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Phytochemical and Pharmacological evaluation of extract of the plant of Hedychium coronarium against paracetamol- induced hepatotoxicity in Rats.

Research Article

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Abstract

In the present study, also it was seen that administration of PCM elevates the levels of serum marker enzymes ALT, SGOT, SGPT, serum Bilirubin, serum Triglyceride and Cholestrol. Extracts of Hedychium coronarium and silymarin treated group exhibited lower levels of all serum parameters as compared to PCM treated group. The stabilization of serum ALT, SGOT, SGPT, Bilirubin, Triglyceride and Cholestrol levels by extracts of Hedychium coronarium is a clear indication of the improvement of the functional status of the liver cells.

The biochemical examination clearly reveals that the hepatic cells are normal in ethanolic extract of Hedychium coronarium treated group (100 and 200mg /kg, p.o) in contrast to group which received PCM. Thus, ethanolic extract of Hedychium coronarium can be considered to be an effective hepatoprotective in nature, as it normalizes the damage caused by PCM to hepatic function. Thus, it was concluded that the extract of Hedychium coronarium possesses hepatoprotective activity.

Keywords: Hepatotoxicity,Hedychium coronarium, Bilirubin

Introduction

Hepatotoxicity

The liver plays a central role in metabolism of a large number of organic inorganic chemicals and drugs which gain access to the body by inhalation, injection or most commonly, via the intestinal tract. The main drug metabolism system resides in the microsomal fraction of the smooth endoplasmic reticulum of the liver cells via P-450 cytochrome and cytochrome reductase enzyme system.

Toxic liver injury produced by drugs and chemicals may Naturally occurring liver disease. Hepatotoxicity from drugs and chemicals is the commonest form of iatrogenic disease. Among the various inorganic compounds producing Hepatotoxicity are arsenic, phosphorus, cooper and iron. An organic agent includes certain naturally occurring plant toxins such as pyrrolizidine alkaloids, mycotoxins and bacterial toxins. A number of risk factors predispose an individual to hepatic drug injury such as pre- existing liver disease, aging, female sex and genetic inability to perform a particular biotransformation.[10]

The drugs can affect the liver into two main categories such as direct or predictable and indirect or unpredictable.

- Direct or predictable: When the drug or one of its metabolites is either directly toxic to the liver or its lowers the host immune defense mechanism and their hepatotoxicity is dose dependent e.g. carbon tetrachloride.
- Indirect or unpredictable or idiosyncratic: when the drugs or one of its metabolites act as a hapten and induces hypersensitivity in the host and the effects are usually not dose related e.g. acetoaminophen.

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The pathologic changes by hepatotoxins include two large categories such as acute liver disease and chronic liver disease.

Acute liver disease: They are characterized by cholestasis, hepatocellular necrosis, fatty changes, granulomatous reaction or vascular disease.

Chronic liver disease: They are characterized by variable degree of fibrosis, cirrhosis or neoplasia

Cause of Hepatotoxicity

1. CCl_4 Induced hepatotoxicity: The drug is metabolized in endoplasmic reticulum and mitochondria with the formation of CCl_3O^-, the reactive oxidative free radical intermediate generated by cytochrome P-450, the nascent oxygen O^- resulted via
2. lipoperoxidation causes rise in intracellular reactive Fe^{+2} ions, aldehyde and depletion GSH, and calcium sequestration. Oxidative CCl_3 O^- , also by direct covalent interaction induces degeneration of Ca^{+2} sequestrations. Failure into sequestration results in increased intercellular Ca^{+2}, aggregation by proteolytic enzymes and causes an increase in Fe^{+2} ions, which in turn by lipid peroxidation precipitates aldehyde cytotoxicity.[11]
3. Paracetamol-induced hepatotoxicity: The liver damage associated with paracetamol overdose is due to the formation of a hepatotoxic metabolite. Therapeutic doses of paracetamol are metabolized mostly to sulphate and glucuronide conjugates. The rest is metabolized to a reactive intermediate which is detoxified by conjugation with glutathione. In overdose, the sulphate and glucuronide conjugation pathways are saturated and more drugs are converted to the reactive metabolite. The glutathione available for its detoxification is rapidly depleted and the metabolite accumulates and binds covalently to liver cell proteins, causing irreversible damage. Liver damage can be prevented by enriching the body with glutathione like substances, such as acetylcysteine, so that the reactive metabolite can be removed by conjugation and the liver cells are protected.[12]
4. Alcohol induced hepatotoxicity: Ethanol produces constellation of dose related deleterious effects in the liver. The primary effects are fatty infiltration of the liver, hepatitis, and cirrhosis. Because of its intrinsic toxicity, alcohol can injure the liver in the absence of dietary deficiencies. The accumulation of fat in the liver is an early event and can occur in normal individuals after the ingestion of relatively small amounts of ethanol. This accumulation results from inhibition of both the tricarboxylic acid cycle and oxidation of fat, in part owing to the generation of excess.

Hepatic diseases

1.Jaundice

Jaundice or icterus refers to the yellow pigmentation of the skin or sclera by bilirubin. Bilirubin pigment has high affinity for elastic tissues and hence jaundice is particularly noticeable in tissues in elastin content. Jaundice is the result of elevated levels of bilirubinin the blood termed hyperbilirubinaemia. Normal serumbilirubin concentration ranges from 0.2 to 0.8 mg/dl, about 80% of which is unconjugated.

A rise of serum bilirubin between the normal and 2 mg/dl is generally not accompanied by visible jaundice and is called latent jaundice.

Features of jaundice

- Increased bilirubin production
- Decreased hepatic uptake
- Decreased hepatic conjugation
- Decreased excretion of bilirubin into bile

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2. Hepatitis

Hepatitis is an infection of liver caused by hepatotropic viruses. Currently there are 5 main varieties of these viruses and a sixth poorly characterized virus, causing distinct types of viral hepatitis.

Hepatitis A virus (HAV): It causes a faecally spread self-limiting disease.

- Hepatitis B virus (HBV): It causes a parenterally transmitted disease that may become chronic.
- Hepatitis C virus (HCV): Non A-Non B (NANB) hepatitis virus involved chiefly in transfusion related hepatitis.
- Hepatitis delta virus (HDV): This is sometimes, associated as superinfection with hepatitis B infection.
- Hepatitis E virus (HEV): It causes water borne infection.
- Hepatitis G virus (HGV): It is a recently discovered parenterally transmitted hepatotropic virus

3.Cirrhosis

Cirrhosis of the liver is a diffuse disease having the following four features:

- It involves the entire liver
- The normal lobular architecture of hepatic parenchyma is dis-organized
- There is formation of nodules separated from one another by irregular bands of fibrosis
- It occur hepato-cellular necrosis

All forms of injury to the liver such as microbiologic, toxic, circulatory or traumatic result in necrosis of liver cells. The extent of involvement of hepatic lobule in necrosis varies. Accordingly, liver cell necrosis is divided into three types.

- Diffuse (submassive to massive) necrosis: when there is extensive and diffuse necrosis of the liver involving all the cells in groups of lobules, it is most commonly caused by viral hepatitis or drug toxicity.
- Zonal necrosis: zonal necrosis is necrosis of hepatocytes in 3 different zones of the hepatic lobule.
- Focal necrosis: this form of necrosis involves small group of hepatocytes irregularly
- Distributed in the hepatic lobule. Focal necrosis is most often caused by microbiologic infections.

4.Hepatic failure

Though the liver has a marked regenerative capacity and a large functional reserve, hepatic failure may develop from severe acute and fulminant liver injury with massive necrosis of liver cells (acute hepatic failure), or from advanced chronic liver disease (chronic hepatic failure).

Two type of hepatic failure are known:

- Acute (fulminant) hepatic failure: It occurs most frequently in severe viral hepatits. Other causes are hepatotoxic drug reactions (e.g. anaesthetic agents, NSAID, antidepressants), carbon tetrachloride poisoning, acute alcoholic hepatits, mushroom poisoning and pregnancy complicated with eclampsia.
- Chronic hepatic failure: It occurs most often due to cirrhosis. Other causes include chronic active hepatitis, chronic cholestasis and Wilson"sdisease.

5.Hepatic tumours and tumour like lesions

The liver is the site for benign tumours, tumour like lesions and both primary and metastatic malignant tumours. However metastatic tumours are much more common than primary tumours and tumour like lesions. Tumour like lesions includes cysts in the liver and focal nodular hyperplasia.

- **Bengin tumours**

Hepatocellular tumours: Hepatocellular (liver cell) adenoma Biliary tumours: Bile duct adenoma (cholangioma) Mesodermal tumours: Haemangioma

- **Malignant tumours**

Hepatocellular tumours: Hepatocellular (liver cell) carcinoma, hepatoblastoma (embryoma)

Biliary tumours: Cholangiocarcinoma combined hepatocellular and cholangiocarcinoma cystadenocarcinoma

Mesodermal tumours: Angiosarcoma, embryonal sarcoma

6.Other infections

- Cholangitis: Cholangitis is the term to describe inflammation of the extrahepatic or intrahepatic bile ducts or both.
- Pyrogenic liver abscess: Most liver abscesses are of bacterial (pyrogenic) origin, less often they are amoebic, hydatid and rarely actinomycotic.
- Amoebic liver abscess: Amoebic liver abscess are less common than pyrogenic liver abscess and have many similar features. They are caused by the spread of Entamoeba histolytica from intestinal lesions.
- Hepatic tuberculosis: Tuberculosis of the liver occurs as a result of military dissemination from primary complex or from chronic adult pulmonary tuberculosis.The diagnosis is possible liver biopsy. The patients may have unexplained fever,jaundice, hepatomegaly or hepatosplenomegaly.

Materials and Methods[6-13]

Selection of plant

Ethanopharmacological survey was conducted among herbal practioner of Jabalpur Madhya Pradesh, the plant *Hedychium coronarium* used for the liver related issues.

Collection and identification of the plant material

The whole plant of *Hedychium coronarium* were collected from the local area of Jabalpur, Madhya Pradesh, in the month of April 2019 and authenticated at Safia College of Science, Peer gate Bhopal, Madhya Pradesh.

Preparation of powder

The whole plant of Hedychium coronarium were dried in shade and then powdered with a mechanical grinder. The powdered was passed through sieve no. 40 and stored in a labelled air tight container for further studies.

Extraction procedure

The collected, cleaned powder of whole plant of Hedychium coronarium was used for the extraction process. 200 g dried powder of whole plant evenly packed in the soxhlet apparatus and extracted with various solvent in increasing polarity like petroleum ether, chloroform, ethyl acetate, ethanol by hot continuous extraction process for about 26 h. The aqueous extraction was carried out by cold maceration process after above extractions. The extracts were filtered while hot through Whatman filter paper to remove any impurities if present. The extracts were concentrated by vacuum distillation to reduce the volume up to 1/10. The concentrated extracts were transferred to 100 ml beaker and evaporated on the water bath. Then extracts were collected and placed in desiccators to remove the excessive moisture. The dried extracts were packed and labelled in air tight container for the further studies such as a phytochemical screening and pharmacological activities.

Pharmacological study

The experiment was carried out on Wistar albino rats of 4 months, of both sexes, weighing between 120 to 180 gm. They were provided from Sapience Bio-analytical Research Lab, Bhopal, (M.P.). The animals were acclimatized to the standard laboratory conditions in cross ventilated animal house at temperature 25±2°C relative humidity 44 –56% and light and dark cycles of 12:12 hours, fed with standard pallet diet and water ad libitum during experiment.

Preparation of Paracetamol and Silymarin

Paracetamol was prepared in 0.5% sodium CMC solution and used for oral administration. Previous experiments for dose finding indicate Paracetamol 2g/kg was selected as the toxicant dose in the present study. Silymarin was dissolved in normal saline and 100mg/kg p.o. dose was selected as a standard.

Experimental Design

In the experiment, a total of 30 rats were used. The rats were divided into 5 groups comprising of 6 animals in each group as follows:

Group I: Normal control rats received 1ml/100gm of 0.5% sodium CMC using an intragastric tube for 7days.

Group II: Negative control rats received paracetamol 2g/kg, p.o. for inducing hepatotoxicity **Group III:** Rats received Silymarin (100mg/kg, p.o.) for 7 days and paracetamol 2g/kg, p.o. on 6th day.

Group IV: Rats received ethanolic extract of *Hedychium coronarium* 100mg/kg once daily for 7 days and paracetamol 2g/kg, p.o. on 6th day.

Group V: Rats received ethanolic extract of *Hedychium coronarium* 200mg/kg once daily for 7 days and paracetamol 2g/kg, p.o. on 6th day.

Sample collection

At the end of the experiment on 7th day, Rats were sacrificed by cervical dislocation. Blood was collected by orbital puncture and allowed to clot for 30 minutes at room temperature. The serum was separated by centrifugation at 3000 rpm at 30°C for 15 minutes.

Biochemical estimation

The serum samples were subjected to biochemical parameters examination like ALT, SGOT, SGPT, Bilirubin, Triglyceride and Cholestrol levels were estimated by using standard kits (Span diagnostics Ltd).

Procedure

In different tubes,1 ml the buffered substrate was added to 0.1 ml of serum and incubated at 37^{O}C for 30 min. Then 1 ml of DNPH reagent was added to arrest the reaction. To the blank tubes, 0.1 ml of enzyme was added only after the addition of DNPH reagent. The tubes were kept aside for 15 min and then 10 ml of 0.4 N sodium hydroxide was added and read at 505 nm in a UV spectrophotometer. The enzyme activity is expressed as UL^{-1} of serum.

Result and Discussion

Hepatoprotective study

The hepatitis associated with liver cirrhosis even hepatocellular carcinoma, which has become one of the most prevalent diseases in the world, can be induced by viruses, alcohol or other toxic chemicals. Increasing transaminase activities and jaundice were significantly observed in most hepatitis sufferers. Liver damage in animal models can be induced by Peracetamol from transaminases and ALP leakage in the blood can be detected, which is often associated with hepatonecrosis. In this study, the protective effect of ethanol extracts of whole plant of Hedychium coronarium on Peracetamol induced hepatotoxicity was evaluated through various biochemical parameters.

Effect of extracts on serum glutamate oxalacetate transminase (SGOT) and serum glutamate pyruvate transamainse (SGPT) levels

The levels of SGOT and SGPT in normal control group were 86.05and 64.24
respectively, while the levels of SGOT and SGPT were elevated in Peracetamol treated rats and found to be 177.37 and 157.48, respectively. The ethanol extracts of whole plant of Hedychium coronarium (100/200 mg/kg) were decreased the levels of SGOT and SGPT, when compared with toxic group. The ethanol extracts of whole plant of Hedychium coronarium showed more significant activity at a dose level of 100 mg/kg.

Effect of extracts on alkaline phosphatase (ALP) level

The level of ALP in normal control group was found to be 68.42 and elevated value was found to be (136.52)Peracetamol treated rats. The ethanol extracts of whole plant of Hedychium coronarium (100 and 200 mg/kg) were decreased the level of ALP, when compared with toxic group.

Effect of extracts on total bilirubin (TB) level

The level of TB in normal control group was found to be 1.3 and Peracetamol treated rats was found to be 4.08. The aqueous stem bark extract of Hedychium coronarium (100 and 200 mg/kg) significantly decreased the level of TB, when compared with toxic group. The TB value of 100 and 200 mg/kg of aqueous extract were 3.44 and 2.87,

Effect of extracts on total cholesterol (TC) level

The level of TC in normal control group was 75.83 and elevated level was found to be Peracetamol treated rats (86.37). The ethanol extracts of whole plant of Hedychium coronarium (100 and 200 mg/kg) were decreased the levels of TC, when compared with toxic group. The ethanol extracts of whole plant of Hedychium coronarium (100 mg/kg) showed more significant activity than the 200 mg/kg.

Effect of extracts on triglyceride level

The decrease the level of Triglyceride was found to be Peracetamol treated rats (173.2), where the level of Triglyceride in normal rat group was found to be127.92. The ethanol whole plant extracts of Hedychium coronarium (100 and 200mg/kg) significantly increase the level of triglyceride, when compared with normal control group rats. The TP level of 100 and 200 mg/kg of ethanol extracts were found to be 156.0, 157.9 and 161.9, respectively.

Conclusion

PCM treatment significantly increased the serum enzyme levels, namely ALT, AST and ALP indicating chemical induced hepatocellular toxicity. Serum levels of these enzymes are very sensitive markers employed in the diagnosis of liver diseases. When the hepatocellular plasma membrane is damaged, the enzymes normally present in the cytosol are released into the blood stream. This can be quantified to assess the type and extent of liver injury.

In the present study, also it was seen that administration of PCM elevates the levels of serum marker enzymes ALT, SGOT, SGPT, serum Bilirubin, serum Triglyceride and Cholestrol. Extracts of Hedychium coronarium and silymarin treated group exhibited lower levels of all serum parameters as compared to PCM treated group. The stabilization of serum ALT, SGOT, SGPT, Bilirub in, Triglyceride and Cholestrol levels by extracts of Hedychium coronarium is a clear indication of the improvement of the functional status of the liver cells.

The biochemical examination clearly reveals that the hepatic cells are normal in ethanolic extract of Hedychium coronarium treated group (100 and 200mg /kg, p.o) in contrast to group which received PCM. Thus, ethanolic extract of Hedychium coronarium can be considered to be an effective hepatoprotective in nature, as it normalizes the damage caused by PCM to hepatic function. Thus, it was concluded that the extract of Hedychium coronarium possesses hepatoprotective activity.

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Groups	No. of animal	Dose (mg/kg)	Result
1.	3	2000	3 death
2.	3	300	1 death
3.	3	300	1 death

Table No 2: Acute toxicity study

S.No	Feature	Observation
1	Leaf type	Simple
2	Margin	Undulate
3	Shape	Oblong
4	Venation	Pinnate
5	Color	Green
6	Leaf length	8-12 inch
7	Flower color	White
8	Fragrance	Pleasant
9	Bark and Stem	Green

Table No: 3: Effect of ethanolic extract of *Hedychium coronarium* on serum biochemical parameters in PCM induced hepatic injury in rats.

GROUP	ALP (IU/L)	SGOT/AS T (IU/L)	SGPT/AL T (IU/L)	TOTAL BILURUBI N (mg/dl)	TRIGLTCE RI DE (mg/dl)	CHOLESTR OL (mg/dl)
Group I (Normal Control)	68.42 ± 0.62	86.05± 3.57	64.25 ± 2.84	1.3 ± 0.32	127.92±5.36	75.83 ± 2.34
Group II (- ve Control PCM)	136.52 ± 0.81	177.37 ± 7.81	157.48 ± 1.25	4.08 ± 0.68	173.2±9.94	86.37 ± 3.15
Group III (Std. Silymarin)	79.65 ± 0.37**	83.71 ± 4.79**	87.39 ± 4.97**	2.06 ± 0.76	156.0±2.63	75.53 ± 0.49
Group IV (EEEA, 100mg/k g)	116.35 ± 0.89*	112.17 ± 5.35**	118.12 ± 5.97*	3.44 ± 0.46	157.9±5.68	71.56 ± 0.68
Group V (EEEA, 200mg/k g)	103.63 ± 0.52**	106.18 ± 2.45**	110.62 ± 4.96**	2.87 ± 0.32	161.9±6.83	78.40 ± 2.69

Fig 1: Silymarin treated

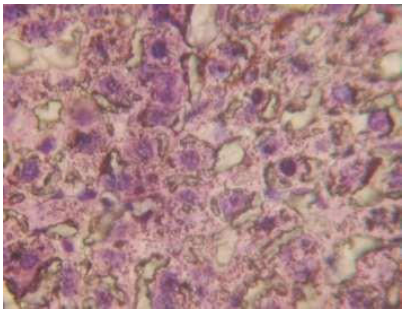

Fig 2 : Ethanolic Extract of *Hedychium coronarium* 200mg/kg treated