INTRODUCTION

Herbal formulations are oldest form of health care which provide rational means for the treatment of many internal diseases which are considered to be stubborn and incurable in other system of medicines. It lays a great deal of emphasis upon the maintenance of positive health of an individual and aims both the prevention and cure of diseases. Quality of herbal medicine directly affects their safety and efficacy.

Many drugs commonly used today are obtained from herbs. Indeed, about 25% of the prescription drugs dispensed in the US contain at least one active ingredient derived from plant material. As per WHO report, 80% of the world population relies on the drug from natural origin. A large number of traditional herbal medical practices have been adopted for the diagnosis, prevention and treatment of various diseases and such practices were experimentally proved depicting the scientific insight behind their traditional adoption.

The liver plays a pivotal role in transforming, clearing chemicals and is susceptible to the toxicity from these agents. Any injury to liver caused by chemicals, drugs, alcohols and microbial agents can result in many disorders ranging from transient elevation in liver enzymes to life threatening liver cirrhosis and hepatic failure. The most common reason for withdrawal of more than 900 drugs from the market is due to their liver damaging effect. Different mechanisms are responsible for either inducing hepatic injury or intensifying the damage process. Several chemicals damage mitochondria, an intracellular organelle that produces energy, its dysfunction causes releases of an excessive amount of oxidants which in turn injures hepatic cells and bile duct cells that leads to accumulation of bile acid inside the liver. Galactosamine is ahexosamine derived from Galactose, is a well-established hepatotoxin, it induces a diffuse type of liver injury closely resembling human viral hepatitis and acute self-limiting hepatitis with necrosis, inflammation and regeneration, resembling drug-induced liver diseases in humans. The amino sugar is a constituent of some glycoprotein hormone such as FSH & LH. Other sugar constituents of FSH & LH include glucosamine, galactose & glucose. Keeping in view of the importance of the disease and also considering the fact that green medicine are safe; we selected an herbal origin drug for this project.

The herbal plant used for the study is Ipomoea marginata, which comes under the family Convolvulaceae. It commonly known as south Indian thiruthali and it is used to promote strength, very well reputed drug to cure sterility in women.

ABSTRACT

Currently available synthetic drugs for the treatment of liver diseases are inadequate due to their side effects. Ayurvedic and herbal medicinal products contain a number of chemical compounds that may give the anticipated activity in combination. Ipomoea species have several properties including hepatoprotective activity. The main aim of the present study was to evaluate the hepatoprotective activity of Ipomoea marginata stem extract by in-vitro MTT assay and formulation herbal tablet containing the extract by wet granulation method. The prepared tablets were subjected to physical evaluation like weight variation, hardness, thickness, friability and disintegration test. All the pharmaceutical evaluations comply with the standards. Stability studies also conducted for a period of two months at various temperatures and there was no significant variation in the properties of the prepared herbal tablet.

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the present study focused to investigate the in vitro hepatoprotective activity of the stem extract of Ipomoea marginata and development of tablet formulation containing the extract.

MATERIALS AND METHODS

Collection and identification of plant material
Ipomoea marginata (desr) verde used in the present study was collected from Athaloor, Palakkad district, Kerala. The plant was authenticated by the Botanist Mr. Bijeshmon P.P, Sreedhareeyam Ayurvedic Research & Development Institute, Koothattukulam and the voucher specimen code:RT018 has been submitted to the Department of Pharmacognosy, Grace College of Pharmacy, Athaloor for future reference. HEP G2 (Human Hepatic Carcinoma) cell line was purchased from NCCS Pune was maintained in Dulbecco’s modified eagles media (HIMEDIA) from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecco’s Modified Eagles medium (DMEM) (Sigma Aldrich, USA). All the chemicals of analytical grade were obtained from Spectrum reagents and chemicals Pvt.Ltd. Edayar, Kochi.

Preliminary phytochemical evaluation
Preliminary phytochemical evaluation involves extraction and identification of active constituents. Maceration is used for extraction by immersing 100 g of crude drug in a stoppered container with about 100ml of chloroform water mixture and allowed to stand for 24 hours. Shake frequently for 6 hours and allowed to stand for 18 hours. The mixture of crude drug containing solvent is filtered and concentrated to dryness at room temperature. Identification of chemical constituents was performed using different chemical tests.

In-vitro Hepatoprotective activity by MTT Assay

Cell lines and Maintenance
The cell line was cultured in 25 cm² tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphotericin B (2.5µg/ml). Cultured cell lines were kept at 37°C in a humidified 5% CO₂ incubator. The viability of cells was evaluated by direct observation of cells by Inverted phase contrast microscope and followed by MTT assay method.

Cells seeding in 96 well plate
Two days old confluent monolayer of cells were trypsin zed and the cells were suspended in 10% growth medium, 100µl cell suspension (5x10⁶ cells/well) was seeded in 96 well tissue culture plate and incubated at 37°C in a humidified 5% CO₂ incubator.

Preparation of compound stock
1mg of the sample was weighed and completely dissolved in 1mL DMEM using a cyclomixer. The extract solution was filtered through 0.22 µm Millipore syringe filter to ensure the sterility. Galactosamine (50mM) was added to induce toxicity.

Cytotoxicity Evaluation
After attaining sufficient growth, Galactosamine (50mM) was added to induce toxicity and incubated for one hour, prepared extracts in 5% DMEM were five times serially diluted by two-fold dilution (100µg, 50µg, 25µg, 12.5µg, 6.25µg in 500µl of 5% DMEM) and each concentration of 100µl were added in triplicates to the respective wells and incubated at 37°C in a humidified 5% CO₂ incubator.

Cytotoxicity Assay by Direct Microscopic observation
Entire plate was observed at an interval of each 24 hours; up to 72 hours in an inverted phase contrast tissue culture microscope and microscopic observation were recorded as images. Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.

Cytotoxicity Assay by MTT Method
15 mg of MTT (Sigma, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization. After 24 hours of incubation period, the sample content in wells were removed and 3 0µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37°C in a humidified 5% CO₂ incubator for 4 hours. After the incubation period, the supernatant was removed and 100µl of MTT Solubilisation Solution (DMSO) was added and the wells were mixed gently by pipetting up and down in order to solublize the formazan crystals. The absorbance values were measured by using microplate reader at a wavelength of 540 nm.

The percentage of growth inhibition was calculated using the formula

\[
\% \text{ of viability} = \frac{\text{Mean OD Samples}}{\text{Mean OD of control group}} \times 100
\]

Formulation of Herbal tablet
The tablets were prepared by using non-aqueous wet granulation method using Ipomoea marginata stem extract100 mg, spray dried lactose 137 mg, Starch 40 mg, Polyvinyl pyrrolidone 10 mg, Talc 10 mg, magnesium stearate 3 mg were triturated into fine powder. Then the granules were prepared by adding sufficient quantity of granulating liquid containing Polyvinyl pyrrolidone and Isopropyl alcohol to make a damp mass. The wet mass was then passed through sieve no 16 and dried to get uniform granules. The prepared and evaluated granules were compressed into tablets with average weight of 300 mg. The tablets were evaluated for the average weight, hardness, thickness, friability and disintegration test.

Flow properties of granules
Angle of repose
It was determined by allowing the granules to flow through a funnel and fall freely on to a surface. Further addition of powder was stopped as soon as the pile has touched the tip of the funnel. A circle was drawn around the pile without disturbing it. The height and diameter of the resulting cone were measured. The same procedure was repeated three times
and the average value was taken. The angle of repose was calculated using the following equation:
\[
\tan \theta = \frac{h}{r}
\]
Where \( h \) = height of the powder cone; \( r \) = radius of the powder.

**Bulk Density**

A 50 g of granules were weighed and poured into a 100ml measuring cylinder and the volume was recorded. The bulk density was then calculated.

Bulk Density \((BD) = \frac{M}{V}\)

Where \( M \) is mass and \( V \) is volume

**Tapped Density**

A 50g weight of granules were and poured into a 100ml measuring cylinder and tapped on a hard surface 30 times from about 2cm height and the volume was recorded.

Tapped Density \((TD) = \frac{M}{V}\)

Where \( M \) is mass and \( V \) is volume

**Carr’s Index**

Carr’s Index (%) was determined using the following relationship
\[
C.I. = (TD – BD/ TD) \times 100
\]

**Hausner’s ratio**

Hausner’s ratio was determined using the following relationship:
\[
H. R=TD/BD
\]
Where TD is Tapped density, BD is Bulk density

**Evaluation of Tablets**

**Weight variation**

The IP weight variation test was performed by taking 20 tablets. It was weighed and the average weight was taken. Then each tablet was weighed individually. The percentage deviation can be determined by using the following formula.
\[
\% \text{ Deviation} = \left( \frac{\text{Average weight} – \text{individual weight}}{\text{Average weight}} \right) \times 100
\]

**Hardness Test**

Pfizer hardness tester was used for measuring the hardness of the formulated Herbal tablets. Five tablets were taken at random and subjected to evaluation.

**Friability**

It is a measure of tablet strength. The friability was determined by using Roche Friabilator. 10 tablets were taken and their weight determined. Then they were placed in the friabilator and allowed to make 100 revolutions at 25rpm. The tablets were then dusted and reweighed. The percentage of weight loss was calculated by using the following formula:
\[
F = 100X(1 - \frac{w}{wo})
\]
Where, \( wo \) = Weight of tablets before friability
\( w \) = Weight of tablets after friability

**Disintegration Test**

Six tablets were taken in the disintegration apparatus. Six glass tubes that are 3 inches long open at the top and held against a 10-mesh screen at the bottom end of the basket rack assembly. To test the disintegration time one tablet was placed in each tube, and the basket rack was positioned in a 1 litre beaker of water at 370C ± 20C such that the tablets remain 2.5 cm from the bottom of the beaker. A standard motor driver device was used to move the basket assembly up and down through a distance of 5-6cm at a frequency of 28-32 cycles per minute. To meet the USP standard all particles of tablet must pass through 10 mesh screens in the time specified.

**Stability study**

Stability studies were carried out at specified conditions of temperature and relative humidity of 25 °C ± 2°C/60% RH ± 5% and 40 °C±2 °C/75% RH±3% for a specific time period of 60 days. The samples were evaluated for physical appearance, hardness and disintegration time after stability studies.

**RESULTS AND DISCUSSIONS**

**Preliminary phytochemical evaluation**

Preliminary phytochemical investigations of all the plant extracts were carried out by standard protocols. The chemical test showed the presence of Alkaloids, Glycosides, Steroids, Flavonoids, Carbohydrate, Tannins and Phenolics in stem extracts.

**In-vitro Hepatoprotective activity by MTT Assay**

The in-vitro hepatoprotective activity was performed by using MTT assay method and the results shown in table.1 and figure 1.

**Table 1 Evaluation of MTT assay**

<table>
<thead>
<tr>
<th>Sample Concentration (µg/ml)</th>
<th>OD I</th>
<th>OD II</th>
<th>OD III</th>
<th>Average Absorbance @ 540nm</th>
<th>Percentage Viability</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>0.8374</td>
<td>0.8518</td>
<td>0.8552</td>
<td>0.8481</td>
<td>100.00</td>
</tr>
<tr>
<td>Galactosamine</td>
<td>0.4816</td>
<td>0.4920</td>
<td>0.4644</td>
<td>0.4793</td>
<td>56.52</td>
</tr>
<tr>
<td>Stem extract</td>
<td>0.625</td>
<td>0.6526</td>
<td>0.6611</td>
<td>0.6630</td>
<td>56.52</td>
</tr>
<tr>
<td></td>
<td>0.7174</td>
<td>0.7043</td>
<td>0.6938</td>
<td>0.7052</td>
<td>56.52</td>
</tr>
<tr>
<td></td>
<td>0.6944</td>
<td>0.6825</td>
<td>0.6875</td>
<td>0.6881</td>
<td>81.14</td>
</tr>
<tr>
<td></td>
<td>0.6536</td>
<td>0.6678</td>
<td>0.6514</td>
<td>0.6576</td>
<td>77.54</td>
</tr>
<tr>
<td></td>
<td>0.6388</td>
<td>0.6514</td>
<td>0.6266</td>
<td>0.6389</td>
<td>75.34</td>
</tr>
</tbody>
</table>

D-Galactosamine inhibits protein synthesis by depletion uridine triphosphate pool causing the generation of reactive oxygen species (ROS) and finally apoptosis of hepatic cells.

From the results it can be observed that the viability of D Galactosamine treated cells was reduced to 56.52% when compared with untreated controlled samples having 100% viability. The stem extracts effectively ameliorated Galactosamine toxicity up to 25µg/ml in a dose dependent manner. 12.5ug/ml effectively restored with cell viability to 83% which can be considered as significant hepatoprotection. Higher concentrations such was 50 and 100 ug/ml shown reduced hepatoprotection than lower concentrations suggesting possible cytotoxicity at higher concentrations.
CONCLUSION

The stem extract of *Ipomoea marginata* was found to contain Alkaloids, Glycosides, Steroids, Flavonoids, Carbohydrate, Tannins and Phenolics, which are the active constituents of the drug. In-vitro hepatoprotective activity was performed using MTT assay and it showed that the stem extract of the stem can be a very good alternative as it has proved to be very effective for their activity. The herbal tablet was formulated and subjected to the known official monographs requirements and were found to comply with the standards of the BP and IP. These tablets which were prepared from local plant that grows well in India and other parts of world can be used as hepatoprotective drug with low price.

Acknowledgments

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Reference


