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Levels of Lactase dehydrogenase, Gamma-glutamyl transferase, Glycogen, Bilrubin and Evaluate the hepatoprotective activity of ethanol Extract of *Mimosa pudica*

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Abstract

Plants that were used for therapeutic purposes in the ancient era are being used in present day as well. The Sox Halation procedure is used in order to obtain the extracts that were undiluted. These extracts were examined by implementing the Gas Chromatography Mass Spectrophotometry (GCMS) to distinguish the biochemical elements. The present study was conducted to evaluate the hepatoprotective activity of ethanol extract of *Mimosa pudica* against alcohol induced albino rats. This was evident from significant levels of Lactate dehyrogenase-LDH, Gamma-glutamyl transferase-GGT, Glycogen, bilirubin, Protein and Cholesterol. *Mimosa pudica*, may be responsible for the significant hepatoprotective activity and the results justify the use of *Mimosa pudica* as a hepatoprotective agent.

Keywords: *Mimosa pudica*, GC-MS, DNA isolation, Hepatoprotective activity, liver homogenate, Serum

1. INTRODUCTION

For medicinal benefits, several plants have been identified as a foundation of various effective and prevalent medicines. From the period of the ancient times until today, these herbs used as traditional medications, have been widespread and have proved to be efficient in curing diseases across the world. It has been demonstrated that *in vitro*

screening techniques may possibly give the required prime perceptions that have been important in selecting crude plant extracts with conceivably valuable characteristics for further chemical, microbiological and pharmacological examinations. Number of medicinal preparations has been advocated in tradition system of medicine especially in ayurvedha for treating liver disorders (Huo H-Z *et al*., 2011). Usage of many plant products in hepatoprotective activity have often claimed to offer significant relief, Indian medicinal plants belonging to about 40 families were investigated as liver protective drug The liver plays vital role in maintenance, performance, regulation of homeostasis, secretions of bile, storage of vitamins (Ahsan *et al*., 2009) and detoxification in the body. It participates in all the biochemical pathways to growth, immune system, nutrient supply, energy provision and reproduction the proper functioning of liver is essential for healthy people.

Hepatic cell injury caused by various toxicants like chemotherapeutic agents, anti tuberculosis drugs, carbon tetrachloride, paracetamol, chronic alcohol consumption and pathogenic microbes are well reported.

2 MATERIALS AND METHODS

2.1 GC-MS Method:

The extracts were obtained distinctly and distinguished by GCMS. The purified extract of *Mimosa Pudica* was freeze dried then mimosine extract and phytochemical's were obtained.

2.2 DNA isolation from plant

Fresh *Mimosa pudica* leafs was washed with sterile water. Leaf sample in mortar pestle is taken. Universal DNA extract 200µl were added and in buffer solution was taken. Add more buffer & make up into 500 µl, incubate to 59-60ºC and Spin down sample at10, 000 rpm for 10min.Transfer supernatant into other sterile microfuge. Equal volume of chloroform isomyl alcohol mixture (24:1) to sample & mix well. Centrifuge at 10,000rpm for 10min & at three different phases. Collected upper aqueous phase in other sterile microfuge tube. Added equal volume pure ice cold IPA (Isopropyl alcohol) to the upper phase. Mix it & incubated to 20º -30ºC.After that precipated DNA was seen. Centrifuge tube for 10,000rpm for 10min.After centrifugation discard supernatant & collected pellets .Added 70% of alcohol & after complete drying DNA appears as water drop .Then 50 µl of sterile water or buffer added and dissolved pellet and stored that DNA in low temperature.

2.3 Experimental studies done in rats

All the end of the experimental period of 30 days the rats were deprived of food for a night and the next day , sacrificed by cervical dislocation .Blood was collected and kept for 30 minutes without disturbing. The clots were then centrifuged for 15 minutes at 2000rpm to separate serum and used for biochemical analyasis.

The liver was removed for histopathology. A part of the liver was washed with ice cold tris buffered saline (pH 7.4) to follow lipid peroxidation rate.15% homogenate were prepared using phosphate buffered saline (pH7.0) in cold condition for the estimation of antioxidants and liver marker enzymes(20% liver homogenate were prepared using 5% trichloro acetic acid to estimate reduced glutathione. The homogenate was centrifuged at 2000rpm for 10minutes and the supernatant was used for the experiment.

2.4 Preparation of extract*:*

The coarsely powdered leaves (300g) of *Mimosa pudica* was extracted to exhaustion in a soxhlet apparatus at 50ºC with 500ml of ethanol. The extract was filtered through a cotton plug, followed by whatsman filter paper and then concentrated by using a rotary evaporator at a low temperature (40-60ºC) and reduced pressure to provide ethanolic extractive of 8.20g.

2.5 Preliminary phytochemical analysis

The ethanolic extract of *M. pudica* was subjected to identify the presence of various phytoconstituents viz. alkaloids (Mayer's test), steroids and terpenoids (Leibermann Burchard test), glycosides (Modified Borntrager's test), tannin and phenolic compounds (ferric chloride test), flavonoids (Lead acetate test), Saponins (Foam test), proteins and amino acids (Xanthoproteic test) etc.

2.6 Experimental design

Wistar albino rats (150-200 g) used in the present studies. The animals were fed with standard pellet diet (Amala medical college-thrissur) . All the animals were acclimatized for a week before use. The animals were received the drug by oral gavages tube. The laboratory conditions duly undertaken by registered veterinary practitioner. The rats were divided into four groups Group 1: Control – Normal healthy Rats., Group 2: Alcohol Control- Rats were given with 40% alcohol orally, Group 3: Simultaneous induction and treatment- Alcohol + Aqueous extract (Simultaneous given) 40% alcohol and aqueous plant (*Mimosa pudica*) extract (1gm/ kg body weight) was given orally and Group 4: Aqueous extract control – Rats given

with aqueous extract (1gm/kg body weight) of the plant *Mimosa pudica* orally.

Assessment of liver function

Blood was collected from all the groups by puncturing the retro-orbital plexus and was allowed to clot at room temperature and serum was separated by centrifugation at 2500rpm for 10 min. The serum was used for estimation of biochemical parameters to determine the functional state of the liver.

Toxicity studies

Healthy Wistar albino rats of either sex weighing 150-200 g maintained under standard laboratory conditions were used for acute oral toxicity test according to Organization for Economic Co-operation and Development guidelines 423 (OECD, 1996). Male albino Wistar rats of body weight 150gms were obtained from Amala medical college, Thrissur. The rat were housed in large spacious cages and were fed on standard pellet diet and water libitium. The animals were well ventilated area. The experimental procedures were carried out as per strictly animal ethics committee's rules and regulations of this institute.

3. RESULT

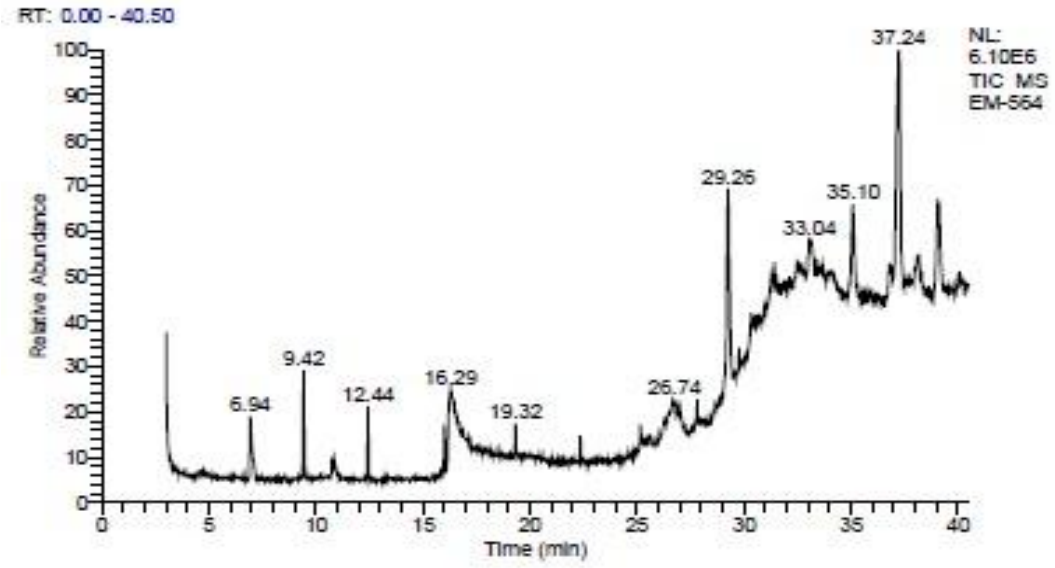

Fig-I: ***Gas Chromatography- Mass Spectrometry Analysis (GC-MS) graph of Mimosa pudica*** **leaves.**

Table-I Levels of lactase dehydrogenase, gamma-glutamyl transferase, glycogen & bilrubin

Groups	Lactate dehyrogenase-LDH		Gamma-glutamyl transferase-GGT		Glycogen	Bilirubin		Protein	Cholesterol
	Serum (Units)	Liver (Units)	Serum (Units)	Liver (Units)	Liver (Units)	DB	TB	Serum	Serum
I	350.34± 3.22	373.24± 23.18	9.45± 0.46	2.34± 0.78	1.57± 0.11	0.50± 0.04	1.64± 0.06	8.19± 0.02	165.23± 0.05
II	403.67± 9.33	543.12± 19.50	15.47± 1.23	4.23± 0.18	1.10± 0.99	1.24± 0.03	2.89± 0.21	6.13± 0.83	240.43± 1.07
III	380.44± 12.23	462.23± 16.26	3.54± 0.34	4.52± 0.15	1.39± 0.07	0.71± 0.23	1.62± 0.08	7.74± 0.92	189.12± 1.37
IV	230.56± 2.39	372.33± 22.91	7.14± 0.19	3.19± 0.28	1.77± 0.30	0.91± 0.07	1.48± 0.06	9.29± 1.10	168.22± 1.09

Statistical comparision:

a = Significant ($p \leq 0.05$) when Group II compared with Group I

b= Significant ($p \leq 0.05$) when Group III compared with Group I1

LDH (serum) – Units : Microgram of pyruvate liberated /min/litre

LDH (Liver) – Units : Microgram of pyruvate liberated /min/mg/protein

GGT – Units :IU/Litre

Glycogen – Units :gm/100mg

Bilirubin – Units : mg /dl

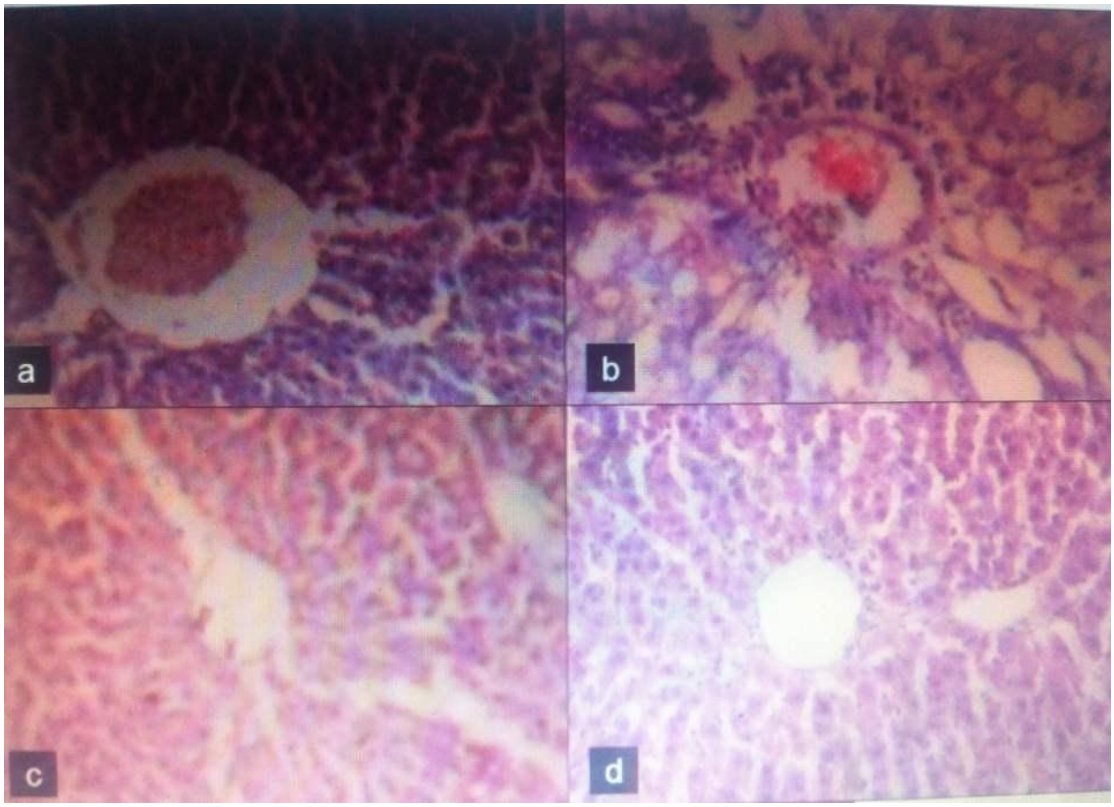

Fig II- Hepatoprotective activity

a. Normal hepatic architecture, b. Alcohol control –Hepatic necrosis

c. Cell regeneration, d. Hepatic regeneration

3.1 Hepatoprotective efficacy of *Mimosa pudica*

The present study on the Hepatoprotective efficacy of *Mimosa pudica* on ethanol induced liver damage in rats was carried out and results obtained are discussed. The activity of the enzyme antioxidants such as superoxide dismutase is 176.83g, Peroxidase is 0.989g, were found to be present in the plant extract of *Mimosa pudica*. Non enzymatic antioxidants like phenols are 27.63g, flavonoids is 0.436g and Reduced glutathione is 216.19g. Vitamin-E is 179.81g, and tannins is 87.678g were analyzed in the aqueous extract of *Mimosa pudica*.

3.2 Estimation of lactase dehydrogenase, gamma-glutamyl transferase, glycogen & bilrubin

The result shows LDH(Lactate dehydrogenase) were assessed in serum showed highest value shows in Group II (403.67±9.33) and liver showed highest value (543.12±19.50).The GGT (Gamma-glutamyl transferase) were assessed in serum –

highest value shows in Group II showed (15.47±1.23) and liver showed highest value (4.23±0.18).

A significant increase (P< 0.05) in ALP, ACP and gamma – GT activity was exhibited in alcohol induced groups when compared to Group I animals treatment with *Mimosa pudica* extract showed a better reduction in Group III(P< 0.05) rats. When liver cell plasma membrane is dam aged variety of enzymes normally located in the cytosol are released. The estimation of these enzymes is useful quantitative marker of the extent and type of heaptocellular damage. Co- administration of *Mimosa pudica* extract with alcohol decreased significantly (P< 0.05) Gamma-GT activity when compared with Group II rats.

All forms of liver disease lead to an increase in membrane bound gamma glutamyl transferase (gamma- GT) in serum and liver. This is due to the release of cell membrane fragments into the circulation. On prolonged contact of bile acids with blue duct epithelia could solubilise and release – gamma –GT.

4. DISCUSSION

In rat treated with toxic dose of alcohol, a significant increase in serum and liver were observed. Simultaneous oral administration of aqueous extract of *Mimosa pudica* reduced the LDH activity to a significant extent compared to control. LDH, cytosolic liver marker enzyme is a regulator of many biochemical reactions in the body tissues and the fluid. Disturbances in cell membrane was estimated by measuring the leakage of LDH .

When liver cell plasma membrane is damaged variety of enzymes normally located in the cytosol are released. The estimation of these enzymes is useful quantitative marker of the extent and type of heaptocellular damage. Co- administration of *Mimosa pudica* extract with alcohol decreased significantly (P< 0.05) Gamma-GT activity when compared with group II rats. All form of liver disease lead to an increase in membrane bound gamma glutamyl transferase (gamma- GT) in serum and liver. This is due to the release of cell membrane fragments into the circulation. On prolonged contact of bile acids with blue duct epithelia could solubilise and release – gamma –GT.

5. CONCLUSIONS

The most important of these biologically active constituents of plants are alkaloids, flavonoids, tannins and phenolic compounds. There are many herbs, which are predominantly used to treat cardiovascular problems, liver disorders, central nervous system, digestive and metabolic disorders. The *Mimosa pudica*, invites attention of

the researchers worldwide for its Biotechnology studies, Pharmacological, Microbiological activities such as antidiabetic, antitoxin, antioxidant and wound healing activities. It is reported to contain alkaloid, glycoside, flavonoid and tannins All parts of the plant are considered to possess medicinal properties and used in the treatment of biliousness, leprosy, dysentery, vaginal and uterine complaints, inflammations, burning sensation, fatigue, asthma, leucoderma, blood diseases and liver disease.

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