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EVALUATION FOR EXTRACTION, PHYTOCHEMICAL ANALYSIS AND HEPATOPROTECTIVE POTENTIAL OF PLANT EXTRACT OF CURCUMA CAESIA

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ABSTRACT

Herbs plays an important role in the treatment of various diseases. Medicinal herbs are significant source of hepatoprotective drugs. Many clinical researchhas also shown that herbal have genuine utility in the treatment of liver diseases. There are many herbal drugslike kali haldi which are hepatoprotective and are used inmany liver diseases. Herbal based therapeutics for liver disorders has been used in india for a long time and has been popularised world over by leading pharmceuticals. Large number of plants and formulations have been claimed to have hepatoprotective activity. Therefore dueimportance has been given globally to develop plant based drugs.

KEYWORDS:- Liver, Liver diseases, Herbal drugs, Hepatoprotective drugs.

1. INTRODUCTION

Since time immemorial, mankind has made the use of plants in the treatment of various ailments. The Indian Traditional Medicine like Ayurveda, Siddha and Unani are predominantly based on the use of plant materials. Herbal drugs have gained importance and popularity in recent years because of their safety, efficacy, availability and cost effectiveness. The association of medicinal plants with other plants in their habitat also influences their medicinal values in some cases. In modern technology world new ideas excels the traditional use in more effective and valuable way.

In this view present project has been designed to improve the traditional efficacy of a crude drug in a new type of formulation. In this formulation important component has been obtained from plant material and combination with the other natural components which itself

promote their bioavailability, effectiveness with required safety and stability. Many herbal drugs and formulations are used for the protection of liver from their toxicity and their improvement in case of infections. Hence, there is an ever in- creasing need for safe hepatoprotective agent is always required.[1]

There is no any such drug or formulations which can assure the cure from that ailment. In search of that one of the most useful and spiritually well known drug kali haldi has been selected. The most effective bioactive components have been separated and formulated in combination with other natural vehicle for instant effect of drug.

1.1 Liver

Liver is considered to be one of the most vital organs that functions as a centre of metabolism of nutrients such as carbohydrates, proteins and lipids and excretion of waste metabolites. Additionally, it is also handling the metabolism and excretion of drugs and other xenobiotics from the body thereby providing protection against foreign substances by detoxifying and eliminating them. The bile secreted by the liver has, among other things, plays an important role in digestion. Liver cell injury caused by various toxicants such as certain chemotherapeutic agents, carbon tetrachlo-ride, thioacetamide etc., chronic alcohol consumption and microbes is well-studied.

Enhanced lipid peroxidation during metabolism of ethanol may result in development of hepatitis leading to cirrhosis.

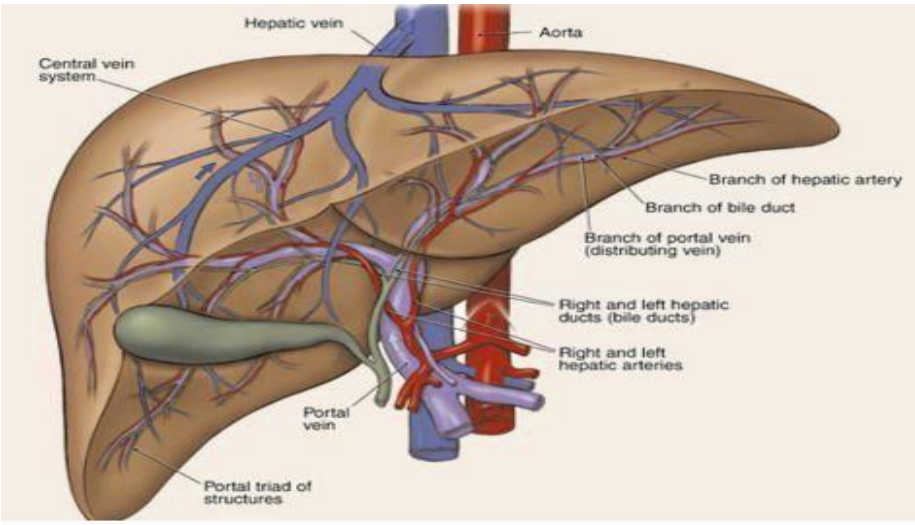

Figure 1.1: Anatomy of the liver.

1.2 Liver diseases

Liver is the largest organ inside our body. It helps our body digest food, store energy, and remove poisons.

There are many kinds of liver diseases

- Diseases caused by viruses, such as hepatitis A, hepatitis B, and hepatitis C.
- Diseases caused by drugs, poisons, or too much alcohol. Examples include fatty liver disease and cirrhosis.
- Liver cancer.
- Inherited diseases, such as hemochromatosis and Wilson disease.

Symptoms of liver disease can vary, but they often include swelling of the abdomen and legs, bruising easily, changes in the color of your stool and urine, and jaundice, or yellowing of the skin and eyes. Sometimes there are no symptoms. Tests such as imaging tests and liver function tests can check for liver damage and help to diagnose liver diseases.

1.3 Treatment of liver disease

Each liver disease will have its own specific treatment regimen. For example, hepatitis A requires supportive care to maintain hydration while the body's immune system fights and resolves the infection. Patients with gallstones may require surgery to remove the gallbladder. Other diseases may need long-term medical care to control and minimize the consequences of their disease.

In patients with cirrhosis and end-stage liver disease, medications may be required to control the amount of protein absorbed in the diet. The liver affected by cirrhosis may notbe able to metabolize the waste products, resulting in elevated blood ammonia levels and hepatic encephalopathy. Low sodium diet and water pills (diuretics) may be required to minimize water retention.

In those with large amounts of ascites fluid, the excess fluid may have to be occasionally removed with a needle and syringe (paracentesis). Using local anesthetic, aneedle is inserted through the abdominal wall and the fluid withdrawn. Operations may be required to treat portal hypertension and minimize the risk of bleeding. Liver is the final option for patients whose liver has failed.

1.4 Herbal treatment

Medicinal herbs are significant source of hepatoprotective drugs. Mono and poly- herbal preparations have been used in various liver disorders. According to one estimate, more than 700 mono and poly-herbal preparations in the form of decoction, tincture, tablets and capsules from more than 100 plants are in clinical use. A drug having beneficial effect on the liver transplant is known as hepatoprotective drug. On the other hand, drugs having toxic affect on the liver are better known as hepatotoxic drugs. Clinical research has also shown that herbals have genuine utility in the treatment of liver diseases.[2-3]

1.4.1 Classification

These are generally classified into 3 categories without any strict delineation amongstthem.

- **Anti hepatotoxic agents:** These generally antagonize the effects of anyhepatotoxins causing hepatitis or any liver disease.
- **Hepatotropic agents:** These generally support or promote the healing processof the liver. In practice these two activities cannot be easily distinguished from each other.
- **Hepatoprotective agents:** These generally prevent various types of liver affections prophilactically. In general any hepatoprotective agent can act as an anti hepatotoxic or hepatotropic agent but the vice versa is always not true.

1.5 Hepatoprotective herbs

Herbal-based therapeutics for liver disorders has been in use in India for a long time andhas been popularized world over by leading pharmaceuticals. Despite the significant popularity of several herbal medicines in general, and for liver diseases in particular, they are still unacceptable treatment modalities for liver diseases. The limiting factors that contribute to this eventuality are:

(i) Lack of standardization of the herbal drugs.
(ii) Lack of identification of active ingredients(s)/principles(s).
(iii) Lack of randomized controlled clinical trials (RCTs).
(iv) Lack of toxicological evaluation.

The use of natural remedies for the treatment of liver diseases has a long history, starting with the Ayurvedic treatment, and extending to the Chinese, European and other systems of traditional medicines. The 21st century has seen a paradigm shift towards therapeutic evaluation of herbal products in liver disease models by carefully synergizing the strengths of

the traditional systems of medicine with that of the modern concept of evidence-based medicinal evaluation, standardization and randomized placebo controlled clinical trials to support clinical efficacy.[1]

A large number of plants and formulations have been claimed to have hepatoprotective activity. Nearly 160 phytoconstituents from 101 plants have been claimed by Pharmacopeia Foundation to possess liver protecting activity. In India, more than 87 plants are used in 33 patented and proprietary multi-ingredient plant formulations. In spite of the tremendous advances made, no significant and safe Hepatoprotective agents are available in modern therapeutics. Therefore, due importance has been given globally to develop plant-based hepatoprotective drugs, effective against a variety of liver disorders.[5]

1.6 Natural Products and Plants as liver protecting drugs

The successful therapy of liver depends on identification of pathogens and elaboration of suitable models for hepatic injuries *in vivo* and *in vitro* test model systems are available to screen the antihepatotoxic activity1 of any substance. For the *in vivo* models. The dose of a known hepatotoxin like CCl4, D-galactosamine (D-gal N), alcohol, thioacetamide etc., which produces a marked and measurable effect, is administered to the animal. The magnitude of toxic effect is measured by some suitable parameters e.g., by determining the activity of serum glutamate oxalacetate transaminase (SGOT) and serum glutamate pyruviate transaminase (SGPT) or by recording the increase in hesobarbital sluptum or by histological examination of liver. *In vitro* models employing primary cultured hepatocytes and using CCl4 or D-Gal N or ethanol as toxins have been devised. Phytoconstituents with elucidated structures or otherwise have been classified under appropriate chemical groups.[4]

Phenyl propanoidsalkaloids

Organic acids and lipidsflavanoids

Quercetin glycosides saponins diterpenoidsterpenoids essential oilsphenols

2. Review of literature

Baranwal *et al.*, (2018) developed a nano curcumin chewable tablet which can provide promising results for curcumin to improve its biological activities. Tablet was evaluated for Weight variation test, Friability, Hardness and Time required for complete chewing and are found to be with inacceptable limits. This study was focused to make a nano curcumin chewable tablet. These tablets are also very cost effective and can give to the children who

have difficulty in swallowing and to the adults who dislike swallowing. Chewable tablets are a help to improved patient acceptance through pleasant taste, patient convenience. This formulation of nano curcumin chewable tablet will increase the bioavailability of curcumin which results in better absorption of curcumin in the human body. This will enhance the effectiveness of nano curcumin chewable tablet was successfully develop. It is yellowish in color with the pleasant odor and taste.[6]

Rachmawati *et al.*, (2014) formulated tablet containing curcumin nanoemulsion for oral delivery. Curcumin nanoemulsion with particle size of 125.7± 1.029 nm and percentage of drug encapsulated of 99.52% was formed. Tablet containing this nanoemulsion has good physical characteristics with hardness of 5.95 kg/cm^2 and 99.79% of curcumin dissolved in 60 minutes. Curcumin nanoemulsion formulated into tablet dosage form still maintained the particle size of 134±1.45 nm when the tablet was dispersed in water.[7]

Bordoloi *et al.*, (2012) studied the antiulcer activity of the ethanolic extract of the rhizome of *C. caesia* on experimental animal models. Four groups of albino rats weighing 150-200 g were taken for the study (n = 5). Group A: Control (3%gum acacia 5 ml/kg/day orally for 7 days). Group B: Experimental control (Aspirin 400 mg/kg orally as single dose on 7th day). Group C: Test (*C. caesia* extract 500 mg/kg/day orally for 7 days plus Aspirin 400 mg/kg orally on 7th day) and Group D: Standard (Ranitidine150 mg/kg orally for 7 days and Aspirin 400 mg/kg orally on 7th day). The stomachs of the sacrificed rats were removed. The ulcer index, pepsin activity, free and total acidity and volume of gastric juice in group III and IV showed significant decrease in comparison to group II whereas there was increase in gastric mucus secretion.[8]

Rajamma *et al.*, (2012) Investigated antioxidant and antibacterial activities of oleoresins isolated from *Curcuma caesia*. Oleoresins were extracted from rhizomes of nine starchy *C. caesia,* using dichloromethane and evaluated for antioxidant and antibacterial activity. Oleoresins from all the species exhibited high DPPH radical scavenging activity and ferric reducing power, which had good correlation with phenolic content The oleoresins inhibited both gram+ve (*Staphylococcus aureus* and *Bacillus subtilis*) and gram-ve (*Escherichia coli*) bacteria. Maximum sensitivity was observed in the case of *B. subtilis*. The results indicated that the oleoresins from these species (most of which are unutilized) would have good potential as additives for food and medicinal applications.[9]

Satija *et al.*, (2011) compared the analgesic and antipyretic activity of different extracts obtained from *C. caesia* and *C. amada* rhizomes. Analgesic and antipyretic activities of the plant extracts was evaluated using chemical model of acute pain and brewer's yeast induced hyperthermia in rats. The writhing and pyrexia were observed at the doses of 250 and 500 mg/kg body weight of rats. Both the plants exerted analgesic and antipyretic activity. Where by *C. amada* showed better response in comparison to *C. caesia.*[10]

Gill *et al.*, (2011) studies two most popular species of genus Curcuma, *C. amada*and *C. caesia* were proved for their anthelmintic activity. In this study, four extracts viz. Petroleum ether, Dichloromethane, ethanol and aqueous extract of rhizomes of *C. amada* and *C. caesia* were investigated for anthelmintic activity at three different concentrations. Three concentrations (50 mg/ml, 100 mg/ml and 150 mg/ml) of each extract were studied which included the determination of paralysis time and time of death of earthworms. All the extracts of both the plants exhibited dose dependant activity. The results indicated that ethanol extract (150 mg/ml) of *C. caesia* was most effective in causing paralysis of earthworms, while the ethanol extract (150 mg/ml) and Dichloromethane extract (150 mg/ml) of both Curcuma species were very effective in causing death of earthworms.[11]

Mangla *et al.*, (2010) Investigated the anti-oxidant activity of methanolic extract of rhizomes of *C. caesia* using DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay. The IC 50 (Inhibitory concentration) was calculated by plotting graph between the percentages of inhibition versus concentration. The IC 50 valueof extract and Butylated Hydroxytoluene was found to be 862.35 µg and 46.25 µgfor 2 ml of 500 µM concentration of DPPH. This suggests that methanolic CC extracthad moderate IC 50 value as compared to Butylated Hydroxyltoluene.[12]

2.1. Review on hepatoprotective activity of flavonoids

Hariharapura *et al.*, (2014) investigated the different parts of the plant for antioxidant and hepatoprotective properties. The protective effect exhibited by *Hypericum mysorense* flowering tops (HMF) and *Hypericum mysorens* leaves (HML) against free radical-induced toxicity could be due to the protection of hepatic drug metabolizing enzymes and their antioxidant activities. The hepatic injury caused by CCl4 is associated with damage to the endoplasmic reticulum, and any compound capable of preventing the toxicity of CCl4 must have some direct or indirect effect on the liver. Both HMF and HML extracts have a maximum quantity of phenols and flavonols in them. The

antioxidant activity of phenolics and flavonoids is well known and widely accepted. HMF and HML showed potent in vitro and in vivo antioxidant and hepatoprotective activity among the various extracts of HM. The antioxidant and hepatoprotective activity of HM may be due toits rich flavonoid content. Two compounds were isolated from the HMF and HML,namely, hyperoside and rutin.[13]

Essam F. Al-Jumaily *et al.*, (2014) investigated the antioxidant activity of flavonoids purified and methanolic extract from propolis on male mice compared to that caused by carbon tetrachloride as a hepatotoxic model by measuring the determination of catalase, glutathione peroxidase and superoxide dismutase changein liver damage.[14]

Khowala *et al.*, (2014) prepared ethanolic and aqueous extract of *A. indica* tuber were prepared to evaluate *in-vitro* antioxidant potential, *in-vivo* hepatoprotective activity and GCMS analysis of the extract. *In vitro* and *in vivo* studies confirmed that the tuber of A. indica can be used as potential, inexpensive, safe and natural antioxidant for pharmaceutical applications. The ethanol extract of the tuber part of the plant contained higher phenolic and flavonoid content and scavenging activities as compared to those in the water extract. The ethanolic extract contained phytosterols as identified in GCMS analysis which must be purified in future for therapeutic uses.[15]

Jing Tong *et al.*, (2015) total flavonoids TFs from *C. glandulosum* seeds exhibited significant anti-oxidant capacity *in vitro* by inhibiting LPO and boosting the antioxidant capacity of the liver. Hepatoprotective effects of TFs were demonstrated in HepG2 cell model and in rats with CCl4-inducedhepatotoxicity in a certain extent. A correlation existed between the lipase inhibition effects and the hepatoprotective activity of TFs from *C. glandulosum* seeds. Results of the present work provide a scientific evidence for the appropriate use of *C. glandulosum* seeds in folk medicinefor the treatment of liver diseases, anti- oxidant capacity and lipase inhibition maybe the main mechanism.[16]

Fernando and Soysa (2016) the results suggest aqueous extract of the whole plant of E. quinquangulare AEQ possess hepatoprotective activity against ethanol induced livertoxicity of porcine liver slices which can be attributed to antioxidant properties and membrane stabilizing effects caused by the plant material.[17]

Qinge Maa *et al.*, (2016) Two new flavonoids named 4, 7-dihydroxy-5- hydroxymethyl-

8-prenylflavonoid and 4, 7- dihydroxy-5-hydroxymethyl-6, 8- diprenylflavonoid, together with seven known flavonoids were isolated from theaerial parts of *Capsella bursa-pastoris* (L.) Medik., Brassicaceae, for the first time. The chemical structures of the purified compounds were identified by theirspectroscopic data and references. Moreover, compounds were evaluated for theirhepatoprotective activities against d-galactosamine induced toxicity in WB-F344 cells by using a MTT colorimetric method. As a result, compounds (10 M) exhibitedmoderate hepatoprotective activities.[18]

3. Plant profile

3.1 Curcuma caesia

Kali haldi (*Curcuma caesia*) is a perennial herb with bluish-black rhizome native to North-East and Central India. Black Turmeric is also sparsely found in Papi Hills of East Godavari, West Godavari, and Khammam Districts of Andhra Pradesh. The rhizomes of kali haldi have a high economical importance because of its putative medicinal properties. The rhizomes are used in the treatment of smooth muscle relaxant activity.[19] haemorrhoids, leprosy, asthma, cancer, epilepsy, fever, wound, vomiting, menstrual disorder, anthelmentic, aphrodisiac, inflammation, gonorrhoeal discharges, and etc.[20]

In Madhya Pradesh, the plant is regarded asvery auspicious and is stated that a person who possess it will never experience shortage of cereals and food. The rhizomes of the plant are aromatic in nature. The inner part of the rhizome is bluish- black in colour and emits a characteristic sweet smell, due to presence of essential oil.[20]

Figure 5.1: Rhizomes and Leaves of *curcuma caesia.*

3.1.1 Morphology

The plant is normally erect with height ranging from 0.5 to 1.0 m. It is divided into underground large ovoid tuberous rhizome often called root-stock and an erect aerial shoot along with leaves and reproductive part.

3.1.1.1 Root

As the plant propagates with rhizome, the primary roots are not seen; however, yellow brown long fibrous and tapering adventitious roots are present all over the surface of rhizome.

3.1.1.2 Rhizome

The rhizome is tuberous with camphoraceous sweet odour, about 2-6 cm in diameter, the shape and size is often variable. It is sessile, laterally flattened and covered with adventitious roots, root scars and warts. It shows longitudinal circular wrinkles on the surface giving the look of nodal and intermodal zones to the rhizomes. The surface (cork) of rhizome is dark brown, bluish black, or buff in colour.

3.1.1.3 Leaves

The leaves are usually present in the groups of 10-20; each leaf is broad oblong lanceolate and glabrous. A deep farraginous purple colour is present in the middle region of the lamina. The petiole is ivory in colour and ensheathing the petiole encircle each other forming pseudoxis. The variation is parallel in nature.

3.1.1.4 Flower

Flowers are pale yellow colour with reddish border Calyx: 10-15 mm long, obtuse and 3 toothed. Collora: long tubular, pale yellow lip-3 lobe semi- elliptic.

3.1.2 Distribution

This plant is widely distributed in north-east and central India. *Curcuma caesia* is sparsely found in Papi Hills of East Godavari, West Godavari, and Khammam Districts of Andhra Pradesh.

Vernacular nameshindi: Kali Haldi

Manipuri: Yaingang Amuba or Yaimu

Marathi: Kala-haldi

Telugu: NallaPasupu

Bengali: Kala haldi

Mizo: Aihang, Ailaihan

Assamese: kalahaladhi

Malayalam: Kari manjal

Sanskrit: Rajani Nishaa, Nishi, Ratri.

3.1.3 Phytochemical constituents of *curcuma caesia.* Roxb

Preliminary phytochemical studies was carried out and reported the presence of alkaloid, steroid, phenolic and tannin as major constituents in successive solvent extraction of rhizome with n-hexane, petroleum ether (60:80), benzene, chloroform, ethyl acetate and water. The volatile oil component of the rhizomes of *Curcuma caesia* was analysed by GC-MS by Pandey *et al.*[56] that resulted in the identification of 30 components, representing 97.48% of the oil, with camphor (28.3%),ar- tumerone (12.3%), (Z)-Ocimine (8.2%), 1-ar-curcumene(6.8%), 1, 8-cineole (5.3%), element (4.8%),borneol (4.4%), bornyl acetate (3.3%) and curcumene (2.82%) as the major constituents.(Pandeyet al 2003). Rastogi et al reported linalool as the major component comprising 20.42% followed by ocimine (15.66%), 1- ar-curcumene (14.84%), zingiberol (12.60), 1, 8-cineole (9.06%), and borneol (7.4%) as major constituent9.The *curcuma caesia* rhizome oil was also analysed by Banarjee et al. and reported almost similar composition consisting of (+) linalool (20.42%), ocimine (15.66%), 1- ar-curcumene (14.84%), zingiberol (12.60), 1, 8-cineole (9.06%), and α-borneol (7) d-camphore (18.88) as major constituent.[21]

3.1.4 Traditional uses of *curcuma caesia*[22-23]

The fresh and dried rhizomes of *Curcuma caesia* are used for treatment of various diseases.

The following uses have been reported-

1. Dried rhizomes and leaves of *Curcuma caesia* Roxb. are used in piles, leprosy asthma, cancer, wounds, impotency, fertility, tooth ache, vomiting, and allergies.
2. Fresh rhizome decoction is used as anti-diarrhoeiaric and to get relief from stomach ache.[6] The fresh rhizome paste of *Curcuma caesia* is applied during the snake bite and scorpion bite. The dried powder used to mixed with seed powder of *Andrographis paniculata* Wall ex. Nees and applied during insect and snake bite.
3. The rhizome is used for the treatment of cough, fever, dysentery, worm infection.
4. The fresh rhizomes are used in leprosy, cancer, epilepsy, anti-helmenthic,aphrodisiac, gonorrhoreal discharge.
5. Rhizome of *Curcuma caesia* is grounded in the form of paste in rheumatic

arthritis.

6. The rhizome of *Curcuma caesia* is grounded and applied in the form of paste in rheumatic arthritis.
7. Crushed rhizome paste is applied against cut or injury to control bleeding and quick healing.
8. The rhizome *Curcuma caesia* is administered during inflammation of tonsils.
9. The roots of the *Curcuma caesia* are grounded into powder and used water to treat gastric disorder.[24-25]

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