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Original Article

Formulation and evaluation of Phytosomesyrup for Hepato-protective activity of *Clerodendroninfortunatum* Linn., root extract

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ABSTRACT: The root portions of the *Clerodendroninfortunatum* Linn. roots extracted with ethanol by cold maceration process. The extract was vacuum dried and subjected to phytochemical screening for the detection of various phyto constituents.Polyphenols in plant extracts react with specific redox reagents (Folin- Ciocalteu reagent) to form a blue complex that can be quantified by visible-light spectrophotometry. Flavonoids are potent antioxidants and have aroused considerable interest recently because of their potential beneficial effects on human health in fighting diseases. The capacity of flavonoids to act as hepatoprotectant depends upon their molecular structure. Preliminary phytochemical screening of Petroleum ether, Ethanol, Ethyl acetate, Water extract of *Clerodendroninfortunatum* Linn., were done which gives information about active constituents such as carbohydrates, starch, mucilage, saponins, flavanoids, tannins, phenolic compounds in the different extract and was more in Ethanolic and,Ethyl acetate extract. The. The extracts exhibited potent activity against hepatic cells. The study reveals that *Clerodendroninfortunatum* possess better hepatoprotective activity.

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INTRODUCTION

Historically, crude plant extracts are used as natural medicine for the remedy of human infectious sicknesses. Flora are rich in a ramification of phytochemical consisting of tannins, terpenoides, alkaloids, and flavonoids that have been located in vitro to have antimicrobial homes. Even though the mechanism of motion and efficacy of these natural extracts in most cases continues to be had to be established scientifically. Worldwide incidence of infectious diseases because of microorganism is a major public health problem. Giant ethno botanical information exists in India from ancient time. The dictionary of Indian folk-medication and ethno botany consists of 2532 flowers. India has about forty-five, 000 plant species and a lot of them had been studied for their medicinal residences. About 2000 figures are available within the literature and typically 500 species are utilized by indigenous structures.

Even though early (4500-1500 BC) origins and a long history of utilization in the final two centuries, the Ayurveda had received very little authentic support and hence less attentions were observed from suitable clinical practitioners and researchers. A large volume of work is now being finished at the Botany, Pharmacognosy, Biotechnology, Chemistry and Pharmacology of natural drug treatments.

The importance of ethno medicine has been realized and paintings is being finished on psycho energetic vegetation, home treatments and vegetation offered via street drug carriers.

Fewer well known ethno medicines have been identified which might beused totreat intestinal, joint, liver and pores and skin diseases.

MATERIALS AND METHODS

Collection and authentication of plant materials

The root of *Linn*, belongs to the family *Verbenaceae*was collected from Pathanamthitta district of Kerala. Taxonomical identification of the plants was done by Dr.Thomas Mathew, Marthoma College Thiruvalla and the voucher herbarium specimen were deposited in college museum asVSCp-14.

Preparation of the extract

Material:

Sample:*Clerodendroninfortunatum*Linn root powder

Method:

By cold Maceration

Procedure

*Clerodendroninfortunatum*Linn., roots washed separately using water and extracted with 90% ethanol as solvent. The macerate collected after one week. The solvents were allowed to evaporate to obtain the dried extracts. It was then weighed and percentage yield was calculated. The extracts were stored properly in a desiccator for further studies.

Phytosomes were prepared by using, extract from *Clerodendroninfortunatum*Linn.

Procedure

Phytosomes were prepared by reaction of natural phospholipid (soy PC) with extract at a molar ratio of 1:1 separately. The reaction is carried out by refluxing in 100 ml round bottom flask with 20 ml, methylene chloride, the mixture was refluxed at a temperature not exceeding 40°C for 2 h. The resultant clear solution was evaporated and 10 ml of *n*-hexane was added to it with continuous stirring, the precipitate was filtered and dried under vacuum to remove traces of solvents, resulting in a thin film formation. *Clerodendroninfortunatum*Linn. extract Phytosome,thin film was separated and kept in an amber coloured glass bottles, flushed with nitrogen, and stored at room temperature.

Phytosome Syrup

Preparation of simple syrup

Simple syrup was prepared according to Indian Pharmacopoeia by adding 667gm of sucrose to purified water and heated until it is dissolved with stirring to produce 100ml

Preparation of Phytosome Syrups

The herbal syrups were prepared by adding one part of formulated phytosomes mixed with five parts of simple syrup (1:5) to 50 ml. Required quantity of methyl paraben was added to the above mixture named as SP1. Solubility was checked by observing the clarity of solution visually. The final developed herbal syrup was then subjected to evaluation of production quality as per official standards.

Evaluation of Phytosomesyrup SP1

Physicochemical parameters: The herbal syrup was evaluated for various physicochemical parameters such as physical appearance (colour, odour, taste), pH, Wt./ml and Specific Gravity.

Colour examination: - Five ml final syrup was taken into watch glasses and placed against white back ground in white tube light. It was observed for its colour by naked eye.

Odour examination: Two ml of final syrup was smelled individually. The time interval among two smelling was kept 2 minutes to nullify the effect of previous smelling.

Taste examination: A pinch of final syrup was taken and examined for its taste on taste buds of the tongue.

Determination of pH: Placed an accurately measured amount 10 ml of the final syrup in a 100 ml volumetric flask and made up the volume up to 100 ml with distilled water. The solution was sonicated for about 10 minutes. pH was measured with the help of digital pH meter.

- **Specific gravity at 250°C**: A thoroughly clean and dry Pycnometer was selected and calibrated by filling it with recently boiled and cooled water at 250C and weighing the contents. Assuming that the weight of 1 ml of water at 250°C when weighed in air of density. The capacity of the Pycnometer was calculated. Adjusting the temperature of the final syrup to about 20°C and the Pycnometer was filled with it. Then the temperature of the filled Pycnometer was adjusted to 25°C, any excess syrup was removed and weight was taken. The tare weight of the Pycnometer was subtracted from the filled weight. The weight per milliliter was determined by dividing the weight in air, expressed in g, of the quantity of syrup which fills the Pycnometer at the specified temperature, by the capacity expressed in ml, of the Pycnometer at the same temperature. Specific gravity of the final syrup was obtained by dividing the weight of the syrup contained in the Pycnometer by the weight of water contained, both determined at 25°C.

- **Stability testing**: Stability testing of the prepared herbal syrup was performed on keeping the samples at accelerated temperature conditions. The final syrup was taken in amber colored glass bottles and were kept at accelerated temperature at, Room temperature, 40C and 470C respectively. The samples were tested for all the physicochemical parameters, turbidity and homogeneity at the interval of 24 hr, 48 hr and 72 hr. to observe any change.

Evaluation of Hepatoprotective activity

Animals

Wistar albino rats weighed about 130-210g were divided into seven groups of six rats each. Animals were housed at a temperature of 23 ± 2°C and relative humidity of 30–70%. All animals were allowed free access to water and fed with standard commercial rat chow pellets.

The study was approved by the Institutional Ethical Committee, which follows the guidelines of Committee for Control and Supervision of Experimental Animals (CPSCEA).

Grouping of Animals

Group I: Control group received normal saline in a (5ml/kg p. o.)

Group II: Toxic Control group received CCl_4 in liquid paraffin (0.7 ml/kg, 1:1, v/v, i. p, on alternate days)

Group III: Standard group receivedLiv 52 in a dose of (100mg/kg p.o)

Group IV: Receive with EECi (200mg/kg orally)+ CCl4, 1.5 ml/kg, i.p

Group V: Receive Formulated phytosome syrup using isolated compound25mg/g p.o +CCl4, 1.5 ml/kg, i.p

Assessment of Hepatoprotective activity

On the seventh day after administration of last dose of test samples the rats were anesthetized by light ether anaesthesia and blood was collected by making intra-cardiac puncture. It was allowed to coagulate for 30 min and serum was separated by cold centrifugation at 2500 rpm for 15 min. The centrifugate was used to estimate the serum SGPT, SGOT, SALP , and total protein content were determined.

Histopathological Studies

Finally, the rat liver was isolated and subjected for histopathological changes.

Statistical analysis

RESULT

Phytochemical analysis of*Clerodendroninfortunatum*Linnrootextract showed a positive response for the presence of carbohydrates, starch, mucilage, saponins,flavonoids, tannins, phenolic compounds.

Acute oral toxicity (LD50) study

In the assessment and evaluation of the toxic characteristics of a substance, determination of acute oral toxicity is usually an initial step. Acute oral toxicity was performed by using OECD guidelines-423 (Organization of Economic Co- Operation and Development) - Fixed dose procedure (FDP). The Fixed Dose Procedure (FDP) is a method for assessing acute oral toxicity that involves the identification of a dose level that causes evidence of non-lethal toxicity (Evident toxicity) which describing clear signs of toxicity following administration of test substance, such that an increase to the next highest fixed dose would result in the development of severe toxic signs and probably mortality LD50 (median lethal dose oral, is a statistically derived single dose of a substance that can be expected to cause death in 50 percent of animals when administered by the oral route. The LD_{50} value is expressed in terms of weight of test substance per unit weight of test animal (mg/kg).

Observation

No toxicity or death was observed for these given dose levels, in the selected and treated animals. So, the LD_{50} of the ethanolic extract of*Clerodendroninfortunatum*Linn. (EECi) as per OECD guidelines-420 is greater than 2000 mg/kg (LD50 > 2000 mg/kg). Hence the biological dose was fixed 200 mg/kg for ethanolic extract.

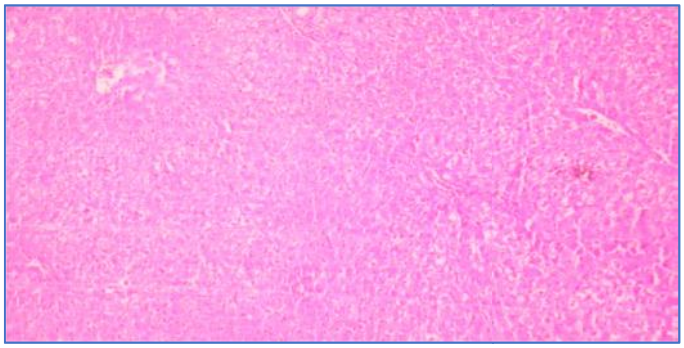
Rats treated with CCl_4 (0.7ml/kg body weight) suffered from hepatotoxicity. The serum levels of SGPT, SGOT and bilirubin level were significantly elevated and protein level was significantly decreased as shown in Table. Treatment with EECp extract 200mg/kg, described Table for 7 days significantly decreased enzyme levels and bilirubin level. Meanwhile it showed increase in protein level in the blood, when compared to control and CCl4 treated group ($P< 0.0001$). Results were also comparable with standard drug Liv-52 (100mg/kg p.o).

Histopathological observations

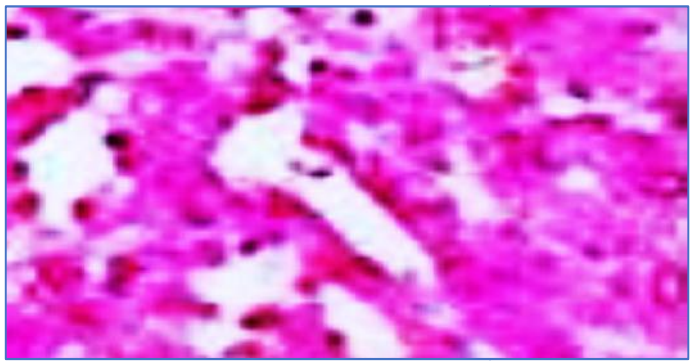
Histopathology of normal rat liver revealed prominent central vein, normal arrangement of hepatic cells (A). Microscopic examination of CCl4 treated liver section shows necrosis and fatty degeneration (B). Liver section treated from silymarin protected the structural integrity of hepatocyte cell membrane and recovery of hepatocyte cells (C).EECi extract 200mg/kg, Extract Phytosome 40mg/kg, shows in Fig. 1. Treated groups showed maximum recovery of hepatocytes, attenuated the fatty degeneration and necrosis and finally exhibited a significant protection against CCl4 induced liver toxicity.

Table 1: Effect of EECi extract, phytosome using Extract on Hepatoprotective activity.

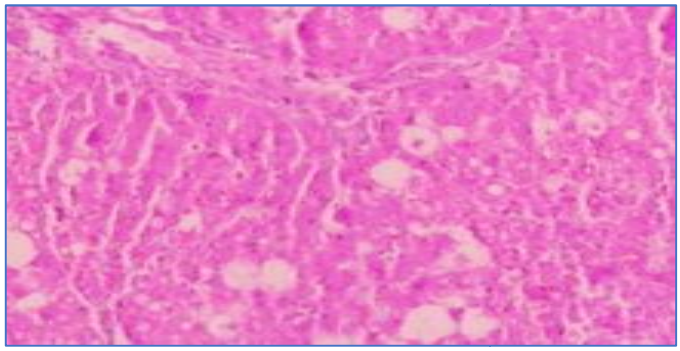
Groups	Treatment	SGPT (µ/ml)	SGOT (µ/ml)	Serum bilirubin (mg/ml)	Total Protein (mg/ml)
1	CONTROL	127.11 ± 0.1282	28.65± 0.0060	1.160 ± 0.0421	3.435 ± 0.0076
11	CCl_4 (0.7ml/kg)i.p	248.20 ± 0.0562	56.62±0.0042	3.235 ± 0.0113	2.580 ± 0.0030
111	Standard Liv -52 (100 mg)+CCl_4	145.52±0.0042	36.53± 0.0055	1.165 ± 0.0049	3.125± 0.0066
1v	EECi (200mg/gm)+CCl_4	131.15± 0.3003	21.53± 0.0055	0.970 ± 0.0030	2.775 ± 0.0033
V	Phytosome syrup using Isolated compound (40mg/gm)+CCl_4	140.17± 0.0056	34.01± 0.0046	1.13 ± 0.0021	2.325±0.0060



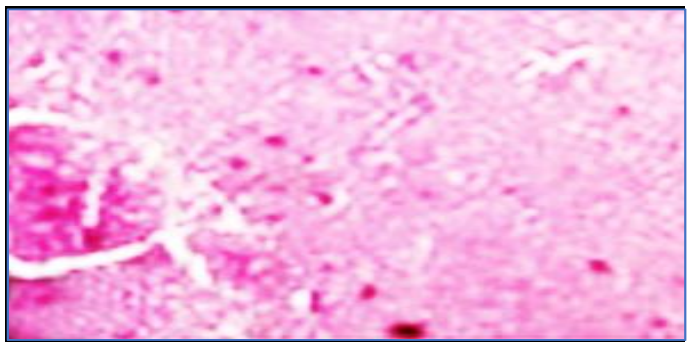
Normal control



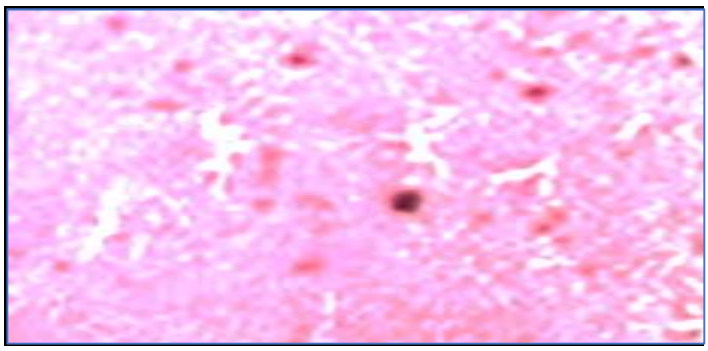
CCl_4 treated live



Standard Liv 52 (100 mg)+CCl_4



EECi (200mg/Kgm)+CCl_4



EECiPhytosome (40mg/Kgm)+CCl_4

Figure 1:Histopathological observations

Significant hepatoprotective activity was observed treated with the *Clerodendroninfortunatum*extract phytosomecompared to the standard. Phytochemical analysis of*Clerodendronpaniculatum*rootextract showed a positive response for the presence of carbohydrates, starch, mucilage, saponins,flavonoids, tannins, phenolic compounds.

CONCLUSION

Hepatoprotective activity of *Clerodendroninfortunatum*shows better activity compared to the standard. Phytochemical analysis of *Clerodendroninfortunatum*rootextract showed a positive response for the presence of carbohydrates, starch, mucilage, saponins, flavonoids, tannins, phenolic compounds. Hepatoprotective Activity of *Clerodendroninfortunatum* root extract phytosome may due to the reason it is a flavonoid.

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