Preclinical evaluation of hepatoprotective activity of *Grewia hirsuta* extract on ethylene glycol induced liver damage in Wister albino rats

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ABSTRACT

The extract of *Grewia hirsuta* were screened for its Hepatoprotective activity in ethylene glycol induced liver damage in Wister albino rats. The extracts at dose of 250, 750 mg/kg were administered orally once daily. The substantially elevated serum enzymatic levels of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transminase (SGPT), serum alkaline phosphatase (ALP) total bilirubin, SOD and catalase were restored towards normalization significantly by the extracts. Cystone was used as standard reference and exhibited significant Hepatoprotective activity against ethylene glycol induced hepatic damage in rats.

KEY WORDS: *Grewia hirsute,* hepatocarcinoma, Transaminase, Cystone.

1. INTRODUCTION

Liver has a prominent role in the regulation of physiological processes. It is involved in varieties of vital functions such as detoxification. Furthermore, detoxification of a variety of drugs and xenobiotic occurs in liver. Hence liver diseases are among the most serious health aliments. They may be classified as acute or chronic hepatitis (inflammatory liver disease, hepatitis, (non-inflammatory disease) and cirrhosis (degenerative disorder resulting in fibrosis of the liver). Liver disease are mainly caused by toxic chemicals (certain antibiotics, chemotherapeutics, peroxidised oil, aflatoxin, carbon tetrachloride, paracetamol, chlorinated hydrocarbons etc.), excess consumption of alcohol, infection and autoimmune disorder. So it has become very much necessary to protect the liver form all these agents.

Ethylene glycol is a highly toxic chemical agent, the most famous drug used to induce liver damage experimentally. It induced fibrosis, cirrhosis and hepatocarcinoma as is the liver is responsible for metabolism and detoxification of the most of components including ethylene glycol that enter the body.

2. MATERIALS AND METHOD

2.1. Collection and authentication of plant material: Plant material was collected from the local hilly region of Kondapalli forest, Andhra Pradesh. It was identified and authenticated by Dr.Khasim, Department of Botany and Micro Biology in Acharya nagarjuna university, Guntur, Andhra Pradesh.

2.2. Preparation of hydro alcoholic extract of *Grewia hirsuta*: The collected fresh plant root materials were dried in shade (3 week) and then dried in a hot air oven at 25°C for three days and they were made in to coarse powder with the use of mixer grinder, the powder of entire plant of *Grewia hirsuta* were weighed separately and transferred to a round bottomed flask and then went to continuous heat extraction with soxhalet apparatus using 95% ethanol and distilled water (1:1) for 24 hours. Then the hydro alcoholic extract was concentrated. Extract obtained was dried by placing it on a petiolate on electric water bath (70°C) and kept in an oven at 30°C for 2hrs. The extract obtained was kept for drying and stored in vacuum desiccators

2.3. Instruments: Auto analyser, Refrigerator centrifuge, UV-sector photometer, Research centrifuge tube, and Homogenizer, Dhona balance, hot air oven. Heating mantle microscope, oral feeding needles, incubator, and test tube.

2.4. Diagnostic kits: Diagnostic kits used for estimation of total protein, albumin, Bilirubin, alkaline phosphatase, SGOT and SGPT (were procured from Royal Diagnostic lab).

2.5. Grouping of animals: Animals were divided into 5 groups, each group consisting of 6 animals. The animals were fasted for 24 h prior to ethylene glycol.

Group A: Normal Control

Group B: Diseased

Group C: Positive Control (systone of about 15 mg/kg), s.c

Group D -medium dose of *Grewia hirsuta* (200mg /kg), p.o

Group E -high dose of *Grewia hirsuta* (300mg/kg), p.o

2.6. Estimation of alkaline phosphates: Phosphates are enzymes which catalyse the splitting of a phosphate from mono phosphoric esters. Alkaline phosphate (ALP), a mixture of iso enzymes from liver, bone, intestine and placenta, has maximum enzyme activity at about P^H 10.5. Serum ALP measurements

are of particular interest in the investigation of hepatobillary and bone diseases.

2.7. Estimation of Serum Bilirubin: Bilirubin is formed from the haem fragment of hemoglobin released by aged or damage red blood cells. Liver, spleen and bone marrow are the sites of bilirubin production. Bilirubin formed in spleen and bone marrow is transported to the liver. In the liver it is converted into bilirubin conjugates — bilirubin mono and diglucuronides. Any liver disease affects the above systems, and hence bilirubin accumulates in serum leading to jaundice.

2.8. Estimation of Total Protein: The serum-total protein, as its name implies, represents the sum total of numerous different proteins, many of which vary independently of each other. Proteins are present in all body fluids but the protein concentration is normally high (> 3g/dl) only in plasma, lymphatic fluids and some exudates. Protein concentration in the cerebrospinal fluids of normal subjects is < 45 mg/dl, whereas the urine contains only trace. Measurement of serum-total protein is useful in conditions relating to changes in plasma or fluids volumes, such as shock, liver disease and dehydration. In these conditions concentration of serum-total protein is elevated indicating hemo concentration. Henio dilution is rejected as relative hypoproteinemia, which occurs with water intoxication or salt retention syndrome, during massive intravenous infusions.

2.9. Estimation of SGOT: SGOT coverts L Aspartate and x ketogutarte to oxaloacetate and glutamate. The oxaloacetate formed react with 2, 4 Dinitrophenyl hydrazine to produce a hydrazine derivate, which is an alkaline medium produce a brown colour complex whose intensity is measured. The reaction dose not obeys beer's law and hence a calibration curve is plotted using a pyruvate standard. The activity of SGOT is read off this calibration curve.

2.10. Estimation of SGPT:

L-Alanine + 2-Oxoglutarate ⟶ ALT ⟶ Pyruvate + L-Glutamate

Pyruvate + NaOH ⟶ LDH ⟶ L-Lactate + NAD

ALT: Alanine aminotransferase

LDH: Lactate Dehydrogenase.

2.11. Estimation of albumin: Albumin binds with the dye bromocresol green in a buffer medium to form a green Coloured Complex. The intensity of coloured form is directly proportional to the amount of albumin present in the sample.

3. RESULTS

Table.1.Effect of hydro alcoholic extract of *Grewia hirsuta* on SGPT, SGOT and ALP

Treated group	SGPT	SGOT	ALP
Normal saline(2ml/kg)	115.8±0.1308	460.0±17.98	174.0±2.769
Control(1ml/kg)	152.1±0.23	541.3±6.893	250.0±0.0
Standard(systone)25/mg/kg	150.5±0.1302	327.5±6.52	174.2±1.887
GH(medium dose) 200 mg	138.5±0.1202	424.0±0.89	172.7±4.187
GH(high dose) 300mg	115.8±0.1308	454.8±2.15	209.5±4.559

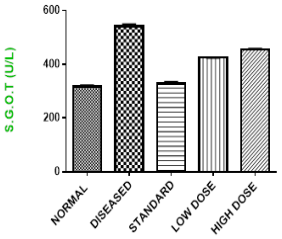

Figure.1.Effect of *Grewia hirsute* on S.G.O.T in female rats

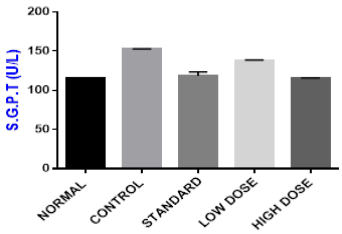

Figure.2. Effect of *Grewia hirsute* on S.G.P.T in female rats

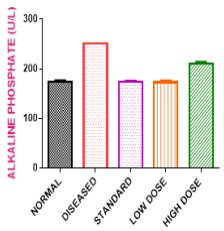

Figure.3. Effect of *Grewia hirsute* on alkaline phosphatase in female rats

Table.2.Effect of *Grewia hirsuta* on total bilurubin and total protein:

Treated group	Total bilirubin	Total protein
Normal saline(2ml/kg)	0.2336±0.0015	6.267±0.1256
Control(1ml/kg)	0.1335±0.0013	5.00±0.00
Standard	0.3004±0.0013	6.767±0.1054
GH(low dose)	0.2164±0.0014	6.350±0.0562
GH(high dose)	0.3504±0.0015	7.033±0.0557

Table.3.Effect of *Grewia hirsuta* on globulin and albumin

Treated group	Globulin	Albumin
Normal saline	3.36±0.088	3.46±1.229
Control	4.30±0.236	4.28±0.1014
Standard	2.61±0.079	3.50±0.1238
GH(low dose)	2.30±0.096	4.41±0.1138
GH(high dose)	2.41±0.135	3.43±0.1054

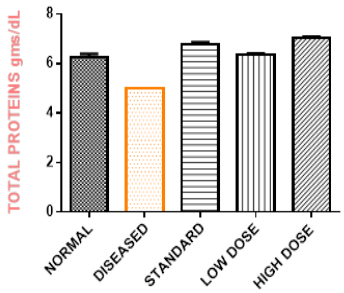

Figure.4. Effect of *Grewia hirsute* on total proteins level in female rats

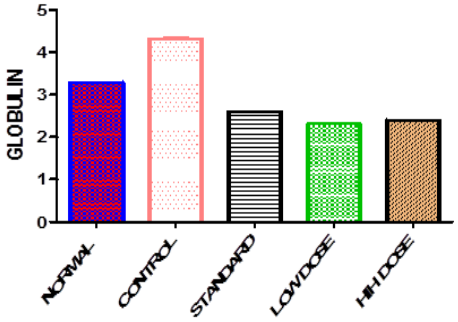

Figure.5. Effect of *Grewia hirsute* on globulin in female rats

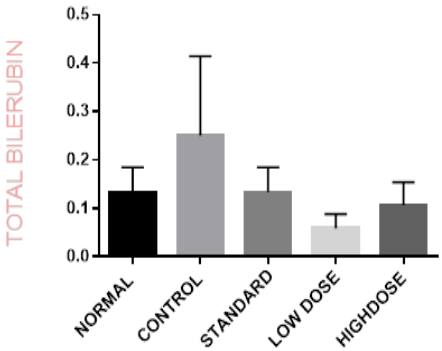

Figure.6. Effect of *Grewia hirsute* on total bilurubin in female rats

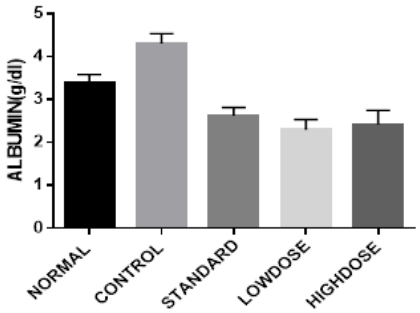

Figure.7. Effect of *Grewia hirsute* on albumin in female rats

DISCUSSION

Ethylene glycol is well known hepatotoxicity agent and hepatotoxicity induced by ethylene glycol is the most commonly used model system for the screening of hepatoprotective activity of plant extracts of drugs. The changes associated with ethylene glycol induced liver damage are similar to that of acute viral hepatitis. Toxicity begins with the changes in endoplasmic reticulum, which results in the loss of metabolic enzymes located in the intracellular structure. Ethylene glycol is a xenobiotic that produces hepaotoxicity in various experimental animals. Ethylene glycol is metabolized by cytochrome p450 to form a reactive trichloromethylperoxyl radical (ethylene glycoloz). Both radicals are capable of binding to DNA, lipids, proteins or carbohydrates, leading to lipid per oxidation, cell necrosis, and excessive deposition of collagen in liver and liver fibrosis. Liver is a vital organ which regulates many important functions in our body such as metabolism, secretion and storage. Furthermore, detoxification of a variety of drugs and xenobiotic also occur in liver. Thus, as it exposes to number of toxicants, chemicals, it gets damaged. In the present study, ethylene glycol is used to induce hepatotoxicity as it is easily available and known hepatotoxicity agent. When liver plasma membrane is damaged variety of enzymes found in the cytoplasm were released into the circulatory system which results in elevated levels of such enzymes in serum.

SUMMARY

The present study is performed to evaluate the Hepatoprotective activity of ethanolic leaf extract of *Grewia hirsute.* The Hepatoprotective activity is studied against ethylene glycol induced liver damage and the reports are summarized as follows:

- The phytochemical screening of GH extract showed the presence of alkaloids, glycosides, tannins, steroids, carbohydrates, reducing sugars.
- Biochemical parameters like Alkaline Phosphates (ALP), direct bilirubin and total protein are also considered.

Ethylene glycol induced hepatotoxicity is significantly prevented by pretreatment of hydro ethanolic leaf extract of *Grewia hirsuta*. Biochemical parameters like Alkaline Phosphates (ALP), total, direct bilirubin and total protein after treating with the hydro ethanolic leaf extract of *Grewia hirsuta* confirmed that the extract that has Hepatoprotective activity.

4. CONCULSION

The Hepatoprotective activity of hydro ethanolic extract of *Grewia hirsuta* is confirmed by following parameters. The isolated liver from the control group resulted in increase of their physical parameter like wet liver and wet liver volume. While treating with GH extract it is found that the values of these parameters were decreased, this indicated that it has Hepatoprotective activity. The animals that are treated with ethylene glycol resulted in the elevated levels of serum enzymes like SGPT, SGOT, ALP, Total bilirubin and reduced levels of total protein. While treating with GH extract it is found that the levels of enzymes were reduced while the level of total protein increased, this indicates that it has Hepatoprotective activity. In ethylene glycol treated animals, there is severe damage to the hepatic cells and also disturbance in the architecture of the liver. In the present study, the animals treated with the GH extract exhibited reduced hepatic damage. Finally, depending upon the improvement of physical, biochemical, parameters and Histopathological studies, it is concluded that the hydro ethanolic leaf extract of GH has Hepatoprotective activity and thus supports the traditional application of the same under the modern science.

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