutions and test samples

Rifampicin+ Isoniazid solution

To induce hepatotoxicity in rats, 50 mg/ kg each body weight, dose of Rifampicin+ Isoniazid was selected. Solution of Rifampicin+ Isoniazid was prepared separately in distilled water 0.1 mol/L of hydrochloric acid were added to the solution to adjust the pH to 3.0.

Carboxymethyl cellulose solution

1% carboxymethyl cellulose in distilled water (10ml/kg body weight).

Aqueous extract of Guava leaf

Stock solution 75mg / ml of aqueous extract of. Guava leaf was prepared in distilled water. Appropriate dilutions were made to administer the doses of 200,400 mg/kg. Rats were weighed in respectable groups based on their body weight.[23] Grouping of animals for Hepatoprotective activity.

Animal are grouped into 5 groups. Six rats were provided to each group.(n=6)

Figure: Group consisted of six rats.

Group I The control group consisted of six rats.
Group II Six rats were used as toxicants
Group III Six rats were used as test subjects.
Group IV Six rats were used as test subjects.
Group V Six rats were used as standards.
Group I (normal control) 1% carboxymethylcellulose (10ml/kg body weight) was administered in distilled water to the solvent control group.
Group II (toxic) Animal received Isoniazid+ Rifampicin (50 mg/ kg each body weight, p.o). For 28 days, RIF+ INH was administered orally. Histopathological investigations and detailed analyses were conducted on these animals after 28 days to confirm hepatotoxicity.
Group III (Test) Animal receives Rifampicin + Isoniazid (50mg/ kilogram body weight each, p.o) 28 days of oral administration of aqueous extract of Guava leaf (200 mg/kg body weight).
Group IV (Test) Animal receive Rifampicin + Isoniazid (50 mg/ kilogram body weight, p.o) and aqueous extracts of A. Guava leaf extract (500 mg/ kg body weight, each p.o)for 28 days.

Group V (Standard) Each animal received 50 mg of Rifampicin + Isoniazid each p.o. and 100 mg of Silymarin p.o. for 28 days.

Under light anesthesia, animals were weighed after they had received treatment, and their serum was collected with a cardiac puncture. The livers were then dissected, after all animals had been sacrificed, they were washed in saline, weighed, and homogenized in 0.1 M Tris-HCL buffer. For the biochemical analysis, homogenates of serum and liver were used. Formalin-preserved livers: a study.[24]

Figure: light anesthesia rats.

Biochemical chemical parameters investigated

A 15-minute spin at 2500 rpm separated the serum after the blood had clotted. These parameters were measured in the serum: The total bilirubin, total protein, alkaline phosphatase, and albumin levels are determined by the serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), and total bilirubin. By using the autoanalyzer, the biochemical parameters were determined as per the manufacturer's instructions in the kit's instruction
manual.

Serums glutamics pyruvic Transaminases (SGPT) determination

Method using SGPT (Spandiagnostic reagent kit)

Principle

SGPT (ALT) catalysis the following reaction

α-Keto glutamate+ L – alanine ⟶
L- glutamate + pyruvate

A hydra zone is formed when pyruvate is coupled with 2,4- Dinitrophenyl hydrazine (2,4- DNPH). In an alkaline medium, colorimetry can be used to determine the shade of brown.

Reagents

Reagent 1: Buffereds alanine α- KG substrate, pH 7.4
Reagent 2: DNPH colour reagents
Reagent 3: Sodium hydroxide 4 ml
Reagent 4: Working pyruvate standard, 2Mm

Preparation of working solution

Table: Determination of Serum glutamic pyruvic Transaminase (SGPT

Tubs No.	1	2	3	4	5
Enzymes activity (units/ml)	0	26	56	96	200
Reagents 1: Buffered alanine ph. 7.4 (ml)	0.5	0.45	0.4	0.35	
Reagents 4: Working pyruvate standard2Mm(ml)	-01	0.05	0.1	0.15	
Purified water(ml)	0.1	0.5	0.1	0.1	
After thoroughly mixing, let it stand at ambient temperature for 20 minutes.		0.5	0.5	5.0	
Solutions 1.ml	5.0	5.0	5.0	5.0	

Test procedure Solution 1: Mix well by inversion. Comparing each of the five tubes' O.D. to cleaned water on an autoanalyzer after allowing standing at room temperature for 10 minutes.

Table: Dilute 1ml of reagent 3 to 10 ml with purified water.

Reagent I Buffere alanines Ph 7.4	0.27 ml
Incubate at 37°C for 5min	
Serum	0.06 ml
Mixed well and incubated at 37°for 30 mintus	
Reagent 2: DNPH color reagents	0.27 ml
Mixed well or allow to stands at room temperture. for 20 mintus	

After mixing, let it rest at the room temperature for ten minutes. The U/I is calculated using a chemo analyzer.

Measurement of glutamic oxaloacetic transcription factors in serum (SGOT)

Method using SGOT (Span diagnostic reagent kit)

Principle

A - amino acid is transferred to a keto acid by transaminases, which catalyzeamino group transfer.

Keto glutaric + aspartic acid → Glutamic + Oxalis acetic acid

During the reaction, pyruvic acid is formed spontaneously from oxaloacetic acid. To determine the rate of reaction, dinitrophenyl hydrazine is used to estimate pyruvic acid. A wavelength of 520 nm is estimated for the formation of dinitrophenyl hydrazine. With color reagent, unreached a - keto glutamate also gives colored product, but its intensity is much lower than that of pyruvate.

Reagent

pH-7.4 standards pyruvate (mm) an ketone glutamate – aspartic acid substrate SGOT, 2,4 dinitrophenyls hydrazines, and 0.6 ml sodiums hydroxides buffers.

Procedure

To prepare SGOT substrate, combine 28.2 mg a-keto glutamate with 2.66 g aspartate in a beaker.Dissolve 1 M sodiums hydroxides in water. Adjust the sodium hydroxide solution pH to 7.4. Dilute to the mark with phosphate buffer after quantitatively transferring to a 100 mlvolumetric flask.

For preparing the standard curve, a number of tubes were set up according to the table below, and 1.0 mL of Dinitrophenylhydrazine solutions was added to each tube. After 15 minutes, observe the optical density (OD) using a spectrum meter using green filter at 520 nm. Connect the point by the smooth curve and plot the result against SGOT concentration units.

Table: Determination of Serum glutamic oxaloacetic.

Tube No	Sodium pyruvate ml	Substrate ml	Water ml	SGOT units
1	0.5	0.5	0.2	0
2	0.4	0.6	0.2	22
3	0.3	0.7	0.2	55
4	0.2	0.8	0.2	95
5	0.1	0.9	0.2	150
6	0.0	1.0	0.2	215
Solution	Test ml	Control ml	Blank ml	Standard ml
Substrate	1.0	1.0	1.0	1.0
Keep for 5min in boiling water bath at 37°C				
Serum	0.2	-	-	-
Incubate at 37°C for 60 min				
Sodium pyruvate	-	-	-	0.2
Dintrophenyl hydrazine	1.0	1.0	1.0	1.0
Serum	-	0.2	-	-
Allow to stand for 20min at room temperature				
Sodium hydroxide	10.0	10.0	10.0	10.0

Let stand for 10 minutes, then estimate with a chemo analyzer and give as U/I.

Determinations of the serum alkaline phosphate (SALP)

King and Armstrong (1965) estimated the alkaline phosphate level using the alkane phosphate method. (Span analytical reagent kit).

Opinion

Alkaline phosphate from serum changes phenyl phosphate to inorganic orthophosphate and phenol. A complex that can be detected at 510 nm is formed when phenol reacts with potassium ferricyanide in the alkaline medium.

Substances

Reagents 1: buffered substrate pH 10.0 Reagents 2: Chromogen reagent Reagents 3: Phenol standard, 10 mg %.

Preparations of working solutions

To one ampoule of buffer substrates, add 4.5 ml of distilled water.

Procedures

Table: Determinations of the serum alkaline phosphate (SALP)

	Blank	Standard	Control	Test
Workings buffer solution distilled water.	0.7 ml	0.7 ml	0.7 ml	0.7 ml
Mixed as well as and incubate for 4 min at 37°C				
Serums	-	-	-	-
Phenols standards, 10 mg%	-	7 ml	-	-
Mixed as well as and incubated for 15min, at 37°C				
Chromogens reagents	2.0 ml	2.0 ml	2.0 ml	2.0 ml
Serums	-	0.75 ml	-	-

Allow the mixture to stand at room temperature for 10 minutes after mixing well. Using a chemo an analyzer, we estimate the amount and express it as U/I.

Determination of the serum total bilirubin

(Agape diagnostic kit) Bombay was used to determine the total bilirubin level in serum.

Bituminoid combine es with sulphonic acid to create azobilirubin, which can be measured at a wavelength of 532-536 nm when there is deoxidized sulphonic acid present. Bituminoid combines with sulphonic acid to create azobilirubin, which can be measured at a wavelength of 532-536 nm when there is deoxidized sulphonic acid present. When methyl sulfoxide is not present. The azobilirubin complex is formed only by direct (conjugated) bilirubin.

Reagents

1. Reagent for total bilirubin, Dimethyl sulfoxide, Sulphatic acid, Stabilizer
2. NaNO3, Activator
3. Reproduction standard = 10 mg/dl

Method Total hematoidin

In equal parts of the total bilirubin reagents, two milliliters of activator were additional, and 0.1 milliliter of serum was added. After thoroughly mixing, the combination was incubated for exactly five minutes at 37 degree C temp.

For the blank samples, 1.0 ml total bilirubin component was mixed with 0.1 ml distilled water and nurtured for exact 5 Min at room dissatisfaction. Calculated using the chemoanalysers and expressed asmilligrams per deciliter.

Determination of albumin serum total protein

Method used (Span diagnostic kit)

Principle

In alkaline medium, the protein reacts with cupric ions to form a violet-color complex. In the same way as the compound at 530 nm, a reagent blank of 0.01 ml was treated.

Procedure for total protein

Span diagnostics kit reagent was use. The absorbance at 530 nm was determined by mixing 1 ml of occupied reagent with 0.01 ml of blood serum. This was also done with the 0.01 ml reagent blank of standard solution. Mix well after adding each reagent estimated by a chemoanalysers and express as dl.

Procedure for albumin

Span diagnostics kit reagent was use and absorb at 630 nm. The same procedure was followed for the reagent blank, which was 0.01 ml of standard solution. Mix well after adding each reagent estimated by a chemoanalysers and expressed asgrams per dl.

Histopathological studies

Fixing liver tissues consisted of dehydrating in gradual ethyl alcohol (50-100%), clearing them in xylene, and putting them in 10% formalin, and then embedding them in paraffin. For photomicroscope observation of fatty changes, hyaline degeneration, cell necrosis, ballooning degeneration, and lymphocyte infiltration, 4- to 5-millimeter-thick sections were stained with hematoxylin then bromeosin (H–E) dye.

Statistical analysis

The mean value was used to represent all the values ± standard error of mean. (S.E.M) We used Graph prism pad software (type 3.0) to analyze six rats. Newman-Keuls multiple comparison test was used to assess the meaning of alterations between control and experimental groups. Statistical implication is defined as P>0.05.

RESULTS

Preliminary Phytochemical Analysis

Table: Chemical tests revealed that the phytoconstituents included several phytoconstituent including sterols, glycosides, tannins, terpenoids, saponins and flavonoids in aqueous extract of Guava Leaf.

S. N	Constituents	Tests	Inference
1	Carbohydrate	Molisch test	+
		Fehling solution test	+
2	Test for Alkaloid's	Dragendorff's test	+
		Mayer test	+
		Wagner test	
3	Test for Fats and fixed oils 4	1% Copper Sulphate, 10% NaOH	+
4	Test for Anthraquinone Glycosides	Borntragor's Test	+
5	Assessment for Cardiacs Glycoside	Keller-Killiani Test	+
		Legal test	+
6	Test aimed at Flavonoids	Alkaline reagent test	+
		Shinoda	+
7	Test for Tannins	Ferric chloride	+
		Gelatin Test	+
8	Test for Protein	Heat test	+
		Hydrolysis	+
9	Triterpenoids	Salkowski	+

Drug Tolerance Report

Based upon the available literature survey, the aqueous extract of Guava leaf did not yield any harmfulness even at the maximum dose particular up to 2000 mg/kg, from that the effective dose (ED50) of aqueous extract of Guava leaf was decide $1/10^{th}$ of maximum quantities (2000 mg/kg). So, the doses of aqueous extracts of Guava leaf were used such as 200 mg, 400 mg/kg rat body weight, p.o for the Antihepatotoxicity activities.

Effect of aqueous excerpt of Guava leaf on body weight, liver weight of RIF+ INH induced hepatotoxicity in rats

The effect of Guava leaf on the body weight of RIF+INH befuddled rats is shown table 5.2. Associated with the supervisor group, the RIF+INH preserved group experienced a higher weight loss perceptually than the control group. A. marvelous group also experienced varying levels of body weight loss during the experiment, although the loss was less pronounced than that in the RIF + INH group.

Table: Effect of aqueous extracts of Guava leaf on body weight, Wt. of RIF+INH transported hepatotoxicity in Rats.

Handling / dosage	Body mass (g)	Liver mass (g)
Controls	186.05 ±5.05	5.02 ± 1.05
RIF+ INH (50mg/kg)	178.33 ± 4.97	7.65 ± 0.15
G. l Extract 200 mg /kg	179.03 ± 5.06	6.95 ± 1.06
G. l Extract 400mg /kg	181.05 ± 4.65	5.90 ± 1.03
Silymarin 100 mg/kg	184.06 ± 4.35	5.25 ± 0.12

Consequence of aqueous extract of Guava leaf Extract on Biochemical parameters (SGPT, SGOT, and ALP) of RIF+INH induced hepatotoxicity:

Control serum value of AST (SGOT), ALT (SGPT) and ALP in rats was found to be171.53 ± 6.50, 25.55 ± 3.04 and 229.50 ± 10.43 (U/L) respective Table, while toxic dose of rifampicin + isoniazid (50 mg/ kg) raised significant the respectively serum enzyme value to 376.40 ± 8.55, 82.33 ± 5.61 and 389.78 ± 8.24^{Z}. In animals pretreated with Guava leaf extracts 200 m/kg,00 mg/kg the serum value of transaminase was found to be AST 234.65 ± 13.22, 191.65 ± 6.95. ALT 64.85 ± 4.21, 51.25 ± 4.25. ALP 298.47 ± 10.51, 246.65 ± 11.45. Which were significant minor ($p < 0.05$), ($p < 0.001$) than the value of the toxic controland quite similar to that of the control groups ($p < 0.001$). Using a one-way ANOVA and the Newman-Keuls various assessment test, the result was found to be significant ($p = 0.001$). For each group of six rats, the values are expressed as Mean ± SEM.

In comparison to respective controls, zip < 0.001, and cp < 0.001 in comparison toRespective RIF+INH controls

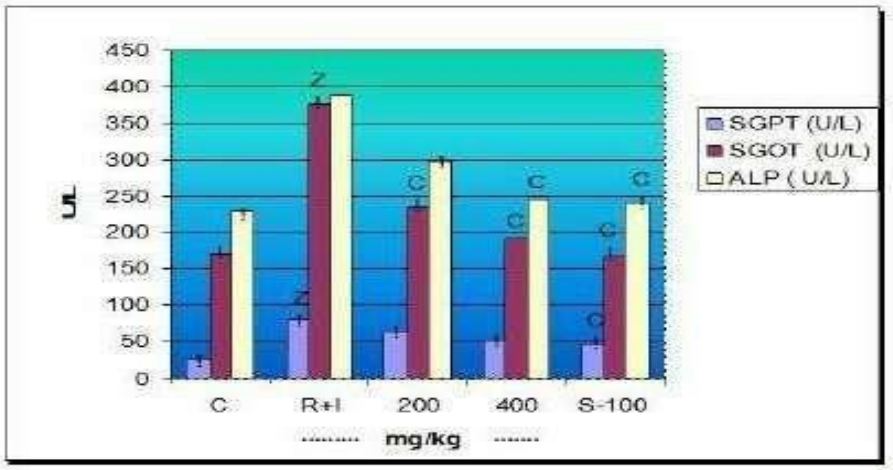

Figure: Effects of aqueous extract of Guava leaf (AAM) arranged liver marker enzymes of RIF + INH induced hepatotoxicity.

Consequence of Aqueous Extract of Guava leaf on Total Protein, Bilirubin and Albumin of RIF + INH Brought Hepatotoxicity in Rats

Control serum value of total proteins, bilirubin and albumin, in rats found to be 6.87 ±0.43, 1.32 ± 0.03 (g/dl) and 0.54 ± 0.03 mg/ dl respectively Table**,** while toxic doses of rifampicin + isoniazid (50mg/ kg each) raised significantly the serum value of bilirubin 1.76 ±0.21 and lower the serum values of total protein, albumin 3.48 ± 0.50, 0.71 ± 0.07 respectively. In animals pretreated with Guava leaf200 mg/ kg, 400 mg/ kg the serum value was found to be 5.59 ± 0.64, 1.03 ± 0.04 and 0.80 ± 0.15, for P. guajava (400 mg/ kg) 7.65 ± 0.31, 1.17±0.13 and 0.51 ± 0.10 which were significantly lower $p < 0.05$, $p < 0.001$ than the values oftoxic group in case of total bilirubin and higher in case of total protein and albumin, which is quite similar to that of control group ($p < 0.001$). Using one-way ANOVA followed by Newman-Keuls multiple comparison test, the result was significant ($p = 0.001$).

Table Effect of aqueous extract of Guava leaf on Total Protein, Albumen and enzymes of RIF+INH induced hepatotoxicity

Treatment/dose	Total protein (g/dl)	Albumen (g/dl)	Total hematoidin (mg/dl)
A G.L Extract (200 mg kg)	Z,4.48 ± 0.50	Z 0.72 ± 0.07	Z,1.66 ± 0.21
A. G.L Extract (400 mg/kg)	C 5.59 ± 0.64	A 1.03 ±0.04	Ca 1.03 ±0.04 0.80±0.15
Silymarin (100 mg/kg)	C 7.65 ± 0.31	C 1.17±0.13	C 0.51±0.10
Control	C7.53 ± 0.28	C 1.29 ± 0.07	C 1.03 ± 0.10

The data is presented as the Callous ± SEM for six rats in each cluster. A comparison between respective controls gave zp< 0.001 and a comparisonbetween respective RIF+ INH controls gave ap< 0.05 and cp< 0.001

Liver section of normal control rates

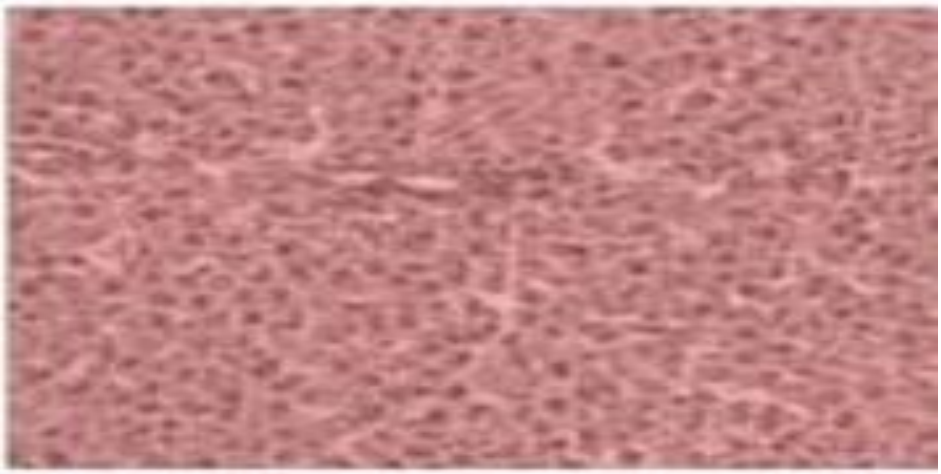

Figure: Group1 control.

Showing normal hepatics cell with fresh-looking cytoplasm well transported out essential vein protuberant nucleus

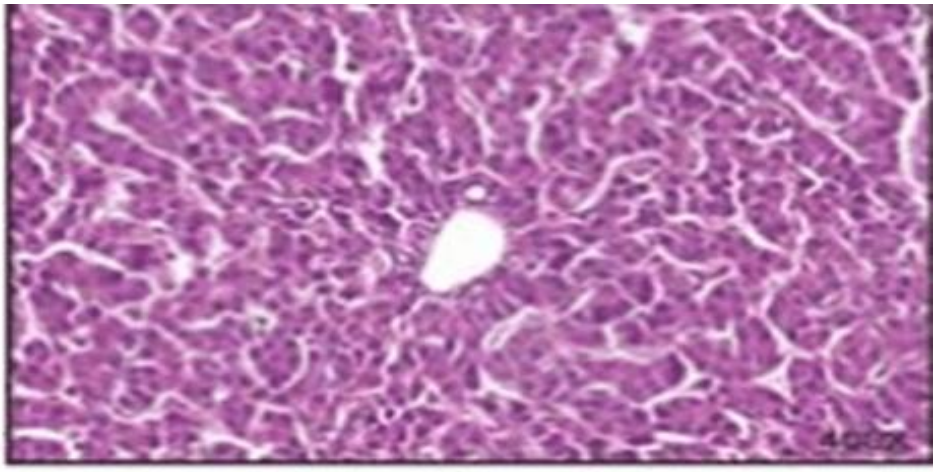

Figure: Groups 2 Toxicant.

Liver section of RIF+ INH (50kgmg/kg show enormous fatty fluctuations necrosis ballooning developments centrals manners congestions loss of cellular limitations and nonnuclear inflammatory infiltration.

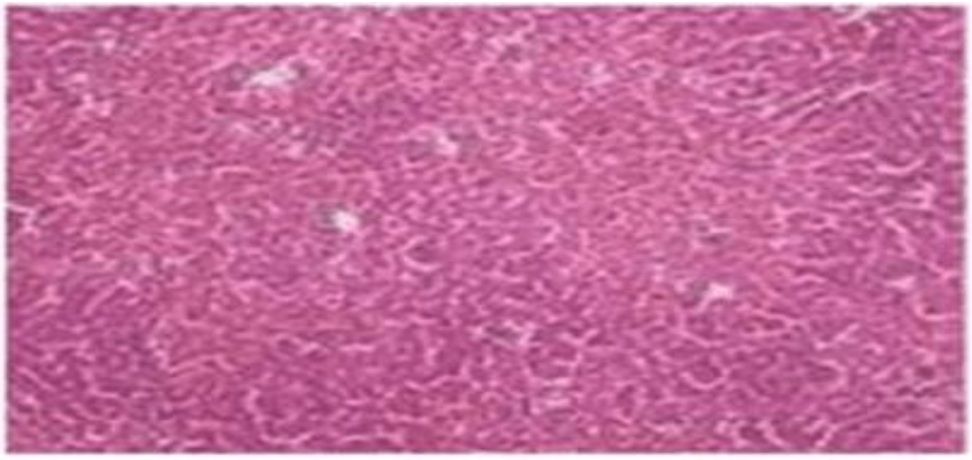

Figure: Group 3 test AAM 200 mg/kg.

Liver section rates RIF+ INH (50mg/kg each po) + guava Leafe extract 200mg/x28days showing hepatic cell well preserved cytoplasm prominenuclus of central vein and sinusoid show congestions.

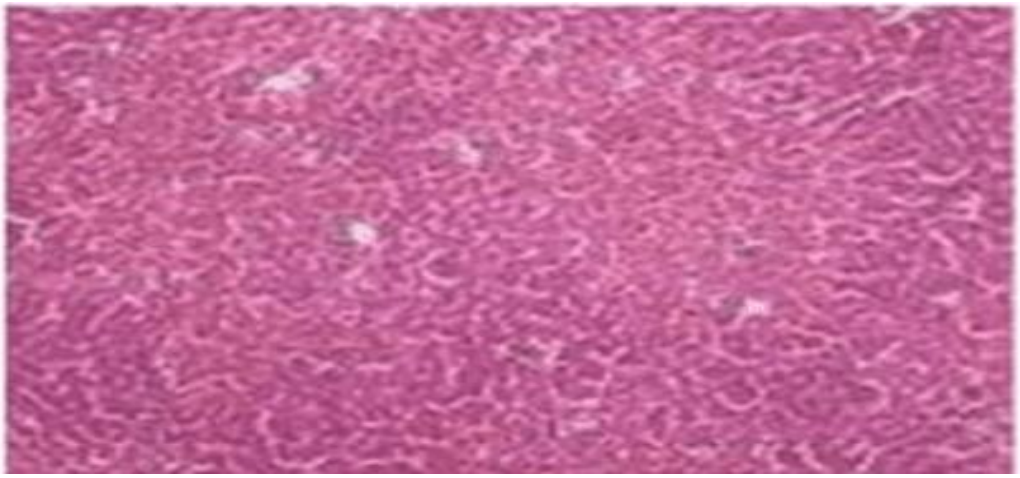

Figure: Group 4 Test (AAM 400 mg/kg.

Liver piece of rats preserved RIF =INH (50 mg/kg. Each p. o) + guava leaf quotation (400mg/kg) x28 presentation as well as brought out centrals veins hepatics cells with well-preserve cytoplasm promintanucleus.

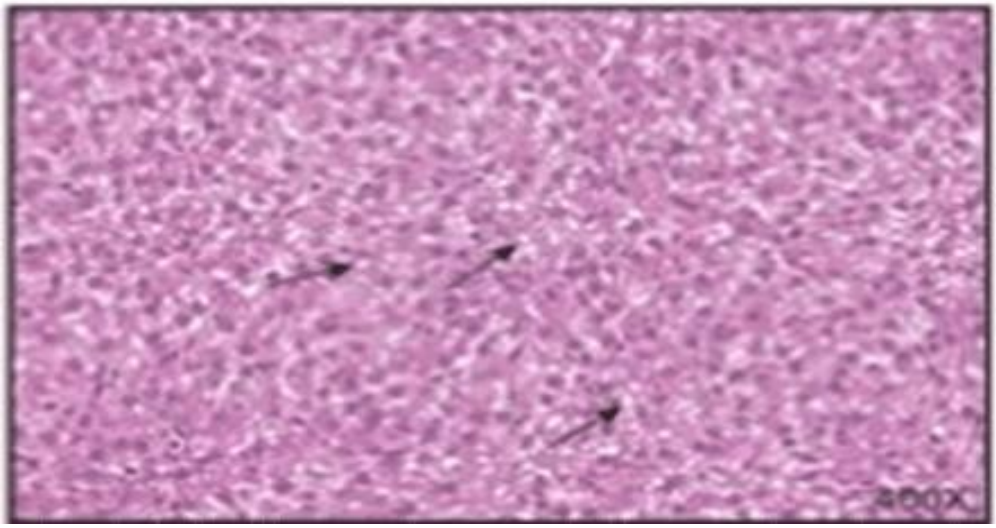

Figure: Group 5 Standard (Silymarin 100 mg/kg).

Liver pieces of rates treat with RIF+INH (60 mg/kg each p. o) x 38 days show as well as brought out centrals veins hepatics cell with well-preserved cytoplasm prominent nucleus near normals livers buildings

SUMMARY AND CONCLUSION

Assessment of hepatology activity of Guava leaf was carried out with aqueous extract at a quantity of 200, 400 mg/kg. Hepatic damage was induced by anti-tubercular drugs, is connected with misrepresentation metabolic purposes. The toxin absorbed from the intestine isa major process of various serious and continuing diseases. Ayurvedic medicinal structure of drugs describes a large variety of herbal resources for ailment of diseases. Ethnopharmacology illustrated that Guava leaf is traditionally being used for cure of hepatoprotective activity. Exhaustive literature survey helped to collect the information about the plant in the direction of hepatoprotective activity the availability and economic factors achieved the concentration to explore the hepatoprotective potential of of the plant. Attempt was taken to establish the scientific validation in this regard. The plant material was collected and authenticated. Dried powder was used for successive extraction with chloroform water which yielded 18.2% extractive values respectively from the 500 gm of initial weight of powder. In aqueous extracts of Guava leaf, various phytoconstituents such as sterols, saponins, tannins, terpenoids and flavonoids were detected by chemical tests. You may rapidly and effectively rewrite and rephrase your text by using paraphrasing tool, which modifies your sentences.

Treatment with aqueous extract of P. guajava significantly prevented the hepatotoxicity caused by isoniazid and rifampicin. This preventive effect was demonstrated by an increase in albumin and total protein in serum and a decrease in elevated biochemical parameter levels such as serum SGOT, SGPT, ALP, and total bilirubin. Hepatic cells were almost completely restored on histopathological slides, with just slight fatty alterations and no necrosis.

Based on improved Guava leaf aqueous extract was found to have considerable hepatoprotective effect in a dose-dependent manner based on serum marker enzyme levels, histological results, and the presence of phytoconstituents., Supporting the traditional application of Guava leaf in light of modern science as a possible cytoprotective agent against drug-induced hepatotoxicity.

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