Investigating The Protective Role of Brassica Juncea Extract Against Ibuprofen-Associated Liver Damage In Rats

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Abstract-
Hepatoprotective activity of the ethanolic of brassica juncea was studied in Wistar rats using the ethanol-induced liver hepatotoxicty. Ethanol administration resulted in significant elevation of physical parameters (Viz. rat liver weight and liver volume), biochemical parameters like (Viz. serum aspirate transaminase (AST); alanine transminase (ALT); alkaline phasphatase (ALP); direct bilirubin and total bilirubin levels, while albumin and total protein were found to be decreased compared to normal group). Pretreatment with silymarin, ethanolic extract of brassica juncea significantly prevented the physical and biochemical changes induced by these hepatotoxins. Histopathology of liver confirmed our finding as the treatment with the extracts resulted in minor liver cell damage compared to toxic control group.

Keywords: Biochemical parameters, Hepatoprotective, Alcoholic Liver Diseases (ALD), Ethanol.

INTRODUCTION:

One of the leading causes of illness and death worldwide is now liver disease. One of the most frequent causal factors that presents a significant clinical and regulatory problem is drug-induced liver damage. Drug-induced hepatotoxicity symptoms are extremely varied, spanning from fulminate hepatic failure to an asymptomatic increase of liver enzymes. Despite the great advancements in modern medicine, there are very few trustworthy medications that either shield the liver from harm or promote hepatic cell regeneration. Numerous potent plant extracts are widely used to treat a range of clinical conditions, including liver disease. Thus, there is ongoing interest in the search for safe and effective medications to treat liver problems(1). Hepatic disorders are a significant global health issue that are typically encountered in underdeveloped nations. The major causes of them are the use of certain medications and large quantities of chemicals. There isn't a medication that can protect the liver from harm, promote liver function, or aid in liver regeneration. liver cells. Effective medications are therefore desperately needed to supplement or replace those now in use. One important source of pharmaceutical medications is medicinal plants. Current patterns indicate that the demand for phytoconstituents from certain medicinal herbs is rising, and these herbs have been found to have hepatoprotective potential (2).

There are a lot of herbal preparations on the market. The goal of the current review is to gather information on potential phytochemicals from therapeutic plants that have been examined in hepatotoxicity models utilizing cutting-edge scientific methodology. Clinical studies conducted in this century have validated the effectiveness of several herbs in treating diseases related to the liver. As a result, this review study advances our understanding of documented native herbs that are frequently used to treat and prevent liver problems (3).

The most recent WHO statistics, released in May 2014, indicates that 216,865 fatalities in India were due to liver disease, or 2.44% of all deaths. An estimated 50 million people die each year from liver cancer and cirrhosis worldwide. Chronic viral hepatitis is the cause of 1.3 million fatalities globally. Every year, alcohol abuse causes 2.5 million deaths worldwide, with more than 5,000 of those deaths occurring in

England and Wales in the previous ten years. According to data from Pune Municipal Corporation's birth and death registration department in India, liver-related issues claim the lives of 35–40 persons on average each month. Unlike what is commonly believed, liver disorders can strike non-drinkers as well (4). Alcohol misuse is still the leading cause of liver disease.

Liver:

One of the biggest glands in the body is the liver. It is the principal organ responsible for preserving the interior environment of the organism. In adult males, the weight of the liver is 1.4–1.8 kg, while in mature females, it is 1.2–1.4 kg. The liver is essential to the secretion of bile, which is necessary for digestion. Nearly every metabolic route leading to development, illness prevention, nutrition supply, energy production, and reproduction is impacted by it. Hepatocytes are uniconfigured epithelial cells that make up the majority of the liver. All compounds that enter the bloodstream from the stomach and intestines are filtered by the liver, which also has the ability to eliminate some hazardous molecules and store others.

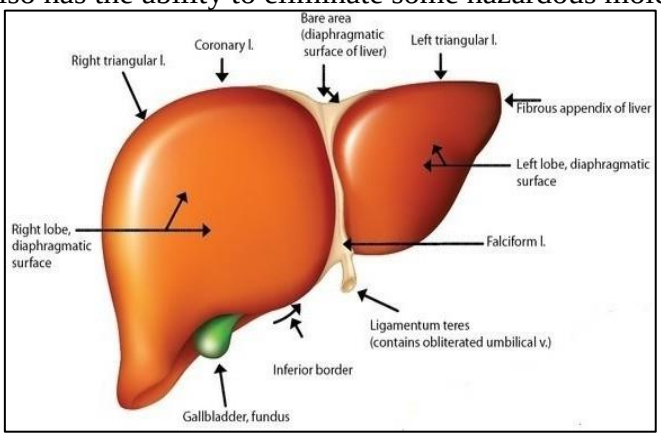

Figure 1: Structure Of Human Liver

The hepatocyte carries out the metabolic processes of the liver, including the production and excretion of bile during the process of bilirubin metabolism; control over the metabolism of cholesterol; lipid synthesis and secretion of plasma lipoproteins; regulation of carbohydrate homeostasis; formation of urea; and the metabolism or detoxification of drugs and other foreign substance (5).

The majority of the liver mass, or roughly 80% of it, is made up of parenchymal cells called hepatocytes. Although it makes up only 6.5% of the overall volume of liver cells, the other kind of non-parenchymal cells accounts for 40% of the total counts of liver cells. Additionally, it releases approximately 2.5 milliliters of bile in its own ducts, which are then transported by the gallbladder through the clogged cystic duct to be stored. By changing their chemical structure, the liver renders a lot of drugs inactive. The liver creates glucose from disaccharides and polysaccharides, such as sugars, starches, and protein molecules, and converts glucose to glycogen as a type of energy storage (6).

LOBES:

The right, left, caudate, and quadrate lobes are the four that are visible on the surface. The liver is bifurcated into the right and left main lobes by the Falciform ligament. The bigger right lobe is further subdivided into the right lobe proper, the caudate lobe, and the quadrate lobe.

When viewed from the front (diaphragmatic) surface, gross anatomy historically separated the liver into two parts: a right and a left lobe; however, the visceral (underside) portion is divided into four lobes and contains the caudate. It is visible on the falciform ligament surface along with quadrate lobes. The liver is divided by the visible on its front into a left lobe that is somewhat larger than the right lobe, and the two extra lobes are situated between the right and left lobes, one in front of the other.Imagine a line dividing the liver and gallbladder into two parts, starting from the left of the vena cava and continuing forward. "Cantlie's line" is the name of this passage. There are further anatomical features that further separate the left side of the liver into two portions, including the round (ligamentum teres) and the liver's ligamentum venosum ligament. This left part is divided into four segments by the porta hepatis, sometimes called the transverse fissure of the liver, an important anatomical landmark. These segments can be named in an

anticlockwise fashion, beginning at the caudate lobe as I. Seven segments are visible from this visceral view; the eighth segment is only discernible from the parietal view (11).

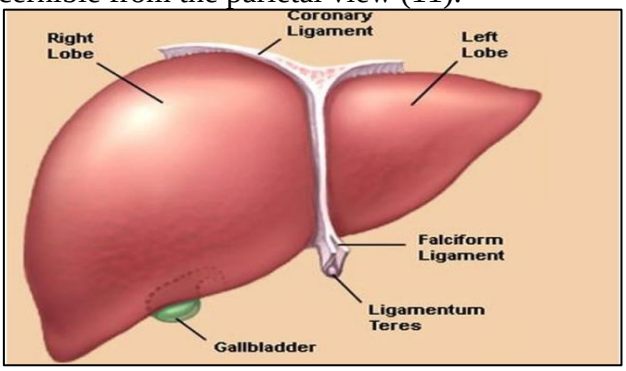

Figure 2: Human Liver lobes

Facts about liver

The liver carries out more than 500 different processes, such as preventing infection, eliminating toxins, producing proteins and hormones, regulating blood sugar, and aiding in blood clotting. The human liver is the largest internal organ with the highest metabolic complexity. There is only one organ: the liver. that is capable of self-renewal, enabling someone to give a portion of their liver to someone else. When a piece of the liver is transplanted, the recipient's liver will grow to the proper size, and the donor liver will regenerate to its original size. Keeping the body's metabolic balance in check is a huge responsibility for the liver (7).This involves the production of serum proteins, the breakdown of dietary amino acids, carbohydrates, lipids, and vitamins, as well as the detoxification and excretion of xenobiotic pollutants and endogenous waste products into bile. Because the liver's metabolic processes are vital to the functioning of other organs, hepatic diseases have far-reaching effects. The signs and symptoms of liver damage often follow recognizable patterns. There are cases where the liver is the predominant site of the disease. In many cases, the involvement of the liver is secondary and frequently results from common human diseases like drunkenness, cardiac decompensation, extra-hepatic infections that lead to the spread of disseminated illness, or from deliberate disruptions of circulation or bile flow (8).

1) Inflammation: Hepatitis is the term used to describe damage to the hepatocytes brought on by an influx of either acute or chronic inflammatory cells into the liver. Liver injury is frequently caused by sensitized T lymphocytes attacking living liver cells that express antigens. The portal tract may be the only area of inflammation, or it may spread to the parenchyma. For instance, hepatitis A virus (HAV), HBV, HCV, HDV, and HEV-caused viral hepatitis.

2) Degeneration: A chemical or immunological insult damages the hepatocytes, causing them to appear edematous. Another type of degeneration is called steatosis, in which fat droplets build up inside the hepatocytes. For example, hepatic degeneration may result from a hereditary illnesses or external materials like alcohol (9).

3) Cell death: Toxic or immunologically driven cell death happens through apoptosis, a process that results in hepatocytes becoming smaller, pyknotic, and highly eosinophilic. As an alternative, hepatocytes could experience osmotically-induced swelling and rupture known as lytic necrosis. The remaining kinds are centrilobular. major necrosis, sub-massive necrosis, bridging necrosis, and necrosis (10).

4) Fibrosis: When the liver is directly exposed to toxicity or inflammation, fibrotic tissue is created. The pattern of blood flow in the liver and the perfusion of hepatocytes are permanently altered by collagen deposition. Fibrosis may initially appear inside the sinusoids, the major vein, or the area surrounding these structures. Bridging fibrosis is the process by which these fibrous threads gradually connect different liver regions (portal-to-portal, portal-to-central, and central-to-central). Most people agree that fibrosis is an irreversible result of liver injury (7).

5) Cirrhosis: Cirrhosis is an end-stage liver condition characterized by ongoing fibrosis and parenchymal damage. The liver is divided into nodules of degenerating hepatocytes encircled by scar tissue (11).

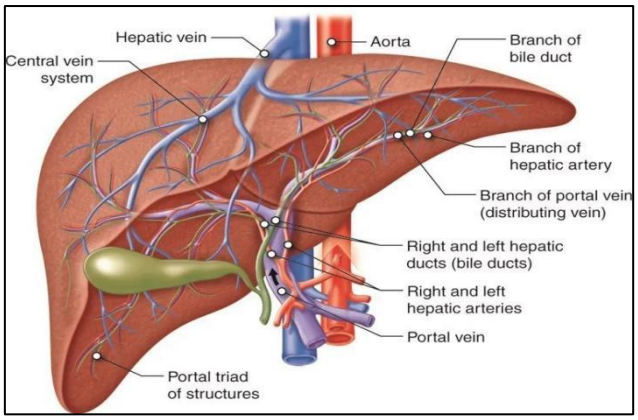

Figure 3: Anatomy Of Human Liver

Hepatic dysfunction, manifested as jaundice, hypoalbuminemia, hyperammonemia, hyperglycemia, factor hepatitis, palmar erythema, spider angiomas, hypogonadism, gynecomastia, weight loss, muscle wasting, and portal hypertension, are the clinical outcomes of liver illnesses. (12,7) Life-threatening consequences such as hepatic failure manifested as hepatic encephalopathy, hepatorenal syndrome, portal hypertension due to cirrhosis, malignancy with chronic illness, and hepatocellular carcinoma will result from these if they are not treated promptly.

❖ **Functions of the liver:**

The liver is said to as the human body's laboratory and performs well over 500 different tasks. Because it filters all incoming nutrients and fluids, the liver is involved in nearly every physiological activity (13).

The liver is an organ with a high metabolic activity that performs numerous essential life tasks. The liver's main jobs include producing bile, carrying out metabolic processes, detoxifying or purifying blood, and storing vitamins and minerals. Up to 500 distinct functions are believed to be carried out by the liver, most of which occur in conjunction with other organs and systems. The gastrointestinal tract, which includes the liver, is in charge of dissolving food into smaller pieces that cells can utilize (14).The liver is located in the abdomen, below the ribcage. It is a large organ with many different functions, including:

• Production and secretion of bile and bile salts to help digestion and absorption.
• Production of insulin-like growth factor (IGF-I).
• Production of clotting factors.
• Release of glucose into the blood to provide energy for cells.
• Production of urea, a waste product.
• Cholesterol production.

The gallbladder, a little organ located behind the liver, is responsible for storing the bile the liver produces and releasing it into the small intestine to facilitate absorption and digestion (15). Most vertebrates' livers generate bile, also known as gall, a dark green to yellowish brown fluid that aids in the small intestine's digestion of lipids. In humans, the gall bladder stores and concentrates bile, which is produced by the liver and stored there as liver bile. The amount released daily is between 600 and 1000 ml, with a PH of approximately (15).

Bile also aids in the emulsification of fats. Bile acids are the source of bile salts. Hepatocytes in the liver need cholesterol to make these.The liver converts cholesterol into the two essential bile acids, chenodeoxy cholic acid and cholic acid.

❖ **Metabolic function:**

1) **Carbohydrate metabolism Maintenance of normal blood glucose level**

The liver releases glucose into the bloodstream by breaking down stored glycogen
when blood glucose levels are low. The liver transforms glucose into triglycerides and glycogen when blood glucose levels are high (for storage).

2) Protein Metabolism

The following are the most important components of liver-based protein metabolism (16)

• The amino acid molecules undergo deamination and transamination, after which the non-nitrogenous portion of the molecules is converted to glucose or lipids. To measure liver damage, serum samples of a

number of the enzymes involved in these pathways—such as alanine and aspartate aminotransferases—are frequently tested.

• The body eliminates ammonia through the urea production process. Because ammonia is so poisonous, it will cause damage to the central nervous system if it is not quickly and effectively eliminated from the bloodstream. Blood supply abnormalities known as portosystemic shunts are a common cause of such hepatic encephalopathy in dogs and cats.

• Amino acid synthesis that is not necessary. The majority of plasma proteins are synthesized by hepatocytes. The primary plasma protein, albumin, is synthesised almost entirely by the liver. Furthermore, the liver produces a lot of the clotting components required in blood to cause coagulation.

3) Lipid metabolism

Lipid metabolism is centered in the liver. It uses a mechanism that links the metabolism of lipids and carbohydrates to produce about 80% of the cholesterol the body synthesizes from acetyl-CoA. Triglycerides can also be produced, stored, and exported by the liver. By means of the fatty acid oxidation route, which links tricarboxylic acid cycle activity with lipid catabolism, the liver is also the location of keto acid synthesis (7).

To regulate the body's triglyceride and cholesterol levels, the liver assembles, secretes, and absorbs different lipoprotein particles. Very low-density lipoproteins, or VLDL, are among these particles that help transfer lipid to other tissues for instant consumption or to adipose tissue for storage as fat. Loss of lipid and protein components alters the shape of VLDL particles during these processes. Because the resultant low-density lipoprotein (LDL) particles have an affinity for the LDL receptor, which is present on the surface of several bodily cells, including hepatocytes, they are subsequently transported back to the liver. The liver produces and secretes more lipoprotein particles, known as high-density lipoproteins (HDL). They return extra cholesterol and triglycerides to the liver for excretion after scavenging them from other tissues and the circulation. Therefore, cholesterol that is not required by different organs is eliminated from the circulation by the processes of HDL secretion and LDL removal (7,11).

Symptom of liver diseases

Symptoms may begin slowly and slowly get worse. They may also begin suddenly and be severe from the start (16). Early symptoms may be mild and include:

• Breath with a musty or sweet odor
• Change in sleep patterns and changes in thinking
• Confusion that is mild, forgetfulness and mental fogginess
• Personality, mood changes, Poor concentration and Poor judgment
• Worsening of handwriting or loss of other small hand movements
• More severe symptoms may include:
• Abnormal movements or shaking of hands or arms
• Agitation, excitement, seizures (occur rarely) or disorientation
• Drowsiness, confusion, strange behavior or severe personality changes
• Slurred speech and slowed or sluggish movement

Causes Of Liver Damage (17)

• **Chemical induced liver damage:** Carbon tetrachloride, alcohol consumption, aflatoxins, 1, 1, 2, 2-tetrachloroethane, carbon tetrabromide, dimethyl formamide, ethylene dichloride.

• **Drug induced:** Liver damage is the most frequent cause of drug withdrawals from the market, with over 900 medications linked to the condition. Fifty percent of all acute liver failures and five percent of hospital admissions are caused by drug-induced liver damage. A few of them are Overdose on acetaminophen, amiodarone, ketoconazole, and rifampicin.

• **Virus induced:** Hepatitis A, B, C, D and E.

• **Other causes**: Non-alcoholic fatty liver, malnutrition, extrahepatic infections, ingestion of poisonous wild mushrooms, haemochromatosis.

• Congenital birth defects, or abnormalities of the liver present at birth
• Metabolic disorders, or defects in basic body processes
• Alcohol or poisoning by toxins
• Nutritional deficiencies

PATHOPHYSIOLOGY OF LIVER DAMAGE

Hepatocellular stress brought on by viruses or hepatotoxins can cause the release of chemokines on the one hand and the activation of liver-resident macrophages on the other. Proinflammatory cytokines are released by both Kupffer cells and natural killer cells. They include interleukins-1β and interferon-gamma (IFN-γ), whose tissue concentration increases early after toxins are administered, and tumor necrosis factor and interleukin-6 in a similar kinetics (11).

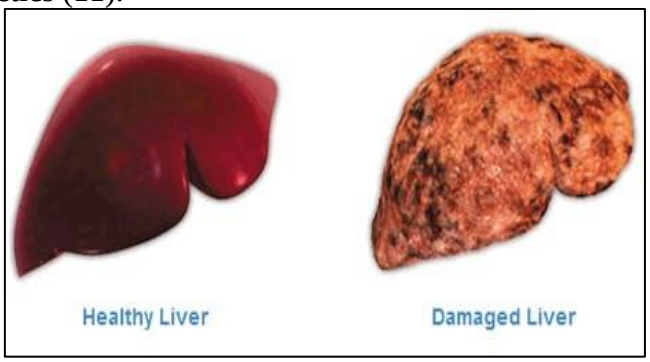

Figure 4: Pathophysiology of liver damage

On the portal or sinusoidal endothelial cells, they cause a down regulation of PECAM 1 and an increase in the expression of cell adhesion molecules such as ICAM-1 and VCAM-1. These chemicals facilitate the inflammatory cells' recruitment and sinusoidal transmigration in the direction of the hepatocyte. As long as harmful nox are present or are given often, inflammation will persist. Leukocytes may reach the liver tissue mostly through the portal route, which is also where the inflammation primarily starts (18).

Pathophysiological Mechanisms (19)

The pathophysiologic mechanisms of hepatotoxicity are still being explored and include both hepatocellular and extracellular mechanisms. The following are some of the mechanisms that have been described:

a) **Disruption of the hepatocyte:** A drop in ATP levels brought on by the drug's covalent binding to intracellular proteins may disrupt actin. Actin fibril disintegration at the hepatocyte's surface results in membrane rupture and bleb formation.

b) **Disruption of the transport proteins:** Bile flow can be disrupted by medications that alter transport proteins at the canalicular membrane. Cholestasis is brought on by the loss of villous processes and the disruption of transport pumps such multidrug resistance-associated protein 3. These factors prohibit bilirubin from being excreted.

c) **Cytolytic T-cell activation:** A medication's covalent attachment to the P-450 enzyme functions as an immunogen, triggering cytokines and T cells as well as a complex immunological response.

d) **Apoptosis of hepatocytes:** The tumor necrosis factor alpha receptor of Fas may initiate the apoptotic pathways, hence initiating the cascade of intercellular caspases and ultimately leading to programmed cell death.

e) **Mitochondrial disruption:** By preventing the synthesis of flavin and nicotinamide adenine dinucleotides, many medications suppress mitochondrial activity by having a dual effect on both beta-oxidation energy production and ATP production (20).

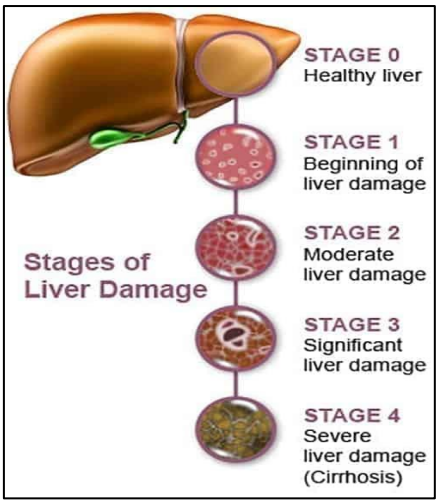

Figure 5: Stages Of Liver Damage

Drug Induced Hepatotoxicity

Drug-induced liver damage is a health concern that is expected to rise with the amount of prescription and over-the-counter medications consumed, as well as the current trend of pharmacologically active substances being used in complementary and alternative medicine. The most well-known justification for the removal of officially authorized pharmaceuticals from distribution is medication-induced hepatotoxicity (21). Furthermore, it accounts for more than half of all cases of severe liver failure in the US.

The exact frequency of medication- or drug-induced liver damage is difficult to determine, and overall, research aiming to quantify its incidence suffer from drawbacks such as under-reporting and the majority of the data coming from review studies. There is frequently a lack of information regarding the use of herbal products for self-healing and the potential for interactions with prescription drugs and over-the-counter drugs (20).

Despite the low recurrence of medication-induced liver injury, the U.S. Centers for Disease Control and Prevention record approximately 1600 new cases of severe liver failure annually, with approximately 41% of these cases being related to paracetamol hepatotoxicity. According to a metaanalysis of about 40 prospective studies, the rate of antagonistic medication responses at the moment where a patient is admitted to the hospital is estimated to be 6.7%, and the rate of fatal hostile medication responses adds up to 0.32%.The number of reports of adverse drug reactions and the deaths associated with them has increased significantly between 1995 and 2005. Many cases of medication-induced liver injury are unique, meaning that the reaction involves arbitrary in light of the medication's established pharmacological characteristics, and from this point on is hardly discernible during preclinical stages of development (22).

However, research suggests that these reactions could be influenced by the patient's increased propensity to respond to the prescribed prescription, depending on factors like coexisting infections or other relevant drugs.Hereditary factors, such as HLA type, can once again increase a person's susceptibility to antagonistic drug reactions. Clinically evident adverse drug reactions typically occur during periods of inactivity, anywhere in the spectrum ranging from one to twelve months (usually within ninety days), and they almost always disappear when the drug is stopped. A few distinct clinical components, such as cholestatic, mixed, or hepatitis/hepatocellular, may be shown by medication that studies liver damage (23).

Drug toxicity mechanisms (24)

Classic division of drug reactions is at least 2 major groups which include:

✓ Drugs which directly affect liver.

✓ Drugs which mediate an immune response.

• **Intrinsic / predictable drug reactions:** This class of drugs' molecules caused reproducible harm in mammals, with dosage being a factor in the harm. Damage may result from the drug itself or from a metabolite. One appropriate illustration of a well-known, consistent hepatotoxin at greater dosages for therapeutic purposes. Tetrachloride of carbon is another example.

• **Idiosyncratic / unpredictable drug reactions:** These pharmacological responses can be further categorised as immunological allergic or hypersensitive reactions and metabolic-idiosyncratic reactions. It manifests in an unpredictable manner and without clear dose dependency (24,20).

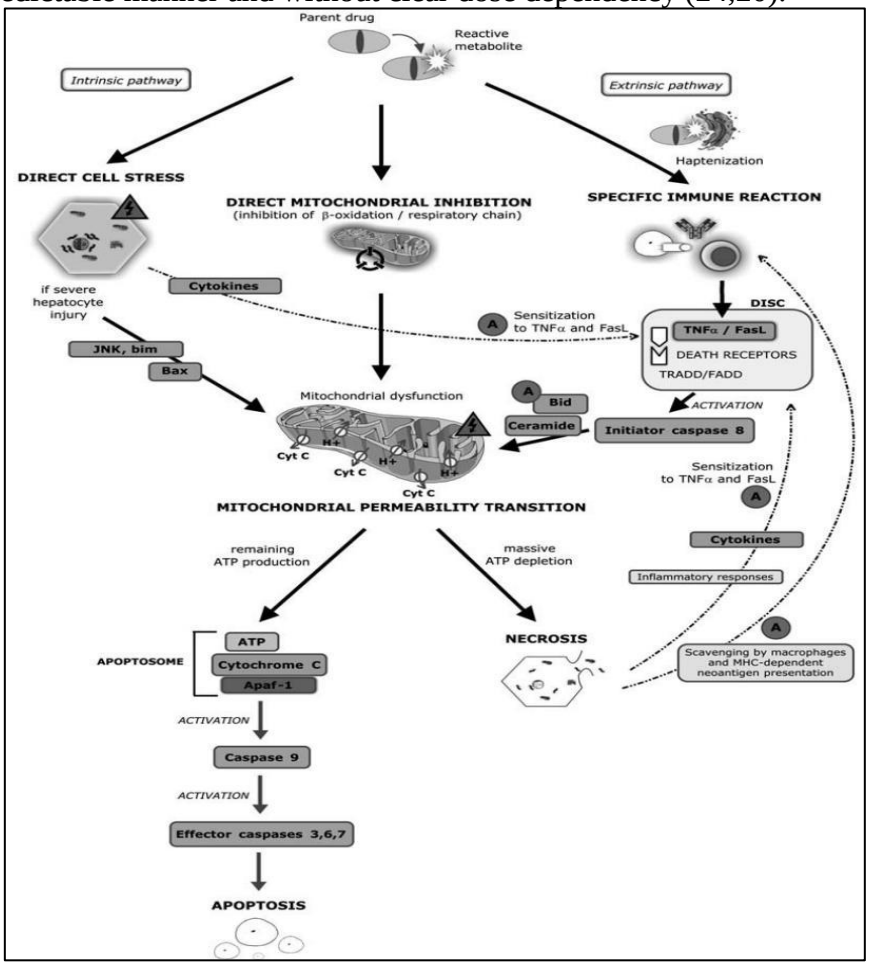

Figure 6: Three Step Mechanistic Working Model Of Hepatotoxicity

Diagnosis (25)

Many further tests may also be used to support the diagnosis. These include blood tests, such as:

• Liver function tests, which are blood tests that check a wide variety of liver enzymes and by products.

• A complete blood count (CBC), which looks at the type and number of blood cells in the body Abdominal X-rays

• Ultrasounds, to show size of abdominal organs and the presence of masses

• An upper GI study, which can detect abnormalities in the esophagus caused by liver disease

• Liver scans with radio tagged substances to show changes in the liver structure

• ERCP, or endoscopic retrograde cholangiopancreatography. A thin tube called an endoscope is used to view various structures in and around the liver.

• Abdominal CT scan or abdominal MRI, which provide more information about the liver structure and function.

Diagnosis of Drug-Related Hepatotoxicity (11)

The diagnosis of medication-related hepatotoxicity cannot be made with a single test, not even a liver biopsy. It is imperative to initially evaluate other causes of hepatic damage by a mix of imaging techniques, serologic testing, and historical information from the patient. CT stands for magnetic resonance imaging. A1AT alpha1-antitrypsin, AST aspartate aminotransferase, ALT alanine aminotransferase, TIBC total iron-binding capacity, and MRCP magnetic resonance cholangiopancreatography are among the tests that can be performed using magnetic resonance imaging and endoscopic retrograde cholangiopancreatography.

Prevention

Some, but not all, liver diseases can be prevented. For example, hepatitis A and hepatitis B can be prevented with vaccines. Other ways to decrease the risk of infectious liver disease include: Practicing good hygiene, such as washing hands well after using the restroom or changing diapers (26).

• Avoiding drinking or using tap water when traveling internationally.
• Avoiding illegal drug use, especially sharing injection equipment.
• Practicing safest sex. Practicing safer sex provides less protection.
• Avoiding the sharing of personal hygiene items, such as razors or nail clippers.
• Avoiding toxic substances and excess alcohol consumption.
• Using medications only as directed and caution around industrial chemicals.
• Eating a well-balanced diet following the food guide pyramid.
• Getting an injection of immune globulin after exposure to hepatitis A.
• Using recommended safety precautions in healthcare and day care work.

Treatment for Hepatotoxicity

The list of treatments mentioned in various sources for hepatotoxicity includes the following (27). Always follow professional medical advice about any treatment or change in treatment plans. Treatment of hepatotoxicity is depending upon causative agent, degree of liver dysfunction and age and general health of patient. Treatments for hepatotoxicity include (28):

✓ Withdrawal of causative medication or removal from exposure to causative agent. ✓ Regular monitoring of patient and review of liver function – where liver dysfunction is mild to moderate and liver function is improving.

✓ Complete avoidance of alcohol and medication that may contribute to further liver damage.

✓ N-Acetylcysteine is used for paracetamol toxicity.

Management of symptoms of liver damage.

• Nutrition – with vitamin supplementation as required
• Regular exercise in order to maintain muscle mass.
• Ursodeoxycholic acid. Management of pruritus
• Cholestyramine
• Antihistamines. Management of ascites
• Low sodium diet.
• Diuretics – furosemide, spironolactone.
• Removal of fluid via a needle in the abdomen – Paracentesis.
• Portosystemic shunting. Management of portal hypertension
• Beta – blockers
• Oesophageal variceal banding
• Portocaval shunt Management of acute liver failure due to hepatotoxicity
• Supportive care always in intensive care unit – airway protection, fluid and electrolyte management.
• Management of complications such as bleeding problems and hepatic encephalopathy.
• Liver transplantation – for acute fulminant liver failure or end stage cirrhosis.

MATERIALS AND METHODS

Materials:

Brassica juncea leaves was selected because of its traditional uses, Ibuprofen, Healthy male Wistar rats (250-300 g) .

Methods:

Ibuprofen induced hepatotoxicity

The method described by Gomaa was adopted to induce liver injuries in the Wistar rats. Liver damage was induced by the administration of ibuprofen orally with single dose (400mg/kg body weight) in rats. The principle is that NSAIDs with short half-lives, such as ibuprofen, may produce renal and liver injury in a matter of days as reported by Bindu, Mazumder and Bandyopadhyay. After the completion of experimental course of therapy, the rats were fasted overnight and blood samples were collected by cervical dislocation under light ether anesthesia. Serum samples were used for the determination of various parameters (12). The serum a marker of oxidative-stress and biochemical parameters of the liver function were measured, while the liver was harvested and the histological examination carried out.

Assessment of hepatoprotective activity(26)
Hepatic enzymes, AST and ALT were used as the biochemical markers of the hepatic damage and were assayed by the method as given in reference. ALP activity was measured using the method as given in reference, and serum bilirubin was estimated by the method as given in reference, to assess the acute hepatic damage caused by ibuprofen.

ESTIMATION OF OXIDATIVE PARAMETER

The activity of superoxide dismutase (SOD) was measured by the modified method as in reference. Catalase (CAT) activity was measured in liver homogenates by the method as given in reference. Reduced glutathione (GSH) activity was assayed according to the method described as given in reference, Malondialdehyde (MDA) and the activity of Glutathione- S-transferase was estimated by the method as given in reference (27, 28).

Histopathological examination

The livers were excised quickly and fixed in 10% formalin and paraffin embedded. Sections of about 4-6μm were stained with hematoxylin and eosin (H&E) for Histological evaluation. In brief 4-6 μm thick sections of paraffin embedded rat liver was dewaxed with distilled water for 2 min. Then the section was stained with hematoxylin for 5 min at room temperature. After 15 min, the section was counterstained with eosin for 2 min, dehydrated with alcohol, washed with xylene and blocked by eosin. Hematoxylin and eosin-stained studies were observed under microscope(29, 30) .

STATISTICAL ANALYSIS

The data are expressed as mean ± Standard Error of the Mean (SEM) (n=6). Analyses are done using the GraphPad Prism version 8 in Stat software. Comparisons of the SEM of control, disease and treated rats were made by repeated -measures one-way analysis of variance (ANOVA) followed by Tukey's test and two-way ANOVA followed by Bonferroni posttest with, $*P < 0.05$ $**P < 0.01$, $***P < 0.001$, and $^{\#} P < 0.05$, $^{\#\#} P < 0.01$, $^{\#\#\#} P < 0.001$ compared with control group, was considered to indicate a statistically significant difference.

CONCLUSION:

The present investigation indicates that ethanolic extract of Brassica juncea exert significant protection against ibuprofen-induced hepatotoxicity by its ability to ameliorate the lipid peroxidation through the free radicals scavenging activity, which enhanced the levels of antioxidant defense system.Brassica juncea appears to be useful in the attenuation of ibuprofen induced lipid peroxidation and showed more prominent effect. The extract showed significant activity against ibuprofen induced hepatotoxicity in rats when compared with thatof standard drug silymarin.

The present study suggested that the Brassica juncea has a preventive and curative effect in Ibuprofen induced hepatotoxicity in Wister rats. From the above study, we can conclude that this plant has medicinal properties. However, further investigations and analysis are required in order to establish the active compounds which are responsible for the hepatoprotectivity.

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