Research Article

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IN VITRO, IN VIVO STANDARDIZATION, FORMULATION AND EVALUATION OF DIRECTLY COMPRESSED POLYHERBAL HEPATOPROTECTIVE TABLETS

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Received on: 11/07/13 Revised on: 07/08/13 Accepted on: 14/09/13

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DOI: 10.7897/2277-4343.04529

Published by Moksha Publishing House. Website www.mokshaph.com

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ABSTRACT

The traditional medicine all over the world is nowadays appraised by an extensive activity of research on plant species and their therapeutic principles. When two or more herbs are used in formulation they are known as polyherbal formulation. A large number of populations suffer due to various reasons from hepatic diseases of unknown origin. The development of antihepatotoxic drugs being a major thrust area has drawn the attention of workers in the field of natural product research because synthetic drugs may cause serious side effects. The present study was aimed to study the *in vitro* antioxidant property by DPPH, superoxide, and hydroxyl radical scavenging assays, *in vivo* hepatoprotective activity by CCl₄ induced hepatotoxicity in albino rats, formulation of polyherbal hepatoprotective tablets containing equal quantities of methanolic extract of roots of *Begonia laciniata* Roxb., whole plant of *Cuscuta epithymum* (L.) L and whole plant of *Dendrobium ovatum* (L.) Kraenzl., which were used traditionally in Chittoor and Khammam districts of Andhra Pradesh, India., developed by direct compression method using super tab-11SD, primojel, talc and magnesium stearate as excipients and then subjected to evaluation of pre compression and post compression parameters. All the selected plants showed dose dependant antioxidant property, highest at 360µg dose and significant dose dependant hepatoprotective activity, highest at a dose of 400 mg/kg b.w compared to standard drug silymarin. The histopathological studies also confirmed protective effects of extracts against CCl₄-induced liver injuries. The observations from formulation support the ideal properties of compressed tablets and its feasibility for large scale commercial production.

Keywords: Antioxidant property, Hepatoprotective activity, Pre formulation studies, Pre compression parameters, Post compression parameters.

INTRODUCTION

Since the introduction of the herbal medicines, many people were impelled to consider the importance of many herbs for treating several forms of disorders. It is no wonder, during the past decade there has been an exponential rise in the application of herbal remedies and such notable increase even continues in these days. However, several herbal products lining in those shelves are not really standardized in terms of its effectiveness and safety. When two or more herbs are used in formulation they are known as poly herbal formulation. Sometimes herbs are combined with mineral preparation. Herbal formulations are usually prepared with the combinations of individually extracted single herbs to get the benefit of synergism or to prevent side effect arising from chief herb¹. Liver has a pivotal role in the maintenance of normal physiological process through its multiple and diverse functions, such as metabolism, secretion, storage and detoxification of variety of drugs. In the absence of reliable liver protective drugs in modern medicine, in India, a number of medical plants and their formulations are used to cure hepatic disorders in traditional systems of medicine². There are numerous plants and traditional formulations available for the treatment of liver diseases. About 600 commercial herbal formulations with claimed hepatoprotective activity are being sold all over the world³. Treating liver diseases with botanical drugs has a long tradition, but evidence for efficacy is sparse. Moreover, synthetic drugs available in the market may cause serious side effects. Keeping this in

mind for giving scientific proof, the present work was designed and screened the three medicinal plants which were traditionally used for treating liver disorders in Chittoor and Khammam districts of Andhra Pradesh, India⁴.

MATERIAL AND METHODS

Materials

The roots of *Begonia laciniata*, whole plant of *Cuscuta epithymum* and whole plant of *Dendrobium ovatum* were collected from Sathupally, Kuppam and surrounding villages (Medaram-Tadavi forest range, Berikonda, Thuvvakonda and Raakasigubbalu) of Khammam and Chittoor districts of Andhra Pradesh, India and authenticated by Dr. Madha Chetty, taxonomist and HOD of Botany, Sri Venkateswara University, Thirupathi, India (Voucher specimen No's SVU-B-12, 13, 14), ascorbic acid (Sigma Aldrich Chemie, Germany), Riboflavin (S.D chemicals, India), 2- deoxyribose (Sigma Chemicals, USA), hydrogen peroxide (SD fine chemicals), carbon tetrachloride (Poona Chemical Laboratory, Pune, India), silymarin, gallic acid, and catechin (Nature remedies, Bangalore, Karnataka, India), SGOT, SGPT, SALP, BILIRUBIN estimation kits (Span Diagnostics, Surat, India), super tab 11SD (Spray dried lactose), primojel (sodium starch glycolate), talc, magnesium stearate and carboxy methyl cellulose (CMC) of pharmacopeial grade were gift samples from DFE Pharma, Bangalore, India; Wistar albino rats (purchased from Mahaveer Enterprises, Hyderabad, India). Hepatotoxicity induced by CCl₄. All

other solvents and chemicals used were of analytical grade purchased from local source.

Preparation of Extract

Before going to preparation, the collected plant materials i.e., roots of *Begonia laciniata*, whole plant of *Cuscuta epithymum* and whole plant of *Dendrobium ovatum* were subjected to standardization according to the guidelines of WHO for organoleptic, physiochemical, heavy metal, microbiological and pathogen analysis⁵ (Table 1). After collection, the plant materials were shade dried, powdered (40 mesh size) to get a coarse powder and then subjected to soxhlet extraction continued for 8 cycles (6 h) using methanol as a solvent. The extract was filtered and concentrated at reduced temperature on a rotary evaporator. The percentage yield was found to be 29.31, 27.52 and 32.46 % w/w respectively and then subjected to preliminary qualitative⁶⁻¹⁰ and quantitative (for phenolics, flavonoids and alkaloids) phytochemical analysis (Table 1-2).

Determination of Total Phenolic Content

The total phenolic content was estimated using the modified Folin-Ciocalteu photometric method¹¹. The appropriate amount of filtered methanol extracts was oxidized with Folin-Ciocalteu's reagents and after 5 minutes the reaction was neutralized with saturated sodium carbonate. The solution was then immediately diluted to the volume of 50 ml with distilled water. The absorbance was measured at 750 nm after 90 minutes of incubation at room temperature against the blank. As the standard was used gallic acid. The total phenolic content is expressed as g Gallic acid equivalents (GAE) per 100 g of dry weight (dw).

Determination of Total Flavonoid Content

The total flavonoid content was measured using a modified colorimetric method¹¹. The appropriate amount of extract was added to a test-tube together with distilled water. Then 5 % NaNO₂ was added, after 5 minutes 10 % AlCl₃ and after another 5 minutes 1 M NaOH followed by the addition of distilled water. The absorbance was measured against the blank at 510 nm after 15 minutes. The standard curve was prepared using different

concentration of catechin. The flavonoid content was expressed as g Catechin equivalents (CE) per 100 g of dry weight (dw).

Determination of Total Alkaloid Content

The total alkaloid content was determined according to UV-Spectrophotometer method¹². This method is based on the reaction between alkaloid and bromocresol green. The part of the plant extract was dissolved in 2 N HCl and then filtered. 1 ml of this solution was transferred to separatory funnel and washed with 10 ml chloroform. The pH of phosphate buffer solution was adjusted to neutral with 0.1 N NaOH. One ml of this solution was transferred to a separating funnel and then 5 ml of bromocresol solution along with 5 ml of phosphate buffer were added. The mixture was shaken and the complex formed was fractioned with chloroform by vigorous shaking. The fractions were collected in a 10 ml volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm. All experiments were performed thrice; the results were averaged and reported in the form of mean $\pm$ S.E.M.

In Vitro Antioxidant Property

The selected plant methanolic extracts were evaluated by DPPH radical scavenging assay, super oxide radical scavenging assay, and hydroxyl radical scavenging assay. There is no detailed study on free radical scavenging activity on each plant. Hence, a detailed study was carried out.

Screening for 1, 1-diphenyl-2-picrylhydrazyl (DPPH) Radical-scavenging Activity

An aliquot of 3 ml of 0.004 % DPPH solution in methanol and 0.1 ml of plant extract at various concentrations (40, 80, 120, 160, 200, 240, 280, 320 and 360 μ g/ml) were mixed. The mixture was shaken vigorously and allowed to reach a steady state at room temperature for 30 minutes. Decolorization of DPPH was determined by measuring the absorbance at 517 nm. A control was prepared using 0.1 ml of respective vehicle in the place of plant extract/ascorbic acid. The percentage inhibition activity was calculated by using the following formula (Figure 1).¹³

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of plant extract/ascorbic acid}}{\text{Absorbance of control}} \times 100$$

Screening for Superoxide Radical Scavenging Activity

Riboflavin photo reduction method¹⁴ was used to screen the different plant methanolic extracts. 0.1 ml of different concentrations (40, 80, 120, 160, 200, 240, 280, 320 and 360 μ g/ml) of plant extract and 0.1 ml of 6 μ M ethylene diamine tetraacetic acid (EDTA) containing NaCN, 0.1 ml of 50 μ M nitroblue tetrazolium, 0.05 ml of 2 μ M riboflavin were transferred to a test tube, and final volume

was made up to 3 ml using phosphate buffer. Then the assay tubes were uniformly illuminated with an incandescent light (40 Watt) for 15 minutes and thereafter the optical densities were measured at 560 nm. A control was prepared using 0.1 ml of respective vehicle in the place of plant extract/ascorbic acid. The percentage inhibition of superoxide production was evaluated by comparing the absorbance values of control and experimental tubes (Figure 2).

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of plant extract/ascorbic acid}}{\text{Absorbance of control}} \times 100$$

Screening for hydroxyl radical scavenging activity

Deoxyribose degradation method¹⁵ was used to screen the different concentrations (40, 80, 120, 160, 200, 240, 280, 320 and 360 µg/ml) of each plant extract. Fenton reaction mixture consisting of 200 µl of 10 mM ferrous sulphate (FeSO₄·7H₂O), 200 µl of 10 mM EDTA and 200 µl of 10 mM 2-deoxyribose and was mixed with 1.2 ml of 0.1 M phosphate buffer (pH 7.4) and 200 µl of plant extract. Thereafter, 200 µl of 10 mM H₂O₂ was added before the incubation at 37°C for 4 h. Then 1 ml of this Fenton reaction mixture was treated with 0.2 ml of 8.1 % sodium dodecyl sulphate, 1.5 ml of 0.8 % thiobarbituric acid and 1.5 ml of 20 % acetic acid. The total volume was then made to 5 ml by adding distilled water and kept in an oil bath at 100°C for 1 h. After the mixture had been cooled, 5 ml of 15:1 v/v butanol-pyridine mixture was added. Following vigorous shaking, the tubes were centrifuged at 4000 rpm for 10 minutes and the absorbance of the organic layer containing the thiobarbituric acid reactive substances was measured at 532 nm. A control was prepared using 0.1 ml of vehicle in the place of plant extract/ascorbic acid. The percentage inhibition of hydroxyl radicals by the extract/compound was determined by comparing the absorbance values of the control and the experimental tubes as calculated for hydroxyl radical assay (Figure 3).

Calculation of 50 % inhibition concentration

IC₅₀ value was calculated for each extract and positive control and obtained by plotting a graph by taking concentration on X-axis and % inhibition on Y-axis. The graph was extrapolated to find the concentration needed for 50 % inhibition (Table 3 and Figure 4).

Acute toxicity study

Acute toxicity study is a method for assessing acute oral toxicity that involves the identification of a dose level that causes mortality. Acute toxicity studies were performed for selected plant methanolic extracts according to the toxic classic method as per guidelines 423 prescribed by OECD¹⁶, 2001 using female albino rats. The selected three extracts showed neither visible sign of toxicity nor mortality. The results clearly indicated non-toxicity of the extracts at a dose of 2000 mg/kg. From this, 1/20th, 1/10th and 1/5th and doses were selected for the experimental study. Hence there is no LD₅₀ and all the extracts tested are considered safe and nontoxic.

In Vivo Screening for Hepatoprotective Activity

Animals used

Wistar albino rats of either sex weighing between 200 - 250 g were obtained from Mahaveer Enterprises, Hyderabad, India. The animals were housed under standard environmental conditions (temperature of 22 ± 1°C with an alternating 12 h light- dark cycle and relative humidity of 60 ± 5 %), one week before the start and also during the experiment as per the rules and regulations of the Institutional Ethical Committee and by animal regulatory body of the government (Regd: No: 516/01/CPCSEA). They were fed with standard pellet laboratory diet supplied by M/s. Rayans biotechnologies Pvt. Ltd., Hyderabad, India and water *ad libitum*.

Experimental procedure

Albino rats of either sex (200 - 250 g) were used in the study. The animals were fed with standard diet and water *ad libitum* two weeks before and during the experimental period. Each selected plant methanolic extract was tested at 400 mg/kg dose level. The animals were divided in to 12 groups (I-XII), each consisting of 6 animals. Group I received 5 % gum acacia suspension and acts as a normal control and Group II received CCl₄ at a dose of 1 ml/kg orally (p.o.) acts as negative control. Groups III-XII were treated with selected drugs (silymarin and plant extracts) for 5 days before the commencement of experiment and on day 6th of the experiment, blood samples were collected (6th day) at 0 h in all groups and CCl₄ was administered to all groups except group I (normal control) one hour after the administration of drugs. On 7th day blood samples were collected from all groups by retro orbital puncture, serum was separated by centrifugation and used for the estimation of blood serum parameters (SGOT, SGPT, SALP and T.BILI.) according to the standard procedures. The liver sections also dissected out subjected to histopathology studies and results are shown (Tables 4-5 and Figures 5-11).¹⁷

Histopathological studies

All the animals were anesthetized with ethyl ether and livers were dissected out quickly by cutting on the ventral side. The isolated liver specimen was trimmed to small pieces and preserved in neutral buffered formalin (10 % formaldehyde in phosphate buffered saline) solution for 24 h. The liver specimen was subjected to dehydration with acetone of strength 70, 80, 100 % respectively, each for one hour. The infiltration and impregnation was done by treatment with paraffin wax twice each time for one hour. Specimens were cut into sections of 3 - 5 µm thickness using microtome and were stained with haematoxylin and eosin and later the microscopic slides of the liver were photographed at 40 X magnification¹⁸⁻¹⁹.

Statistical analysis

The mean ± SEM values were calculated for each parameter. Percentage reduction in biochemical parameters with the test samples was calculated by considering the difference between the hepatotoxin treated group and the control group as 100 % reduction. For the determination of significant inter group difference, each parameter was analyzed separately using one way analysis of variance (ANOVA) followed by Dunnet's test.

Development and Evaluation of Polyherbal Hepatoprotective Tablets

When two or more herbs are used in formulation they are known as poly herbal formulation. Sometimes herbs are combined with mineral preparation. Herbal formulations are usually prepared with the combinations of individually extracted single herbs to get the benefit of synergism or to prevent side effect arising from chief herb. For formulation of polyherbal tablets, direct compression method²⁰ was selected because direct compression method is simplest means of production of a pharmaceutical tablet and high dose formulations²¹. It

requires only that the active ingredient is properly blended with appropriate excipients before compression²². Three key factors for successful tableting are flow and compactability of the compression mix, and drug content uniformity in the mix and the final tablets²³. The biologically potent methanol extract was used for developing of herbal tablet formulation. All the selected herbal extracts showed dose dependent significant activity, hence equal proportions of extracts were used for the development of formulation. The plant extracts were mixed with super tab 11 SD, primojel, magnesium stearate and talc as excipients according to the formula (Table 6) and compressed into round shaped tablets each weighing 500 mg (Figure 12) by using Remek 10 station automated punching machine and then subjected to various post compression parameters for evaluation. All the excipients are of pharmaceutical grade.²⁴

Pre formulation studies on biologically active extracts

Prior to the development of major dosage forms, it is essential that pertain fundamental physical and chemical properties of the drug molecule and other divided properties of the drug powder are determined. This information decides many of the subsequent events and approaches in formulation development. This first phase is known as pre formulation. All the extracts were characterized for their organoleptic properties, solubility²⁵⁻²⁶, loss on drying²⁷, compatibility with excipients²⁸ and micromeritic properties like bulk density²⁹, carr's index, hausner ratio and angle of repose³⁰⁻³¹ according to the prescribed standard procedures (Table 7).

Evaluation of powder blends for pre compression parameters

After characterization of individual extracts, the final blend was also characterized for precompression parameters like percentage of fines³², bulk density, carr's index, hausner ratio and angle of repose according to the standard procedures. All the parameters are within the limits and shown good flow properties (Table 8).

Evaluation of poly herbal tablets for post compression parameters

The tablets prepared by direct compression method were subjected to various quality control tests (post compression parameters) such as general appearance like size, shape and thickness; organoleptic properties like color, odor and taste; uniformity of weight, hardness, friability and stability studies³⁵ according to the standard procedures. The data within the range of pharmacopeial specifications was shown (Table 9).³³⁻³⁴

RESULTS AND DISCUSSION

The methanolic extract of *Begonia laciniata*, *Cuscuta epithymum* and *Dendrobium ovatum* were investigated for antioxidant property in comparison with the known antioxidant ascorbic acid following *in vitro* studies. The quantities of the extracts required for the *in vitro* inhibition of radical such as DPPH, superoxide and hydroxyl were compared to the known antioxidant ascorbic acid. All the extracts showed dose dependent

scavenging activity. The standard drug ascorbic acid also showed similar dose dependent activity and produced maximum scavenging activity at a dose of 360 µg (Figures 1-3). The selected plant methanolic extracts at dose levels of 100 mg/kg b.w, 200 mg/kg b.w and 400 mg/kg b.w, were tested by taking silymarin as a standard. The tested doses exhibited significant hepatoprotective activity against CCl₄-induced liver intoxicated rats by reduction in increased serum levels of SGOT, SGPT, SALP and Total Bilirubin. A slight decrease was found after the treatment with 100 mg/kg b.w dose when compared with the CCl₄ group. However administration of doses at 200 mg/kg b.w and 400 mg/kg b.w produced significant decreasing at serum levels of SGOT, SGPT, SALP and Total Bilirubin. (Tables 4-5, Figures 5-8). Histopathological examination of the liver sections of the control group showed normal architecture of the liver with distinct hepatic cells. The liver section of CCl₄ intoxicated group showed complete disarrangement of normal hepatic cells with intense centrilobular necrosis, vacuolization, fatty changes, sinusoidal hemorrhages and dilatation. The liver sections of silymarin treated rats showed a normal hepatic architecture with normal hepatocytes. Whereas the rats treated with test methanolic extracts of *Begonia laciniata*, *Cuscuta epithymum* and *Dendrobium ovatum* at doses of 100 mg/kg b.w 200 mg/kg b.w and 400 mg/kg b.w showed recovery from CCl₄ induced liver damage as evident from normal hepatocytes and with higher dose of 400 mg/kg b.w showed significant attenuation of inflammatory and necrotic changes and cellular architecture of liver was preserved indicating a marked protective activity similar to that observed in silymarin treated rat liver sections and the effect was found to be dose dependant (Figures 9-11). Phytochemical studies on the three selected plants revealed flavonoids, alkaloids, triterpenoids, glycosides, steroids and carbohydrates. The presence of above constituents in selected plant extracts alone or in combination might be responsible for the observed antioxidant and hepatoprotective activity. It is also supported by quantitative estimation of phytoconstituents (Table 2). Poly herbal hepatoprotective tablets were developed according to the formula (Table 6). Preformulation studies are performed on individual methanolic extract according to the standard procedures (Table 7). The precompression parameters were determined for final powder blend and the results (Table 8) of physical appearance, percentage of fines, bulk density, angle of repose, carr's Index and hausner's ratio indicated that the poly herbal powder blend possess good flow, compaction properties and good packing ability. After development of tablets by a direct compression method using Remek 10 station automated punching machine tablets were subjected to measuring of post compression parameters like physical appearance, uniformity of weight, hardness, friability, thickness, and disintegration time by standard pharmacopeial procedures (Table 9). All the parameters of the test products are complied with the pharmacopeial requirements. The poly herbal tablets were also tested for their accelerated stability at 40 ± 2°C / 75 ± 5 % RH and the results (Table 10) are reproducible.

Table 1: WHO standardization of roots of *Begonia laciniata*, whole plant of *Cuscuta epithymum* and whole plant of *Dendrobium ovatum* and qualitative preliminary phytochemical analysis

S. No.	Parameter	<i>Begonia laciniata</i>	<i>Cuscuta epithymum</i>	<i>Dendrobium ovatum</i>
1.	Organoleptic characters			
	Colour	Pale brownish white	Pale pinkish red	Pale greenish white
	Odour	Characteristic	Characteristic	Characteristic
	Taste	Characteristic	Characteristic	Characteristic
	Physical appearance	Free flowing powder	Free flowing powder	Free flowing powder
2.	Physiochemical characters			
	Water soluble extractive	57.13 %	61.11 %	72.45 %
	Alcohol soluble extractive	79.27 %	82.67 %	80.00 %
	pH 1% w/v solution	5.21	4.28	6.14
	Loss on drying	4.32 %	5.56 %	4.50 %
	Ash content	4.02 %	7.02 %	6.32 %
	Acid insoluble ash	0.63 %	2.13 %	0.63 %
	Moisture content by Karl Fischer	3.41 %	2.86 %	1.21 %
	Foreign organic matter	1.0 %	1.92 %	3.73 %
3.	Heavy metals			
	Lead	5 ppm	6.04 ppm	5.00 ppm
	Arsenic	1 ppm	1 ppm	1 ppm
	Cadmium	0.2 ppm	0.3 ppm	0.2 ppm
	Mercury	1 ppm	1 ppm	1 ppm
4.	Microbiological analysis			
	Total aerobic count	280 CFU/g	327 CFU/g	410 CFU/g
	Yeast and mould	25 CFU/g	42 CFU/g	30 CFU/g
5.	Pathogen analysis			
	<i>E. Coli</i>	Absent	Absent	Absent
	<i>Salmonella</i>	Absent	Absent	Absent
	<i>Pseudomonas aeruginosa</i>	Absent	Absent	Absent
	<i>Staphylococcus aureus</i>			
6.	Qualitative preliminary phytochemical analysis			
	Alkaloids	+	+	+
	Carbohydrates	-	+	+
	Flavonoids	+	+	+
	Glycosides	+	+	+
	Phytosterols	+	+	+
	Proteins and amino acids	-	-	-
	Saponins	-	-	-
	Tannins	-	-	-
	Triterpenoids	+	+	+

‘+’ Present, ‘-’ Absent

Table 2: Total Phenolic, flavonoid and alkaloidal content of *Begonia laciniata*, *Cuscuta epithymum* and *Dendrobium ovatum* plant extracts

S. No.	Plant methanolic extract	Phenolic content (g GAE/100 g dw)	Flavonoid content (g CE/100 g dw)	Alkaloid content (mg/100 g plant material)
1.	<i>Begonia laciniata</i>	2.23 ± 0.12	0.92 ± 1.12	32.24 ± 0.54
2.	<i>Cuscuta epithymum</i>	4.12 ± 1.14	3.76 ± 0.68	41.74 ± 0.86
3.	<i>Dendrobium ovatum</i>	4.51 ± 0.4	5.34 ± 0.93	48.16 ± 0.46

*Values are means of triplicate determination ± Standard deviation

Table 3: IC₅₀ values (µg/ml) of methanolic extracts of *Begonia laciniata*, *Cuscuta epithymum*, *Dendrobium ovatum* and ascorbic acid

Extract/Positive control	DPPH	Superoxide	Hydroxyl
<i>Begonia laciniata</i>	214.33	246.97	199.36
<i>Cuscuta epithymum</i>	244.88	236.54	213.69
<i>Dendrobium ovatum</i>	225.75	227.76	244.67
Ascorbic acid (positive control)	201.75	176.95	211.61

Table 4: Percentage reduction of various serum biochemical parameters due to treatment with methanolic extract of roots of *Begonia laciniata*, whole plant of *Cuscuta epithymum* and whole plant of *Dendrobium ovatum* against CCl₄ - induced hepatotoxicity in albino rats

Parameter	Methanolic extract of <i>Begonia laciniata</i>				Methanolic extract of <i>Cuscuta epithymum</i>				Methanolic extract of <i>Dendrobium ovatum</i>			
	Silymarin 50 mg/kg b.w	BLME 100 mg/kg b.w	BLME 200 mg/kg b.w	BLME 400 mg/kg b.w	Silymarin 50 mg/kg b.w	CEME 100 mg/kg b.w	CEME 200 mg/kg b.w	CEME 400 mg/kg b.w	Silymarin 50 mg/kg b.w	DOME 100 mg/kg b.w	DOME 200 mg/kg b.w	DOME 400 mg/kg b.w
SGOT	91.24	43.46	73.47	88.88	89.38	53.28	71.23	89.38	87.15	44.20	66.73	84.15
SGPT	92.96	60.21	83.28	90.45	91.20	62.14	80.97	88.83	88.29	39.66	64.63	86.63
SALP	90.23	53.34	69.85	86.60	74.25	33.05	56.83	69.58	86.44	36.47	60.67	82.69
T.BILI.	82.90	21.93	54.04	73.21	71.68	27.72	41.59	65.48	77.85	43.35	78.78	71.09

BLME: Methanolic extract of *Begonia laciniata*; CEME: Methanolic extract of *Cuscuta epithymum*;DOME: Methanolic extract of *Dendrobium ovatum***Table 5: Percentage reduction of various serum biochemical parameters due to treatment with polyherbal methanolic extract containing *Begonia laciniata*, *Cuscuta epithymum* and *Dendrobium ovatum* against CCl₄ - induced hepatotoxicity in albino rats**

Treatment	SGOT	SGPT	SALP	Total Bilirubin.
Silymarin 50 mg/kg	86.58	81.06	89.78	73.27
Polyherbal methanolic extract (150 mg/kg b.w p.o.)	67.33	50.39	67.92	48.61
Polyherbal methanolic extract (300 mg/kg b.w p.o.)	91.43	86.80	85.52	81.10

Table 6: Formula for development of polyherbal hepatoprotective tablets by direct compression method

S. No.	Ingredients	PHF (mg)
1.	PHME (API)	300
2.	Super Tab 11 SD (Diluent-Binder)	144
3.	Primojel (Super disintegrant)	50
4.	Magnesium Stearate (Lubricant)	3
5.	Talc (Glidant)	3
	Total Weight	500

PHME: Polyherbal methanolic extract containing equal quantities of *Begonia laciniata*, *Cuscuta epithymum* and *Dendrobium ovatum*

PHF: Polyherbal formula

Table 7: Pre formulation studies on biologically active extracts

Methanolic extracts	Color and Odor	Solubility	Loss on drying (%)	Angle of repose (°)	Bulk density (g/cm ³)	Hausner ratio	Carr's index (%)
<i>Begonia laciniata</i>	Pale brown and Characteristic	Soluble in water	1.43	37.35 ⁰	0.56	1.21	32.28
<i>Cuscuta epithymum</i>	Brownish black and Characteristic	Soluble in water	0.93	39.61 ⁰	0.48	1.28	34.94
<i>Dendrobium ovatum</i>	Dark brown and Characteristic	Soluble in water	1.12	41.27 ⁰	0.51	1.31	31.26

*Values are means of triplicate determination

Table 8: Pre formulation studies on final powder blend for pre compression parameters

S. No	Pre compression parameter	Observation/Result
1.	Physical appearance	Pale brown colored, free flowing with characteristic odor
2.	Percentage of fines	17 % w/w
3.	Bulk density	0.46 g/cm ³
4.	Angle of repose	32.42 ⁰
5.	Hausner ratio	1.156
6.	Carr's index	13.35 %

*Values are means of triplicate determination

Table 9: Evaluation of poly herbal tablets for post compression parameters

S. No	Post compression parameter	Observation/Result
1.	Physical appearance	Pale brown, round shaped tablets with characteristic odor
2.	Hardness	5.2 ± 0.15 kg/cm ²
3.	Friability	0.72 ± 0.37 %
4.	Uniformity of weight	499 ± 0.71 mg
5.	Thickness	4.18 ± 0.52 mm
6.	Disintegration time	9.31 ± 0.26 minutes

*Values are means of triplicate determination ± standard deviation

Table 10: Accelerated stability studies of the developed poly herbal tablets

Time (Months)	Storage condition: 40 ± 2°C / 75 ± 5 % RH				
	Parameters evaluated				
	Physical appearance	Uniformity of weight (mg)	Hardness (cm ²)	Friability (%)	Disintegration time (min)
I	Pale brown, round shaped tablets with characteristic odor	500 ± 1.21	5.2 ± 0.29	0.83 ± 1.24	9.67 ± 0.71
III	Pale brown, round shaped tablets with characteristic odor	501 ± 0.25	5.4 ± 0.15	1.01 ± 1.11	12.23 ± 2.15
VI	Pale brown, round shaped tablets with characteristic odor	501 ± 1.68	5.4 ± 1.28	1.11 ± 0.65	13.10 ± 0.23

Figure 1: Bar diagram of concentration-dependent percentage inhibition of DPPH radical scavenging activity

BLME: Methanolic extract of *Begonia laciniata*
 CEME: Methanolic extract of *Cuscuta epithymum*
 DOME: Methanolic extract of *Dendrobium ovatum*

Figure 2: Bar diagram of concentration-dependent percentage inhibition of superoxide radical scavenging activity

BLME: Methanolic extract of *Begonia laciniata*
 CEME: Methanolic extract of *Cuscuta epithymum*
 DOME: Methanolic extract of *Dendrobium ovatum*

Figure 3: Bar diagram of concentration-dependent percentage inhibition of hydroxyl radical scavenging activity

BLME: Methanolic extract of *Begonia laciniata*
 CEME: Methanolic extract of *Cuscuta epithymum*
 DOME: Methanolic extract of *Dendrobium ovatum*

Figure 4: Bar diagram of IC₅₀ values (µg/ml) of methanolic extracts of *Begonia laciniata*, *Cuscuta epithymum*, *Dendrobium ovatum* and ascorbic acid

BLME: Methanolic extract of *Begonia laciniata*
 CEME: Methanolic extract of *Cuscuta epithymum*
 DOME: Methanolic extract of *Dendrobium ovatum*

Figure 5: Percentage reduction of various serum biochemical parameters due to treatment with methanolic extract of roots of *Begonia laciniata* against CCl₄-induced hepatotoxicity in albino rats
BLME: Methanolic extract of *Begonia laciniata*

Figure 6: Percentage reduction of various serum biochemical parameters due to treatment with methanolic extract of whole plant of *Cuscuta epithymum* against CCl₄-induced hepatotoxicity in albino rats
CEME: Methanolic extract of *Cuscuta epithymum*

Figure 7: Percentage reduction of various serum biochemical parameters due to treatment with methanolic extract of whole plant of *Dendrobium ovatum* against CCl₄-induced hepatotoxicity in albino rats
DOME: Methanolic extract of *Dendrobium ovatum*

Figure 8: Percentage reduction of various serum biochemical parameters due to treatment with polyherbal methanolic extract (PHME) containing *Begonia laciniata*, *Cuscuta epithymum* and *Dendrobium ovatum* against CCl_4 - induced hepatotoxicity in albino rats
PHME: Poly herbal methanolic extract

Figure 9: Effect of methanolic extract of *Begonia laciniata*, *Cuscuta epithymum* and *Dendrobium ovatum* against CCl_4 - induced hepatotoxicity in albino rats

Histopathology: Photomicrographs of liver sections

CV-Central Vein; PV-Portal Vein; V-Vacuole; N-Necrosis; SS –Sinusoidal Spaces; FC- Fatty Changes

Figure 10: Effect of methanolic extract of *Begonia laciniata*, *Cuscuta epithymum* and *Dendrobium ovatum* against CCl_4 - induced hepatotoxicity in albino rats

CV-Central Vein; PV-Portal Vein; V-Vacuole; N-Necrosis; SS –Sinusoidal Spaces; FC- Fatty Changes

Histopathology: Photomicrographs of liver sections

Figure 11: Effect of polyherbal methanolic extract containing *Begonia laciniata*, *Cuscuta epithymum* and *Dendrobium ovatum* against CCl_4 - induced hepatotoxicity in albino rats
CV-Central Vein; PV-Portal Vein; V-Vacuole; N-Necrosis; SS-Sinusoidal Spaces; FC- Fatty Changes PHME: Poly herbal methanolic extract

Figure 12: Prepared polyherbal hepatoprotective tablets

No significant difference in the physical appearance, uniformity of weight, hardness and friability and disintegration time was observed during accelerated stability studies. It is therefore, concluded that the formulated tablets are stable at specified storage conditions.

CONCLUSION

All these scientific observations support the traditional use of *Begonia laciniata*, *Cuscuta epithymum* and *Dendrobium ovatum* for treating liver disorders. The free radical scavenging and antioxidant properties of phytoconstituents may be the possible mechanisms of its hepatoprotective potential. The developed formulation is more safe and effective similar to the commercial herbal formulas containing silymarin.

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Cite this article as:

Seru Ganapaty, Maddi Ramaiah, Kanuri Yasaswini, Padarthi Mahesh Babu. In vitro, in vivo standardization, formulation and evaluation of directly compressed polyherbal hepatoprotective tablets. *Int. J. Res. Ayurveda Pharm.* 2013;4(5):769-780 <http://dx.doi.org/10.7897/2277-4343.04529>

Source of support: Nil, Conflict of interest: None Declared