Journal of Advances and Scholarly Researches in Allied Education
Vol. 16, Issue No. 6.1, May-2019 (Special Issue), ISSN 2230-7540

Screening and Characterization of Ficus Species for Hepatoprotective Potential

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ABSTRACT

Liver plays a vital role in almost all physiological processes and, injury to liver can affect health. There are various endogenous and exogenous agents present in the environment which directly or indirectly affect the function of liver like drugs, alcohols, xenobiotic compounds etc. Liver cells have ability to repair the damage because of its ability to regenerate new cells and it has various system reduces the toxic effects of chemicals. Due to continuous exposure of these agents, leads to cease the ability of cell to repair and produces ROS in the cells which again cause damage to cells. Liver cirrhosis is the final stage in which it cannot be cured but can be treated. But in the early stage of disease treatment can be possible. Various allopathy medicines are available in the market and are most expensive and have various side effects. Developing a drug from medicinal plants or the use of plant's part is cost-effective and have less or no side effects. So, in the current study, we evaluate and compare the antioxidant, antiinflammatory and hepatoprotective activities of selected Ficus species of Karnataka namely F. parasitica, F. tsiela, F. microcarpa, F. heterophylla, F. dalhousiae, F. drupacea and F. mollis. Bark and leaf samples were used for the present studies extracted with different solvent hexane, ethyl acetate and methanol. Total phenolic content of selected Ficus species were determined which suggested that F. dalhousiae bark methanolic (FdBM) extract contained high amount of total phenolics compared to all other plant samples. The bioactive molecule isolated was naringenin which belongs to flavonoid group and has very good bioactivity. Again naringenin was used for in vivo hepatoprotective studies in paracetamol-induced hepatotoxicity on rats. Naringenin inhibited the activity of serum biomarkers. So, inhibition of these cytokines revealed the protection of hepatic cells from injuries. In silico studies also revealed the protective role of naringenin as the molecular docking studies suggested that naringenin inhibits the expression of MMP-2, H-RAS GTPase, Human Arginase-2, PROMMP- 2/TIMP-2 complex and NF-kappaB, which are responsible for the activation of hepatic stellate cells (HSCs) that stimuli the expression of other cytokines and lead to liver damages. Molecular docking also revealed that the binding of naringenin with IL-10 and IL-13 was very weak and have very low XP GScore, it showed that naringenin doesn't affect the expression of cytokines which helps in repair mechanism of liver during liver disease as compared with positive control silibilin.

Article submitted at :
National Conference on Advances in Multidisciplinary Research (NCAMR-2019)
Organized by : Kurukshetra Institute of Professional Studies, Kurukshetra, Haryana

Keywords – *Ficus Species, Liver, Liver Cirrhosis, Liver Diseases*

INTRODUCTION

In human healthcare, medicinal plants and its secondary metabolites plays an important role for the treatment of many diseases. Traditional medicine plants have been mentioned and widely used in ancient medicinal system and in natural health care practices including tribal or folk medicines and have been used for treatments and cures of various ailments (Subramoniam and Pushpangadan, 1999). The therapeutic effects of medicinal and herbal medications are marvelous and have permissible medications for various kinds of diseases and for the reduction of its symptoms. Amusingly, in developing countries, the demand for medicinal plants are progressively increasing from last few decades (Abere et al., 2010). World Health Organization (WHO) has published that nearly 65% of the total world population and population from developing countries rely on plant-based medicines (Cragg And Newman, 2013). Medicinal properties of bioactive compounds has been practiced from past many years for the discovery and development of new drugs. More than 500 drugs of well-known structure have been screened and identified that are extracted from various plants and also used in allopathy medicine (Farnsworth, 1990; Cox and Balick, 1994). Isolation of new compounds from medicinal plants, which in turn serve as lead molecules and these molecules can be synthesized or semi-synthesized which helps in enhanced activity with reduced toxicity. Also, these novel and bioactive compounds can be used as patentable entities (Lahlou, 2013).

Scientific communities' effort to develop and identify a novel drug or medicine, ethno-botanical, phytochemical, clinical studies etc. are very essential and important aspects to identify any traditional medicinal plants. Discovery and authentication of any novel drug or traditional drug is very important in ethno pharmacology. Therapeutic activity of any compound or molecule mostly depends on an empirical evaluation of plants having medicinal properties and which often is allied to traditional knowledge. Standard herbal formulation is an approach in which the toxicity of any plant is reduced and cost-effective. This helps to develop and identify useful and novel drug.

India is rich in plant medicinal traditions, which ensures the health security of many people in the country (Wakdikar, 2004). More than 1.5 million practitioners are using medicinal plants for preventive and curative purposes (Ramakrishnappa, 2002). Because of therapeutic properties, the use of medicinal plants will continue in future for better affordability. Recently developed countries are also using traditional medicinal plant systems (Wakdikar, 2004). Most of the drugs from western medicine are isolated or extracted from natural products: digitoxin, taxol, warfarin, atrococaine, hyoscine, pilocarpine, morphine, pinequinine, reserpine, codeine, menthol, ephedrine, etc. Active compound from natural sources may not always be suitable for developing an effective drugs (Bell and Charlwood, 1980). Medicinal plants are significantly useful and economically important. Now-a-days, medicinal plants are more popular than synthetic or modern drugs because in case of medicinal plants, the rate of infections, antibiotic resistance in microorganisms are having very less side effects.

OBJECTIVE OF THE STUDY

1. To the study Screening of Ficus species for hepatoprotective properties.

2. To the study Studies on pathway-intermediates in inhibition of liver injury by isolated compounds.

MATERIALS AND METHODS

Materials

1,1-diphenyl, 2-picrylhydrazyl radical (DPPH), 2, 2-Azinobis (3-ethyl benzothiazoline-6-sulfonic acid) (ABTS), 15-Lipoxygenase (15-LO), Linoleic acid and 2- deoxyribose from Sigma–Aldrich, St. Louis. Gallic acid, ascorbic acid, Quercetin, rutin and Sodium nitroprusside were purchased from Himedia (Bangalore, India), dichlorofluorescein diacetate (DCF-DA), thiobarbituric acid (TBA), bovine serum albumin (BSA), 5, 5-dithiobis (2-nitrobenzoic acid) (DTNB), 2, 4-dinitrophenyl hydrazine (DNPH) and glutathione (GSH) were purchased from Sigma–Aldrich Inc. (Mumbai, India) trichloroacetic acid (TCA), hydrogen peroxide (H2O2; 30%), thiobarbituric acid (TBA), folin-ciocalteu phenol reagent (FC Reagent), sodium citrate, ethylene diamine tetra acetic acid (EDTA), pyrogallol, Solvents (hexane, ethyl acetate and methanol, HPLC grade) and other chemicals and solvents used were purchased from various suppliers with highest purity grade.

Test Kits

Human Cox-2 inhibition kit was obtained from Cayman, Ann Arbor, USA, TNF-α and interlukin-6 assay kit from ebiosciences. Commercial test kit for serum biomarkers such as ALP, LDH ALT and AST, were obtained from Aggape, Kerala, India.

Collection and identification of plant materials

Plant materials including leaves and stem bark belonging to the Ficus species (Moreceae family) were collected from various parts of Karnataka, India. Plant materials were identified and authenticated by a Plant taxonomist at University of Mysore, Mysuru (Table 3.1). The leaves and bark were washed under running tap water followed by distilled water, shade -dried and coarsely powdered.

LIVER AND ITS ROLE

Liver is one of the vital organ in our body and plays a very important role in regulating, maintaining and carry out several physiological functions such as metabolization of nutrients like fat, protein and carbohydrates, manufacturing of essential amino acids, excretion of waste metabolites, detoxification of waste metabolites. Additionally, it also serves to metabolize and eliminate drugs and other xenobiotics from our body. It also protects body against various foreign substances (Saleem et al., 2010).

During oxygen metabolism Reactive oxygen species (ROS) are generated and at certain level it performs various physiology functions. Generation of ROS is a part of normal physiological process and is responsible for the manifestation of several cellular processes including various signal transduction pathways. It also works as a defense mechanism invading microbes and expression of many genes which are responsible for growth and ageing (Finkel and Holbrook, 2000). Superoxide dismutase, Catalase, and glutathione etc., are antioxidant enzymes, which act

as natural antioxidant defense systems and protect its own cells against oxidative damages (Kaplowitz, 2000). Both enzymatic and non-enzymatic systems are necessary to deal with oxidative stress of cells. Generally, drugs, chemicals or xenobiotics first metabolize in liver and forms toxic intermediates. So, liver is the main targeted site for damage or oxidative stress. Hepatic cells metabolize most of the exogenous chemicals including xenobiotic compounds (Ajith et al., 2007). During the process of detoxification, reactive oxygen species (ROS) are generated which causes oxidative stress that leads to hepatic damage (Cohen and Nyska, 2002).

LIVER DISEASES

Liver disease is one of the foremost problem worldwide and is associated with significant rate of morbidity and mortality. In developed countries, excessive alcohol intake and viral-related liver diseases are the main cause of liver diseases whereas in developing countries the most common causes are xenobiotics, hepatitis B and C viruses, some antibiotics, various chemotherapeutic agents, thioacetamide (TAA), paracetamol, carbon tetrachloride (CCl4) (Schuppan and Afdhal, 2008). There are 90% chances of hepatocellular carcinoma and can be related with liver cirrhosis (Okazaki et al., 2001). Fig. 1.2 shows normal and cirrhotic liver. Even with the advancement of modern medicine, effective drugs are still not available that can protect the damage of liver and restores the function of the liver (Chattopadhyay, 2003). Therefore, it is necessary to search alternative medicines or drugs against liver disease.

OXIDATIVE STRESS

The oxidative stress severely affects the hepatic cells by inducing irreversible changes in proteins, DNA, RNA and lipids contents, which directly or indirectly affects the pathways of normal physiological metabolism. These pathways are very important because it helps in the regulation of various genes involved in transcription, expression of various protein, apoptosis of cell, and activation of hepatic stellate cell. Oxidative stress plays a key role for the initiation and progression of liver diseases (Feng et al., 2011; Singal et al., 2011). The common mechanism of oxidative stress-induced by various factors on liver disease is outlined in Fig. 1.3. Oxidative stress also damages some extra-hepatic organs such of kidney and brain (Palma et al., 2014).

MAJOR FUNCTIONS OF LIVER

Homeostasis of various physiological processes are generally maintained by liver (Adi and Alturkmani, 2013; Sherwood, 2015) and it also performs various vital function in the body.

1. Vascular functions: The storage capacity of liver in liver sinusoids is 200-200 ml. kuffer cells removes 90% of the bacteria and hepatocytes help in the synthesis of plasma protein.

2. Metabolic Functions: Liver cells have high metabolic rate.

a) Carbohydrate metabolism: Under the various effects of hormones, liver acts as a glucostat (glycogenesis, gluconeogenesis etc.). In liver glucose is converted into glycogen and also stores inside the liver, and with the demand of energy this glycogen is again converted into glucose (Hall, 2015).

b) **Metabolism of Protein:** Liver cells synthesizes various essential amino acids and other plasma protein. Liver produces most of the blood clotting factors. It also produces some immune factors like phagocytes and in response to microbes these phagocytes start producing acute phase proteins which helps the body to fight against various infection. These proteins are also associated with tissue repair mechanism and activation of immune cells. It also helps in amino acid deamination and urea formation (Reichen, 1999)

CAUSES OF LIVER DISEASES

- Damage of liver tissues by chemicals or infiltrated by abnormal cells etc.
- Flow of blood in the liver may be compromised.
- Cholesterol or triglycerides accumulation in case of steatosis.
- Bile flow can be obstructed. Example- cholestasis.
- Cells may become inflamed (such as in hepatitis).

TREATMENT FOR LIVER DISEASES

Liver diseases have their own precise treatment regime. There is no general treatment available for the disease. In hepatitis A, body needs to maintain the water balance because the body's immune system fights against infection. In case of gallstone disease, patient has to operate and remove gall bladder. For other types of liver diseases, it require long-term medication to control the consequences of disease.

In case of cirrhosis, patients are not able to metabolize waste products, which elevates ammonia in the blood that leads to hepatic encephalopathy. Water pills and low sodium diet should be given to patients to minimize water retention of the body. In portal hypertension, operation is necessary to reduce the bleeding. Patients whose liver have failed liver transplantation is the final option (Subramoniam and Pushpangadan, 1999).

COMPLICATIONS OF LIVER DISEASES

Most of the liver diseases can be managed but not cure completely except viral infection and gallstone disease. Hepatic disease progress to fibrosis that leads to cirrhosis and lastly liver failure. Many complications are accompanied with liver disease such as malnutrition, infection, loss of body weight, bleeding and decreased in various metabolic function. Some liver diseases are associated with high risk of developing liver cancer (Heidelbaugh and Sherbondy, 2006).

PLANTS USED IN THE TREATMENT OF LIVER DISEASES

Medicinal plants plays a very important role to maintain the human health and vitality including animals and it cures numerous diseases, including liver disorders. India is called as the "Botanical Garden of the World" because India is one of the richest producer of medicinal plants. Over the past few years, traditional medicinal system has been accepted globally, and has great impact on both world health and international trade. WHO estimated that approximately

3/4 of the world's population use herbal phytomedicines and other traditional medicines for the treatment of various diseases (Govind and Madhuri, 2008; Madhuri, 2008; Madhuri and Pandey, 2009; Pandey and Madhuri, 2010).

Still treatments of liver diseases are not very reliable. Scientific communities are working on bioactive compounds from plants and trying to evaluate hepatoprotective potential of various plants against liver diseases. Various dietary antioxidants has been given for the treatment of liver damage. For developing appropriate drugs against liver disease, then assessment of medicinal plants are very important aspects (Kumar et al., 2011).

In allopathy, there is no satisfactory hepatoprotective drugs available for the treatment of liver disease. Large number of plants have been identified and may possess hepatoprotective activities, but still there is no scientific evidences available to validate the hepatoprotective properties of medicinal plants (Suky et al., 2011).

Many poly-herbal formulations and plant-based medicines are being used for the treatment and prevention of hepatic diseases. The medicinal plants has numerous secondary metabolites possessing strong antioxidant activities and these antioxidant compounds may be flavonoids, terpenoids, polyphenols, saponins, alkaloids, minerals, vitamins, carotenoids. The damage caused by free radicals can be cured by using antioxidants, which help to protect liver cells from further disruption.

Ficus Plants

Ficus plants are found throughout the world and it is moderate woody plants or trees. In indigenous system of medicine like ayurveda, unani, siddha and homoeopathy, it has vast traditional role. Ficus species are very important plants in many traditional formulations and use in the treatment of various diseases, such as diabetes, dysentery, skin diseases, ulcers, diarrhea etc. It also possesses very good anti-inflammatory, antioxidant and anticancer properties (Nadkarni et al., 1976). Ficus species is one of the important medicinal plant having large number of secondary metabolites or bioactive compounds having a wide range of pharmaceutical attributes.

CONCLUSION

Plant-based medicines or plant-derived natural compounds are becoming popular now a days over allopathy or synthetic medicines because of less toxic effects and low cost. Synthetic drugs have therapeutic activities against liver disease but in the meantime they also have adverse side effects. Natural antioxidants are the compounds can be used to overcome this problem as they maintain the homeostasis of cell and redox potential of ROS because of the concentration of ROS crosses the threshold it activates stellate cell by activating various cytokines and ultimately it will damage hepatocytes. Medicinal plants have natural antioxidant activity and majority of the world's population are using for the treatment of various diseases and, antioxidant property of any plant is the backbone for the treatment of various ailments. Ficus species are used as traditional medicinal system and it belong to Moraceae family. Hence, in the current study, we evaluated and compared the antioxidant, anti-inflammatory and hepatoprotective activities of selected Ficus species of Karnataka namely F. parasitica, F. tsiela, F. microcarpa, F.

heterophylla, F. dalhousiae, F. drupacea and F. mollis because not much studies on the bioactivities have been done.

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