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A Study on Polyherbal Formulation for Kidney Protection

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Abstract - Polyherbal formulations have long been used for liver protection, kidney protection, hepatic dysfunction treatment, and regeneration. They may help enhance appetite and protect the gastrointestinal system. The purpose of the current research was to analyse the potential of herbal formulation made up of Wedeliachinensis leaves and Boerhaaviadiffusa roots in having activities like nephroprotectivity and hepatoprotectivity. Nephroprotective and hepatoprotective efficacy was studied using MTT cytotoxicity test employing mammalian cell culture. The findings of the hepatoprotective and nephroprotective activities demonstrated that the formulation combination of herbs Wedeliachinensis and Boerhaaviadiffusa roots is a great source of organ stimulant with high therapeutical relevance. The hepatoprotective and nephroprotective qualities make this formulation a unique one concentrating on liver and kidney illnesses.

Keywords - Herbal formulation, Polyherbal Formulation, Kidney, Kidney Protection

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INTRODUCTION

Non-synthetic, natural medications originating from plant/herbal sources are becoming more popular throughout the world since they are more tolerable and have fewer side effects. Herbal medications, utilised in Indian systems of medicine are nonetheless reported to be effective and safe in such diseases. These medications are safe to use and have a special place in the treatment of long-term illnesses. To get the most out of a plant medication, it's more common to utilise it in combination than than alone. (1)

There has been a surge in the popularity of plant-based foods and beverages in the industrialised world in the last few years. These items are increasingly being sought for as medical treatments, nutraceuticals and cosmetics. Around 6,000 herbal producers operate in the country of India. There are around 4000 ayurvedic drugs created each year. Most of these manufacturers produce their items on an exceedingly speculative basis because to a lack of adequate infrastructure, qualified personnel, reliable procedures, and strict regulatory requirements (2).

Developing dependable, precise and sensitive quality control systems utilising a mix of classical and contemporary instrumentation has become vital to provide (3) a good coordination between the quality of raw materials, in process materials and the finished products. In order to ensure the quality control of herbal medicines, standardisation is needed.

In manufacturing and quality control, the term "standardisation" is used to define all efforts required to ensure a consistent product quality. From the beginning of a plant through its eventual use in medicine, it covers it all. It also entails altering the herbal medication preparation to a specific content of a component or a set of compounds with proven therapeutic action either by adding excipients or by combining herbal medicines or herbal drug preparations (4) A medicine's quality, purity, as well as the presence or absence of adulteration, are all evaluated as part of the process of going through the review procedure.

Many variables impact the bioefficacy and repeatable therapeutic effect of herbal medications, making standardisation a difficult process. The appropriate identification of plants, harvest season, and collecting location, as well as their extraction and purification processes and rationalising the combination of polyherbal medications are all necessary steps in obtaining high-quality herbal goods (5).

Ayurvedic practitioners have relied on three herbs for centuries: Ferula asafoetida, Momordica charantia, and Nardostachys jatamansi. Abdominal discomfort, constipation, diarrhoea, diabetes, epilepsy, hysteria, and mental weakness are among the conditions for which these medications are prescribed. It was thus decided to investigate the effects of petroleum either, chloroform or benzene on experimental hepatotoxicity as well as to develop and evaluate

polyherbal suspension formulations of the above extracts that showed significant activity and to compare these effects with LIV-52 as the standard marketed product. As a result, this research examined the effects of petroleum chloroform and benzene on experimental hepatotoxicity and developed and evaluated polyherbal suspension formulations of the aforesaid extracts that shown considerable efficacy. (6-8).

Ferula asfoetida oleo gum resin from Pragati Ayurvedic Drug store in Belgaum was used to authenticate the Momordica charantia fruit at the Foundation for Revitalization of Local Health Traditions (FRLHT) in Bangalore. Experimenters used analytical or laboratory-grade chemicals and solvents. The Wistar rats were provided by Venkateshwara Enterprises in Bangalore. They were kept in a typical lab environment, where they had access to commercial laboratory animal food and water at all times. The research was approved by the IACUC (Res. No. 34/2005 CPCSEA) (8).

OECD updated draught guidelines 423 October 2000 were used to conduct acute toxicity investigations. Doses for hepatoprotective efficacy and suspension formulations were determined based on these investigations. Using the maceration method, extracts of Ferula asafoetida, Momordica Charantia, and Nardostachys jatamanii were obtained, and their hepatoprotective activity was tested in Wistar rats exposed to carbon tetrachloride-induced liver toxicity. These extracts were found to be effective in protecting the liver against the toxicity. A total of seven extracts were used to manufacture the suspensions after this research (9).

PROCESSING AND POLYHERBAL FORMULATIONS

TCM relies heavily on processing and polyherbal formulations. These methods, when used in conjunction with physical or chemical interactions, are thought to enhance results, lessen toxicity, or provide synergistic or balanced effects. TR has been shown to have a variety of processing and compatibility issues. According to the Chinese Pharmacopoeia's 2015 edition, the "typical" technique of processing TR is frying it with licorice water extract and then "stir-baking" it in ginger juice, vinegar, salt, rice wine, or Coptidis Rhizoma water extract, as described in ancient medical texts and local traditions. Using licorice, salt, and other similar ingredients is called "stir-frying" in the parts that follow. It is possible to change the effects of TR using various processing methods. Many additional herbs, such as Angelica sinensis radix for blood deficiency and Pinellia and Coptidis rhizomes for recurrent vomiting, are used therapeutically in conjunction with TR to treat various diseases.

MATERIALS AND METHODS

Sample Collection

From an FSSAI-approved herbal powder factory in Coimbatore, Tamil Nadu, Wedeliachinensis leaves and Boerhaaviadiffusa root powder were procured. To keep the herbs fresh for future use, they were placed in an airtight, lightproof container. Wedeliachinensis leaves (WC), Boerhaaviadiffusa (BD) and Formulation mix (FM) were identified as the samples.

Sample extraction

A total of 20g of powdered herb mixture was extracted with 150 ml of distilled water. The aqueous extracts are then allowed to evaporate in the open air. Membrane filters were used to filter the extracts.

Chemicals used

Chemicals used in the study were Fetal Bovine Serum (FBS), DMEM medium, Saline, Trypsin,H_2O_2.

Determination of Hepatoprotective activity

Human liver HepG2 cells were treated to different doses of the formulation (100, 200, 300, 400, and 500 mg/ml) in a medium containing H2O2(1mM). The vitality of HepG2 cells was then determined using the MTT reduction test to determine cytotoxicity. HepG2 cells were cultured in DMEM culture media, single-cell suspension was produced, and 1 104 cells per well were planted onto a 96-well flat bottom plate. After a 48-hour incubation period, 100 litres of 1 mM H2O2 were added to each well, followed by 100 litres of diluted extract at various concentrations, and the plates were incubated for another 48 hours at 37°C in a humidified incubator with 5% CO2. Each well's supernatant was collected, and 100 mL of MTT (0.5 mg/mL) was added. MTT is converted to an insoluble, pigmented (dark purple) formazan product in the cell's mitochondria. The cells were then solubilized in 100 litres of DMSO, and the released, solubilized formazan reagent was spectrophotometrically quantified.

The hepatoprotective (12) effects of the formulation extracts were tested against the toxicity of H2O2 on liver cells using a 540 nm wavelength.

Determination of in-vitro nephroprotective activity

Cells were grown in DMEM culture conditions and a single-cell suspension was made, and 1104 cells per well were seeded into bottom plate. After a 24-hour incubation period, 100 litres of 1 mM H2O2 were added to each well, followed by 100 litres of diluted extract at various concentrations, and the plates were incubated for another 24-hour period at 37°C in a humidified incubator with 5% CO2. The supernatants that were collected received 100 mL of MTT at a concentration of 0.5 mg/mL. In the

mitochondria, MTT is transformed into an insoluble, purple-colored formazan product that cannot be dissolved in water. To measure the formazan released and solubilized, the cells are dissolved in 100 litres of DMSO (13). The formulation extracts' nephroprotective effects were tested against the toxicity produced by H2O2 on liver cells, with readings taken at 540 nm.

RESULTS AND DISCUSSION

Determination of Hepatoprotective activity

H2O2 caused hepatotoxicity in HepG2 cell lines. Hepatoprotective properties of formulation extract have been studied. The induced hepatotoxicity was decreased by the presence of phytoconstituents in the formulation mix extract. Table I indicates the percentage of cells that died as a result of toxicity caused by H2O2 in the presence or absence of various concentrations of Formulation extracts.

Table 1 demonstrates that when the concentration of formulation increases, cell death decreases, indicating that the formulation has protective properties.

The PC50 (Protective Concentration 50%) in HepG2 cells is 381.48g/ml, indicating that it has stronger hepatoprotective action than H2O2 toxicity. Table 2 shows that at a concentration of 381.48g/ml, effective protection of roughly 50% against toxicant control has been achieved. Both Boerhaaviadiffusa and Wedeliachinensis have hepatoprotective properties and include phytoconstituents with this property (13).

Determination of nephroprotective activity

H2O2 produced nephrotoxicity in Vero cell lines. The nephroprotective properties of formulation mix extract have been studied. Induced nephrotoxicity was prevented by the inclusion of different phytoconstituents and minerals in formulation mix extract. Table 3 indicates the percentage of cells that died as a result of toxicity caused by H2O2 in the presence or absence of various concentrations of formulation mix extracts. Table 3 shows that the H2O2 toxicity was reduced when the formulation concentration was increased (14).

Table 4 shows that at a concentration of 732.06g/ml, effective protection of around 50% above toxicant control has been achieved. Boerhaaviadiffusa has nephroprotective properties and includes phytoconstituents with nephroprotective properties, resulting in modest nephroprotective efficacy in Vero cells against H2O2 toxicity (14).

Table 1: Percentage cell death upon H_2O_2toxicity in HepG2 cell lines

	Test Concentration	% of cell death in presence of
S.No	(µg/ml)	formulation + H_2O_2
1	100	66.27 ± 2.68
2	200	54.25 ± 2.63
3	300	49.85 ± 3.51
4	400	43.98 ± 5.3
5	500	35.19 ± 5.85
H_2O_2 toxic control		88.56 ± 1.75

Table 2: Hepatoprotective activity of formulation mix over toxicant in HepG2 cell line

S.No	Test Concentration (µg/ml)	% Protection offered over toxicant control
1	100	25.16 ± 3.03
2	200	38.74 ± 2.98
3	300	43.70 ± 3.97
4	400	50.33 ± 5.98
5	500	60.26 ± 6.61

Table 3: Percentage cell death upon H2O2 toxicity in Vero cell lines

S.No	Test Concentration (µg/ml)	% of cell death in presence of formulation + H_2O_2
1	100	89.63 ± 0.44
2	200	88.34 ± 0.77
3	300	83.41 ± 0.44

4	400	67.09 ± 1.18
5	500	63.21 ± 1.18
H_2O_2 toxic control		93.26 ± 0.44

Tabe 4: Nephroprotective activity of formulation mix overtoxicant in Vero cell line

S.No	Test Concentration (µg/ml)	% Protectionoffered over toxicantcontrol
1	100	3.89 ± 0.48
2	200	5.28 ± 0.83
3	300	10.56 ± 0.48
4	400	28.06 ± 1.27
5	500	32.22 ± 1.28

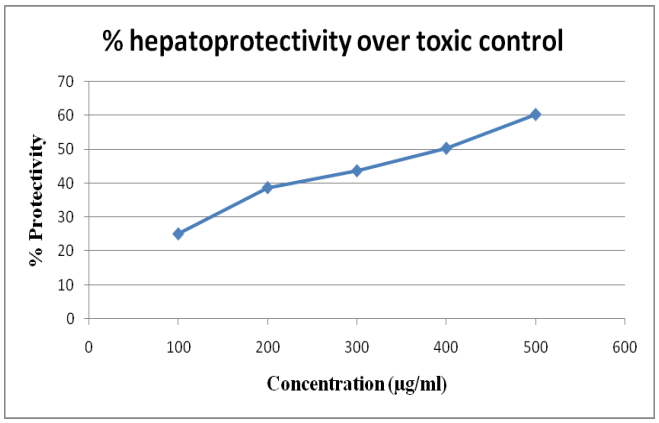

Figure 1: Percentage hepatoprotectivity of formulation mixover toxic control

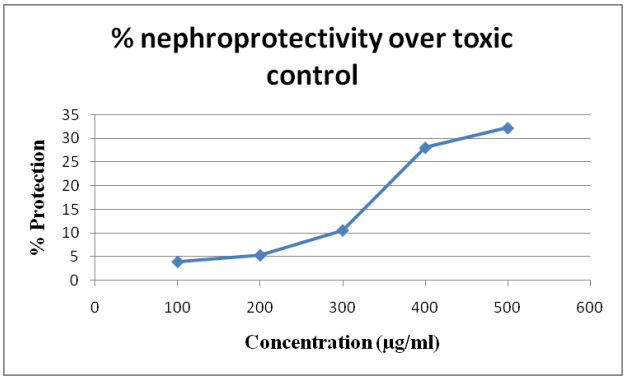

Figure 2: % nephroprotectivity of formulation mix overtoxic control

CONCLUSION

From the foregoing findings, it can be inferred that herbal medicine formulations have significant hapatoprotective effect in therapeutic and preventive modes in acute toxicity models, and that the results are equivalent to those of silimarin. This can only be proven in further investigations on chronic hepatotoxicity and hepatoprotection mechanisms.

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