In-vitro Hepatoprotective Activity of Glycyrrhizia Glabra Root extract on Hepg2 cell line

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Abstract-Background-In Vitro assays are important in the evaluation of the plant with hepatoprotective activity. this study compares the D-galactosamine and without D-galactosamine with glycyrrhiza glabra extract root on Hepg2 cells. Dexamethasone was used as the positive control. Then determine the cytotoxicity.
Aim-This study was undertaken by evaluating the in-vitro Hepatoprotective Activity of glycyrrhiza glabra Root extract on Hepg2 cell line.
Material & Method-Four doses of the test solution (50, 100, 150, 200ug/ml) were tested on D-galactosamine induced Hepg2 cells & without D-galactosamine induced Hepg2 cells. MTT assay was performed to determine the hepatoprotective activity.
Result-Hepg2 cells were treated with D-galactosamine (10mM standardized with experiment) for 24 hours to induce hepatic toxicity Dexamethasone was kept a positive control, to check if it drug is inducing any toxicity blank for the same were also maintained. The cells treated with only the drug showed no toxicity but also supplemented the growth of the cells. It was observed that the cell number of cells treated with D-galactosamine (10mM) increased with a sudden group of cells at a higher concentration
Conclusion-The result of the present study indicates that the glycyrrhiza glabra root extract demonstrated significant Hepatoprotective activity.

Keywords- Hepatoprotective, Hepg2 cell line, cytotoxicity, glycyrrhiza glabra.

1.INTRODUCTION

Hepg2 cell is one of the most common worldwide diseases and there is no treatment to date liver cancer is the second most common disease of cancer death worldwide, it causing about 746,000 deaths in 2012. Liver disease is one of the major and serious health diseases. Liquorice is a traditional plant that has been used in the food and treatment of various diseases. Liquorice is the triterpenoid, saponin from the glycyrrhiza glabra root. It consists of one molecule of glycyrrhizic acid and another two molecules of glucuronic acid. glycyrrhiza glabra is the various pharmacological activity such as anti-inflammatory, anticancer, antimalarial, and anti-oxidant. Phytochemical investigation of aqueous extract of glycyrrhiza glabra root extract showed the presence of the flavonoid, saponin, tannins, glycoside, and other chemical constituent. According to Ayurveda, the plant glycyrrhiza glabra root extract is used for cancer, liver disease, and blood disease. And also used in antipyretic, laxative, and diuretic. D-galactosamine is well established as a hepatotoxin, it induced the liver injury of human hepatitis and inflammation, resembling drug-induced liver disease in humans. The toxicity of D-galactosamine is related to the uridine pools that are associated with ribonucleic acid (RNA) and protein synthesis, thus the hepatocellular function. In the present study, dexamethasone is a positive control, it has been evaluated for its hepatoprotective activity against D-galactosamine induced the Hepg2 cell line. Therefore, this study in this study, Dexamethasone was used as the positive control to compare the cytotoxicity of glycyrrhiza glabra against the D-galactosamine-induced hepatotoxicity.

2. EXPERIMENTAL WORK

2.1. Material and method

Plant material-
The plant material was purchased from Mankarnika Aushadhalya, Sadashiv Peth nagnath park, Pune. The plant would be authenticated by Regional Ayurveda Research Institute for fundamental research centre kothrud, Pune.

Hepg2 cell culture & dose determination
Hepg2 cell lines were obtained from the National Canter cell science, Pune, India. Cells were

incubated in humidified atmosphere & 5% co2 in a 370C incubator. The cell was grown in Minimum essential medium (MEM) from- and Fetal bovine serum (FBS) was from-, Dimethyl sulfoxide (DMSO) trypsin obtained from Hi Media, 3-(4,5-dimethyl thiazol 2-yl)-2,5- diphenyl tetrazolium bromide (MTT) was purchased from Hi Media (Mumbai, India), D-galactosamine (Hi media lab Limited), Dexamethasone (Hi media lab Limited), MTT assay determined the dose rang for hepatoprotective study. four doses of glycyrrhiza glabra that is 50,100,150, 200ug/ml.

Preparation of the aqueous extract
50gm dried Liquorice root macerated in 1000ml of distilled water for 24 hours. Shaking frequently for six hours and being allowed to stand for eighteen hours by the rotary incubator. Under the temperature 370c & 80 rpm. Further extraction the aqueous extract was filtered through double filter paper or Whatman filter paper. The filtrate was collected in a sterile flask, and the extracts were concentrated using a lyophilization process and then form the dried powder.

D-galactosamine induced Hepg2 cell line
Hepg2 cells were adjusted to be 4x106 cells/well in MEM supplemented with 10% FBS and 200ul of cell suspension were plated into 96 well culture plates kept for overnight incubation at 370C with 5% co2. When the confluent monolayer was formed, the medium was removed, and washed once with plane medium (MEM without serum). Then the cells were treated with different concentrations (50,100,150,200ug/ml) of the test substance & plate was again incubated for 24 hours. After completion of incubation, the cell was treated with 10Mm of D-galactosamine & plate was further incubated at 370C with 5% co2 for 3 to 4 hours. The supernatant was removed carefully and 100µl of DMSO (100%) was added to dissolve the crystals. Then, the plate was kept for shaking at 180 rpm for 2 to 3 minutes on a plate shaker. Finally, readings were recorded at 550 nm with the help of the ELISA plate reader

Without D-galactosamine induced Hepg2 cell line
Hepg2 cells were adjusted to be 4x106 cells/well in MEM supplemented with 10% FBS and 200ul of cell suspension were plated into 96 well culture plates kept for overnight incubation at 370C with 5% co2. When the confluent monolayer was formed, the medium was removed and washed once with plane medium (MEM without serum). Then the cells were treated with different concentrations (50,100,150,200ug/ml) of the test substance & plate was again incubated for 24 hours. After completion of incubation, the cell was treated with 10Mm extract solution & plate was further incubated at 370C with 5% co2 for 3 to 4 hours. The supernatant was removed carefully and 100µl of DMSO (100%) was added to dissolve the crystals. Then, the plate was kept for shaking at 180 rpm for 2 to 3 minutes on a plate shaker. Finally, readings were recorded at 550 nm with the help of the ELISA plate reader

3. RESULT

On the treatment with the different concentrations (50-200ug/ml) for 24hrs. D-galactosamine induced cytotoxicity & the activity was comparable with the without D-galactosamine. Hepg2 cells were treated with D-galactosamine (10mM standardized with experiment) for 24 hours to induce hepatic toxicity. At the same time, the hepatoprotective drug was also added in a gradient to check its activity against the toxic effect of D-galactosamine. Dexamethasone was kept a positive control, to check if it drug is inducing any toxicity Blanks for the same were also maintained. The cells treated with only the drug showed no toxicity but also supplemented the growth of the cells. It was observed that the cell number of cells treated with D-galactosamine (10mM) increased with a sudden group of cells at higher concentrations.

4. CONCLUSION

Based on the result the glycyrrhiza glabra root extract demonstrated significant hepatoprotective activity against D-galactosamine-induced cytotoxicity.

5. DISCUSSION

In this study, we used the Hepg2 cell line to evaluate the hepatoprotective activity of glycyrrhiza glabra root extract against the liver damage induced by the D-galactosamine. on the treatment with the different concentrations (50-200ug/ml) for 24hrs for D-galactosamine induced cytotoxicity and the activity is comparable with the without D-galactosamine**.** Phytoconstituent such as flavonoids, terpenoids, tannins, and steroids. In recent years due to their diverse pharmacological activity including hepatoprotective activity. In presence of such

glycyrrhiza glabra root extract is responsible for the Hepatoprotective activity.

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