PHYTOCHEMICAL INVESTIGATION AND HEPATOPROTECTIVE ACTIVITY OF LEAVES OF *JATROPHA CURCAS* LINN.

By

MR. SACHIN. M. MAHAJAN.

B.PHARM.

Dissertation

Submitted to the Rajiv Gandhi University of Health Sciences, Karnataka, Bangalore
In partial fulfillment of the requirements for

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Master of Pharmacy

in

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Under The Guidance Of

MR. S. S. JALALPURE.

M.PHARM.

DEPARTMENT OF PHARMACOGNOSY & PHYTOCHEMISTRY,
K.L.E.S's COLLEGE OF PHARMACY,
BELGAUM- 590 010, KARNATAKA, INDIA.

APRIL - 2006.

RAJIV GANDHI UNIVERSITY OF HEALTH SCIENCES,
KARNATAKA, BANGALORE.

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This is to certify that the dissertation entitled "PHYTOCHEMICAL INVESTIGATION AND HEPATOPROTECTIVE ACTIVITY OF LEAVES OF *JATROPHA CURCAS* LINN." is a bonafide research work done by ***MR. SACHIN M. MAHAJAN*** in partial fulfillment of the requirement for the Degree of ***MASTER OF PHARMACY IN PHARMACOGNOSY & PHYTOCHEMISTRY.***

Date :
Place : Belgaum.

Mr. S. S. JALALPURE.
M.Pharm.
Assistant Professor,
Dept. of Pharmacognosy &
Phytochemistry,
K.L.E.S's College of Pharmacy,
Belgaum - 590 010.
Karnataka.

RAJIV GANDHI UNIVERSITY OF HEALTH SCIENCES,
KARNATAKA, BANGALORE.

Endorsement By The HOD, Principal/Head of The Institution

This is to certify that the dissertation entitled "PHYTOCHEMICAL INVESTIGATION AND HEPATOPROTECTIVE ACTIVITY OF LEAVES OF *JATROPHA CURCAS* LINN." is a bonafide research work done by ***MR. SACHIN M. MAHAJAN*** under the guidance of ***MR. S. S. JALALPURE,*** Assistant Professor, Department of Pharmacognosy and Phytochemistry, K.L.E.S's College of Pharmacy, Belgaum.

Dr.(Mrs.) KALPANA S. PATIL.
M.Pharm., Ph.D.
Professor & Head,
Dept. of Pharmacognosy & Phytochemistry,
K.L.E.S's College of Pharmacy,
Belgaum - 590 010.
Karnataka.

Date :
Place : Belgaum.

Dr. F.V. MANVI.
M.Pharm., Ph.D.
Principal,
K.L.E.S's College of Pharmacy,
Belgaum - 590 010.

Date :
Place : Belgaum.

RAJIV GANDHI UNIVERSITY OF HEALTH SCIENCES,
KARNATAKA, BANGALORE.

Declaration By The Candidate

I hereby declare that this dissertation entitled "PHYTOCHEMICAL INVESTIGATION AND HEPATOPROTECTIVE ACTIVITY OF LEAVES OF *JATROPHA CURCAS* LINN."is a bonafide and genuine research work carried out by me under the guidance of ***MR. S. S. JALALPURE,*** Assistant Professor, Department of Pharmacognosy and Phytochemistry, K.L.E.S's College of Pharmacy, Belgaum.

Date :
Place : Belgaum.

MR. SACHIN M. MAHAJAN
B.Pharm.
Dept. of Pharmacognosy & Phytochemistry,
K.L.E.S's College of Pharmacy,
Belgaum - 590 010.
Karnataka.

RAJIV GANDHI UNIVERSITY OF HEALTH SCIENCES,
KARNATAKA, BANGALORE.

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Date :

Place : Belgaum.

MR. SACHIN M. MAHAJAN
B.Pharm.
Dept. of Pharmacognosy & Phytochemistry,
K.L.E.S's College of Pharmacy,
Belgaum - 590 010.
Karnataka.

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ABSTRACT

Back ground and objectives

Number of medicinal preparation have been advocated in traditional system of medicine especially in Ayurveda siddha for treating liver diseases. Various liver diseases has affected human being since its antiquity. Inspite of tremendous advances in the field of the medicine there is no truly satisfactory drug for the treatment of liver disorders. In the indigenous system of medicine, the leaves of Jatropha curcas Linn. (Family-Euphorbiaceae) are reported to be useful in the treatment of jaundice. Hence in the present study, the leaves of Jatropha curcas have been selected for their hepatoprotective activity on experimentally induced hepatotoxic rats.

Material and Methods

The leaves of Jatropha curcas were dried in shade, powdered and then extracted with Petroleum ether (40-60°C), Alcohol and Aqueous successively. The extracts were subjected to preliminary phytochemical investigation. The isolated compounds from aqueous extract and petroleum ether subjected to chromatographic and spectral studies

Acute toxicity study was carried out as per OECD guidelines. Female Wistar albino rats were used for assessment of hepatoprotective activity. Hepatotoxicity was induced by administration of Carbon tetrachloride (2ml/kg) 1:1 with Olive oil subcutaneously on 2^{nd} 3^{rd} day. Liv 52 was used as standard drug. Petroleum ether extract, Alcoholic extract, Aqueous extract (200 mg/kg b.w., p.o.) were administered on 1^{st} ,2^{nd} ,3^{rd} ,4^{th} days. On 5^{th} days blood are collected by cervical decapitation and various biochemical parameters (SGOT,SGPT, SALP and Serum Bilirubin) was studied. Histopathology of liver also studied.

Result and Discussion

Preliminary phytochemical investigation revealed the presence flavonoids carbohydrates, steroids, triterpenoids, saponins in petroleum ether extract, alcoholic extract, and aqueous extract. LD_{50} cut off dose was found to be 200mg/kg b.w. for the extracts of *J. curcas*. Hence, therapeutic dose was taken as 200mg/kg b.w. for all three extracts.

Treatment with aqueous extract of leaves of *Jatropha curcas* significantly lowers the elevated levels of SGOT,SGPT, SALP AND Serum bilirubin. The histopathologocal study also supports the above results. The results were compared to that of standard drug (Liv 52)

Conclusion

The present data indicate that administration of *Jatropha curcas* leaves extracts to rats with experimentally induced hepatotoxicity lowers the elevated levels of SGOT, SGPT, SALP and Serum bilirubin, supporting folk information regarding hepatoprotective activity of the plant.

Key words: Hepatotoxicity; Liv 52; Carbon tetrachloride; *Jatropha curcas* Linn.; Euphorbiaceae

INTRODUCTION

1.1 INTRODUCTION TO MEDICINAL PLANTS

"In the light of this knowledge,

there is no substance in this world

that may not be used as medicine in

this or that manner,

and for this or that purpose".

In the foregoing works of wisdom by renowed ayurvedic experts Charak have been proved repeatedly by human experience all over the world.

Man since time immortal has been using herbs or plant products as medicine for developing immunity or resistance against cold, coryza, joint pain, fever etc. A vast majority of our population, particularly those living in villages depend largely on traditional remedies. [1]

The nature has provided a complete storehouse of remedies to cure all ailments of mankind. Since the down of civilization, in addition to food crops, man cultivated herbs for his medicinal needs. The knowledge of drugs has accumulated over thousands of years as a result of man's inquisitive nature, so that today we possess many effective means of ensuring health care.[2]

With the changing pattern of life style most of the diseases are now becoming lifestyle diseases. The traditional systems of medicine based on ancient cultures are primarily concerned with building the body strength which can help in healing the ailments and these systems rely largely on the nature cure. The Ayurvedic system has described a large number of such medicines based on plants or plant product and the determination of their morphological and pharmacological or pharmacognostical

characters can provide a better understanding of their active principles and mode of action. Contribution of the traditional medicine to human health in the 21st Century is of paramount importance. WHO emphasized that with the changing pattern of life style most of the diseases are now becoming life style diseases. Natural medicines improve the inner strength of the body.

Some of the oldest traditional medical systems include Chinese, Ayurvedic, Unani, Japanese and recently added homeopathy and chiropathy that is also around 200 years old. The use of traditional medicine includes (i) medication by use of medicinal plant, minerals, animal material and (ii) non medication: acupuncture and yoga[3].

The wonderful reference and treatises on herbal cure that have been available in India mention the work of Dhanwantari, Nagarjun and Charak. Most of the Bhikshus of Buddhists monasteries actually maintained the nursery of medicinal herbs around them and when went abroad they carried these herbs for the welfare of common man in those lands[4]. A new herbal 1551 by William Turner was the earliest English book which gave a truly scientific account of plants[5].

Out of 2, 50, 000 plant species only 10,000 or so have been exploited during the course of human civilization[3]. It has been estimated that from 25000 to 7500 species of higher plant about 10% has been used in traditional medicine. However perhaps only about only 1% of these (250-750) are acknowledge through scientific studies to have therapeutic value when used in extract form by human[3, 6].

Modern medical science is currently in the throes of a revolution which is likely to have a dramatic impact on both the theory of medicine and the way it is practiced. The mechanics models which served biomedicine well for many years is gradually collapsing according to Svoboda (1998). Efforts of dedicated researcher are

looking beyond that model's flaws. We now know that networks of chemical communication exist between the nervous and immune systems and that prayer at a distance can positively affect the conditions of those who are seriously ill, even when the prayer and patient are not known to one another. Another participant in this exciting climate of change and ferment is Ayurveda, india's ancient medical system. While Ayurveda has already contributed much to modern medicine (reserpine, gugulipid, plastic surgery), its real contributions are yet to made. While some of these are likely to come in matters of materia medica and technique, most will likely to be derived from Ayurveda's vision is likely to facilitate medicine's ability to teach people not just how to avoid disease but how proactively develop and maintain healthy state[7].

In modern medicine also, plants occupy a very significant place as raw materials for some important drugs, although synthetic drugs brought about a revolution in controlling different diseases. But these synthetic drugs are out of reach to millions of people those who live in remote places depends on traditional healers, whom they know and trust, judicious use of medicinal herbs can even cure deadly disease[8].

1.2 HISTORY OF HERBAL MEDICINE:

We can certainly assume, however that the healing properties of some plants were discovered by primeval humans fairly early and they learned to use them. By collecting and using medicinal plants, people gained valuable experience good as well as bad and handed down their knowledge to future generations.

One of the first written records concerning curatives drugs and narcotic substances was found on a clay tablet in Assyrian cuneiform script dating back to

2,700 BC. The tablet mentions a brown drug, daughter of poppy meaning opium. In ancient Egypt medicinal science and the use of medicinal substances have an age-old tradition. The Egyptian pharmacopoeia always had a supply of medication of plant and animal, as well as mineral origins. There were 25 types of medicinal plants, as basic nutritional and medicinal plants, onion, garlic, lettuce, lenthils, olives and caraways were used. The knowledge of Indian physician is documented by the so-called Bower manuscript found in 1889 in the ruins of MINGSI in central Asia. The document and its author praise the garlic as a panacea claiming it to prolong life to 100 years. In ancient Chinese pharmacology and herbal medicine were the most extensive fields of medicine, they contained 8160 prescription for the use of various drugs, with instructions on how to use how to collect and prepare various drugs from medicinal plants[9].

1.3 MODERN DRUGS FROM AYURVEDA:

Ayurveda offers a holistic lifestyle, caring for the individuals mind and spirit as well as their body. It treats each person as an individual taking in to consideration their unique body constitution and mental disposition[10]. Ayurveda, a traditional indigenous system of medicine, has been practiced in India for centuries[11]. Ayurveda is an ancient science of life that has a strong philosophical basis. Although there is no definite evidence that suggest its exact period of origin, the Vedic period in Indian philosophy goes back to over 5000 years.

Some of the prominent commercial plant Derived Medicinal compounds include: Colchicum, Colchicine, betulinic acid, Camptothecin, topotecan (Hycamtin, CPT-11(irinotecan, Campesterol) ,9-aminocamptothecin, delta -9- tetrahydrocannabi

nol (dronabinol, Marinol, beta lapachone, lapachol, Podophyllotoxin, etoposide, podophyllinic acid, vinblastin (Velben), vincristine (Leurocristine, Oncovin), vindesine (Eldisine, Fildesin), vinorelbine (Navelbine), docetaxel (Taxotere), paclitaxel (Taxol), Tubocurarine, Pilocarpine, Scopolamine. The possibilities for developing new drugs from forest resources should figure heavily in any calculation of the forests' true worth. All 119 plant derived drugs used worldwide in 1991 came form fewer than 90 of the 250,000 plants species that have been identified. Each such plant is a unique chemical factory is correctly mentioned by Norman R. Farnsworth of the University of Illinois at Chicago that are capable of synthesizing unlimited numbers of highly complex and unusual chemical substances whose structures could otherwise escape the imagination. Scientist may be able to synthesize these plant compounds in the laboratory, but dreaming them up, rather than plucking them from the forest and then replicating them, is quite different[6,12].

1.4 ETHANOPHARMACOLOGY:

There are 119 drugs of known structure that are still extracted from higher plants and used globally in allopathy medicine. About 74% of these were discovered by chemist attempting to identify the chemical substances in the plant that were responsible for their medical uses by the humans. These 119 plant derived drugs are produced from less than 90 species of higher plants. Since there are at least 250,000 species of higher plants on earth, it is logical to presume that many more useful drugs will be found in the plant kingdom if the search for these entities is carried out in a logical and systematic manner. The ultimate goal of Ethanopharmacology according to Farnsworth (1990) should be to identify drugs to alleviate human illness via thorough analysis of plants alleged to be useful in human cultures throughout the world. By integrating the sciences of ethanobotany, medicine and plant natural

product chemistry, it is possible to achieve time and cost saving for the identification of active compounds and preclinical development of its initial products. Numerous drugs have entered the international pharmacopoeia via the study of ethanobotany and traditional of medicine. There are many similarities in traditional system of medicine as well as ethanomedicines being connected to each other as 'great traditions and little traditions'. It is from this knowledge future considerations of Ethanopharmacology need to be determined. In view of the progress of Western medicine not only new synthetic drugs but also botanical drugs will have to fulfill the international requirements on quality, safety and efficacy.

IN INDIA

India has vast ethnobotanical knowledge since ancient time. Origin of all such knowledge since ancient time. Origin of all such knowledge in India is from the great tradition of Ayurveda which is a living tradition of practice even today. Indian medical care consist of medical pluralism and Ayurveda still remain dominating even as compared to the modern medicine particularly for treatments of variety of chronic diseases conditions. India is one of 12 leading bio-diversity centres with presence of over 45,000 different plant species; medicinal properties have been assigned to several thousand. About 2000 figure in literature; indigenous systems commonly employ about 500. Among those only about 7,000 plants are used in Ayurveda, 600 in Siddha, 700 in Unani [6,13].

1.5 HERBAL DRUG MARKET:

Commercially, plant derived medicines are worth about $14 billion a year in the United state and $40 billion worldwide. The average turnover of Indian herbal medicine industry is about 2,300 crore against the pharmaceutical industry is turned

about Rs.14,500 crore with a growth rate of 15%. However to achieve the goal of major exporter of herbal remedies several steps need to be taken:

A. Systematic study of world market demand and short listing of medicinal herbs with good potential.
B. Systematic cultivation of medicinal herbs on a large scale.
C. Encouragement for agro-based phytochemicals and pharmaceutical industries to manufacture value added herbal products.
D. Strict legislation to control quality and purity.
E. Upgradation of cultivation and collection process.
F. Documentation of research work and standardisation for quality[14].

The increasing demand for herbal medicines inevitably led to the issue of obtaining and maintaining their quality and purity based on internationally recognized guidelines.

Current Status of Standardization:

WHO has emphasized on the need to ensure the quality control of herbs and herbal formulations by using modern techniques. Several pharmacopoeias like British Herbal Pharmacopoeia, Japanese Pharmacopoeia, United States Pharmacopoeia, British Herbal Compendium, German Commission-E etc. lay down monographs for herbs to maintain their quality. Ayurvedic pharmacopoeia of India which recommends basic quality parameters for 80 common Ayurvedic herbal drugs. BHP contains 233 monographs and quality control tests, Chinese Herbal Pharmacopoeia contain 1751 monographs of substances and articles, BHC contains 84 monographs of medicinal plants. German Commission E has 330 monographs for drug used in German folk medicine.

1.6 STANDARDIZATION BY MARKER COMPOUND:

The best tool developed for standardization is by chromatography. It describes botanical identity and chemical sanclity of herb. One of such technique is marker compound testing and finger print 1010 analysis.

Secondary metabolites present in herb are considered as marker compounds:-

Name of Herb	***Marker Compound***
1. *Andrographis paniculata*	Andrographolides
2. *Boerhaavia diffusa*	Punarnovine
3. *Curcuma longa*	Curcuminoides
4. *Eugenia caryophylata*	Eugenol
5. *Glycyrihiza glabra*	Glycyrrhizine
6. *Withani somnifera*	Withanolides
7. *Tinospora cardifolia*	Giloin
8. *Piper longum*	Piperine
9. *Ocimum basilicum*	Eugenol
10. *Zingiber officinale*	Gingerol

Different chromatographic methods are used to analyze the marker compounds in herbs with the help of modern sophisticated tools like HPTLC, HPLC etc[15].

1.7 PLANTS WITH HEPATOPROTECTIVE ACTIVITY:

Medicinal plants commonly included in ayurvedic recipes for liver ailments have drawn much attention as no reliable hepatoprotective drug is available in modern medicine. Research investigations conducted on several natural plant products used as liver protectives is well documented. Hepatoprotective effect of some of these like *Picrorhiza Kurroa, Trinospora cordifolia, Withania somnifera, Ricinus communis, Tephrosia purpurea* against carbon tetrachloride and galactosamine induced hepatic injury have been confirmed experimentally by various workers. Search for liver

protectives has been a promising field of investigations and number of formulations containing some of these plant products is being routinely used for hepatic disorders.

Here some important herbal drugs which are effective in liver disorders which are proved experimentally.

Allium sativum contains volatile oil contains allicins allin-s-allylmercaptocystein which shows antihepatotoxic activity in ccl4 and galactosamine induced cytotoxicity in cultured rat hepatocytes.

Handa S.S. and Sharma A. works on Aqueous and alcoholic extracts of *Andrographis paniculata* which contains diterpenoid andrographolide shows protection against infective hepatitis induced by ccl4.

Chatopadhaya R. R. and co workers have evaluated leaf extract of *Azadirachta indica* against paracetamol induced hepatotoxicity which normalized necrosis in liver.

Wagner H. and co-workers reported hepatoprotective activity of aqueous extract of *Butea monosperma* due to the presence of flavonoids butarin and isobutarin against CCl4 and galactosamine induced toxicity.

Sony K. B. and Kuttan R. gives detail about *Curcuma longa* were found to protect rats against CCl_4 induced injury. From it curcuminoids and curcumin shows a significant activity against CCL4 and galactosamine induced activity. Liberti and co-workers have carried out detailed evaluation of corms of *Colchium autumnale.* It contains Colchicines alkaloid which decreases collagen degradation there by slowing diseases progression and fibrosis and survival time.

Flavonoids coumestans and wedelolactone isolated from alcoholic extract of leaves of *Eclipta alba* protected rats against CCl_4 induced liver injury. According to Saraf and co-workers, ethanolic extract and its fractions of *Euphorbia syphilitoca* provides significant protection to rats against CCl_4 induced functional, histopathological and morphological changes.

Glycyrrhiza spp. Contains saponins Glycyrrhizain shows protection against hepatitis C virus induced liver damage reversed. *Ocimum sanctum* also protected rats against CCl_4 induced liver injury. It is reported to contain ursolic acid, apigenin luteolin, apigenin-7-0-glucuronide and molludistin. Rege and co-workers in a series of detailed studied measuring biochemical, morphological and histopathological parameters of *Tinospora cordifolia*, Cyanidanol (+) an isolate from *Acacia catechu* and milk extract (decoction with milk) of *Piper longum* for hepatoprotective activity against CCl_4 induced hepatic injury.

An interesting study Mehrotra and co-workers have reported hepatitis-B virus inactivating activity in the alcoholic extract of *Phyllanthus amarus* in an in-vitro test system. Roy and co-workers have reported that aqueous extract of *Phyllanthus emblica* fruit could prevent toxic effect of lead nitrate and aluminium sulphate on liver parenchymal cells. Phyllanthus niruri shows potent hepatoprotective activity shows against CCL4 induced hepatotoxicity. Ethanol extracts and butanol fractions of Ricinus communis which contains ricinine and N-demethylricinine showed protection against CCl4 stimulated conditions of liver damage. Methanolic extracts of defatted parts of *Swertia chirata* contains lignan, syringaresinol effective against CCL4 induced suppression. Alcoholic extract of *Withania somnifera* leaves was found to be

significantly inhibit the CCl_4 induced liver damage. It shows significant alterations in transaminase activity and pentobarbitone sleeping time.

A withanolide, 3-β-hydroxy-2, 3-dihydrowithanolide F, isolated from *Withania coagulans* fruit, also showed significant hepatoprotective activity against CCl_4 induced liver injury. The activity was assessed by measuring phenobarbitone sleeping time, serum transaminase activity and histopathological study.

Lin and Co-workers have reported hepatoprotective activity in solasodine, solamargine, solasonine and ursolic acid isolated from the fruit of *Solanum inacum* as assessed by noting their effect on CCl_4 induced pentobarbitone (PBN) sleep prolongation and elevation of transaminase activity. Carpesterol another isolate, completely prevented pentobarbitone sleeping besides lowering transaminase activity to almost normal level.

CDRI group, through an elaborate study employing number of biochemical parameters, has evaluated hepatoprotective activity in picroliv, a standardized freaction of *Picrorhiza kurroa* root, containing about 60% of a mixture of picroside I and Kutkoside in ratio 1 : 1.5. The overall hepatoprotective activity was calculated by measuring 'PAV' value i.e. Percentage proportion of Abnormal Value, which was found to be significant in picroliv treated group in comparison to CCl_4 control rats.

Ethanol extract of *Withania frutescens* leaves has been reported to have prevented CCl_4 induced alterations in pentobarbitone sleep and biochemical parameters were studied. Avadhoot and Rana have reported significant hepatoprotective effect in the alcoholic extract of *Vitex negundo* seeds.

Patel and co-workers have screened eleven medicinal plants for hepatoprotective activity against CCl_4 induced liver damage, ethanol extract of *Vanda roxburghii*, flowers of *Calotropis gigantea*, fruit of *Quercus infectoria* and rhizome of *Curcuma longa* were found to afford significant protection. Recently got ethanol extract of *Ocimum sanctum* leaf has been reported to possess hepatoprotective effect against paracetamol induced liver damage in rats.

Review also reveals that plants belonging to different families possess antihepatotoxic activity. Such as *Abrus precatorius, Allophyllus edulis, Ampelopisis brevipendunculata , Angelica dahurica ,Artemisia abrotanum, Aphanamixis polystachya, Asteracantha longifolia, Baccharis trimera, Boerhavia repanda, Canarium manii, Carica papaya, Calotropis procera, Combretum glutinosum, Enantia chlorantha, Garcinia kola, Hypericum japonicum, Hypoestes triflora, Mikania cordata, Pueraria tuberosa, Pluchea indica, Rosmarinus tomentosus, Raphnus sativus, Salsola collina, Schizandra chinensis, Silybum marianum, Tamarix dioicia, Vanda rox burghi, Wedelia calendulacea.*

Reports of activity in extract, fractions or isolates are available. Though hepatoprotective activity has been reported in active principles belonging to different groups like flavonoids, triterpenes, steroids, lignans, polyphenols, glycosides, saponins, volatile oils, coumarins etc. Report on acute principles belonging to triterpene, flavonoid, steroid and lignan are comparatively more[1, 16].

1.8 HERBAL FORMULATION:

According to one survey nearly 40 poly herbal commercial preparations are available. Some of the recently tested drugs which shown promising antihepatotoxic

activities are Dextrina, Jigrine, (containing 14 medicinal plants)icterine and indigenous herbal formulations IHF. Ayurvedic drugs like, Kalmegh, Kamilari, Kumari Asav, Argyovardini Kumar Kalp showed protection against CCL_4 induced clinopathologic states (hepatonecrosis) due to physiological alterations of lipophylic enzymes. Kolaviron a mixture of *Garcinia kola* bioflavonoid prevent thioacetamide induced liver toxicity. Hepatogard protect ethanol induced liver damage by reversing the rise in liver transaminase. Other formulations such as Stimuliv, Ceruntine T-60 and serunite GBX are also shown antihepatotoxic property. Four patent herbal formulations available in India market code names: LV, LM, JG and LX revealed moderate to good hepatoprotective action against liver damage induced by CCl_4 and paracetamol. The enzymatic alterations in serum (ALT, AST, SALP, and SACP) and liver (GSH) and histopathological changes were partly of fully normalized in treated animals[16].

4.1 PHARMACOGNOSTIC INVESTIGATION

- Collection & Authentification of *Jatropha curcas* plant (leaf)
- Analysis of powder characteristics
- Determination of physical constants.
    1. Ash Value.
    2. Loss on Drying.
    3. Fluorescence analysis of drug.
    4. Extractive Values
        a) Water Soluble extractive.
        b) Alcohol Soluble extractive.
        c) Pet ether Soluble extractive.

Collection & Authentification of *Jatropha curcas* Linn. Plant (leaf)

The leaves of *Jatropha curcas* Linn. were collected in the month of May from local area of Belgaum (Rakscope). The leaves were authenticated by Dr (Mrs.) M.Vasundara, Associate Professor (Hort.) & Principal Investigator (M&AP), UAS, GKVK, Banglore-65. The leaves are also authenticated by Mr. R. S. Gaudar , Senier scale lecturer, R.L.S. College, Dept. Of Botany, Belgaum. The certificates are given in annexure A-1.

Preliminary Pharmacognostic Characteristics: [51,52]

In present study the leaves *Jatropha curcas* Linn. was investigated for its macroscopic and microscopic characteristics.

Table No.1

Macroscopic Characteristic of leaves of *Jatropha curcas* Linn.

	Leave
Color	Greenish yellow
Odour	Characteristic
Taste	Tasteless
Shape	Long petioled, Orbicular and cordate
Size	10-15 cm in length

Microscopic Characteristics:

Powder Characteristic:-

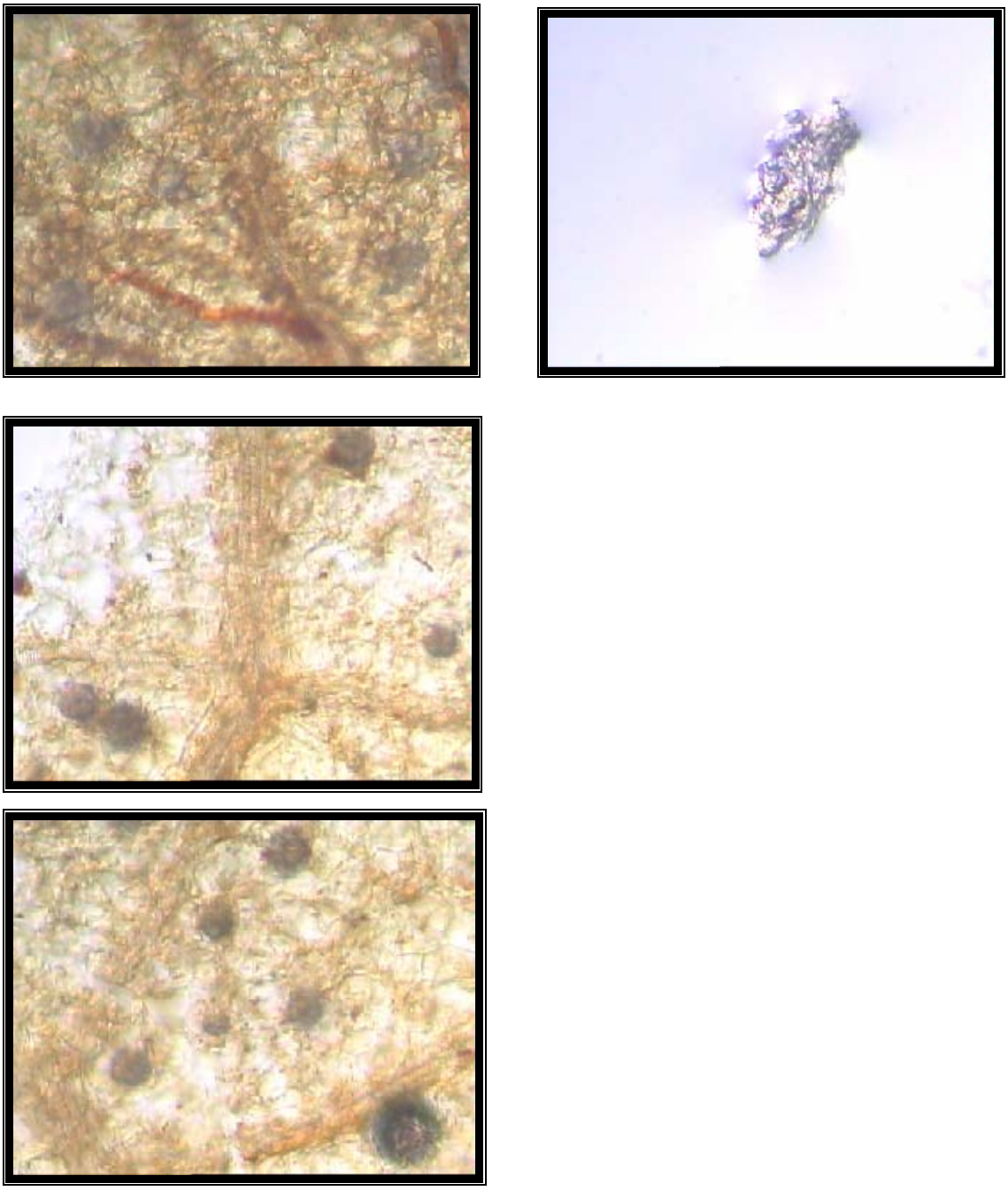
In present study the dried leaves of *Jatropha curcas* Linn. were pulverized into fine powder separately. The powder was investigated for their microscopic characteristic.

Procedure:

The pulverized powder of leaves was boiled separately with chloral hydrate solution in small quantity. Remove cleaved powder in three separate watch glass respectively and stain with one drop each of phloroglucinol and concentrated hydrochloric acid.

Mount a little of the treated powder in dilute glycerine and observed the slide under microscope at low power.

Fig. 2: Microscopic Characteristic of Leaves of *Jatropha curcas* Linn.



The characteristic structures observed for the powdered leaves of *Jatropha curcas* Linn. were tabulated in table no.2.

Table No.2

Microscopic Characteristic of Leaves of *Jatropha curcas* Linn.

	Leaves
1.	Calcium Oxalate
2.	Vascular bundles
3.	Stone cells
4.	Mucilaginous matter

DETERMINATION OF ASH VALUES[53]

1) Total Ash Value:

Method:- Weigh accurately 2 to 3gm of air–dried leaves in a tared platinum or silica dish and incinerate at a temperature not exceeding 450^0C until free from carbon, cool and weigh. If a carbon free ash cannot be obtained in this way exhaust the charred mass with hot water, collect the residue on an ashless filter paper, incinerate the residue and filter paper until the ash is white or nearly so, add the filtrate, evaporate to dryness and ignite at a temperature not exceeding 450^0C. Calculate the percentage of ash with reference to the air – dried drug.

$$\text{Ash\%} = \frac{\text{Loss in Weight}}{W} \times 100$$

2) Determination of Acid Insoluble Ash:

Method: - Boil the ash with 25ml of 2M Hydrochloric acid for 5min, collect the insoluble matter in a Gooch crucible or on an ashless filter paper, wash with hot water, ignite, cool in a desiccator and weigh. Calculate the percentage of acid insoluble at with reference to the air – dried drug.

$$\text{Ash\%} = \frac{\text{Loss in Weight}}{W} \times 100$$

3) Determination of Water Soluble Ash:

Method:-Boil the ash for 5 min. with 25 ml of water, collect the insoluble matter in a Gooch crucible or on an ashless filter paper, wash with hot water, and ignite for 15 min at a temperature not exceeding 450^0C. Subtract the weight of insoluble matter from the weight of the ash, the difference in weight represent the water-soluble ash. Calculate the percentage of water-soluble ash with reference to the air-dried drug. The results were given in (Table no.3)

$$\text{Ash\%} = \frac{\text{Loss in Weight}}{W} \times 100$$

FLUORESCENCE ANALYSIS OF THE DRUG[54]

Many crude drugs show the fluorescence when the sample is exposed to ultraviolet radiation. Evaluation of crude drugs based on fluorescence in daylight is not much used, as it is usually unreliable due to the weakness of the fluorescence effect (Umbelliferae test used for galbanum and asafoetida is, however, an exception). Fluorescence lamps are fitted with suitable filters, which eliminate visible radiation from the lamp and transmit ultraviolet radiation of definite wavelength. Several crude drugs show characteristic fluorescence useful for their evaluation.

Objective: To examine the crude drugs under ultraviolet radiation and report their authenticity.

Materials

Ultraviolet lamp (366nm), crude drugs (entire and powder).

The results are given in (Table no.3)

LOSS ON DRYING[55]

Loss on drying of the air-dried roots of *Jatropha curcas* Linn. was analyzed.

Accurately weighed quantity of sample was taken in a tared glass bottle and initial weight was taken. The sample was heated at 105^0C in an oven and weighed. This procedure was repeated until a constant weight was obtained. The moisture content of the sample was calculated with reference to air-dried drug and the results are in (Table no. 3).

Loss on drying (%) = loss in weight x 100/ w

Where w = weight of the leaf powder in gms.

EXTRACTIVE VALUES[56]

The extractive values for various solvents of air-dried sample were evaluated.

i) Water-soluble extractives.

ii) Alcohol soluble extractives.

iii) Pet ether soluble extractives.

Water-soluble extractive value:

5 grams of dried leaves of leaves of *Jatropha curcas* Linn. were added to 50ml of boiled water at 80ºC in a stoppered flask separately. It was then shaken well and allowed to stand for 10 minutes so as to cool it and filtered. 5ml of filtrate was transferred to an evaporating dish, which was 7.5 cm in diameter, the solvent was evaporated on water bath, allowed to dry for 30 minutes, finally dried in an oven for 2 hours at 100ºC and residue was weighed. Percentage of water-soluble extractives was calculated with reference to the air-dried drug.

Alcohol soluble extractive value:

5 grams of dried leaves of *Jatropha curcas* Linn. were macerated with 100 ml of Alcohol in a closed flask, shaking frequently during the first 6 hours and allowed to stand for 18 hours separately. Thereafter, it was filtered rapidly taking precaution

against loss of methanol. Evaporated 25ml of filtrate to dryness in a tared flat bottom shallow dish dried at 105^0c and weighed. Percentage Alcohol soluble extractive was calculated with reference to the air-dried leaves.

Pet ether soluble extractive value:

5 grams of dried leaves of leaves of *Jatropha curcas* Linn. was macerated with 100 ml of pet ether (40-60^{o}C) in a conical flask, shaking frequently during the first 6 hours and allowed to stand for 18 hours separately. Thereafter, it was filtered rapidly taking precaution against loss of pet ether. Evaporated 25ml of filtrate to dryness in a tared flat bottom shallow dish dried at 105^{0}C and weighed. Percentage pet ether soluble extractive was calculated with reference to the air-dried leaves

Table No.3

Physical Constants for leaves of *Jatropha curcas* Linn.

Sl. No.	Physical Constants	Leaves
1.	Ash Value (%w/w) • Total Ash • Acid Insoluble Ash • Water Soluble Ash	 7.60% 4.52% 5.95%
2.	Fluorescence Analysis • At 254 nm • At 366 nm	 No fluorescence
3.	Loss on Drying (%w/w)	1. 84%
4.	Extractive Values (% w/w) • Water-soluble Extractive • Alcohol soluble Extractive • Pet ether soluble Extractive	 15.2% 11.2% 2.4%

4.2 EXTRACTION:[57-61]

Leaves of *Jatropha curcas* were collected from local areas of Belgaum and authenticated. The leaves were shade dried and coarsely pulverized after sufficient

shade drying. The pulverized powder (200gm) were extracted with petroleum ether (40-60^0 c) followed by alcohol in a Soxhlet extractor and then macerated with water. Extracts were concentrated in a rotary flash evaporator. The residue was dried in a desicator over sodium sulphate. The percentage yield obtained were shown in (Table no.4)

Table No. 4

Percentage Yield of Leaves of *Jatropha curcas* Linn.

SR. NO.	*EXTRACTS*	*NATURE EXTRACT*	*COLOUR*	*WEIGHT (G)*	*%YIELD (W/W)*
1.	**PETROLEUM ETHER (40-60^{0}C) 200GM**	**SEMISOLID AND TASTELESS**	**DARK YELLOW**	**04**	**2%**
2.	**ALCOHOL 190GM**		**DARK BLACK**	**22**	**11.2%**
3.	**AQUEOUS 170GM**		**GREENISH BROWN**	**26**	**15.2%**

4.3. PHYTOCHEMICAL INVESTIGATION

- Extraction of pulverized leaves of *Jatropha curcas* Linn. with petroleum ether (40-60^0) followed by alcohol and then lastly macerated with water.
- Qualitative chemical tests.
- Chromatographic studies
    - Thin layer Chromatography.
    - High Performance Thin Layer Chromatography (HPTLC)
- Spectroscopic studies.
    - U.V. Spectroscopy
    - FT – IR Spectroscopy
    - P – NMR Spectroscopy

QUALITATIVE CHEMICAL TESTS [62]

1. Tests for Phytosterols

The extract was refluxed separately with alcoholic solution of potassium hydroxide till complete saponification. The saponified extract was diluted with water and Unsaponifiable matter was extracted with diethyl ether. The ethereal extract was evaporated and the residue (Unsaponifiable matter) was subjected to the following test by dissolving the residue in the chloroform.

a) ***Salkowaski's test*** To the test extract solution few drops of conc. sulphuric acid was added, shaken and allowed to stand, lower layer turns red indicating the presence of sterols.

b) ***Liebermann-Burchard test*** The test solution was treated with few drops of acetic anhydride and mixed, conc. sulphuric acid was added from the sides of the test tube, a brown ring at the junction of two layers was formed and the upper layer turns green, indicating the presence of steroids.

c) ***Sulphur test*** Sulphur when added in to the test solution, it sinks in it.

2. Tests for Steroidal Glycosides

The solution of extract was prepared with water and 1 volume of 10 % v/v solution of sulphuric acid solution was added, heated on the water- bath for half an hour and extracted, with the chloroform. Chloroform fraction was separated and concentrated. Concentrated fraction was tested for steroids/phytosterol.

3. Tests for Triterpenoids

The test extract solution was prepared by dissolving extracts in chloroform.

a) ***Salkowaski test*** Few drops of concentrated sulphuric acid was added to the test solution of the extract, shaken and on standing lower layer turns golden yellow.

b) ***Liebermann-Burchard test*** To the test solution of the extract, few drops of acetic anhydride were added and mixed well. 1 ml of concentrated sulphuric acid was added from the sides of the test tube, a red colour is produced in the lower layer indicates the presence of triterpenes.

4. Tests for Glycosides

The test solution was prepared by dissolving extract in alcohol 90% or aqueous alcoholic solution.

a) ***Baljet's test*** The test solution treated with sodium picrate gave yellow to orange colour.

b) ***Keller-Killiani test for digitoxose*** The test solution was treated with few drops of ferric chloride solution and mixed, then sulphuric acid containing ferric chloride solution was added, it forms two layers. Lower layer showed reddish brown while upper layer turns bluish green.

5. Tests for Saponins

The test solution was prepared by dissolving extract in the water.

a) ***Foam test*** Test solution on shaking shows formation of foam, which was stable at least for 15 min.

b) ***Haemolysis test*** 2 ml of 18% sodium chloride in two test tubes is taken. To one test tube added distilled water and to other 2 ml test solution. Few drops of blood is added to both the test tubes, mixed and observed for haemolysis under microscope.

6. Tests for Carbohydrates

The test solution was prepared by dissolving test extract with water, hydrolysed with 2N hydrochloric and subjected to following tests.

a) ***Molisch's test*** Test solution with few drops of reagent and 2 ml of conc. sulphuric acid added slowly from the sides of the test tube shows a purple ring at the junction of two liquids.

b) ***Barfoed's test*** Test solution treated with reagent, boiled on a water-bath, shows brick red colour precipitate.

c) ***Benedict's test*** The test solution treated with reagent and boiling on water-bath shows reddish brown precipitate.

d) ***Tollen's phloroglucinol test*** 2.5 ml of concentrated HCl and 4 ml of 0.5% phloroglucinol are treated with 1-2 ml test solution. Heat, yellow to red colour appears.

e) ***Cobalt-Chloride test*** 3 ml of test solution is treated with 2 ml cobalt chloride. Boil and cool. Add few drops of sodium hydroxide solution. Solution appears greenish blue (glucose) or purplish (fructose) or upper layer greenish blue and lower layer purplish (mixture of glucose and fructose).

7. Tests for alkaloids

The test solution was prepared by dissolving extracts in dilute Hcl acid.

a) ***Mayer's test*** Test solution with reagent (potassium mercuric iodide) gives cream coloured precipitate.

b) ***Hager's test*** The acidic solution with reagent (saturated picric acid solution) gives yellow precipitate.

c) ***Dragendroff's test*** The acidic solution with reagent (potassium bismuth iodide) shows reddish brown precipitate.

8. Tests for Flavonoids

The flavonoids are all structurally derived from the parent substance called flavone. The flavonoids, which occur in the form of free, as well as, bound to sugars called glycosides. For this reason, when analysing flavonoids, it is usually better to examine the flavonoids in hydrolysed plant extracts.

Preparation of test solution

To small amount of extract equal volume of 2M hydrochloric acid is added and heated the test tube for 30-40 min at $100^{O}C$, allowed to cool, filtered and extracted with ethyl acetate. The ethyl acetate extract was concentrated to dryness, followed the test for flavonoids to ethyl acetate fraction by dissolving the residue with ethyl acetate.

a) ***Shinoda test*** Test solution with few fragments of magnesium ribbon and conc. hydrochloric acid shows pink to magenta red colour.
b) ***Zn/Hcl reducing test*** Test solution with zinc dust and few drops of hydrochloric acid shows magenta red colour.

9. Test for Phenolics / Tannins

The extract is dissolved in 90% alcohol.

a) ***Ferric chloride test*** Test solution treated with few drops of ferric chloride solution gave dark colour.
b) ***Gelatin test*** Test solution treated with gelatin gives white precipitate.

10. Tests for Proteins

The extract is dissolved in water.

a) ***Millon's test*** Test solution is treated with reagent and heated on a water-bath, protein is stained red on warming.

b) ***Xanthoproteic test*** Test solution treated with conc. nitric acid and boiled gave yellow precipitate.

c) ***Biuret test*** Test solution treated with 40% sodium hydroxide and dilute copper sulphate solution have given blue colour.

Table – 5
Results of Phytochemical Investigation of *Jatropha curcas* leaves extract

Sl. No.	Name of the Test	Pet. ether extract	Alcoholic extract	Aqueous extract
1.	**Test for sterols**			
	a. Test solution + Sulphur (Sulphur powder test)	+	-	+
	b. Libermann Reaction	-	-	+
2.	**Test for glycosides**			
	a. Keller – Killiani Test	-	+	+
	b. Baljet's Test	-	-	-
	c. Legal test	-	-	-
	d. Raymond test	-	+	+
3.	**Test for saponins**			
	a. Haemolytic test	-	+	+
	b. Foam test	-	+	+
4.	**Tests for proteins**			
	a. Xanthoprotein test	-	-	-
	b. Millon's test	-	-	-
	c. Biuret test	-	-	-
	d. Ninhydrin test	-	-	+
5.	**Test for tannins**			
	a. Gelatin test	-	-	-
	b. Ferric chloride test	-	-	-
	c. Lead acetate test	-	-	-
	d. Dil HNO_3 test	-	-	-
6.	**Test for alkaloids**			
	a. Dragendroff's test	-	-	-
	b. Mayer's test	-	+	-
	c. Hager's test	-	+	-
	d. Wagner's test	-	-	-
7.	**Test for carbohydrates**			
	a. Barford's test	-	-	-
	b. Benedict's test	-	-	+
	c. Molisch's test	+	+	+
8.	**Test for Triterpenoids**			
	a. Libermann Burchardt's Test	-	-	+
	b. Salkowaski Test	+	-	+
9.	**Test for flavonoids**			
	a. Shinoda test	-	-	+
	b. Alkaline reagent test	-	-	+
	c. Lead acetate test	-	-	+
10.	**Test for Lipids**			
	a. Sudan III reagent	+	-	-
	b. Solubility test	+	-	-

4.4. SEPERATION AND ISOLATION OF ACTIVE PRINCIPLES.

CHROMATOGRAPHIC STUDIES

The thin layer chromatography studies of various extracts of leaves of *Jatropha curcas* Linn. were carried out to confirm the presence of phytoconstituents.

THIN LAYER CHROMATOGRAPHY [63-66]

The various extracts were subjected to thin layer chromatography for the presence of phytoconstituents in each extract.

In this technique the Silica gel-GF_{254} (for TLC) was used as an adsorbent and plates were prepared by spreading technique, then air dried for an over-night and activated for one hour at 110^0C and used.

TLC of Saponin

Stationary phase : Silica gel GF_{254}

Mobile Phase : Chloroform: Methanol: Water

Proportion : 7:3:4

Detection : Anisaldehyde Sulphuric acid

Preparation of spray reagent:

Anisaldehyde-sulphuric acid reagent was freshly prepared by adding 0.5 ml anisaldehyde into 10 ml glacial acid followed by 85 ml methanol and 5 ml concentrated sulphuric acid and used as a spraying reagent.

Table No. 6

TLC profile of Saponin

Extract	Observation		R_f values
	No. of spots	Colour of spots	
Aqueous	2	Blue	0.5, 0.7

ISOLATION OF PHYTOCONSTITUENTS

Isolation of saponins:

Saponins are usually extracted from dry or fresh plant material with aqueous, methanol and in alcohol. The aqueous extract or aqueous methanolic extract is partitioned against ethyl acetate (to remove non polar constituents) and then against n-butanol to extract saponins (together with other polar constituents). After removal of the solvent, the saponins can be separated by TLC Or HPLC, Or Column chromatography.

Preparative Thin Layer Chromatography: [67]

A thick layer of silica gel GF-254 was coated on the square shaped plate and activated at 110^0C for one hour. The broad band (2mm width) of extracted sample was applied on the plate.

The details of plate were as follows:-

Plate dimension	:	10 x 20 cm
Adsorbent	:	Silica gel GF 254
Activation	:	110^0C for 1 hr
Band parameter	:	Width – 2mm
		Length – 8cm

The plate was developed in a saturated chamber having desired solvent system. After developing the plate was dried and if the band gives fluorescence then it

can be easily scraped. Otherwise a small portion of the band was sprayed with detecting agent, by taking care to avoid the exposure of remaining plate to spray reagent. Then the band is scraped by measuring the height of sprayed band.

The scraped band was then suspended in desired solvent and filtered on Whatman filter paper no.1 and washed several times with same solvent. The filtrates were combined and concentrated and reduced to dryness. This procedure was followed for several scrapings.

Then the resulted compound was run with original sample to confirm the isolation and subjected to spectroscopy studies for identification and partial characterization.

ISOLATION OF SAPONIN

Table No.7

TLC of Isolated Saponin

	Solvent system	Visualizing agent	No. of spots Observed	R_f value
Isolated saponin	Chloroform: Methanol: Water (7:3:4)	Anisaldehyde Sulphuric acid reagent	01	0.7

Isolation of Active Principles:

The petroleum ether extract was subjected to thin layer chromatograph to detect various constituents present in it. The petroleum ether extract has shown presence of steroids

TLC of Steroid

Stationary phase : Silica gel GF_{254}

Mobile Phase : Chloroform: Methanol

Proportion : 8:2

Detection : Anisaldehyde Sulphuric acid

The spots were observed in day light as blue after derivatization respectively.

For separation of active constituents, it was decided to follow the Thin layer chromatography with Chloroform: Methanol (8:2)

Preparation of spray reagent:

Anisaldehyde-sulphuric acid reagent was freshly prepared by adding 0.5 ml anisaldehyde into 10 ml glacial acid followed by 85 ml Methanol and 5 ml concentrated Sulphuric acid and used as a spraying reagent..

Table No. 8

TLC profile of Steroid before derivatization

Extract	Observation		R_f values
	No. of spots	Colour of spots	
Pet ether extract	2	Yellow	0.7,0.9
Isolated Compound	1	Yellow (Blue)	0.7

Preparative Thin Layer Chromatography:

A thick layer of silica gel GF $_{254}$ was coated on the square shaped plate and activated at 110^0c for one hour. The broad band (2 mm width) at petroleum ether extract was applied on the plate. The details of plate were as follows:-

Plate dimension	:	10 x 20 cm
Adsorbent	:	silica gel gf 254
Activation	:	110^0c for 1 hr
Band parameter	:	width – 2 mm
		length – 8 cm

The plate was developed in a saturated chamber having solvent system Chloroform : Methanol (80 : 20). After developing the plate was dried and small portion

of the band was sprayed with Anisaldehyde Sulphuric acid reagent, by taking care to avoid the exposure of remaining plate to spraying reagent. Since compounds are coloured thus can be detected by visual detection but for confirmation was sprayed. The respective colours were observed after heating at 110^0c for 5 mins in hot air oven. The yellow coloured band which was just below solvent front was carefully scrapped and collected separately in container. The scrapped silica gel was suspended in petroleum ether (40-60^0c) and filtered on Whatmann filter paper no.1 and washed several times with petroleum ether. The filtrates were combined and reduced to dryness. This procedure was followed for several scrappings. Each concentrated fraction was subjected for TLC to identify steroids.

Then the resulted compound was run to confirm the isolation and subjected to U.V. absorption and I.R. spectroscopy studies for identification and partial characterization.

Spectral Characterization of Isolated Compounds[68, 69.]

- **UV Spectrum of isolated compounds**

UV spectrum was recorded in JASCO UV530 Spectrophotometer for isolated Saponin A in AR grade DMSO, which gave a sharp peak at 255 nm and Saponin B in AR grade DMSO, which gave a sharp peak at 250 nm. Spectra are given in annexure A-3.

- **FT-IR Spectroscopy of Isolated compounds**

FT-IR spectrum was recorded in “Themro Nicolet” IR-200 spectrometer. The FT-IR spectrum of isolated compound has shown characteristic peaks as listed in Table No. 9,10. Spectra are given in annexure A- 4.

Table No.9

FT-IR of Isolated compounds from aqueous extract

Spectral Peak (cm^{-1})	Mode of vibration	Spectral Peak (cm^{-1})	Mode of vibration
3370.60	Free-OH stretching, Phenolic	3411.08	Free-OH stretching, Phenolic
2926.24	C-H stretching	2924.24	C-H stretching
1596.95	C=O group	2213.90	Carboxyl
1405.37	O-H deformation	2104.11	Ester group
1343.20	C-O ether linkage	1596.95	C=O group
1116.67, 762.62	C-H deformation	1405.37	O-H deformation
	C-O Stretching	1343.20	C-O ether linkage
		1116.67, 762.62	C-H deformation

Table No.10

FT-IR of Isolated compounds from petroleum ether(40-60^0) extract

Spectral Peak (cm^{-1})	Mode of vibration
3470.07	Free-OH stretching, Phenolic
2924.24, 2733, 2612.20	C-H stretching
1456, 1240.16,	CH_2 stretching
930.93	-CH=OH bending
1051.71	C-O stretching

- **P - NMR Spectroscopy:**

Isolated compound from aqueous extract dissolved in DMSO and isolated compound from Petroleum ether (40-60°C) dissolved in CDCl3 and the NMR spectrum was recorded on Bruker AmX -30 MHz. Tetramethylsilane was used as an internal standard and chemical shifts were measured as the relative distance from peak of TMS. Spectra are given in annexure A-5.

HPTLC of Extracts and Isolated Compound:

Aqueous extract of leaves as well as petroleum ether extract of leaves and isolated compounds were subjected to High Performance Thin Layer Chromatography.

The details of HPTLC were as follows:-

Plate	: Aluminium plate precoated with Silica gel GF254
Thickness	: 0.2mm
Plate size	: 10 x 10 cms
Sample application	: 10 µl
Solvent system	: Chloroform : Methanol: (8:2) for steroids. Chloroform: Methanol: Water (70:30:40) for saponins
Detection	: U.V. (366 nm)
Instrument	: CAMAG TLC Scanner 3 & LINOMAT-V

CAMAG TLC Scanner 3 & LINOMAT-V densitometric evaluation system with WINCAT software was used for scanning of thin layer chromatogram objects in reflectance or transmission mode by absorbance or by fluorescence at 254 and 366nm respectively.

The fingerprint of HPTLC profile of all extracts and isolated compound were taken using computer. R_f value of various samples was evaluated using following formula:

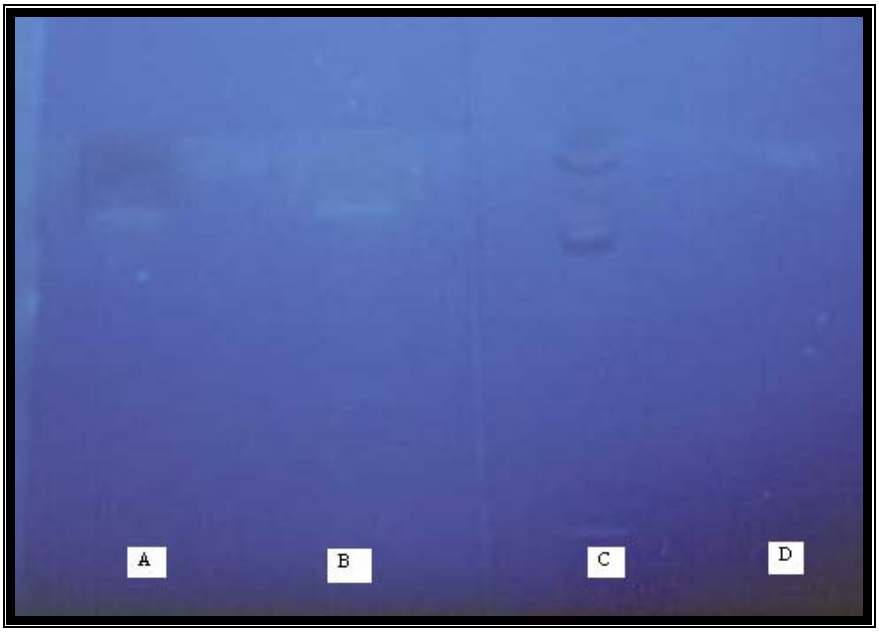
$$Rf = \frac{\text{Distance traveled by sample from base line}}{\text{Distance traveled by solvent from base line}}$$

HPTLC profile of various extract of leaves of *Jatropha curcas* given in annexure A-2.

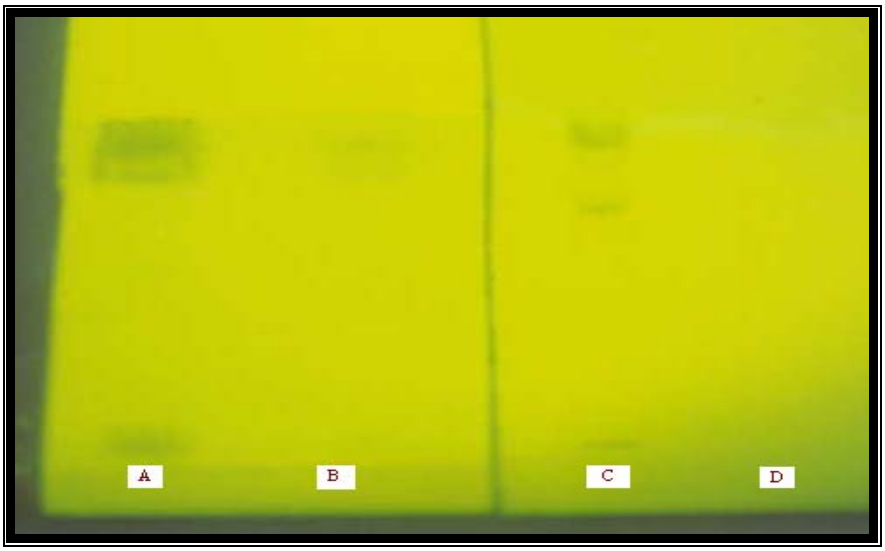
Fig. 3 : HPTLC photograph of isolated compound

A- Aqueous extract **B- Isolated compound from aqueous extract**

C- Petroleum extract **D- Isolated compound from Pet ether extract**



At 366 nm



At 254nm

4.5. PHARMACOLOGICAL SCREENING FOR HEPATOPROTECTIVE ACTIVITY.

4.5.1. INTRODUCTION TO HEPATOTOXICITY

Role of plant in treatment of Hepatotoxicity

Liver plays a vital role in the metabolism and elimination of various exogenous and endogenous compounds. As a result of its continuous involvement, it is susceptible to toxic injuries caused by certain agents and any damage to hepatic cells disturb body metabolism. In recent times lot of interest has been generated to find out a natural remedy for hepatic disorders caused by toxins like alcohol and hepatitis virus[70]. The agent should protect against such damage, especially of one which facilitates regeneration by proliferation of parenchymal cells after damage and arrest growth of fibrous tissue[71].

There is not remedy for liver diseases which are so prevalent in the population. The treatment is mainly symptomatic[72]. Scientists and some industrialists deliberated on various prospective plant remedies for ailments of liver disorder management. In the decade 70s, the world scientific community concentrated on a herbal plant *Vinca rosa*. Then in 80s the attention was focused on *Panax ginseng*. Now, the news of multifarious activities of the Neem tree, indicates that it may become centre for research in 90s. Indian Council of Medical Research, New Delhi, in its revived research on traditional medicine, had adopted liver diseases as one among six thrust areas and for multidisciplinary study. Screening of active constituents from Kutki *(Picrorhiza Kurroa),* Bhoomyamalaki *(Phyllanthus niruri)* have shown marked protection against jaundice. Hepatitis continues to be a major health problem in urban areas in India, and several studies in viral hepatitis were under investigation by the ICMR. For example, extracts of milk thistle *(Silybum marianum)* fruits under investigation for the treatment of alcoholic hepatitis. According to Indian Society of Gastroenterology, Mulethi *(Glycyrrhiza glabra)* prevents multiplication of viruses

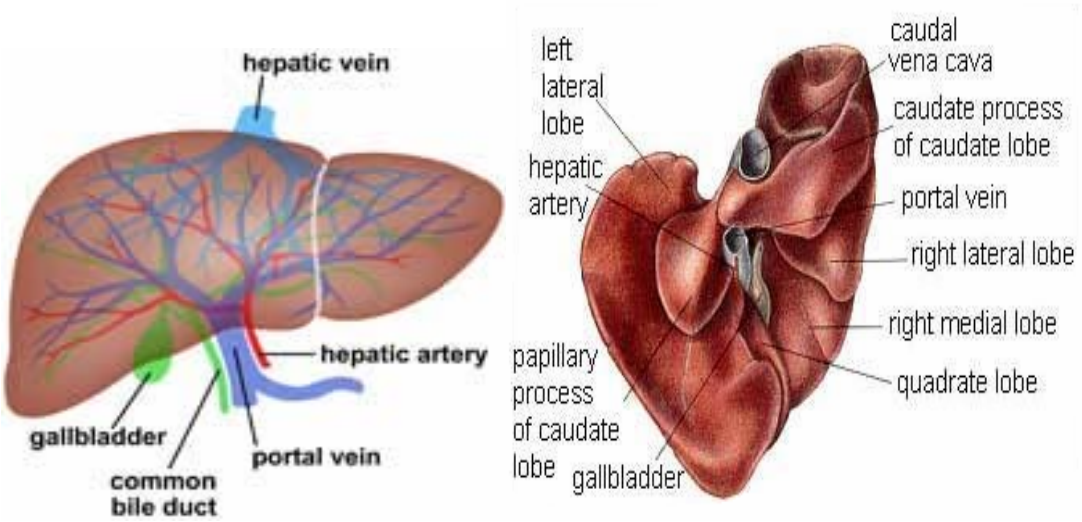
inside liver cells. The disorder of liver may be acute or chronic hepatitis (inflammatory liver diseases), hepatosis (non-inflammatory liver diseases) and liver cirrhosis (fibrosis of the liver). Liver enzymes act as an index of sub-clinical hepatic damage. Serum glutamic pyruvic transaminase (SGPT), serum glutamic-oxaloacetic pyruvic transaminase (SGOT), Serum bilirubin and Serum alkaline phosphatase are reported as an index of hepatic injury and cholestasis[73].

4.5.1.1. ANATOMY AND HISTOLOGY OF LIVER:

Anatomy:

The liver is a large, solid, gland situated in the right upper quadrant of the abdominal cavity. Liver is reddish brown in colour, soft in consistency and very friable. It weighs about 1600 g in males and about 1300 g in females. The liver occupies the whole of the right hypochondrium, the greater part of the epigastrium and extends into the left hypochondrium reaching up to the left lateral line.

The liver is the largest gland in the body. It secretes bile and performs various other metabolic functions[74].



Histology of the Liver[75]:

The liver is divided into two principle lobes – a large right lobe and smaller is left lobe. The lobes of the liver are made up of many functional units called lobules. A lobule is typically six sided structure (hexagon) that consists of specialized epithelial cells called hepatocytes (hepat=liver, cytes=cells), arranged in irregular, branching interconnected plates around a central vein. Instead of capillaries, the liver has larger, endothelium-lined spaces called Sinusoids, through which blood passes. Also present in the sinusoids are fixed phagocyte called Stellate reticuloendothelial (Kupffer) cells, which destroy, worn-out white blood cells and red blood cells, bacteria and other foreign matter in the venous blood draining from the gastrointestinal tract.

Blood supply of the liver:

The liver receives blood from two sources, from hepatic artery it obtains oxygenated blood and from the hepatic portal vein it receive deoxygenated blood containing newly absorbed neutrients, drugs and possibly microbes and toxins from gastrointestinal tract. Branches of both the hepatic artery and the hepatic portal vein carry blood into liver sinusoids, where oxygen, most of neutrients and certain toxic substances are taken up by the hepatocytes.

Functions of Liver:

- ***Carbohydrate metabolism:***

The liver is especially important in maintaining a normal blood glucose level. When blood glucose is low, the liver can break down glycogen to glucose and release glucose into the bloodstream, when blood glucose is high it converts glucose to

glycogen and triglycerides for storage. Liver can also convert certain amino acids and lactic acid to glucose.

- ***Lipid Metabolism:***

Hepatocytes store some triglycerides, breakdown fatty acids to generate ATP, synthesize cholesterol and use cholesterol to make bile salts.

- ***Protein Metabolism:***

Hepatocyte deaminate amino acids so that the amino acids can be used for ATP production or converted to carbohydrates or fats, resulting toxic ammonia is converted to less toxic urea, which is excreted in urine. Hepatocyte synthesize most plasma proteins, such as alpha and beta globulin, albumin, prothrombin and fibrinogen.

- ***Processing of drugs and hormones:***

The liver can detoxify substances such as alcohol or excrete drugs such as penicillin, erythromycin and sulfonamides into bile.

- ***Storage:***

In addition to glycogen, the liver is a prime storage site of certain vitamins (A, B12, D, E and K) and minerals (iron and copper), which are released from liver.

4.5.1.2 BASIC HEPATIC HISTOPATHOLOGY[76]:

Hepatocellular changes: Hydropic change is a descriptive term applied to the hepatocyte with pale, watery cytoplasm and a normal nucleus. A wide variety of conditions produce this relative lack of cytoplasmic staining. Increased eosinophilia may occur with drug-related hydroperplasia of the smooth endoplasmic reticulum. Active regeneration of hepatocytes after necrosis as in several viral hepatitis or recovery phase

of fatty liver produces a widespread hepatocellular hydropic change as well as a cobblestone pattern of the liver cords. Hydropic change is also an indicator of hepatocellular damage and is noted in acute viral hepatitis and drug induced hepatic injury including alcohol injury.

Hepatocellular fat accumulation may be either large cytoplasmic bodies of foamy fat. Fatty liver occurs because of 1)Sudden increase in mobilization of fat from the periphery to the liver, 2) Relative lack of protein necessary for hepatocellular fat release, 3) Increased hepatocellular fat formation by metabolic changes and 4) Decrease hepatocellular fat degradation. Fatty liver is common in alcohol ingestion, parenteral nutrition, tuberculosis, starvation, certain drugs, diabetes mellitus and obesity. Electron microscopy shows that the cytoplasmic fat is not membrane bound and lysosomes are greatly increased.

Hepatocellular Necrosis: Necrosis may be classified in many ways, including location (Zonal, periportal, perivenular and so on), mechanism (lytic, coagulative), amount (submassive versus focal), cellular type (lymphocytotoxic veruses hyline necrosis) and various patterns are associated with different etiologic factors. Zonal necrosis is a common pattern of injury after an acute hepatic injury. Sharply demarcated perivenular (zone 3) coagulation necrosis is typical of several anoxic injury or acetaminophen injury and may be explained by differences in oxygenation and activity of drug-metabolizing enzyme. Periportal necrosis (Zone 1) is not common and is noted in eclampsia. Midzonal injury is reported for yellow fever.

Hepatocellular necrosis (hepatocytolysis) of the lytic type, with the associated macrophage activity that is so complete and rapid that dead hepatocytes are rarely noted, such necrosis is common in viral hepatitis, alcoholic liver disease and many

hepatotoxic reactions, and the type of inflammatory reactions varies in these conditions. Coagulative necrosis in the liver is characterised by dying hepatocytes that retain some staining of the cytoplasm and the nuclei lose basophilia and gradually disappear. The cells become shrunken and slowly disappears because of the action of inflammatory cells.

Acidophilic injury usually occurs in an isolated hepatocyte and is similar to coagulative necrosis except that the cytoplasm becomes more eosinophilic and waxy and the nucleus may be retained and be a dark. These bodies are common in acute viral hepatitis, chronic active hepatitis, severe burns and other liver disorders. Confluent necrosis is attributable to fusion of focal or zonal necrosis and may result from intensive necrosis those bridges between different zones. Submassive hepatic necrosis is recognized by confluent necrosis that usually involves many perivenular areas and occurs most commonly in severe acute viral hepatitis, drug injury etc. Massive hepatic necrosis is distinguished from submassive hepatic necrosis by the presence of a thin rim of viable-appearing hepatocytes around each portal tract.

Regeneration: The liver has a remarkable capacity for regeneration and during recovery from submassive hepatic necrosis. In normal adult rat liver, the hepatocytes have an annual turnover of one mitosis per year; weight doubles 48 hours and has returned to normal weight at 3 to 6 days after partial hepatectomy. In man liver, regeneration also occurs rapidly and even in cirrhotic liver. After major hepatic resection for tumor, regeneration of normal hepatic volume occurs by 3 to 6 months, and liver function appears normal at 2 to 3 weeks after surgery.

Fibrosis: Hepatic fibrosis is the most important fracture of chronic liver disease and leads to cirrhosis and irreversible physiologic changes in the liver that

account for many clinical manifestations of fatal liver disease. Fibrosis of the liver is a common response to chronic inflammatory conditions. Experimental cirrhosis in the carbon tetrachloride treated rat and mouse have served as models, but the adequacy of the morphologic and biochemical lesions has been doubled compared to human cirrhosis.

Patterns of fibrosis: Simple hepatocellular necrosis does not result in collagen formation, but in severe hepatic necrosis a collapsed stroma may form a framework for collagen retention. Repeated or continuous necrosis is associated with fibrosis, and the most striking fibrosis within the lobule is noted in chronic alcoholic liver disease. This fibrosis occurs even with a very mild inflammatory response. Another common pattern of fibrosis occurs in the portal areas in chronic active hepatitis, which tends to be more confirmed to the portal tracts and does not extend into the lobule as much as portal fibrosis does in alcoholic. Non-collagen matrix components have been investigated for a possible role in fibrosis. Fibronectin both is a plasma protein and may produce a cellular form in injury.

Cirrhosis: The currently accepted definition of Cirrhosis requires the term be applied to a liver with a diffuse fibrosis (that is the entire liver and not focal) and contain regenerative nodules, which are masses of hepatocytes lacking the normal blood flow because of the lack of terminal hepatic venules. Regenerative or hyperplastic nodules are required for the identification of cirrhosis because altered blood flow and portal hypertension correlates with thin presence. In contrast hepatic fibrosis, which is usually a precursor to cirrhosis, is not associated as frequently with portal hypertension. The development of hepatocellular carcinoma is relatively common in

cirrhosis with the required regenerative nodules and is not as frequent as it is in fibrosis.

Cirrhosis is classified as Micro nodular cirrhosis applies to the liver which nearly all the nodules are less than 3 mm diameter though some have used 1.5 mm as the minimum diameter because that is the diameter of a normal lobule. Examples include – alcoholic cirrhosis, binary tract obstruction and hepatic venous obstruction.

Macro nodular cirrhosis applies if most of the nodules are greater than 3mm diameter, and it occur in two forms. The more common form has nodules divided by thin septa that are often incomplete and have linking portal tracts. This pattern common in so-called post-hepatitis cirrhosis. The median time interval for conversion of micro nodular cirrhosis to macro nodular type is 2.25 years and majority of patients have such progression. Alcoholic cirrhosis often contains fats within the hepatocytes and the parenchyma is increased in weight. Etiologic factors related to cirrhosis include alcoholism, hepatitis B virus, various metabolic diseases such as hemochromatosis, Wilson's disease etc. Cirrhosis without recognizable cause is called as "Cryptogenic cirrhosis". The irreversibility of cirrhosis has been emphasized in several experimental and clinical studies. If cirrhosis is reversible, it must be very rare, though the most convincing patients have biliary obstruction that has been corrected surgically. Functionally more important than nodular size in cirrhosis is the size of the entire hepatic mass, which can be estimated with radioisotope scans.

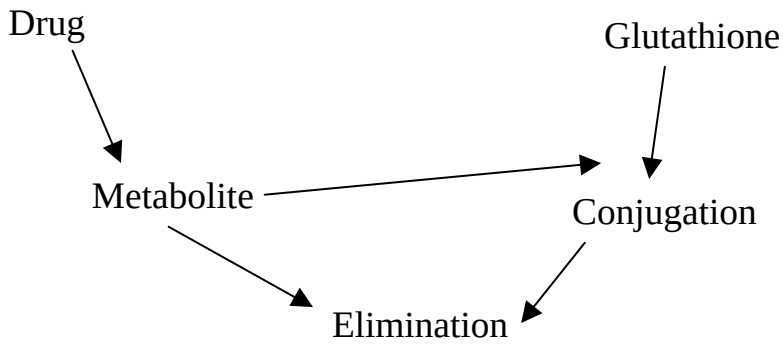
Steatosis[77]: Steatosis may be produced by a wide variety of drugs and toxins, by far the commonest cause being alcohol. In most instances drug produce steatosis through inhibition of lipoprotein synthesis or secretion, or through interference with fatty acid oxidation.

Types of steatosis are, Macrovesicular steatosis takes the form of large vesicles due to Coalescence of small lipid droplets within the cytosol and the eventual displacement and indentation of the nudeus. Corticosteroids may cause this type of steatosis. Agents which produce zonal liver necrosis, in particular industrial solvents such as carbon tetrachloride, phosphorous, toxins also show significant macro vesicular fatty infiltration. Chronic occupational exposure to organic solvents has been associated with steatosis.

Micro vesicular steatosis, in contrast mimics acute fatty liver of pregnancy and Reye's syndrome. In case of liver damage by tetracycline hepatocytes are filled with finely divided fat droplets that do not displace the nuclei, initially the fat is confined to zone 3 but progressively become panacinar in distribution. There is no significant necrosis and little cholestasis. The liver damage probably results from inhibition of lipoprotein production increased uptake of fatty acids, increased formation of triglycerides and/or impaired mitochondrial oxidation of fatty acids leading to severe intra hepatocytic lipid retention.

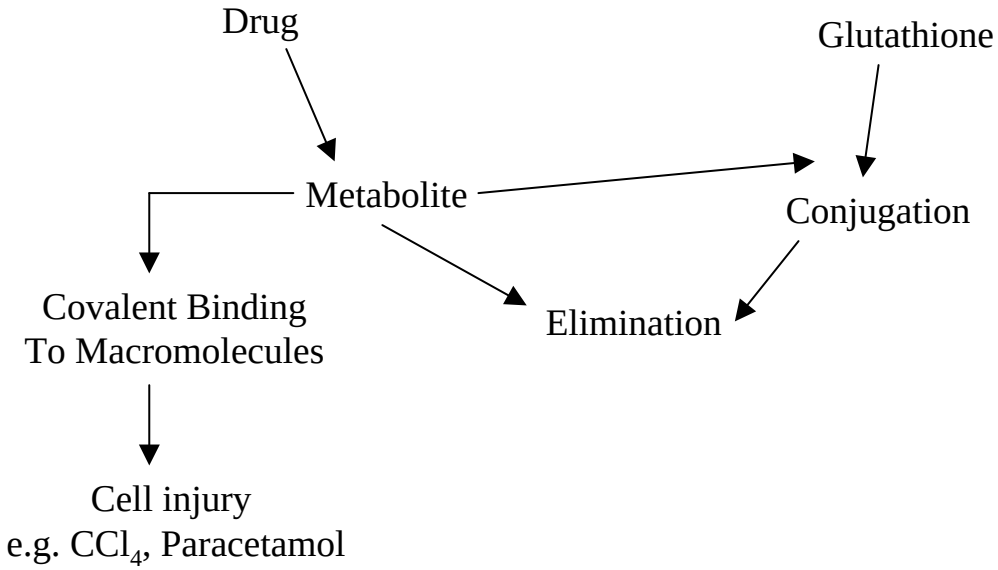
4.5.1.3. ENVIRONMENTAL INJURY – DRUG AND TOXINS:

Water soluble drugs and chemical substances is polar molecules, can be excreted by the Kidneys, whereas those which are not water-soluble i.e. non-polar molecules are handled mainly by the liver. The main drug metabolizing enzymes increase the polarity of the molecule in one of the three ways :- a) Oxido-reductase, of which the enzyme cytochrome P450 is important, b) Hydrolase and Transferase and c) Conjugation reaction.



Traditionally, drug injury to the liver was classified into direct, where injury is predictable and dose-dependent, and indirect, where it is not dose-dependent and is unpredictable. It is now clear that this was a great oversimplification, and most drugs undergo metabolism within the liver cell before causing injury. Substances such as tannic acid, ethionine, ferrous sulphate, white phosphorus all cause liver damage directly causes cell injury. In contrast, most of the traditional liver poisons such as carbon tetrachloride are in fact harmless in an animal such as Chicken which cannot metabolise them. Paracetamol in overdose and halothane probably operate in a generally similar way.

Alcoholic liver injury too may be the result of a reactive metabolite. Although



both zonal necrosis and viral hepatitis-like liver cell necrosis caused by drugs and toxins usually have a mechanism similar to that outlined above, very occasionally reactive metabolite may create new cellular antigens which elicit an immunological response-free drug hypersensitivity e.g. Methyldopa.

Cell injury by CCl_4[78]: Toxic injury of liver induced by carbon tetrachloride (CCl_4) is a model system of toxic injury. Toxic injury of liver by CCl_4 is the result of their metabolic conversion by a complex of enzymes bound to membranes of the smooth-surfaced endoplasmic reticulum. Action of these enzymes is a major mechanism by which toxic compounds are converted to less toxic ones. In some instance non-toxic substances are metabolized to toxic ones such as in case of CCl_4.

Carbon tetrachloride is converted by homolytic cleavage to a highly reactive haloalkane free radical and a chlorine free radical in the following reaction:-

CCl_4 ----------> CCl_3 + Cl.

These in turn reacts with a variety of intracellular molecules, notably the unsaturated fatty acids.

Polyenolic fatty acids for example are converted to organic free radicals which in turn react with molecular oxygen to form organic peroxides. These compounds are highly unstable and decompose spontaneously to form aldehydes, ketones and other products.

CCl_4 reacts with sulfahydryl groups which mediate the function of the many cell proteins including a number of important enzymes and this reaction leads to their alkylation and subsequent loss of function. The free radicals formed react rapidly with other molecules to form additional free radicals; such reactions are autocatalytic and tend to spread from a small focus to involve large areas of cytoplasm. The

earliest change that has been detected in rat liver cells is a functional one that occurs 30 min after the intragastic administration of a single dose of 0.25ml of CCl_4. It consists of rapid decrease in synthesis of the export protein albumin as well as the cytochromic. The diminution of protein synthesis after intoxication appears to be linked to disaggregation of the polysomes and probably represents a physical disruption of their association with messenger RNA.

Significantly in this early phase of carbon tetrachloride induced injury, mitochondria appear morphologically intact and are capable of normal oxidative phosphorylation and fatty acid oxidation among their many functions.

Within few hours after administration of CCl_4 neutral lipids (triglycerides) begin to accumulate in the cytoplasm making their first appearance as osmiophilic droplets ultimately fill the entire cytoplasm. Approximately 10 to 12 hours after CCl_4 administration the liver is grossly enlarged and pale because of accumulated fat. Lipid can also accumulate in the liver by mechanisms such as by increased mobilization of free fatty acids from depot fat.

Shortly after the ingestion of as little as 5 ml to as much as 100 ml of carbon tetrachloride, swelling and hydropic degeneration of the centrilobular hepatic cells develop. These changes progress to a diffuse fatty degeneration and necrosis in the centrilobular parenchyma with collapse of the reticulum network, followed shortly by haemorrhage and leukocytic infiltration.

Autoradiographic studies have shown a raid uptakes of carbon tetrachloride by the cytoplasm and nuclei of the cells of centrilobular areas. Autoradiographic evidence shows that radioactive ^{14}C and carbon tetrachloride remain in the centrilobular areas as long as 2 days after ingestion.

Endoplasmic reticulum, is damaged 30 minutes of the administration of carbon tetrachloride, whereas the mitochondria survive unaltered for several hours. Protein synthesis is reduced within 2 hours of poisoning. Fatty acids are mobilized from peripheral fat depots to the liver. In the liver cell they are oxidized to triglycerides.

Experimentally, CCl_4 has been widely used to study toxic hepatic necrosis and it is still a favoured model of cirrhosis, which regularly develops after repeated injection of CCl_4 into rats[79].

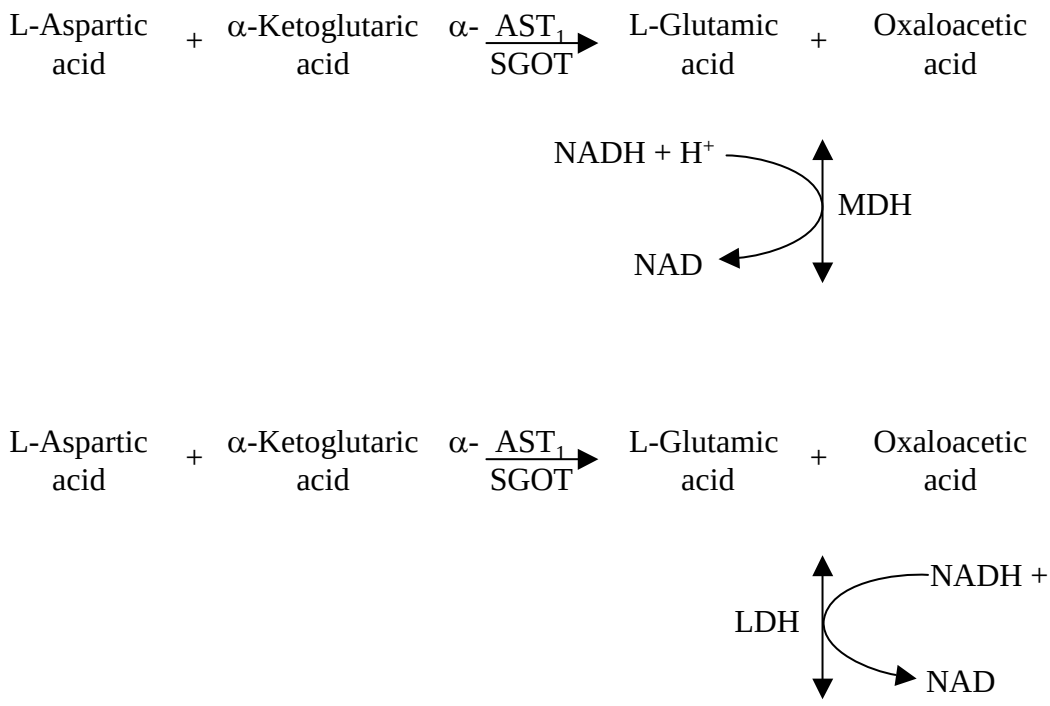
4.5.1.4. ENZYME THAT DETECT HEPATOCELLULAR NECROSIS[80]

The liver contains thousands of enzymes, some of which are also present in serum in very low concentrations. These enzymes have no known function in serum and behave like other serum proteins. They are distributed in plasma and intestinal fluid and have characteristic half-lives of disappearance, usually measured in days. The elevation of a given enzyme activity in serum is thought to primarily reflect its increased rate of entrance into serum from damaged liver cells. Serum enzyme tests can be grouped into two categories:- 1) enzymes whose elevation in serum reflects generalized damage to hepatocytes and, 2) enzymes whose elevation in serum primarily reflects cholestasis.

Aminotransferase: The serum aminotransferases (formerly called transaminases) are sensitive indicator of liver cell injury, and most helpful in recognizing acute hepatocellular diseases such as hepatitis. Alanine aminotransferase [(ALT, serum glutamic-pyruvic transaminase (SGPT)] and Aspartate aminotransferase [(AST, serum glutamic-oxaloacetic transaminase (SGOT)], activities in serum are the most frequently measured indicator of liver disease. These enzymes catalyze the transfer of the α-amino groups of alanine and aspartic acid

respectively to the α-keto group of ketoglutaric acid. This results in the formation of pyruvic acid and oxaloacetic acid. Of the numerous methods developed for measuring ALT and AST activity in serum, the most specific method couples the formation of pyruvate and oxaloacetate the products of the aminotransferase reactions, to their enzymatic reduction to lactate and malate. The reduced form of nicotinamide-adenine dinucleotide (NADH), the cofactor in this reduction is oxidized to NAD. Because NADH, but not NAD, absorbs light at 340 nm, the event can be followed spectrophotometrically by the loss of absorptivity at 340nm. Both aminotransferase normally are present in serum in low concentrations, less than 30 to 40 IU/L.

AST is found in the liver, cardiac muscle, skeletal muscle, the kidneys, the brain, the pancreas, the lungs, leucocytes and erythrocytes, in decreasing order of concentration, whereas ALT is present in highest concentration in the liver. The increase in ALT and AST serum values is related to damage to or destruction of tissue rich in the aminotransferases or to changes in cell membrane permeability that allow ALT and AST to leak into serum.



Aminotransferases typically are elevated in all liver disorders. These include all types of acute and chronic hepatitis, cirrhosis, infectious mononucleosis, alcoholic liver diseases etc. Elevations up to eight times the upper limit of normal are non-specific and may be found in any of the mentioned disorders. The highest elevations occur in disorders associated with extensive hepatocellular injury, such as drug and viral hepatitis, exposure to hepatotoxins such as carbon tetrachloride. ALT is somewhat higher than AST.

The aminotransferases may be falsely elevated or diminished under certain circumstances. Drugs such as elevated aminotransferases values if older colorimetric tests are used. Conversely low values of AST may be seen in uremia. These values increases after dialysis.

Serum Bilirubin: Bilirubin is a major breakdown product of hemoglobin. Hemoglobin is derived from red cells that have outlived their natural life and

subsequently have been removed by the spleen. During splenic degradation of red blood cells, hemoglobin (the part of the red blood cell that carries oxygen to the tissues) is separated out from iron and cell membrane components. Hemoglobin is transferred to the liver where it undergoes further metabolism in a process called conjugation. Conjugation allows hemoglobin to become more water-soluble. The water solubility of bilirubin allows the bilirubin to be excreted into bile. Bile then is used to digest food.

As the liver becomes irritated, the total bilirubin may rise. It is then important to understand the difference between total bilirubin, which has undergone conjugation (that is hepatic cell metabolism), and at portion of bilirubin which has not been metabolized. These two components are called total bilirubin and direct bilirubin. The direct bilirubin fraction is that portion of bilirubin that has undergone metabolism by the liver. If the direct bilirubin is low, while the total bilirubin is high, this reflects liver cell damage or bile duct damage within the liver itself.

1. **Formation:** About 250 to 350 mg of bilirubin forms daily; 70 to 80% derives from the breakdown of senescent RBCs. The remaining 20 to 30% (early-labeled bilirubin) comes from other heme proteins located primarily in the bone marrow and liver. The heme moiety of Hb is degraded to iron and the intermediate product biliverdin by the enzyme heme oxygenase. Another enzyme, biliverdin reductase, converts biliverdin to bilirubin.

2. **Plasma transport:** Because of internal hydrogen bonding, bilirubin is not water-soluble. Unconjugated (indirect-reacting) bilirubin is therefore transported in the plasma bound to albumin and cannot pass through the glomerular membrane; thus, it does not appear in urine. The binding weakens under certain conditions (eg,

acidosis), and some substances (eg, certain antibiotics, salicylates) compete for the binding sites.

3. **Liver uptake:** The details of liver uptake of bilirubin and the importance of intracellular binding proteins (eg, ligandin or Y protein) are unclear. Uptake of bilirubin is via active transport and is rapid, but it does not include uptake of the attached serum albumin.

4. **Conjugation:** Free bilirubin concentrated in the liver is conjugated with glucuronic acid to form bilirubin diglucuronide, or conjugated (direct-reacting) bilirubin. This reaction, catalyzed by the microsomal enzyme glucuronyl transferase, renders the bilirubin water-soluble. Under some circumstances, glucuronyl transferase forms only bilirubin monoglucuronide, with the second glucuronic acid moiety added at the bile canaliculus via a different enzyme system, but this reaction is not widely considered physiologic. Bilirubin conjugates other than the diglucuronide are also formed; their significance is uncertain.

5. **Biliary excretion:** Conjugated bilirubin is secreted into the bile canaliculus with other bile constituents. Other organic anions or drugs can affect this complex process. In the intestine, bacterial flora deconjugate and reduce bilirubin to compounds called stercobilinogens. Most are excreted in the feces and give the stool its brown color; substantial amounts are absorbed and re-excreted in the bile, and small amounts reach the urine as urobilinogen. The kidney can excrete bilirubin diglucuronide but not unconjugated bilirubin. This explains the dark urine typical of hepatocellular or cholestatic jaundice and the absence of urinary bile in hemolytic jaundice.

Abnormalities at any of these steps can result in jaundice. Increased formation, impaired liver uptake, or decreased conjugation can cause unconjugated

hyperbilirubinemia. Impaired biliary excretion produces conjugated hyperbilirubinemia. In practice, both liver disease and biliary obstruction create multiple defects, resulting in a mixed hyperbilirubinemia. Moreover, when conjugated bilirubin builds up in plasma, a portion becomes covalently bound to serum albumin. This protein-bound fraction (δ-bilirubin) is not measurable by routine techniques but is often a major component of circulating bilirubin, especially during recovery from jaundice.

Serum alkaline phosphatase: Alkaline phosphatase is an enzyme, which is associated with the biliary tract. It is not specific to the biliary tract. It is also found in bone and the placenta. Renal or intestinal damage can also cause the alkaline phosphatase to rise. If the alkaline phosphatase is elevated, biliary tract damage and inflammation should be considered. However, considering the above other etiologies must also be entertained. One way to assess the etiology of the alkaline phosphatase is to perform a serologic evaluation called isoenzymes. Another more common method to asses the etiology of the elevated alkaline phosphatase is to determine whether the GGT is elevated or whether other function tests are abnormal (such as bilirubin)

Alkaline phosphatase may be elevated in primary biliary cirrhosis, alcoholic hepatitis, PSC, gallstones in choledocholithiasis.

4.5.2. MATERIALS AND METHODS

1. Animal Selection
2. Extracts Used.
3. Acute toxicity study.
4. Method of Screening

1. ANIMAL SELECTION:

Female albino Wistar rats weighing 120-150g breed in the animal house, Sri. Venkateshwara Enterprises, Bangalore (CPCSEA Reg. No.276), were used in this study. They were employed for assessing hepatoprotective activity and acute toxicity study respectively. The animals were allowed free access to food and tap water. Rats were housed in a group of six in clean cages at 25^0C and 12 hr photo period. The bedding materials of the cages were changed everyday.

2. EXTRACTS USED:

Extracts	Code Used
Petroleum ether (40-60^0C)	D
Alcohol	E
Aqueous	F

Above all extracts were taken for the present study.

Chemicals Used:

Olive oil – Seven ships brand, Nirmal Chemicals, Bangalore. Carbon tetrachloride (CCl_4) – 95% Poona Chemical Laboratory, Pune. Liv-52 Syrup – The Himalaya Drug Company, Bangalore.

Estimation Kit (SGOT ,SGPT ,SALP, and Serum Billirubin):

SGOT Kit – Crest Biosystem Ltd. Santacruz.
SGPT Kit – Crest Biosystem Ltd. Santacruz.
SALP Kit – Crest Biosystem Ltd. Santacruz.
Serum Bilirubin Kit – Crest Biosystem Ltd. Santacruz.
All other regents were of analytical grade.

3. ACUTE TOXICITY STUDY[81].

Acute oral toxicity – Acute toxic class method:

The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD), received draft guidelines 423, received from committee for the purpose of control and supervision of experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.(See annexure A-8)

Principle of Test:

It is the principle, which is based on a stepwise procedure with the use of a minimum number of animals per step; sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex (normally females). Absence or presence of compound related mortality of the animals dosed at one step will determine the next step.

- No further testing is required.
- Dosing of three additional animals with the same dose.
- Dosing of three animals at the next higher or the next lower dose level.

The method enables a judgment with respect to classifying the test substances to one of the series of toxicity classes defined by fixed LD_{50} cut off values.

Description of the Method:

1) Selection of Animal Species:

Healthy young female Albino Wistar rats weighing between 120-150 gms (8 to 10 weeks old) were used for acute toxicity study to determine LD_{50} of various extracts. Totally they were three groups, each groups consists of three animals.

2) Housing and Feeding Condition:

The temperature in the experimental room was around 25^0C. Lightning was artificial, the sequence being 12 hours dark, 12 hour light. The conventional laboratory diet was fed, with an unlimited supply of drinking water.

3) Preparation of Animals:

The animals were randomly selected, marked to permit individual identification, and kept in their cages for 5 days prior to closing to allow for acclimation of the laboratory condition.

4) Preparation of doses:

All the extracts were prepared as a suspension by triturating with either water or 2% of gum acacia or 1% twean 80.

5) Administration of doses:

The test substances are administered in a single dose by gavage using an intragastric tube. Animals were found prior to dosing, following period fasting, the animals were weighed and test substance was administered. After dosing, food was withheld for a further 3-4 hrs in rats.

6) Number of animals and dose levels:

In each steps three animals were used in each group. Since there was no information on the substance to be tested (i.e. extracts), starting dose was 300 mg /kg body weight up to 2000 mg/kg body weight.

The procedure of dose selection and finalizing LD_{50} cut off values are shown in the Table No. 11.

The following LD_{50} values were obtained for various extracts:-

Table No.11

Dose Selection and Finalizing LD_{50} Cut off value of Extracts

Name of Extract	LD_{50} cut off mg/kg b.w.	Therapeutic Dose*	GHS classification
Aqueous	2000mg/kg	200mg/kg	Class 4
Alcohol	2000 mg/kg	200mg/kg	Class 4
Petroleum ether(40-60^0)	2000 mg/kg	200mg/kg	Class 4

1/10th of this lethal dose was taken as effective dose (therapeutic dose) for subsequent wound healing activity.

7) Observations:

Animals were observed initially after dosing at least once during the first 30 minutes, periodically during the first 24 hours. In all cases death was observed within first 24 hours. Additional observations like changes in skin and for eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems and behaviour pattern. Attention was also given to observation of tremors and convulsions.

3. METHODS OF SCREENING:

Evaluation of Hepatoprotective Activity:

The method according to Handa S.S. and Anupam Sharma has been used in this study[82,83].

The above extracts were administered on group of 6 female albino Wistar rats, weighing about 120-150g, for recording enzymatic levels and histopathology during the evaluation. Animals were administered with carbon tetrachloride (2 ml/kg) i.p. to induce hepatotoxicity. Marked increased in the serum level or SGOT, SGPT, SALP, and Serum Bilirubin was taken as indication of hepatotoxicity.

The procedure consists of:-

- Group A – Served as Control and received single daily dose of 1 ml/kg i.p. of sucrose solution for 4 days along with 1 ml/kg s.c. of olive oil on 2nd and 3rd days.
- Group B – Also received single daily dose of 1 ml/kg i.p. aqueous sucrose solution for 4 days with 2 ml/kg of Carbon tetrachloride by subcutaneous route dissolved in an equal volume of olive oil on 2nd and 3rd days.
- Group C– Received standard drug Liv-52 as a single daily dose of 5 ml/kg of oral route for 4 days with 2 ml/kg of carbon tetrachloride by subcutaneous route on 2nd and 3rd days.
- Group D, E and F received single daily dose of 500 mg/kg of extracts by oral route for 4 days respectively, with 2 ml/kg of carbon tetrachloride by subcutaneous route on 2nd and 3rd days.

Schedule for Carbon tetrachloride Model:

Sr. No.	Group	Days				
		1	2	3	4	5
1.	A-Control	SS	SS, OO	SS,OO	SS	Animals were sacrificed
2.	B- Carbon tetrachloride	SS	SS, CCl_4	SS, CCl_4	SS	
3.	C- Std drug Liv-52	TS	TS, CCl_4	TS, CCl_4	TS	
4.	D- Petroleum ether extract	TS	TS, CCl_4	TS, CCl_4	TS	
5.	E-Alcohol extract	TS	TS, CCl_4	TS, CCl_4	TS	
6.	F- Aqueous extract	TS	TS, CCl_4	TS, CCl_4	TS	

[SS – Sucrose solution, OO – Olive oil, CCl_4 – Carbon tetrachloride in olive oil in 1 : 1 ratio, TS – Test Solution and SD – Standard Drug (Liv-52)].

All the rats in all the groups were sacrificed on 5^{th} day under light anaesthetic ether. Blood from each rat was collected through cardiac puncture under ether anaesthesia for biochemical investigation i.e. SGOT, SGPT, SALP, and serum Bilirubin estimation. Blood was allowed to coagulate at 37^0C for 30 min and the serum was separated by centrifugation at 2500 rpm for 10 minutes. The liver of all the experimental animals were removed and processed immediately for histological investigation.

Histological Investigations:

The liver from each animal was removed after dissection. The liver lobes were fixed for 48 hrs in 10% formalin and were embedded in paraffin. Subsequently, 5 μ sections of livers were stained with haematoxylin and eosin. These sections were observed under light microscope for histological changes and compound to normal liver physiology.

BIOCHEMICAL INVESTIGATION:

Estimation of SGPT and SGOT:

Serum glutamate pyruvate transaminase activity as an index of hepatic damage has been widely used. Human serum contains several transaminase of which aspartate transaminase (AST) also known as glutamate oxaloacetate transaminase (GOT) and alanine transaminase (ALT) also known as glutamate pyruvate transaminase (GPT) are of diagnostic significance. Elevated levels of serum transaminase has been observed in hepatic injury and are widely used both clinically and in experimental model to reveal the extent of hepatic injury. They are useful for diagnosis of acute viral hepatitis, liver damage due to drugs or chemicals and for the differentiation of these conditions from obstructive jaundice. Thus the capacity of different extracts to reduce the elevated serum transaminase levels in carbon tetrachloride induced acute hepatic failure model has been used as a biochemical parameter to expose its hepatoprotective capacity.

Estimation of Serum Glutamate Pyruvate Transaminase (SGPT):

SGPT or ALT is located in the cytosol of the liver cell. During liver cell inflammation, they are released into circulation due to increased permeability of cell membrane break down of liver cells. Hence, determination of SGPT as index of the extent of liver damage.

Diagnostic reagent kit was used for determination of SGPT also called as "Alanine amino transaminase" (ALT) activity by method of Reitman and Frankel[84].

Principle:

SGPT (ALT) Catalyses the following reaction:

$$\alpha\text{-Ketoglutarate} + \text{L-Alanine} \longleftrightarrow \text{L-glutamate} + \text{pyruvate}$$

Pyruvate so formed is coupled with 2,4-Dinitro phenyl hydrazine (2, 4 DNPH) to give the corresponding hydrazone, which gives brown color in alkaline medium and this can be measured colorimetrically.

Reagents:

Reagent 1 : Buffered alanine α-KG substrate, pH 7.4.

Reagent 2 : DNPH Colour Reagent.

Regent 3 : Sodium hydroxide, 4N.

Reagent 4 : Working pyruvate standard, 2mm.

Reagent Preparation:-

Solution I : Dilute 1 ml of Regent, 3 to 10 ml with purified water.

Precautions:

1. Serum samples must be completely free from haemolysis, since RBCs are very rich in this enzyme. This may result in erroneous results.
2. The use of detergents was avoided to clean glasswares.
3. Cleaned and dry glasswares were used.
4. Reagent 3 is corrosive, contact with skin was avoided.

Advantages:-

1. Highly popular and simpler method.
2. This method gives reproducible results.
3. Substrate and standard are specially stabilized.
4. Very economical.

Stability: 2-8^{0}C till expiry date.

Wavelength: On spectrophotometer at 505 nm, on photocolorimeter using green filter.

Cuvettes: 1 cm light path.

Standardisation of Kit:

To avoid variations due to inter-laboratory assay conditions, standardization of kit was done before assay .

Assay:-

All solutions were pipette out into clean dry test tube labeled as Test (T) as shown below :

Reagents	*Test (T)*
Reagent 1: Buffered alanine, pH 7.4.	0.25 ml
Was incubated at 37^0C for 5 minutes.	
Serum	0.05 ml (50 μl)
Mixed well and incubated at 37^0C for 30 minutes.	
Reagent 2: DNPH colour Reagent	2.5 ml
Mixed well and allowed to stand at room temperature for 20 minutes	

Solution 1:

Was mixed well and allowed to stand at room temperature for 10 minutes and the absorbance was read at 505 nm.

Estimation of SGOT:

SGOT (AST) are located on the cytosol of liver cell. In addition, it is also found in the mitochondria. It is also found in many tissue such as heart, liver, skeletal muscle and kidney which are rich source of SGOT in that order, liver being the second richest source of SGOT the importance of SGOT levels in hepatic damage of hepatic cells leads to increased levels of SGOT in blood serum.

Introduction (Methodology):

SGOT Kit is based on Reitman and Frankel's method[84]. SGOT catalyzes the transfer of the amino group of L-aspartate (ASP) to α-ketoglutarate of the (α-KG) resulting in the formation of oxaloacetate (OAA) and L-glutamate (L-Glu). The

oxaloacetate so formed, is allowed to react with 2,4-DNPH to form 2,4 dinitrophenyl hydrazone derivative which is brown coloured in alkaline medium. The hydrazone derivative of oxaloacetate similar to pyruvate is considerably more chromogenic than that of α-KG. The final colour developed does not obey Beer's law.

Principle:

L-Aspartate + α-Ketoglutarate $\xrightarrow{\text{SGOT, pH 7.4}}$ Oxaloacetate + L-glutamate

Oxaloacetate + 2,4-Dinitrophenylhydrazine $\xrightarrow{\text{Alkaline Medium}}$ 2,4 dinitrophenyl hydrazone

Reagents:

Reagent 1: Buffered substrate - 2 x 12.5 ml

Reagent 2: DNPH colour regent – 2 x 12.5 ml

Reagent 3: Sodium hydroxide – 1 x 25.0 ml

Reagent 4: Pyruvate standard 2mM – 1 x 3.0 ml

Reagent Preparation:-

Dilute sodium hydroxide (3) 1 : 10 with distilled water before use.

Precautions:

1. SGOT (AST) kit is for in-vitro diagnostic use only.
2. Clean and dry glasswares were used; presence of impurities or detergent interferes with enzyme activity.
3. Reagents were stored as 2-8^{0}C, it is necessary that working reagents reaches the temperature of measurement before adding the sample. If to serum cold reagents are added, it would reduce enzyme activity.
4. Haemolysis should be avoided as red cells contain GOT.

Stability : 0-4^{0}C for 1-3 days., -20^{0}C for more than 3-4 days

Wavelength: On spectrophotometer at 505 nm. On photocolourimeter using green filter.

Cuvettes : 1 cm light path.

Standardisation of Kit:

To avoid variations due to inter-laboratory assay conditions, standardisation of kit was done.

Assay:

All solutions were pipette out into two clean dry test tubes labeled blank (B) and Test (T) as shown below;

Reagents	***(B)***	***(T)***
Buffered substrate (1) ml	0.5	0.5
Was incubated at 37^0C for 3 minutes		
Serum (ml)	--	0.1
Mixed well and incubated at 37^0C for 60 minutes		
DNPH Color Reagent (2) ml	0.5	0.5
Mixed well and allowed to stand at room temperature for 20 minutes		
Distilled water (ml)	0.1	--
Working NaOH (ml)	5.0	5.0

All test tubes were shaked to mix and allowed to stand at room temperature for 10 minutes and measure absorbance of Test (T) against blank (B) was measured on spectrophotometer at 505 nm.

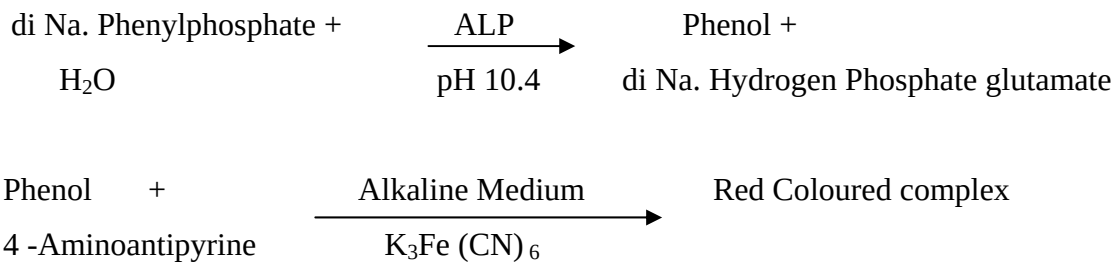
Linearity:

If the enzyme activity of the specimen was above 190 units/ml, the test was repeated using dilute serum with normal saline and multiplied the final results with appropriate dilution factor.

Introduction (Methodology):

SALP Kit is based on Kind and King method[85]. Alkaline phosphatase (ALP) at an alkaline pH hydrolyses di Sodium Phenylphosphate to form phenol. The Phenol formed reacts with 4 -Aminoantipyrine in the presence of Potassium Ferricyanide, as an oxidizing agent, to form a red colored complex. The intensity of the colour formed is directly proportional to the activity of ALP present in the sample.

Principle:



Reagents:

Reagent 1: Buffered reagent – 60 ml

Reagent 2: Substrate regent –6 ml

Reagent 3: Colour Reagent – 60 ml

Reagent 4: Phenol Standard (10 mg/dl) – 5ml

Precautions:

1. SALP kit is for in-vitro diagnostic use only.
2. Clean and dry glasswares were used; presence of impurities or detergent interferes with enzyme activity.

3. Reagents were stored as 2-8^{0}C, it is necessary that working reagents reaches the temperature of measurement before adding the sample. If to serum cold reagents are added, it would reduce enzyme activity.

Stability: 2-8^{0}C till the expiry mentioned

Wavelength: On spectrophotometer at 510 nm. On photocolourimeter using green filter.

Cuvettes : 1 cm light path.

Standardisation of Kit:

To avoid variations due to inter-laboratory assay conditions, standardisation of kit was done before assay.

Assay:

All solutions were pipette out into two clean dry test tubes labeled blank (B),Standard (S), Control (C) and Test (T) as shown below;

Reagents	***(B)ml***	***(S)ml***	***(C)ml***	***(T)ml***
Distilled Water	1.05	1.00	1.0	1.0
Buffered Reagent	1.0	1.0	1.0	1.0
Substrate Reagent	0.10	0.10	0.10	0.10
Was incubated at 37^{0}C for 3 minutes				
Serum (ml)	--	--	--	0.05
Phenol Standard (S)	--	0.05	--	--
Mixed well and incubated at 37^{0}C for 60 minutes				
Color Reagent	1.0	1.0	1.0	1.0
Sample	--	--	0.05	-

All test tubes were shaked to mix and allowed to stand at room temperature for 10 minutes and measure absorbance of Blank (Abs B), Standard (Abs S), Control (Abs. C), and Test (T) against distilled water, was measured on spectrophotometer at 510 nm.

Calculation :

$$\text{Total ALP activity in K.A. units} = \frac{\text{Abs. T- Abs. C}}{\text{Abs. S – Abs. B}}$$

Linearity:

If the enzyme activity of the specimen was above 60 K.A. Units dilute the sample with distilled water and repeat the assay. Multiply the value with the proper dilution factor.

Introduction (Methodology):

Serum Bilirubin kit is based on Jendrassik and Grof's method[86]. Bilirubin reacts with diazotized sulphnilic acid to form a coloured compound. The unconjugated bilirubin couples with the sulphanilic acid in the presence of caffeine – benzoate accelerator. The intensity of the colour formed is directly proportional to the amount of bilirubin present in the sample.

Principle:

Bilirubin + Diazotized sulphnilic acid $\longrightarrow$ Azobilirubin compound

Reagents:

Reagent 1: Direct Bilirubin content reagent – 75 ml

Reagent 2: Direct Nitrate reagent – 4ml

Reagent 3: Total Bilirubin Reagent – 75 ml

Reagent 4: Total Nitrate reagent– 4ml

Reagent 5: Artificial Standard (10 mg/dl) – 10 ml

Precautions:

1. Serum bilirubin kit is for in-vitro diagnostic use only.
2. Clean and dry glasswares were used; presence of impurities or detergent interferes with enzyme activity.
3. Reagents were stored as 2-8^{0}C, it is necessary that working reagents reaches the temperature of measurement before adding the sample. If to cold reagents are added, it would reduce enzyme activity.

Stability : 2-8^{0}C till the expiry mentioned

Wavelength: On spectrophotometer at 540 nm, on photocolorimeter using yellow-green filter

Cuvettes : 1 cm light path.

Standardisation of Kit:

To avoid variations due to inter-laboratory assay conditions, standardisation of kit was done before assay.

Assay:

All solutions were pipette out into two clean dry test tubes labeled blank (B) and Test (T) as shown below;

Reagents	***(B)ml***	***(T)ml***
Total Bilirubin reagent	1.0	1.0
Total Nitrite reagent	--	0.05
Sample	0.1	0.1

All test tubes were shaked to mix and allowed to stand at room temperature for 10 minutes and measure absorbance Test (T) against their respective Blank (Abs B) measured on spectrophotometer at 540 nm.

Calculataion: Total or Direct bilirubin in mg/dl = Abs. t X 13

Linearity:

If the enzyme activity of the specimen was above 20 mg/dl dilute the sample with distilled water and repeat the assay. Multiply the value with the proper dilution factor.

Statistical Analysis:

Results of biochemical estimation were reported for determination of significant intergroup difference each parameter was analysed separately and one way analysis of variance (ANOVA) was carried out[87]. The calculated F ratio been tabulated along with the critical value of F ratio. Dunnet's test was used for individual comparisons[88,89].

Fig.1: Photograph Showing the plant *Jatropha curcas* Linn.

Jatropha curcas plant

Jatropha curcas fruit

Jatropha curcas flower

Jatropha curcas seed

INTRODUCTION TO *JATROPHA CURCAS* (LINN.) PLANT AND REVIEW OF LITERATURE

INTRODUCTION TO FAMILY EUPHORBIACEAE:

The Euphorbiaceae is a large family of about 300 genera and 6000 or more species. Most numbers are trees or shrubs, a few herbs. Some genera (e.g. Euphorbiaceae) xerophyteic. The genera includes *Euphorbia* (about 200 species), *Phyllanthus* (about 550 species), *Mallotus* (2 species), *Ricinus* (1 species), *Croton* (750 species), *Hevea* (12 species), *Jatropha* (175 species), *Manihot* (170 species), *Sapium (*170 species), *Poranthera, (*10 species), *Securinega* (25 species), *Aleurites* (2 species) and *Hippomane* (5 species).

Botanical Name: *Jatropha curcas* Linn.

Synonym: Physic nut, Janglierandi, Mogalierandi,

Family: Euphorbiaceae.

The plant is known by various names in different languages as:-

Sanskrit	-	Kananaeranda, parvataranda.
Hindi	-	Bhagbherenda, Jangalierandi.
Bengali	-	Erandagachh
Marathi	-	Mogalieranda
Gujarathi	-	Ratanjyota
Telugu	-	Nepalamu
Tamil	-	Kadalamanakku
Kannada	-	Adaluharalu
English	-	Physic nut

3.1 DESCRIPTION:

A genus of large shrubs, 3-4 m high, with three species, commonly found in the tropics, *J.curcas,* linn., (Physic nut) *J. Gossypifolia* Linn. (Bellyache bush) and J.glandul fera. Found wild in tropical America it is now wide spread. Now cultivated in tropical and sub-tropical parts of the world for its seeds.

Occurrence and Distribution:

A glabrous erect branched shrub of the Euphorbiaceae family is reported to have been introduced into Asia and Africa by the Portuguese as an oil yielding plant. It is cultivated to a certain extent as an oil seed crop in Cape Verde Island. In Madagascar and parts of French West Africa, where plant is grown as a support for the vanilla plant.

In India, *J. curcas* is found in India in a semi- wild condition in the vicinity of villages. It is reported to be cultivated in central and western parts in India like Madhya Pradesh, Maharashtra, Rajasthan, and Gujarat similarly there are reports of its cultivation in southern states like Andhra Pradesh and Tamilnadu. Gradually its area is increasing day by day in different parts of country.

3.2. MORPHOLOGY:

- Leaves are orbicular-ovate, angular or somewhat three or five lobed, 10 to15 cm long, acuminate, base cordate with long petioles.
- Flowers in loose panicles of cymes, yellowish green, c. 7 mm across.
- Fruits 2.5 cm long, ovoid, black, breaking into three 2 valved cocci.
- Seeds are ovoid, oblong dull brownish black[24,25].

3.3. REVIEW OF LITERATURE

Sthulairanda: gunadhya: syat rasavuryavipaktisy (rajnighantu). According to above shlok *Jatropha curcas* is most potent in respect to *rasa veerya* and *vipaka*. Rasa, Viryas and Vipaka directly influenced the Tridosha, Nutrition and tissue development of the SaptaDhatu: Rasa (plasma), Rakta(blood), Mansa(muscle), Meda(adipose), Asthi(bones), Majja (nerves) and Shukra(semen). The various parts of Pomegranate plants used are flowers, fruit rind, bark, stem, root, leaves and These parts are mainly used as depurative and purgative, chronic rheumatism and skin diseases. Lactoguage and suppurative, styptic, toothache.

***Jatropha curcas* contains:**

Amino acids: arachidic, linoleic, myristic, oleic, palmitic, and stearic acid: arabinose, galactose, glucose, rhamnose, xylose, galacturonic acid and β-sitosterol in seeds: β-D- glucoside of β-sitosterol also in stem bark, seed coat, wood; dulcitol and sucrose in kernel; vitexin isovitexin campesterol β-sitosterol-3 β,7 β-diol and – tricontanol in leaves. B-sitosterol, β-amyrin and taraxerol in the stem-bark[26].

Leaves of *Jatropha curcas* contains following chief constituents:-

1) Alpha amyrin
2) Apigeninlin
3) Campesterol
4) Daucosterol-0.014%
5) Isovitexin
6) J. curcas flavonoid II 0.04%
7) J. curcas triterpene 0.05%
8) J. curcas flavonoid I 0.065%
9) N-1 tricontanol
10) Stigast-5-ene-3-β beta, 7-α-diol,

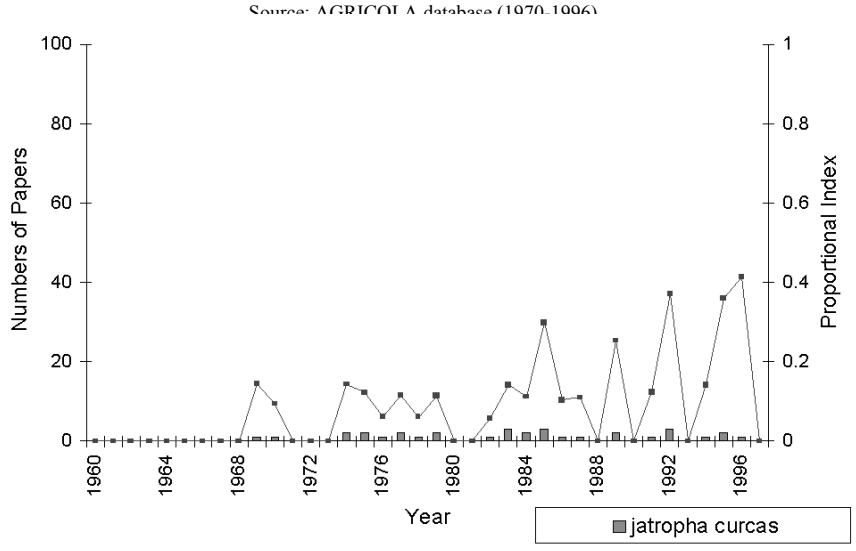
11) Stigast-5-ene-3-β beta, 7- β-diol,

12) Stigasterol 0.025%

13) Vitexin [21]

It also contains four novel diterpenes- curcusones A,B,C and D-isolated and their structure elucidated *(Tetrahedron lett.* 1986,27,2439); isolation of new lathyranes- curculathyranes A and B- and determination of their structures by NMR and X rays diffraction (*tetrahedran lett*.1986,27,5675); new diterpenoids –jatrophal – isolated from roots together with jatrophin (coumarionolignan(I), *Phtochemistry* 1984,23,867);jatropholones A and B, taraxerol, β-sitosterol and its glucoside; jatrophol characterized as 16-hydroxyjatropholone (*Zhiwu Xuebao* 1988, 30, 308;*Chem.Abstr.*1989,110,21068h);oleic(46.72),linoic(30.31), palmitic(14.10) and stearic (7.68%) acids presents in seed oil(Pakistan J. Sci. Ind. Res. 1988, 31, 566; Chem.Abstr.1989,110,54512b)[27].

NEW COMPOUNDS



Analysis of numbers of papers/mentions over time (Agricola database1970-1996)

Anti-metastatic effect of curcusone B, a diterpene from Jatropha curcas was investigated against 4 human cancer cell lines. Treatment with non- cytotoxic doses of curcusone B resulted in a strong reduction of in vitro invasion, motility and secreation of matrix metalloproteinase(MMP) of cancer cells[28].

A complex of %-hydroxypryrrolidine 2-one and pyrimidine -2,4- dione was isolated from the leaves of Jatropha curcas L. by extraction with ethyl acetate and subsequent fractionation of the extract by column chromatography by Ruth staubmann and coworkers[29].

Catherine Auvin, et al., isolated curcacycline B (1), a cyclic nonapeptide from *Jatropha curcas* latex which shows rotamase activity of human cyclophilin B[30].

Three deoxypreussomerins, palmarumycins CPI, JC1and JC2 have been isolated from stem of *Jatropha curcas*. The second and third compounds show antibacterial activity. Isolated compound was characterized by using NMR and X-ray analysis[31].

Aerial parts of the *Jatropha curcas* was examined by Nasi Ravindr Ananth et al., New Lathyrane and Podocarpane Diterpenoids from successive extraction with n hexane and CHCL3-Methanol (1:1)Chemical investigation on *Jatropha curcas* resulted in the isolation of twenty constituents among which four diterpenoids were unknown and sixcompounds, tetradecyl-(*E*)-ferulate,3-*O*-(*Z*)-coumaroyloleanolic acid, heudelotinone ,*epi*-isojatrogrossidione,2a-hydroxy-*epi*-isojatrogrossidione, and 2-methyanthraquinone had not been reported earlier from this species. The structures of the new compounds were established by extensive studies of their 1D- and 2D-NMR spectra[32].

Matsuse IT and coworkers examined the water extract of the branches of Jatropha curcas (Euphorbiaceae) tested for the inhibition of HIV-induced cytopathic effects in cultured cells, HIV-reverse transcriptase (RT) and HIV-protease (PR) enzymes. Inhibited strongly the HIV-induced cytopathic effects with low cytotoxicity[33].

Vitexin and isovitexin have been isolated from the leaves of *Jatropha curcas* by S. Sankara and co workers[34].

Villegas LF et al, detected significant wound-healing activity in *Peperomia galioides, Anredera diffusa* and *Jatropha curcas*[35].

The AM-1 is formulated by Nii-Ayi Ankrash from *Jatropha curcas*, *Gossypium hirsutum*, *Physalis angulata* and *Delonix regia*. In addition male and female Sprague Dawley rats were used to evaluate the acute and sub-chronic toxicity effects of AM-1. The AM-1 eliminated malaria parasites (*Plasmodium falciparum* and *Plasmodium malarie*) from the peripheral blood of patients with malaria. In addition the AM-1 did not show any undesired effects in the patients as well as in laboratory rats[36].

The methanol extract from *Jatropha curcas* was found to produce a moderate cytoprotective effect against HIV in cultured human lyphoblastoid CEM-SS cells[37]. Foetal resorption was observed with methanol, Petroleum ether, and dichloro methane extract of fruit of *Jatropha curcas* indicating the abortifacient property[38].

Pharmacognostic and ethanobotanical studies on the bark of Jatropha curcas was reported by Ahmed J[39]. Methanolic extract of Jatropha curcas leaf was screen for

brine shrimp toxicity, crown gall tumor inhibition, cytotoxicity and DNA interaction. It is most active in cytotoxicity and DNA interaction[40].

The Methanolic extract of defatted roots of Jatropha curcas was fractionated by solvent and yield JC fraction. This JC fraction (100mg/kg) showed activity against castor oil and magnesium sulphate induced diarrhea[41]. Four globulins and seven albumins were identified and isolated from crude water extract of *J.curcas*. Three alkaloids are obtained from the alcoholic extract of the *Jatropha curcas* seeds [42]. The aqueous extract of the latex of Jatropha curcas showed antimicrobial activity[43].

The butanol fraction of latex of *Jatropha curcas* (further extracted with ethanol, ethyl acetate, aqueous) possess both procoagulation and anticoagulant activities former will being evident at high concentration of latex and while the latter is exhibited the lower concentration of the latex[44].

A.M. Majumdar and co-workers reported the anti-inflammatory activity of the Methanolic extract of *Jatropha curcas* against carrageen-induced rat paw oedema, It also shows activity against formalin induced rat paw edema, turpentine induced exudative changes and cotton pellet- induced granular tissue formation after oral treatment of 7 days[45].

Pandit S, Sur TK, Jana U, Debnath PK, Sen S, Bhattacharyya D used ethanolic extract of the leaves of *Adhatoda Vasica* shows Prevention from carbon tetrachloride-induced hepatotoxicity in rats due to the presence of flavonoids, tannins, alkaloids, reducing sugars and saponins. Flavonoids, tannins and microelements have been

suggested to act as antioxidants and exert their antioxidant activity by scavenging the lipid peroxidation[46].

Aqueous leaf extract of *Ocimum gratissimum (Linn),* shows hepotoprotective activity in rabbits because of the presence of flavonoids and saponins (terpene glycosides) [47]. Aqueous extract of fruit pulp of *Annona reticulate* Linn. shows hepatoprotective activity against CCL_4 induced toxicity due to the presence of the steroids glycosides(steroidal saponins), tannins, Flavonoids, triterpenoids proteins and Amino acids[48].

Triterpenoids saponins from the roots and flower buds of *Panax notoginseng* (Burk.) F.H.Chen (Araliaceae) showed potent hepatoprotective effects on liver injury induced by d-galactosamine and lipopolysaccharide (Yoshikawa et al., 2003). The major saponins isolated from the buds, ginsenosides-Rb3 and -Rc, showed stronger hepatoprotective activity than the major saponins isolated from the roots, ginsenoside-Rb1 and -Rg1. Sixteen triterpenoids saponins from *Panax vietnamensis* Ha & Grushv. (Araliaceae) were also found to posses hepatocytoprotective effects on d-galactosamine/tumor necrosis factor-alpha-induced cell death in primary cultured mouse hepatocytes (Tran et al., 2001a). From these results it was concluded that the hepatocytoprotective effect of Vietnamese ginseng is due to dammarane-type triterpene saponins that have an ocotillol-type side chain. Tran et al. (2002) investigated the hepatoprotective effect of majonoside R2 the major saponin constituent from *Panax vietnamensis*. The saponin was tested in vivo on d-galactosamine/lipopolysaccharide-induced hepatic apoptosis and subsequent liver failure in mice[49].

(+)-Dehydrovomifoliol (1), 3-hydroxy-5alpha,6alpha-epoxy-beta-ionone (2), vitexin 7-O-beta-D-glucopyranoside (3), and vitexin 2''-O-beta-D-glucopyranoside (4) were isolated as new constituents from the aerial parts of Beta vulgaris var. cicla. Compounds 3 and 4 demonstrated hepatoprotective activity with values of 65.8 and 56.1%, respectively, in primary cultured rat hepatocytes with CCl_4-induced cell toxicity, compared to controls[50].

OBJECTIVES OF THE STUDY

The liver holds a position of singular importance in the overall functions of the human body. Besides its secretary and excretory functions, it effectively controls numerous vital metabolic processes. The liver is the largest organ in the body and performs diverse functions. It is actively concerned with reticuloendothelial activity and is a storehouse of important nutrients, vitamins and minerals. Its metabolic functions include biotransformation and active participation in metabolism of proteins, fats and carbohydrates.

A large portion of the liver is functionally essential for regulating and maintaining its various activities. Though it possesses a large capacity for regeneration slight damage to the liver by alcohol, toxins or infective agents can hamper its functioning. Thus the normal functioning of liver can be disturbed by various infections, infiltrations or toxic agents such as alcohol, drugs and environmental factors[17].

No effective measures are available for treatment of liver disease in modern medicine. The herbal drugs used in Indian System of medicine are however, claimed to be effective and safe in liver ailments. The efficacy of a particular medicinal herb depends on its chemical constituents either organic or inorganic or combines effect[18].

2.1 NEED FOR THE STUDY:

The major clinical manifestation of liver disorders is jaundice. Despite the extraordinary capacity of regeneration of this organ, a slight injury may lead to fatal complications.

Though liver diseases are among the important diseases affecting mankind, no remedy is available to majority of them at present. However, numbers of medicinal preparations have been advocated in traditional systems of medicine, especially in Ayurveda, for treating liver disorders. Their usage is in vogue since centuries and are quite often claimed to offer significant relief[19]. *Jatropha curcas* Linn. commonly known as ratanjyot, mogalieranda, janglieranda, bhagerendra, physic nut. Family-Euphorbiaceae is widely acclaimed in the old-age Ayurvedic drug with a wide range of biological activity. Fruits, seeds, leaves, flowers, latex, sap and root bark are mainly attributed with various medicinal properties like antidiarrhoeal, stomachic, antidysentric, anthelmentic, thirst, tridosha urinary discharges, abdominal complaints, biliousness, anemia, fistula, diseases of heart(Ayurveda), purgatives, toothache, fever, wounds and refractory ulcers[20].

Jatropha curcas is one of the oldest drug known, it is traditionally used not only in India but world wide.

Brazil: Dried entire plants is taken orally for sinusitis, hot water extract of root is taken orally as an anthelmintic, toothache , fever, and headache.

Cambodia: Seeds extract taken orally as an abortifacient

Cape Varde Islands: Leaf decoction used as lactoguage.

Colombia: Leaf decoction used as febrifuge.

Egypt: Hot water extract of seed is taken orally, for jaundice.

Fiji: Fresh leaf juice taken orally for diarrhea, fever, and as a haemostasis. Fresh stem juice is used externally for sores and sprains.

Guatemala: Hot water extract of the leaf is taken orally as a treatment for dysentery.

Guinea- Bissau: Leaf decoction used as lactoguage.

Haiti: Dried leaf decoction is taken orally, for edema, flu and for cough.

India: Dried branches are applied externally for joint pains. Dried entire plant is taken orally as purgative. Fresh latex is used for toothache. Teeth are cleaned with stem or leaf juice is applied to the painful parts tooth. Fresh leaf juice is taken orally for epilepsy. Hot water extract of seed is taken orally as abortifacient.

Indonesia: Hot water extract of stem is taken orally, to treat matrix cancer and stomach cancer.

Ivory Coast: Fresh leaves are used as a hemostatic.

Mexico: Fresh sap is taken orally for mouth sores, whooping cough.

Nepal: Hot water extract of the leaf is taken orally as a lactoguage.

Nigeria: Leaf decoction used orally as to treat diarrhea, fever, as a rectal injection to treat jaundice, Hot water extract of fresh root is taken orally for jaundice. The infusions are used orally to treat antipyretic and anticonvulsant.

Peru: Hot water extract of seed is taken orally, as a purgative.

Philippines: Fresh bark is used to treat fractures and sprains.

Senegal: Hot water extract of dried leaves is taken orally as treatment for lung disease.

South Africa: Decoction of dried seed is taken orally as a purgative.

Sudan: Seeds are used as oral contraceptives and as an anthelmintic.

Thailand: Seed oil administered orally as a laxative. Seed oil is taken and mixed with a little chilli. The entire plant is taken orally as a purgative.

Tonga: Infusion of dried leaves is taken orally to treat vaginal bleeding.

Venda: Decoction of dried leaves is taken orally as a treatment for toothache. The decoction is used to rinse the oral cavity. The decoction is also taken orally for sore throat.

Vietnam: Seed oil is taken orally as an abortive and as an emmenaguage.

Virgin Island: Hot water extract of the entire plant is administered orally as a treatment for the common cold, either alone or in combination with other plants.

West Africa: Hot water extract of dried leaves are used externally for guinea worm. Hot water extract of seed oil is used externally for parasitic skin diseases as a rubefacient.

West Indies: Hot water extract of the leaf is taken orally for heart troubles.

Zaire: Infusion of dried leaf is taken orally for diarrhea chest pains coughs anemia urinary tract infections diabetes dental caries and infected wounds. Externally, the infusion is used for infected wounds, and for skin diseases[21].

According to Hartwell, the extracts of *Jatropha curcas* are used in folk remedies by Barbadians uses the leaf tea for marasmus, Panamanians for jaundice. In Rajasthan, Leaves are galactagogue, rubefacient, suppurative, insecticidal and are used in foul ulcers, tumors and scabies, given internally in jaundice. In Ghana, *Jatropha curcas* is used in herbal mixture to treat jaundice[22,23].

A folk remedy about *Jatropha curcas* leaves of has not been scientifically investigated for the hepatoprotective activity. As per literature survey the chemical constituents like glycosides, tannins, phytosterol (β-sitosterol, campesterol,

stigmasterol), flavonoids (vitexin, isovitexin), and steroidal sapogenines, possessing hepatoprotective activity, since these are also present in *Jatropha curcas* leaves, hence leaves of *Jatropha curcas* were selected for the chemical investigation and hepatoprotective activity.

In present study the authenticated leaves of *Jatropha curcas* were used. Shade dried leaves of *Jatropha curcas* were powdered and subjected to extraction with petroleum ether (40-60^{0}C), ethanol and water successively. Each time before extracting with next solvent the powdered material was air dried in hot air oven below 50^{0}C. Extracts obtained were subjected to quantitative chemical test, preparative TLC and spectroscopic studies.

All the extracts were screened for the hepatoprotective activity against (CCl_4) carbon tetrachloride induced hepatotoxicity on groups of 6 albino female rats. The results were compared with an Ayurvedic marketed preparation of Liv-52. The hepatoprotective activity was carried out as per the method of S.S. Handa and Anupam Sharma.

The serum enzyme levels, such as SGPT, SGOT, SALP, Serum Bilirubin and Histopathological observation of rat liver tissue were taken as criteria to assess the hepatoprotective activity of the drug.

RESULTS AND DISCUSSION

To assess the hepatoprotective activity of *Jatropha curcas* Linn. leaves, carbon tetrachloride induced hepatotoxicity was produced in female albino rats and parameters like enzyme study (SGOT, SGPT, SALP and Serum Bilirubin) and histopathological studies were carried out and the extent of regenerative changes were observed.

The petroleum ether (40-60^{0}C), alcohol and aqueous extracts were tested for hepatoprotective activity as per the method reported by S.S. Handa and Anupam Sharma[82].

Acute Toxicity Study:

Acute toxicity study was carried out according to OECD guidelines. The following LD_{50} values were obtained for various extracts.

Extracts	*LD_{50} Cut-off*
Petroleum ether extract(40-60^{0}C)	2000 mg/kg, b.w., p.o.
Chloroform extract	2000 mg/kg, b.w., p.o.
Methanol extract	2000 mg/kg, b.w., p.o.
Aqueous extract	2000 mg/kg, b.w., p.o.

1/10th of this lethal dose was taken as effective dose (therapeutic dose) for subsequent hepatoprotective activity i.e. 200 mg/kg b.w., p.o.

Enzyme Level Observation:

The degree of hepatotoxicity developed can be known by elevated levels of SGOT, SGPT, SALP and Serum Bilirubin activity which is attributed to generation of CCl_3 free radical during metabolism by hepatic microsomes which in turn causes

peroxidation of lipids of cellular membrane. In the Table no.12, 13, 14, 15 and Fig. no.4 and it is clear that when CCl_4 was used to induce liver toxicity there is a substantial increase in enzyme activity of SGOT, SGPT, SALP and Serum Bilirubin. Any decrease in the activity of above enzymes would indicate reversed of induced toxicity of liver.

The results indicated that the aqueous extract showed significantly reduced the elevated levels of SGOT SGPT SALP and Serum Bilirubin when compared to other test extracts. Petroleum ether extract and alcohol extract has reduced the elevated levels of SGOT SGPT SALP and Serum Bilirubin to lesser extent compared to aqueous extract Petroleum ether extract has not reduced elevated levels of SGOT,SGPT, SALP and Serum Bilirubin thus was insignificant. The aqueous extract has reduced the increased SGOT levels from 152.2 IU/L to 62.17 IU/L, SGPT levels from 142.8 IU/L to 48.67 IU/L, SALP levels from 90.35 K.A. units to 47.68 K.A. units and serum bilirubin levels from 12.5 mg/dl to 5.85 mg/dl. Alcohol extract has reduced the increased SGOT levels from 152.2 IU/L to 76.5 IU/L, SGPT levels from 142.8 IU/L to 61.33 IU/L, SALP levels from 90.35 K.A. units to 57.43K.A. units and Serum bilirubin levels from 12.5 mg/dl to 5.853 mg/dl. Petroleum ether extract has reduced the increased SGOT levels from 152.2IU/L to 96 IU/L, SGPT levels from 142.8 IU/L to 68.67 IU/L, SALP levels from 90.35 K.A. units to 80.5 K.A. units and Serum bilirubin levels from 12.5 mg/dl to 10.87 mg/dl. While the standard drug Liv-52 has reduced increased SGOT levels from 152.2 IU/L to 59.33 IU/L, SGPT levels from 142.8 IU/L to 42.8 IU/L, SALP levels from 90.35 K.A. units to 44.13 K.A. units and Serum bilirubin levels from 12.5 mg/dl to 5.232 mg/dl.

The enzymatic levels of SGOT, SGPT are indicated in the Table no.12, 13 14 and 15 respectively.

Histopathological Observations:

The microscopic histopathological evaluation of livers from control group, CCl_4 treated group, petroleum ether extract, alcoholic extract, aqueous extract and standard drug Liv-52 have revealed the following observations.

Control (A) : Observed under 100 x H.E of magnification, showed liver tissue with typical lobular arrangement. Individual lobules consist of hepatocytes arranged as cords radiating around centrally placed terminal hepatic veins. Hepatocytes seen are uniform in size, polyherbal in shape, with centrally located large nuclei. The cytoplasm is strongly eosinophilic with a fine basophilic granularity. Portal tracts containing terminal branches of the hepatic portal vein and hepatic artery at the periphery in fibrous stroma are also seen.

Impression : Normal liver tissue

CCl_4 treated (B) : Observed under 100 x H.E of magnification showed liver tissue with disturbances in the lobular arrangement. Degenerative and early necrotic changes extending across lobules. Hepatocytes show ballooning degeneration and steatotic changes. Some amount of fibrosis seen in portal tracts.

Impression: Liver with cytotoxic injury showing mild necrosis and fibrotic changes.

Standard Liv-52 (C) : Observed under 400 x H.E of magnification, showed liver tissue with typical lobular arrangement. Hepatocytes shows variable size. There is a mild increase in fibrous connective tissues.

Impression : Liver with mild sign of hepatotoxicity.

Petroleum ether extract (D)	:	Observed under 100 x H.E of magnification showed liver tissue with disturbances in the lobular arrangement. Degenerative and early necrotic changes extending across lobules. Apoptotic cells and Mallory's bodies are seen. PMN's are seen along dilated blood vessels. Some amount of fibrosis along the portal tract and sinusoids are seen. Impression: Liver with cytotoxic injury
Alcohol extract (E)	:	Observed under 400 x H.E of magnification, showed liver tissue with typical lobular arrangement. Hepatocyte shows variable size. Ballooning changes, steatotic accumulation. There is a mild increase in fibrous connective tissues. inflammatory cells are seen within the parenchyma. Impression : Liver with sign of hepatotoxicity.
Aqueous extract (F)	:	Observed under 100 x H.E of magnification, showed liver tissue with typical lobular arrangement. Few hepatocytes shows steatotic accumulation. Impression : Liver with minimal sign of hepatotoxicity.

The above histopathological observations revealed that aqueous extract treated livers were found to have normal hepatocytes and normal architecture which signifies better hepatoprotective activity as compared to other groups.

Alcohol extract treated liver was found to have moderate hepatocytic degeneration which shows moderate hepatoprotective activity as compared to other groups.

The details of Histopathological observations are shown in Fig .no.4

The enzyme levels and histopathological observation of rat liver indicated that aqueous extract and alcoholic extract have exhibited prominent hepatoprotective

activity to standard Liv-52. While alcoholic extract has exhibited moderate hepatoprotective activity and Petroleum ether has not shown any hepatoprotective activity.

Chromatographic studies

The qualitative TLC/ HPTLC showed a blue colour band for saponin and steroids at R_f value 0.76 and 0.76 using anisaldehyde sulphuric acid reagent respectively for saponin and steroid. Whereas, the isolated compound showed a blue coloured band at R_f value 0.77 and 0.76 with the above reagent.(See annexure A-2). The saponin and steroid were further characterized by spectral studies.

- **UV Spectrum of isolated compounds**

UV spectrum was for isolated Saponin A from aqueous extract which gave a sharp peak at 255 nm and Saponin B from aqueous extract which gave a sharp peak at 250 nm. UV spectrum of isolated compounds from petroleum ether extract gave a sharp peak at 249 nm Spectra are given in annexure A-3.

- **FT-IR Spectroscopy of Isolated compound**

The FTIR spectrum of isolated compounds (saponin A) from aqueous extract showed characteristic absorption at 3370.60 cm^{-1}(Free-OH stretching, Phenolic) , 2926.24 cm^{-1} (C-H stretching), 1596.95 cm^{-1} (C=O group), 1405.37 cm^{-1} (O-H deformation), 1343.20 cm^{-1} (C-O ether linkage), 1116.67 cm^{-1},762.62 cm^{-1} (C-O Stretching, C-H deformation).

The FTIR spectrum of isolated compounds (saponin B) from aqueous extract showed characteristic absorption at 3411.08 cm^{-1} (Free-OH stretching, Phenolic)

2924.24 cm^{-1} (C-H stretching) ,2213.90 cm^{-1} (Carboxyl), 2104.11 cm^{-1} (Ester group) ,1596.95 cm^{-1} (C=O group),1405.37 cm^{-1} (O-H deformation),1343.20 cm^{-1} (C-O ether linkage), 1116.67 cm^{-1}, 762.62 cm^{-1} (C-H deformation).

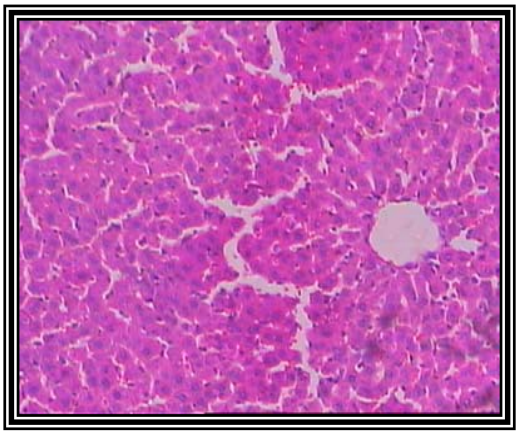
The FTIR spectrum of isolated compound (steroid) from Petroleum ether extract showed characteristic absorption at 3470.07 cm^{-1} (Free-OH stretching, Phenolic), 2924.24 cm^{-1} , 2733 cm^{-1} , 2612.20 cm^{-1} (C-H stretching), 1456 cm^{-1}, 1240.16 cm^{-1}, (CH_2 stretching) 930.93 cm^{-1} (-CH=OH bending), 1051.71 cm^{-1} (C-O stretching). Spectra are given in annexure A-4.

- **P - NMR Spectroscopy:**

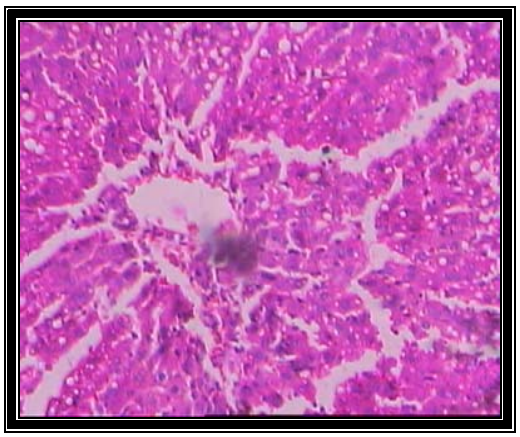
The ^{1}H- NMR spectrum of the isolated compound (Saponins A) from aqueous extract showed chemical shifts signals in DMSO at δ 2.506 (doublet), δ 2.504 (doublet), δ 1.22(singlet); chemical shift between δ 2.08- δ 1.22 (m) indicates aliphatic nature of compound and δ 8.262 (s) indicates OH group. The ^{1}H- NMR spectrum of the isolated compound (Steroid A) from pet ether extract showed chemical shifts signals in $CDCL_3$ at δ 2.17(triplet), δ 1.680 (doublet), δ 1.25 (singlet), δ 0.025 (singlet), δ 0.8620 (singlet); chemical shift between δ 2.38- δ 1.25 (m) indicates aliphatic nature of compound, δ 5.123 (s) indicates OH group. Spectra are given in annexure A-5.

From this data indicate that the compounds isolated from aqueous extract and petroleum ether extract may be saponin and steroid respectively this claims demands further spectral studies for its structural elucidation and identification.

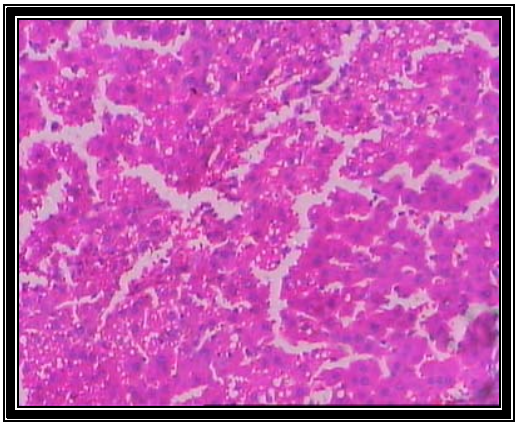
Fig. 4. Histopathological Microphotograph of Rat liver tissue a) Control group b) CCl_4 Treated group c) Standard Liv-52 group d) Pet. ether group e) Alcoholic group f) Aqueous group



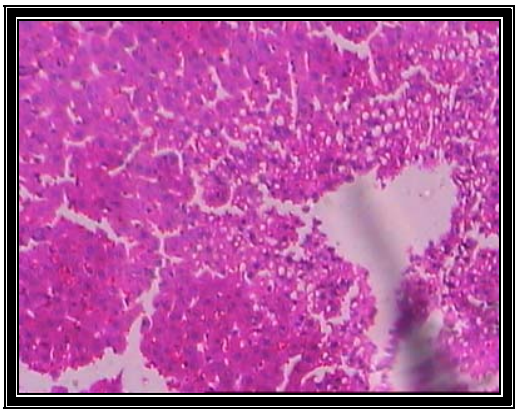
a) Control group



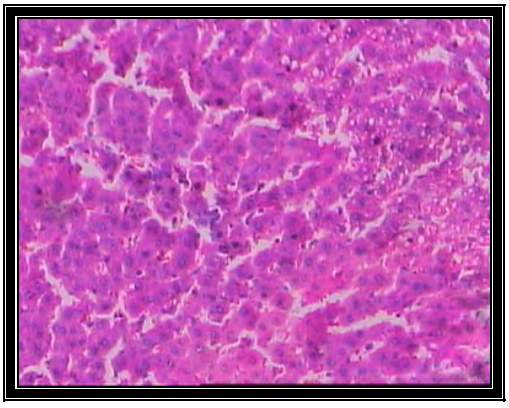
b) CCl_4 Treated group



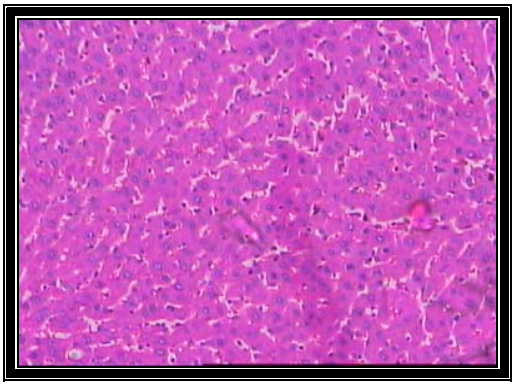
c) Standard Liv-52 group



d) Pet. ether group



e) Alcoholic group



f) Aqueous group

SUMMARY AND CONCLUSION

In the present study, powdered shade dried leaves of *Jatropha curcas* Linn of family *Euphorbiaceae* were subjected to soxhlet extraction successively using solvents Petroleum ether (40-60^{0}C), Alcohol and Water extracts were subjected for preliminary Phytochemical investigations and Pharmacological screening.

The Petroleum ether extract shown the presence of steroids and carbohydrates.

The Alcoholic extract shown the presence of glycosides, saponins, carbohydrates, and alkaloids.

Aqueous extract shown the presence of carbohydrates, flavonoids, steroids, saponins, amino acids, glycosides, and triterpenoids.

The petroleum ether and aqueous extracts was subjected to thin layer Chromatography. The Chromatogram was developed in the mobile phase like Chloroform: Methanol (80: 20) system for petroleum ether extract and Chloroform: Methanol: Water (70: 30: 40) system for aqueous extract. The anisaldehyde H_2SO_4 was used as a spraying reagent. The isolated compound by preparative thin layer chromatography was subjected to thin layer Chromatography by side of extract, in same mobile phase and using same spraying reagent.

The petroleum ether extract showed two spots of which spot just below solvent front was at same Rf of that isolated compound R_f i.e. petroleum extract showed R_f = 0.77 and Rf of isolated compound was 0.76. See annexure A-2.

The aqueous extract also showed two spots of which spot just below solvent front was at same R_f of that isolated compound R_f i.e. petroleum extract showed R_f = 0.77 and Rf of isolated compound was 0.77. See annexure A-2.

The results of the phytochemical investigation have led to the conclusion that aqueous extract extract of *Jatropha curcas* Linn. leaves contain steroids, glycosides carbohydrates, flavonoids saponins, amino acids, and triterpenoids as major active principles. The presence of saponins and steroid compound was confirmed by TLC study.

The petroleum ether (40-60^{0}C) extract, alcoholic extract and aqueous extracts were screened for the hepatoprotective activity. In the present study, CCl_4 was used as hepatotoxic agent. It is well established that hepatotoxicity by CCl_4 is due to enzymatic activation to release CCl_3 radicals in free state, which in turn disrupt the structure and function of lipid and protein macromolecules in the membrane of the cell organelles.

After the treatment with CCl_4 there was significant rise in serum GOT, SGPT, SALP and serum bilirubin levels as compared to control animals. The hepatoprotective activity of all extracts was compared with marketed preparations Liv-52. The Himalaya Drug Company, Makali, Bangalore. The said measurements of SGOT, SGPT, SALP and serum bilirubin levels are recorded in the histopathological observation of rat liver tissue.

The results obtained from the pharmacological screening have led to the conclusion that aqueous extract showed more prominent hepatoprotective activity, alcoholic showed moderate hepatoprotective activity while petroleum ether (40-60^{0}C) extract had not shown any activity from parameters studied i.e. enzymes levels estimation and histopathoogical studies.

The aqueous extract of *Jatropha curcas* Linn. shown decrease in enzyme activity of SGOT, SGPT, SALP and serum bilirubin which has been shown to be

inducer of the microsomal enzymes, supporting the folk information regarding hepatoprotective property. Thus, hepatoprotective action of this drug is likely to be due to its ability to induce microsomal enzymes, thereby accelerating the excretion of CCl_4 or could be due to inhibition of lipid peroxidation induced by CCl_4. The hepatoprotective activity may be due to the combine effect of carbohydrates, flavonoids, steroids, saponins, amino acids, glycosides, and triterpenoids.

However, this claim demands further study of isolation of individual components and observing their effect in the protection of liver against hepatotoxins. Till date, *Jatropha curcas* has been studies exhaustively; especially on its tremendous potential to produce biodiesel from seeds. However, it can be evaluated for its other medicinal properties as per the literature.

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A-2 HPTLC POFILE OF LEAVES OF *JATROPHA CURCAS* LINN.

a) 3D views of all tracks at 366 nm before derivatization

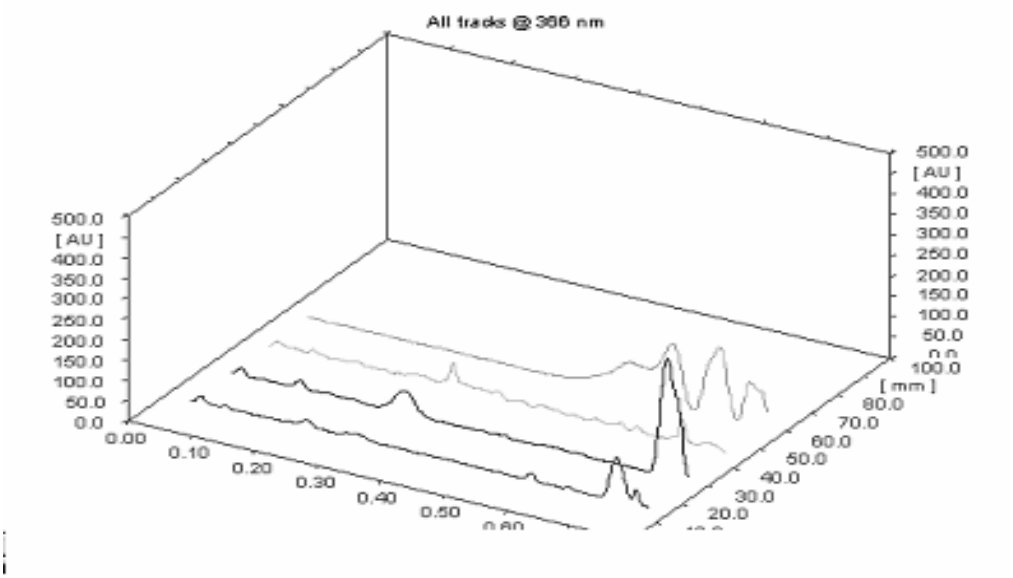


Fig-5 HPTLC Profile (3d View) of the extracts and isolated compounds at and 366 nm

a) Aqueous JC

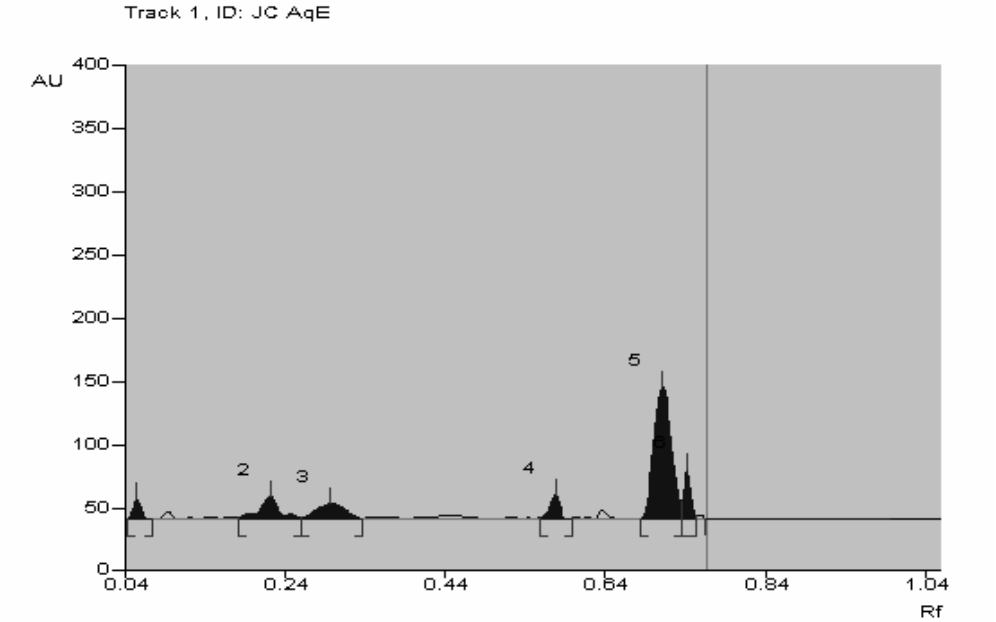
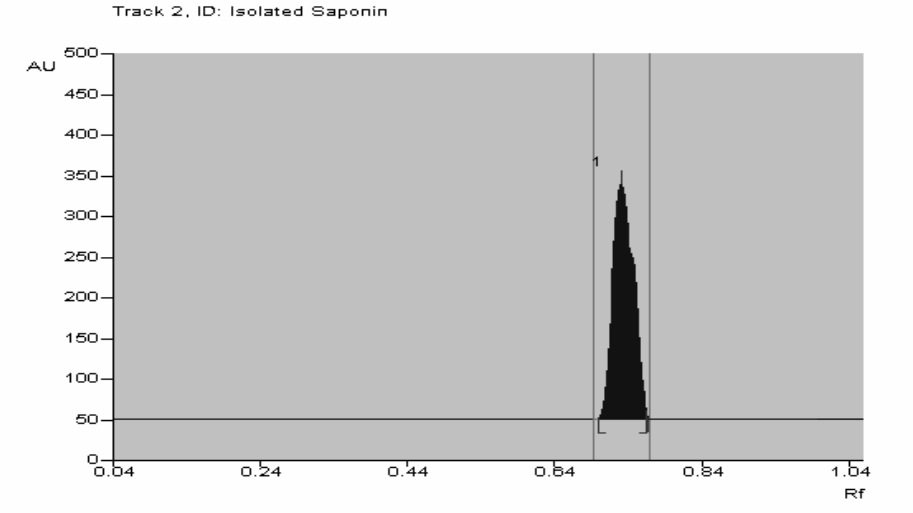
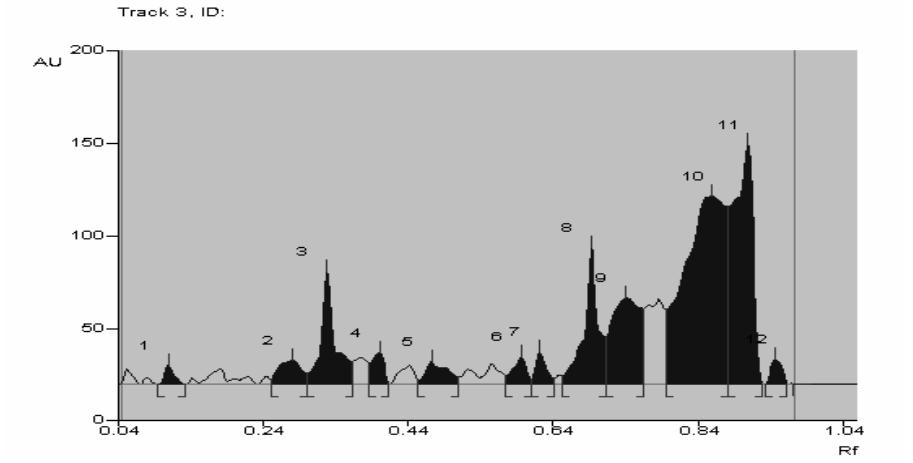


Fig-6 HPTLC screening of the extracts and isolated compound at 366 nm.

b) Isolated compound



c) Pet. Ether JC



d) Isolated compound

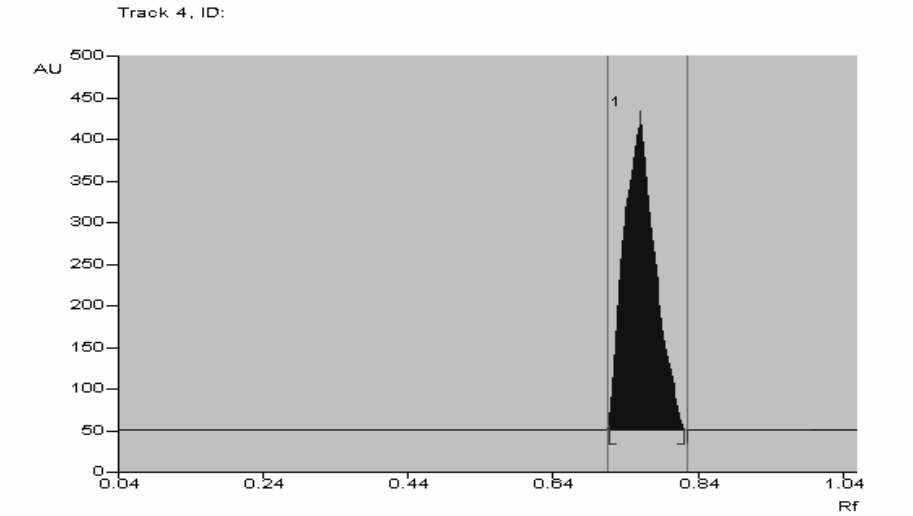
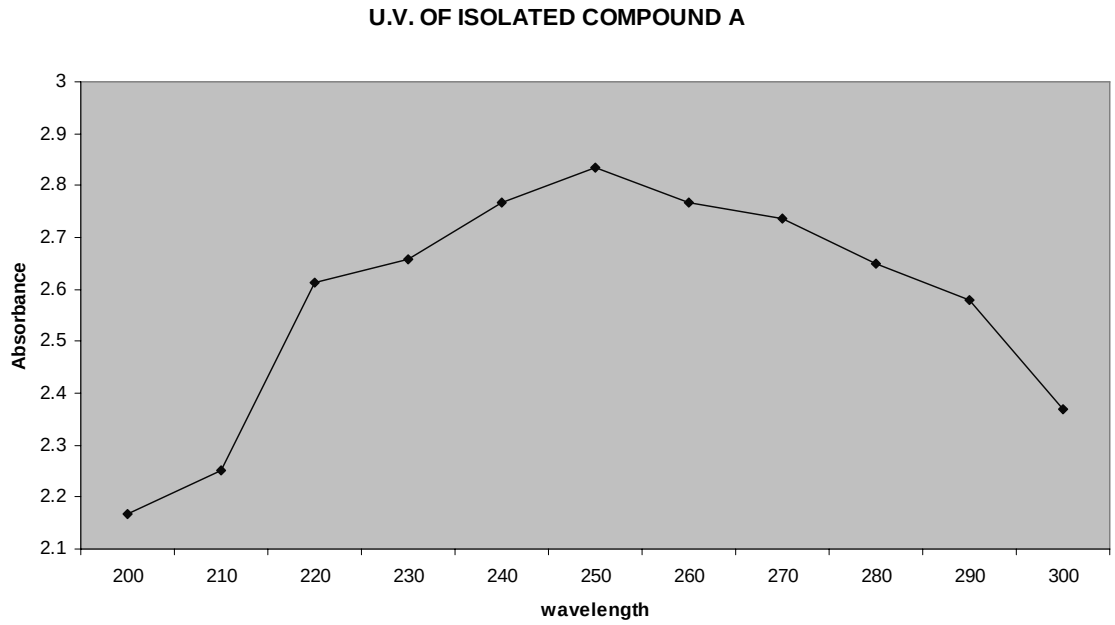


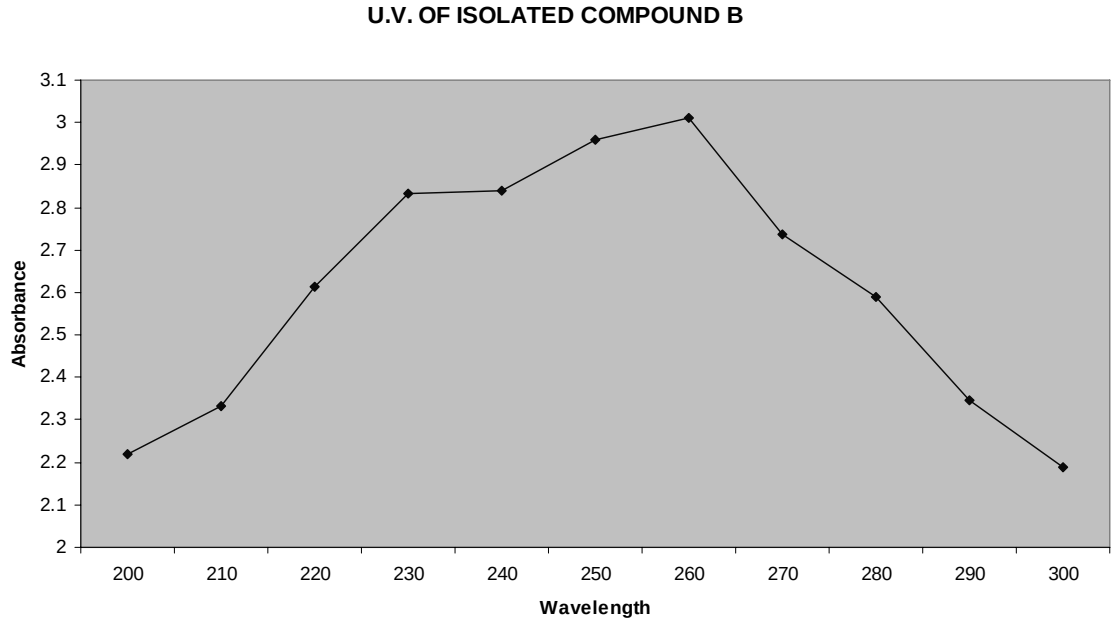
Fig-7 HPTLC screening of the extracts and isolated compound at 366 nm.

Table No. 16: HPTLC profile (at 366 nm) of the extracts of the *Jatropha curcas* Linn. and isolated compound

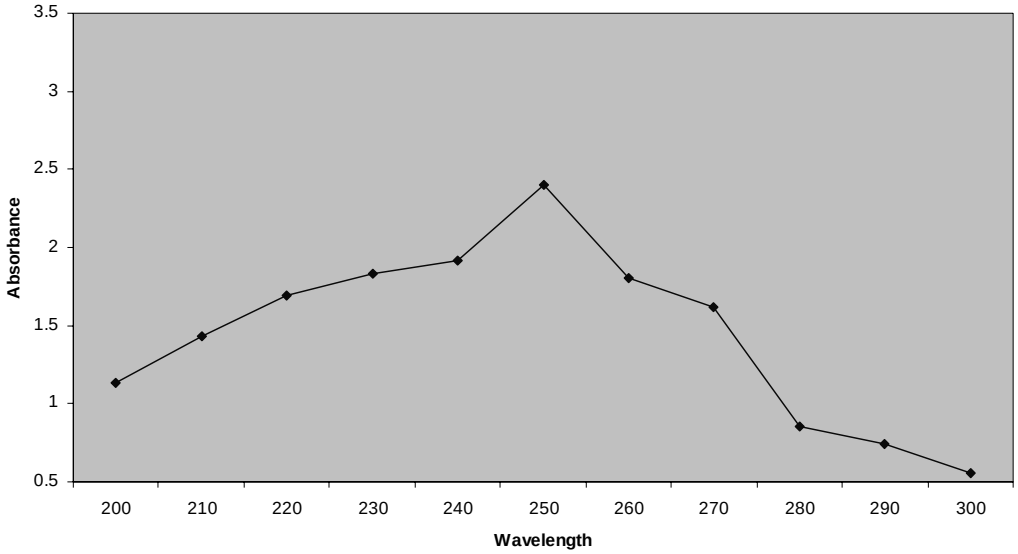
Sr.No.	Track	Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
Track 1 ID Aqueous extract JC											
1.	1	1	0.04	0.1	0.06	15.4	7.41	0.08	0.1	92.8	4.18
2.	1	2	0.18	0.9	0.22	17.8	8.56	0.26	1.4	243.3	10.96
3.	1	3	0.26	1.4	0.30	12.3	5.90	0.34	0.1	247.6	11.15
4.	1	4	0.56	0.8	0.58	19.2	9.26	0.60	0.9	129.5	5.83
5.	1	5	0.69	0.1	0.71	104.8	50.40	0.74	13.3	1301.6	58.61
6.	1	6	0.74	13.3	0.74	38.4	18.47	0.76	2.9	206.0	9.28
Track 2 ID Aqueous isolated											
2.	2	1	0.70	0.2	0.73	284.2	75.05	0.77	10.2	4521.3	76.55
Track 3 ID Pet. Ether JC											
1.	3	1	0.10	3.9	0.11	13.9	6.39	0.13	3.1	139.6	6.13
2.	3	2	0.25	4.5	0.28	13.9	6.39	0.30	7.0	273.7	12.02
3.	3	3	0.30	7.0	0.33	62.2	28.50	0.36	12.7	674.5	29.62
4.	3	4	0.39	11.3	0.40	17.3	7.94	0.41	0.7	167.6	7.36
5.	3	5	0.46	1.4	0.47	11.6	5.30	0.51	2.6	185.3	8.14
6.	3	6	0.58	2.6	0.60	12.5	5.73	0.61	0.5	114.0	5.01
7.	3	7	0.61	1.4	0.62	14.7	6.74	0.64	0.2	95.2	4.18
8.	3	8	0.67	2.4	0.69	57.2	26.23	0.71	1.9	401.3	17.62
9.	3	9	0.71	1.9	0.74	14.8	6.79	0.76	2.7	225.7	9.91
Track 4 ID Pet. Ether Isolated											
1.	4	1	0.72	1.5	0.74	97.5	20.08	0.75	81.0	1071.4	11.02

A-3 U.V. ABSORBANCE OF ISOLATED COMPOUND



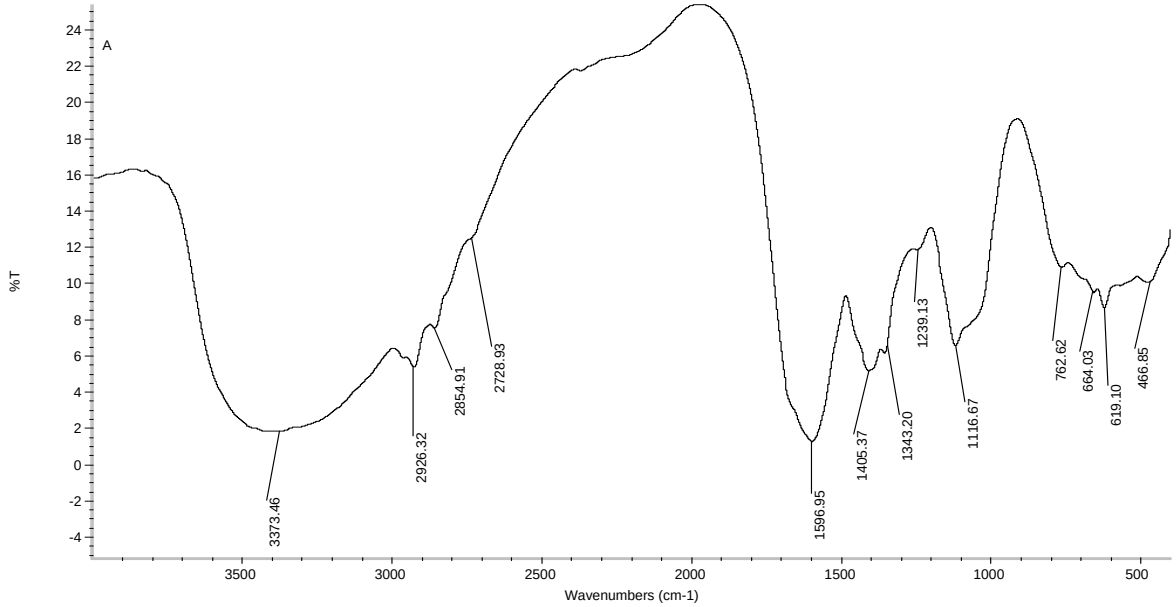


U.V. OF ISOLATED COMPOUND C(PET ETHER EXTRACT)

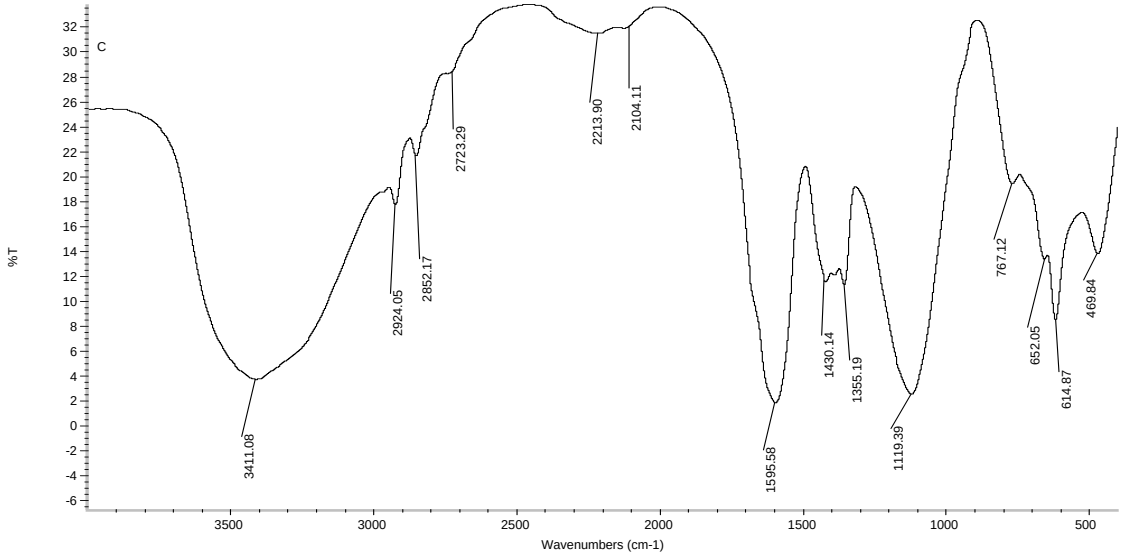


A-4 I.R. SPECTRUM OF ISOLATED COMPOUND

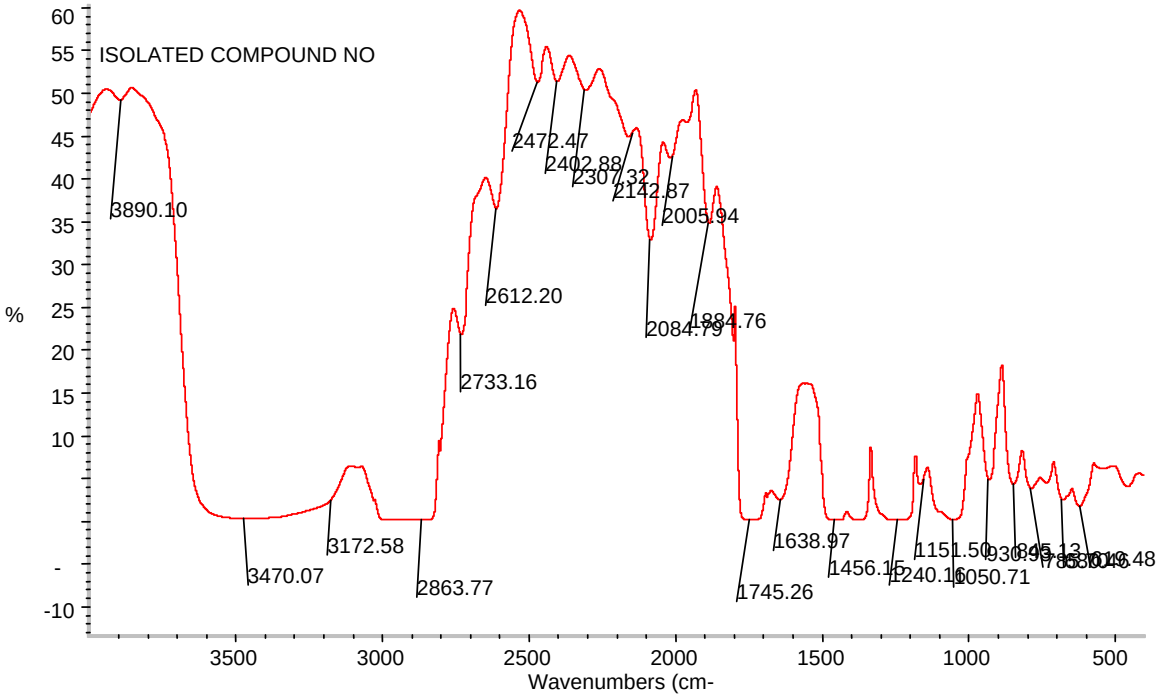
Isolated compound : Saponin A



Isolated Compound : Saponin B



Isolated Compound Pet. ether



GRAPH NO. 4: ENZYMATIC LEVEL OF SERUM BILIRUBIN

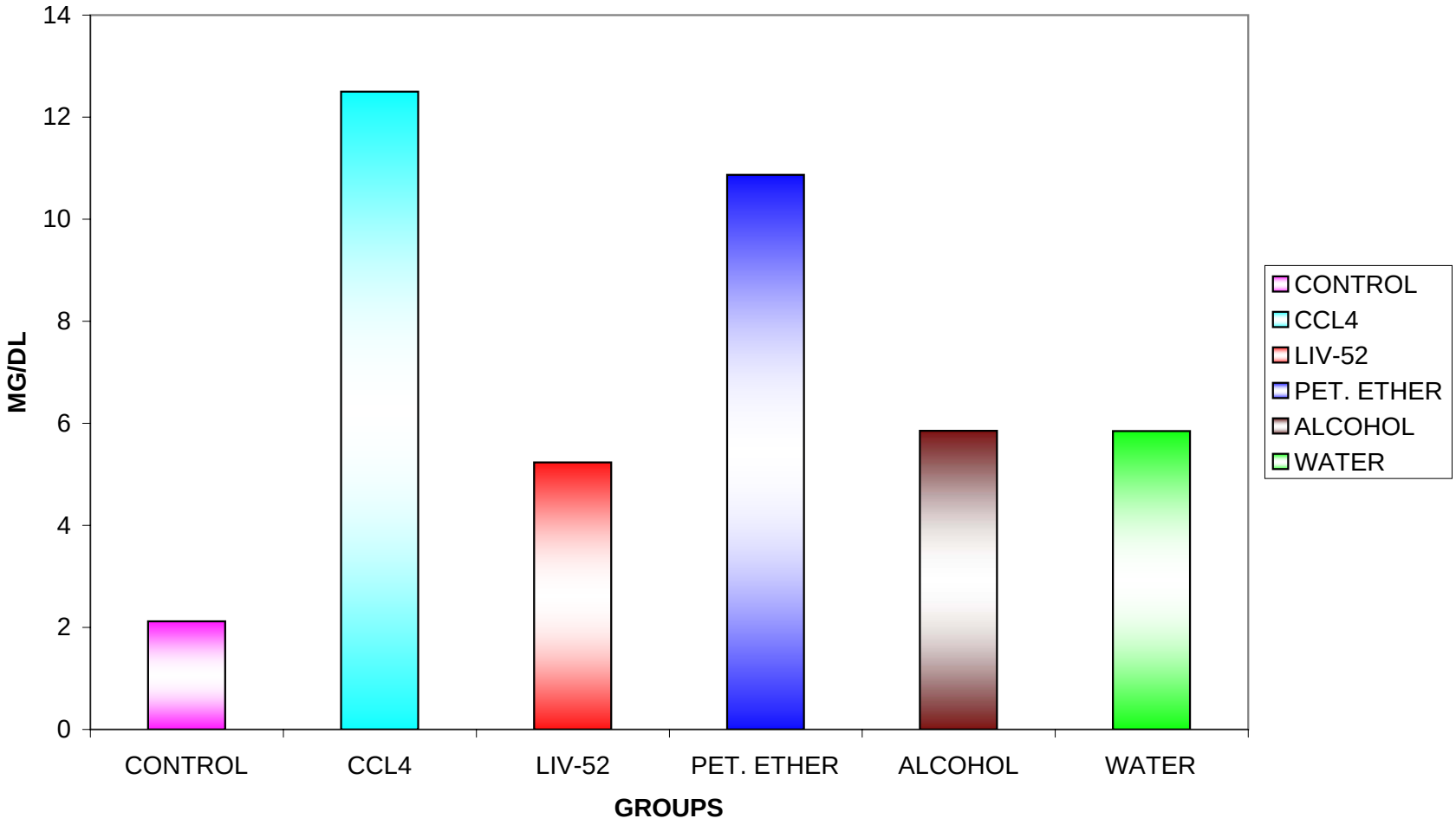


Table No.12 : Table Showing Enzymatic (SGOT) Levels

Sl.No.	Control A IU/L	CCl_4 B IU/L	Standard C IU/L	Pet. Ether D IU/L	Alcohol E IU/L	Aqueous F IU/L
1	45	147	52	91	88	62
2	42	151	47	100	68	50
3	47	155	62	95	83	65
4	42	148	62	108	79	67
5	44	160	68	89	72	60
6	45	152	65	93	69	69
Mean	44.17	152.2	59.33	96	76.5	62.17
S.D.	1.941	4.792	8.09	6.986	8.118	6.795
S.E.	0.7923	1.956	3.303	2.852	3.314	2.774
F ratio *	213.03					
p value			p<0.01	p<0.05	p<0.01	p<0.01
Significance			S	IS	S	S

S - Significance p<<0.01

IS - Insignificance

HS - Highly significance p<0.001

Table No.13 : Table Showing Enzymatic (SGPT) Levels

Sl.No.	Control A IU/L	CCl_4 B IU/L	Standard C IU/L	Pet. Ether D IU/L	Alcohol E IU/L	Aqueous F IU/L
1	41	136	36	65	60	49
2	39	138	42	62	56	48
3	38	144	48	70	58	38
4	30	148	44	72	68	50
5	32	141	35	75	64	52
6	35	150	52	68	62	55
Mean	35.83	142.8	42.83	68.67	61.33	48.67
S.D.	4.262	5.529	6.65	4.719	4.32	5.785
S.E.	1.74	2.257	2.713	1.926	1.764	2.362
F ratio *	330.37					
p value			p<0.01	p<0.05	p<0.01	p<0.01
Significance			S	IS	S	S

S - Significance IS - Insignificance HS - Highly significance

p<<0.01 p<0.001

Table No.14 : Table Showing Enzymatic (SALP) Levels

Sl.No.	Control A IU/L	CCl_4 B IU/L	Standard C IU/L	Pet. Ether D IU/L	Alcohol E IU/L	Aqueous F IU/L
1	28.1	82.2	42.9	75	59.2	42.2
2	25.1	97.2	46	87	37.4	51.1
3	26.2	100.2	45.8	90	62.8	62
4	24.9	80.9	42.8	72	64	45.9
5	25.8	92.5	46.4	80	66.2	44
6	26.4	89.1	40.9	79	55	40.9
Mean	26.08	90.35	44.13	80.5	57.43	47.68
S.D.	1.151	7.821	2.24	6.892	10.58	7.867
S.E.	0.47	3.193	0.9157	2.814	4.317	3.212
F ratio *	71.56					
p value			p<0.01	p<0.05	p<0.01	p<0.01
Significance			S	IS	S	S

S - Significance p<<0.01

IS - Insignificance

HS - Highly significance p<0.001

Table No.15 : Table Showing Enzymatic (SERUM BILIRUBIN) Levels

Sl.No.	Control A IU/L	CCl_4 B IU/L	Standard C IU/L	Pet. Ether D IU/L	Alcohol E IU/L	Aqueous F IU/L
1	2.34	9.52	3.25	10.1	5.2	5.2
2	2.21	12.6	4.17	9.1	7.38	5.1
3	2.31	13.8	6.08	10.5	6.08	4.9
4	2.01	12.6	5.08	11.5	5.06	7.5
5	2.16	13.6	7.01	12.2	5.8	6.8
6	2.1	12.9	5.8	11.8	5.6	5.6
Mean	2.188	12.5	5.232	10.87	5.853	5.85
S.D.	0.1254	1.547	1.36	1.174	0.8373	1.056
S.E.	0.05121	0.6314	0.5562	0.4794	0.3418	0.4311
F ratio *	71.54					
p value			p<0.01	p<0.05	p<0.01	p<0.01
Significance			S	IS	S	S

S - Significance IS - Insignificance HS - Highly significance

p<<0.01 p<0.001