

Research Article



Standardization and evaluation of hepatoprotective activity of polyherbal capsule

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ABSTRACT

In traditional systems of medicine, many plants have been documented to be useful for the treatment of liver disorders. Most of the traditional system of medicine is effective but they lack of complete standardization which is one of the important challenge faced by herbal formulations. The present study was aimed to standardize the developed polyherbal capsule and evaluation of its pharmacological activity, recommended as hepatoprotective agent. Selective combination of herbs such as *Andrographis paniculata*, *Phyllanthus amarus*, *Asparagus racemosus*, *Tinospora cordifolia*, *Boerhaavia diffusa* and *Eclipta alba* for the treatment of hepatic disorder by exploiting the knowledge of traditional system of medicine in which individually proven for their efficacy in hepatoprotective activity were formulated as capsule. Formulation were developed with standardised raw materials as per WHO guidelines, and optimised the formulation with pre-formulation parameters such as bulk density, tap density, Carr's index, Hausner's ratio and angle of repose for blended powder. The powder were capsulated and the designed formulation were standardized with improved formulation parameters such as Organoleptic characters, physical, physicochemical parameters such as ash values, extractive values, qualitative and quantitative phytochemical screening were checked for finished capsules. Quantitative estimation of Andrographolide content of the polyherbal formulation was done by HPTLC method. The designed formulation was in conformity to the properties evaluated for capsules. Pharmacological studies of polyherbal capsule were studied for its therapeutic efficacy, found safe up to 2000mg/kg body weight by acute toxicity study as per OECD guidelines and showed significant hepatoprotective activity at the doses of 200mg/kg and 100mg/kg which is comparable with the standard drug silymarin 25mg/kg body weight by *in vivo* method using CCl₄ induced hepatotoxicity method in rats.

Keywords: Hepatoprotective, HPTLC, Polyherbal capsule, Standardization, WHO.

INTRODUCTION

Liver disease is one of the major causes of morbidity and mortality in public, affecting humans of all ages. About 20,000 deaths occur every year due to liver disorders.¹ Some of the commonly known disorders are viral hepatitis, alcohol liver disease, non-alcoholic fatty liver disease, autoimmune liver disease. Liver is quantitatively the most important site of drug metabolism. However, many drugs are known to cause hepatic injury like paracetamol, pain killers and antibiotics. Synthetic drugs used in the treatment of liver diseases are sometimes in adequate and can have serious adverse effects.

Current medical treatments for these liver diseases are often ineffective, and therefore efforts are being made to seek new effective medications from herbal sources.² Developing therapeutically effective agents from natural products has become a new trend by virtue of their little toxicity or few side effects.

Though the modern allopathic system of medicine has evolved phenomenally the remedy for hepatic disease still depends on herbal and herbal medicine. Polyherbal formulation are preferred than a single herb by the traditional healers for the management of liver disorders. When herbs are combined together, they become more potent and effective within the body than single herb due to their activating or catalyzing influence upon one another. Hepatoprotective polyherbal formulation helps

the liver in clearing away the toxins, regenerates the liver cells and prevents liver failure.

Many herbs and herbal medicines are being used since ages to treat hepatic diseases. However the scientific basis for such is not completely established. Therefore it is necessary to screen the various herbs and herbal products for their claimed hepatoprotective potential.

A polyherbal hepatoprotective capsule was therefore developed using dry extracts of herbs such as *Andrographis paniculata*, *Phyllanthus amarus*, dry powders of *Asparagus racemosus*, *Tinospora cordifolia*, *Boerhaavia diffusa* and *Eclipta alba* for the treatment of hepatic disorder by exploiting the knowledge of traditional system of medicine.

MATERIALS AND METHODS

Preparation of formulation

Dry extracts of herbs such as *Andrographis paniculata* (Whole plant), *Phyllanthus amarus* (Whole plant), dry powders of *Asparagus racemosus* (Root), *Tinospora cordifolia* (Stem), *Boerhaavia diffusa* (Root) and *Eclipta alba* (Whole plant) were selected for the formulation. All the raw materials were standardized as per WHO guidelines.

The pulverized crude drugs of *Asparagus racemosus*, *Tinospora cordifolia*, *Boerhaavia diffusa* and *Eclipta alba* were sieved in 40# and dry extracts of *Andrographis*



paniculata, Phyllanthus amarus were dried in tray drier at 60°C for 20 minutes. Diluents like Dicalcium phosphate, Microcrystalline cellulose, Colloidal silicon dioxide, Magnesium stearate, Talc, Starch, and Gelatin (both in Granulation form) were dried separately in tray drier at 100°C for 30 minutes. All active ingredients were weighed according to the formula, mixed and lubricated with magnesium stearate followed by diluents and preservatives like Methyl paraben sodium, Propyl paraben sodium and Bronopol as specified in formula were mixed well.

The mixture was blended thoroughly for 30 minutes. Then the powder was transferred to the polythene bags and labelled for further studies.³⁻⁶

Pre-formulation studies

Various trial batches were formulated by varying the composition of excipient proportions and the powder were evaluated for its flow characteristics.

The blended powder of all five trial batches were analyzed for its flow characteristics like bulk density, tap density, compressibility index, Hausner's ratio and angle of repose. From the five trial batches one optimized batch is selected for formulation based on above test results.^{7,8}

Development of capsule

The optimized batch were filled in "0" size capsules to an average net content weight of 500 mg. Capsules were then dedusted transferred into polybags, labeled and the samples were evaluated as per testing requirements.^{9,10}

Standardization of polyherbal capsules

The developed capsules were evaluated for the following parameters for the purpose of standardization: Description of capsules, physical and physicochemical parameters, qualitative and quantitative phytochemical studies, heavy metal analysis and microbial load analysis.

Capsule evaluation

The polyherbal capsules were evaluated for its description; average weight & uniformity of weight, disintegration test, pH and moisture content, the observations were compared with Indian Pharmacopoeial standards.¹¹

Quantitative estimation of heavy metals

Quantitative estimation of heavy metals was tested for detection of arsenic, cadmium, mercury and lead as per WHO procedures.¹²

Phytochemical studies

I. Qualitative phytochemical analysis were carried out to detect the presence of phyto-constituents in the polyherbal formulation.^{13,14}

II. Quantitative Estimation of phytoconstituents in Polyherbal Formulation

A. Estimation of Total Alkaloids¹⁵

Preparation of solution : Bromocresol green solution were prepared by heating 69.8mg of bromocresol green with 3ml of 2N NaOH and 5 ml distilled water until completely dissolved and the solution was diluted to 1000 ml with distilled water. Phosphate buffer solution (pH 4.7) was prepared by adjusting the pH of 2 M sodium phosphate (71.6 g Na₂HPO₄ in 1 L distilled water) to 4.7 with 0.2 M citric acid (42.02 g citric acid in 1 L distilled water).

Standard solution

Atropine standard solution was prepared by dissolving 1mg of atropine in 10 ml distilled water. Accurately measured aliquots of (0.4, 0.6, 0.8, 1 and 1.2 ml) atropine standard solution were transferred each to different separatory funnels. Then 5 ml of phosphate buffer pH 4.7 and 5ml BCG solution were added and shaken each mixture with 1, 2, 3 and 4 ml of chloroform. The extracts were collected in a 10-ml volumetric flask and diluted to volume with chloroform. The absorbance of standard solutions was measured at 470 nm against blank prepared as above but without atropine.

Poly herbal capsule – Sample solution

10mg of sample dissolved in 100ml water. 1 ml of the solution were transferred to a separatory funnel and washed with 10 ml chloroform (3 times). The pH of this solution was adjusted to neutral with 0.1 N NaOH. Then 5 ml of BCG solution and 5 ml of phosphate buffer were added to this solution. The mixture is shaken and the complex formed is extracted with 1, 2, 3, and 4 ml chloroform by vigorous shaking. The extracts 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.

B. Estimation of Total Phenol¹⁶

The total phenolic content in the polyherbal capsule was estimated by Folin-Ciocalteu assay method.

Polyherbal capsule – Sample

The reaction mixture were prepared by mixing 0.5 ml of hydroalcoholic solution of sample, 2.5 ml of 10% Folin-Ciocalteu's reagent in water and 2.5 ml 7.5% sodium bicarbonate (1mg/ml). The samples were prepared in triplicate and the mean value of absorbance was obtained.

Blank: 0.5ml Ethanol, 2.5 ml 10% Folin-Ciocalteu's reagent in water and 2.5 ml of 7.5% of sodium bicarbonate.

Standard: The reaction mixture was prepared by mixing (0.5ml, 1ml, 1.5ml, 2.0ml and 2.5ml) of Gallic acid solution and mixed to each tubes with 2.5 ml of 10% Folin-Ciocalteu's reagent in water and 2.5 ml 7.5% sodium bicarbonate.

The samples were heated at 45°C for 45 min, cooled measured the absorbance using colorimeter at a wavelength 765 nm.

C. Estimation of Total Flavonoids¹⁶

The content of flavonoids in Polyherbal capsule was determined using colorimetric method.

Polyherbal capsule (Sample): The solution mixture contains 5ml of 1mg/ml hydroalcoholic solution of sample was mixed with 2% aluminium chloride solution in methanol. The samples were prepared in triplicate for each analysis and the mean value of absorbance is obtained.

Blank: 2% aluminium chloride solution in methanol

Standard: The Quercetin solution mixture containing 100ml of 1mg/ml were prepared. From this solution mixture (0.2, 0.4, 0.6, 0.8, 1.0 ml) was mixed each with 1.5ml of 2% aluminium chloride solution in methanol.

The samples were warmed for an hour at room temperature and the absorbance was measured using colorimeter at a wavelength of 415nm.

D. Estimation of Total Tannins¹⁷

Prior the quantitative estimation of the tannin content in the samples, the presence of tannins were identified using the classic FeCl_3 and Gelatin tests.

The Total tannin content in Polyherbal capsule was estimated by method of Price and Butler with some modifications.

Polyherbal capsule (Sample): 1g of sample were taken transferred to 100ml flask; 50ml water is added and boiled for 30min. Filtered the solution with cotton filter, filtrate is further transferred to 500ml flask and made to 500ml with water. 0.5ml aliquot of sample solution is treated with 1ml of 1% potassium ferricyanide solution and 1ml 1% Ferric chloride solution and made to 10ml with water.

Blank: 1ml of 1% potassium ferricyanide solution and 1ml 1% Ferric chloride solution and made to 10ml with water.

Standard solution: Gallic acid solution of various concentrations (5, 10, 15, 20 and 25 $\mu\text{g/ml}$) is prepared using water. The reaction mixtures are prepared by treating each solution with 1ml of 1% potassium ferricyanide solution and 1ml 1% ferric chloride solution and made to 10ml with water.

After five minutes, the solutions were measured at a wavelength of 700nm colorimetrically.

E. Estimation of Total Saponins¹⁸

The total saponin content in polyherbal capsule was determined gravimetric method.

Procedure: 20 g of the sample was weighed and 100ml of 20 % aqueous ethanol was added. Then the sample was heated over a hot water bath for 4 hours with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extract was reduced to 40 ml over water bath at about 90°C. The concentrate was treated with 20

ml of diethyl ether and the aqueous layer was recovered while the ether layer was discarded. This process of purification was repeated three times and then 60ml of n-Butanol was added and extracted. The n-Butanol extract obtained was then washed two times with 10 ml of 5% aqueous Sodium chloride. The remaining solution was heated in a water bath for evaporating the solvent. After evaporation the samples were dried in the oven to a constant weight and the saponin content was calculated as percentage.

F. Quantitative estimation of Andrographolide content by HPTLC method

HPTLC serves as a convenient tool for finding the distribution pattern of phyto-constituents which is unique to each plant. HPTLC technique is helpful in order to check the identity, purity and standardize the quantity of active principles present in the herbal formulation.

Andrographis paniculata a principle ingredient of the polyherbal formulation contains a high amount of andrographolide, which is one of the main phytoconstituent responsible for Hepatoprotective activity was identified and quantified using HPTLC.

Instrument conditions

Sample used	: Polyherbal capsule
HPTLC Applicator	: CAMAG LINOMAT IV
HPTLC Scanner	: CAMAG TLC SCANNER II
Sample dilution	: 25.4 mg of sample extracted with 1 ml of methanol
Volume of injection	: 20 μl
Mobile phase	: Methanol: Ethyl acetate: Hexane (1:5:4)
Lambda max	: 254nm
Lamp	: Deuterium
Stationary phase	: TLC Silicagel 60 F ₂₅₄ (Merck)

HPTLC methodology

Silica gel 60 F₂₅₄ precoated plates (20 x 10cm) were used with Methanol: Ethyl acetate: Hexane (1:5:4) as mobile phase. 20 μl of standard andrographolide was spotted on pre-coated HPTLC plates. The bandwidth applied on plate was 6mm and ascending mode was used for development of thin layer chromatography. The saturation time was 20mins at humidity level 65% \pm 5% RH and room temperature at 25°C \pm 2°C. TLC plates were developed up to 8cm. The plates were then scanned at 254nm for quantification purpose.¹⁹

Microbial load analysis

For the safe use of polyherbal capsules microbial count were done and checked whether Total aerobic viable count, Yeasts and moulds were within the prescribed limits and the microorganisms *Escherichia coli*, *Clostridia*,

Salmonellae, Shigella, Pseudomonas, Staphylococcus were absent in the finished formulation.

Procedure: The tests were carried out as per WHO procedures for microbial contamination.¹²

Pharmacological Studies

This study was approved by Institutional Ethical Committee and the Ethical Committee approval no. was 7/243/ CPCSEA, Madras Medical College, Chennai. dated 23.08.2012.

Acute toxicity study

The acute toxicity study was designed as per the OECD Guideline for Testing of chemicals, Acute Oral Toxicity (Acute Toxic Class Method), Guideline 423.²⁰

Hepatoprotective activity

Method- Carbon tetrachloride induced Hepatotoxicity in rats

Animals

Adult male Albino Wistar rats were selected for the study. Animal divided into five groups of six each. During the experimental period all the animals were maintained under normal diet and water.

Grouping of animals

Group I: Normal control - Received water as vehicle orally for nine days.

Group II: CCl₄ control - Received 5ml/kg of water orally for nine days and hepatotoxicity was induced by single dose of 1ml/kg of 50%v/v of CCl₄ in paraffin oil (1:1) intraperitoneally on the seventh day.

Group III: Standard drug – Treated with Silymarin 25mg/kg once daily for nine days orally.

Group IV: Test drug (Low dose) – Treated with hepatoprotective poly herbal capsule 100mg/kg body weight once daily for nine days orally.

Group V: Test drug (High dose) - Treated with hepatoprotective poly herbal capsule 200mg/kg body weight once daily for nine days orally.

On the seventh day hepatotoxicity was induced to all the group of animals except normal control by administration of single dose of 1ml/kg of 50%v/v CCl₄ in paraffin oil (1:1) intraperitoneally.

After the experimental period all the animals were overnight fasted, on the 10th day blood samples were collected from retro orbital plexus through the capillary tube, since many of the biochemical and histological changes are known to manifest after 24 hours of CCl₄ administration. Blood was subjected to centrifugation (2000rpm for 10mins) and the serum was collected for the determination of hepatic marker enzymes like SGOT, SGPT, ALP, Total bilirubin and Total protein using semi auto analyzer and diagnostic kit.^{21, 22}

Animals were anaesthetized on the same day and sacrificed by cervical decapitation. Immediately after sacrifice, the liver were removed from the animals and preserved in the liver homogenate solution i.e. 10% formalin solution. Liver was subjected to histo pathological studies.^{23, 24}

Statistical analysis

Statistical calculations were done by using one way ANOVA analysis followed by Dunnet multiple comparison test, P < 0.05 were considered as significant.

RESULTS AND DISCUSSION

Formulation Development

Five trial batches of the polyherbal formulation were prepared and tested for preformulation parameters like bulk density, tapped density, Carr's index, Hausner's ratio and angle of repose.

As per the standards, the flow property of the blend to be filled in the capsules should be in good range and was confirmed by the above parameters. Among the five trials, Trial batch – V showed excellent flow characters and the results are Bulk density (0.45±0.82g/cm) Tap density (0.50 ± 0.002), Compressibility index (8.20 ± 1.032%w/w), Hausner's ratio (1.03 ± 0.022), Angle of repose (27.16 ± 0.92°) and hence that batch was taken for capsule filling.

Standardization of polyherbal capsule

The final batch was tested for Organoleptic characters, Physical, physicochemical parameters and Microbial load analysis, results observed are shown in Table 1.

Table 1: Standardization of polyherbal capsule

Name of test	Observations
Organoleptic characters	
Description	Greyish white powder contained in Blue cap/ Red body "0" size capsule.
Colour	Greyish white powder
Odour	Characteristic odour
Taste	Bitter
Physical Parameters	
pH (1% aqueous solution)	7.33 ± 0.21
Moisture content	3.2% ± 0.14
Average weight	574mg ± 6.06
Uniformity of weight	RSD: 3.8
Disintegration time	2' 32secs ± 0.34
Physicochemical parameters	
Total ash	39.3% ± 0.33
Acid insoluble ash	18.1%± 0.46
Sulphated ash	43.7% ± 0.24
Water soluble extractive	7.4% ± 0.53
Ethanol soluble extractive	0.13% ± 0.11
Heavy metals	Less than 20ppm

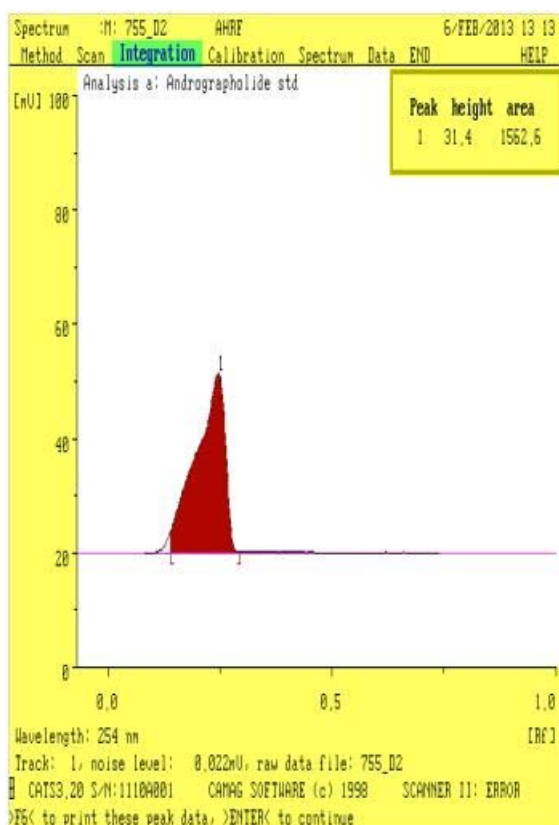
Results are reported as Mean ± Standard deviation. (n=3)



Table 2: Heavy metals & Microbial load analysis

Heavy metals		
Heavy metals	Observation (in ppm)	Limits (in ppm)
Arsenic	0.044	5
Cadmium	0.022	0.3
Iron	4.487	10
Mercury	Not detected	0.5
Microbial load analysis		
Name of organism	Observations	Who limits
Total aerobic viable count	210cfu/g	NMT 1000cfu/g
Yeasts and moulds	Nil	NMT 100cfu/g
<i>E.coli</i>	Absent	Should be absent
<i>Clostridia</i>	Absent	Should be absent
<i>Salmonella</i>	Absent	Should be absent
<i>Shigella</i>	Absent	Should be absent
<i>Pseudomonas</i>	Absent	Should be absent

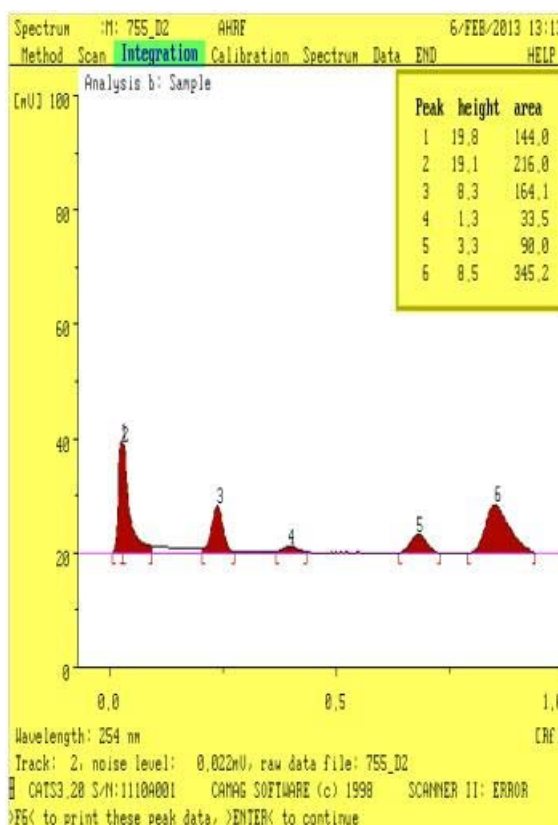
(cfu – Colony forming units)

**Figure 1:** HPTLC Chromatogram for Standard – Andrographolide.

The Polyherbal formulation shows the presence of various phytochemical constituents like Triterpenoids, Flavonoids, alkaloids, carbohydrates, Glycosides, phenols, proteins, saponins, Tannins and steroids qualitatively and hence it is desirable to quantify few of them in order to establish a standard to maintain its quality. Among them the following constituents were estimated and the results are reported as mean \pm standard deviation (n=3), total alkaloids ($1.27 \pm 0.37\%$ w/w), phenolic compounds ($1.56 \pm 0.71\%$ w/w), flavonoids ($3.11 \pm 0.47\%$ w/w), saponins ($0.21 \pm 0.74\%$ w/w) and tannin ($0.16 \pm 0.56\%$ w/w).

Quantification of Andrographolide

High performance thin layer chromatography (HPTLC) finger printing was performed with the methanol extract of the polyherbal capsules with the chromatographic conditions as detailed in material and methods of this study. There were 5 peaks observed with different RF values and different heights. Percentage of areas obtained from the chromatogram used for the qualitative and quantification of the compound comparing with respect to standard present in the capsules. % of Andrographolide in polyherbal capsule was found as 1.323%

**Figure 2:** HPTLC Chromatogram of sample – Polyherbal Capsule

Heavy metal and Microbial load analysis

The presence of heavy metals was tested quantitatively for the following metals and it was found that the formulation was within the WHO limits. Microbial screening is done for the polyherbal formulation from

the fresh packs of the same container and the results of both were detailed in Table 2.

From the results it is shown that the Polyherbal formulation complies with the heavy metals and microbial contamination limits of the WHO guidelines and hence it is safe to be taken internally.

Pharmacological Studies

Acute toxicity study

The acute toxicity of poly herbal hepatoprotective capsule were determined as per the OECD guideline no. 423 (Acute Toxic class method). It was observed that the test dose was not mortal even at 2000mg/kg dose. Hence 1/20th (100mg) were selected as low dose and 1/10th (200mg) as high dose for further study.

Hepatoprotective activity

Method: Carbon tetrachloride induced hepatotoxicity in rats.

The levels of SGOT, SGPT, ALP, Total bilirubin, Total protein in the serum were studied and the results were given in table 3.

Administration of CCl₄ resulted in a significant elevation of marker enzymes like SGOT, SGPT, ALP, Total bilirubin and reduced protein content when compared to normal control group. Administration of polyherbal capsule decreases the elevated levels of marker enzymes and increases the level of total protein in a dose dependent manner, which shows that polyherbal capsule have significant (P<0.001) hepatoprotective activity which was comparable with standard drug. The results therefore suggests that the developed polyherbal capsule able to alleviate the hepatic damage induced by CCl₄ significantly, which is attributed to the presence of one or more phytoconstituents in the formulation.

Histopathological studies

The normal control group has normal cellular architecture with distinct hepatic cells. In carbon tetra chloride treated group the normal structure of liver was lost with sinusoidal distension, congestion, multifocal fatty degenerative changes and bile duct hyperplasia. Standard and test groups showed protective effect by inhibiting the degeneration of hepatocytes and fatty changes whereas test groups showed dose dependent protective effect.

Group – I (Control) – The histological view of rat liver with normal hepatocytes.

Group –II (CCl₄ induced animal) – The normal structure of liver was lost with sinusoidal distension, congestion, multifocal fatty degenerative changes and bile duct hyperplasia.

Group-III (Standard) – Pretreatment with standard inhibits the degeneration of hepatocytes and fatty changes.

Group – IV (Test -Low dose) - Pretreatment with test drug at low dose shows protective effect by inhibiting the mild degeneration of hepatocytes and fatty changes

Group – V (Test- High dose) - Pretreatment with test drug at high dose shows dose dependent protective effect by inhibiting the degeneration of hepatocytes and fatty changes.

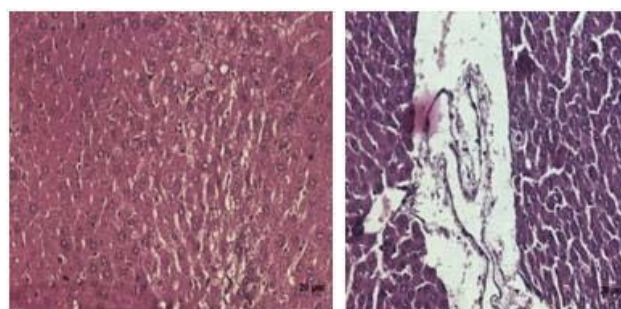


Figure 3

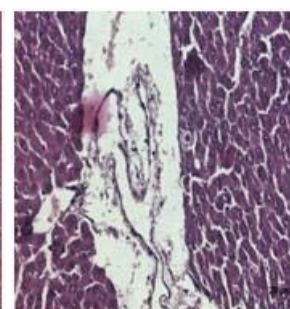


Figure 4

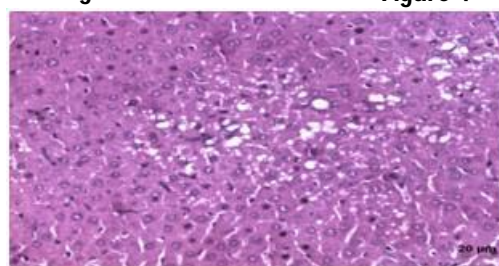


Figure 5

Figure 3: Histopathology of Rat liver- Normal control

Figure 4: Histopathology of Rat liver – CCl₄ control

Figure 5: Histopathology of Rat liver – Test drug (High dose)

Table 3: Hepatoprotective activity parameters

Group	Name of groups	Observation				
		Parameters				
		SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Total Bilirubin (mg/dL)	Total Protein (mg/dL)
I.	Control	23.77 ± 3.08	51.22 ± 2.21	153.31 ± 2.54	0.47 ± 0.05	6.23 ± 0.30
II.	CCl ₄ control	62.84 ± 2.17*	112.70 ± 1.71*	215.54 ± 2.32*	2.13 ± 0.11*	4.83 ± 0.34*
III	Standard	29.46 ± 1.88**	82.06 ± 2.01**	168.12 ± 2.87**	0.94 ± 0.17**	6.0 ± 0.42**
IV.	Test drug 1 (Low dose - 100mg/kg)	38.03 ± 2.19**	84.44 ± 1.45**	186.81 ± 2.03**	1.66 ± 0.28**	5.39 ± 0.22**
V.	Test drug 2 (High dose - 200mg/kg)	29.05 ± 3.31**	79.73 ± 3.0**	179.43 ± 2.13**	0.90 ± 0.17**	5.95 ± 0.46**

Results are reported as Mean ± SEM; (n=6); *P< 0.05 when compared with control (Group I); **P<0.001 when compared with CCl₄ control (Group II) data were analysed by using one way ANOVA f-test followed by Dunnet multiple comparison test.

CONCLUSION

The polyherbal formulation containing six herbs was formulated and standardized as per the WHO guidelines. The results suggested that the developed polyherbal capsule were consistent with various identity, quality and purity. It also gives the surety about the product which was formulated using improved formulation parameters. Hence the formulation may be beneficial for the treatment of liver disease. A detailed preclinical and clinical study is required to establish the use of capsule as Hepatoprotective agent.

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REFERENCES

- Gupta AK, Misra N, Hepatoprotective activity of aqueous ethanolic extract of *Chamomile capitula* in paracetamol intoxicated albino rats, American Journal of Pharmacology and Toxicology, 1, 2006, 17-20.
- Seeff LB, Lindsay KL, Bacon BR, Kresina TF, Hoofnagle JH, Complementary and alternative medicine in chronic liver disease, Hepatology, 34, 2001, 595-603.
- Shah VK, Zalavadiya V, Sheth NR, Raval NK, Golwala DK, Standardization and assessment of preformulation parameters of rasayana Tablet, International Journal of Pharmaceutical Sciences and Drug Research, 2, 2010, 58-62.
- Remington's Pharmaceutical Sciences, 17th Ed, Mack Publishing Co, Easton PA, 1985, 1633-1643.
- Michael, Irene Ash, Handbook of Fillers, Extenders, and Diluents, 2nd Ed, Synapse information Resources Inc, New York, 2007, 8-22.
- Michael, Irene Ash, Handbook of Preservatives, Synapse information Resources Inc, New York, 2004, 108-145.
- Anonymous, Powder Flow, In United States Pharmacopoeia NF-25, 30th Ed, 1174, The official standard of Compendia, 2007, 1123-1156.
- Anonymous, Bulk Density and Tapped Density, In United States Pharmacopoeia NF-25, 30th Ed, 616, The official standard of compendia, 2007, 1012-1102.
- Jone BE, Pharmaceutical capsules, 2nd Ed, Edited by Podczek F. Pharmaceutical press, 2004, 91, 242, 449.
- Van Hostetler, Leon Lachman, Herbert AL, Joseph LK, Hard Capsules, In: The Theory and Practice of Industrial Pharmacy, 3rd Ed, Varghese Publishing House, Bombay, 1991, 374.
- Anonymous, Indian Pharmacopoeia, Vol I, Govt of India, Ministry of Health and Family Welfare, Controller of Publication, Ghaziabad, 2007, A53- 54.
- WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues, World Health Organization, Geneva, 2007, Anex.3, 5 & 6.
- Harborne JB, Phytochemical Methods, A Guide to Modern Techniques of Plant Analysis, 2nd Ed, Chapman and hall, London, 1973, 434.
- Kokate CK, Purohit AP, Gokahle SB, Pharmacognosy, 24th Ed, Vallabh Prakashan, Pune, 2003, 108-109.
- Fazel Shamsa, Hamidreza Monsef, Rouhollah Ghamooshi, Mohammadreza Verdian-rizi, Spectrophotometric determination of total alkaloids in some Iranian medicinal plants, Thailand Journal of Pharmaceutical Sciences, 3, 2008, 17-20.
- Pattanayak SP, Mitra Mazumder P, Sunita P, Total phenolic, flavonoid content and *in vitro* antioxidant activities of *Dendrophthoe falcata* (L.f.) Ettingsh, International Journal of Pharm Tech Research, 3, 2011, 1392-1406.
- Urve Paaver, Vallo Matto, Ain Raal, Total tannin content in distinct *Quercus robur* L. galls, Journal of Medicinal Plants Research, 4, 2010, 702-705.
- Obdoni BO, Ochuko PO, Phytochemical studies and comparative efficacy of the crude extracts of some Homostatic plants in Edo and Delta States of Nigeria, Global J. Pure Appl.Sci, 8, 2001, 203-208.
19. HPTLC analysis of hepatoprotective diterpenoid andrographolide from *Andrographis paniculata* nees (*kalmegh*), Indian Journal of pharmaceutical sciences, 69, 2007, 473-475.
- OECD Guideline for testing of chemicals, Acute Oral Toxicity – Acute Toxic Class method, Original Guideline 423, March 1996, 1-14.
- Bhattacharya D, Pandit S, R Mukherjee, N Das and TK Sur, Hepatoprotective effect of himoliv, a polyherbal formulation in rats, Indian Journal of Physiol Pharmacol, 47, 2003, 435-440.
- Kandasamy CS, Shimna Thasnim PS, Mohammed Basil E, Arul Raj P, Gopal V, Venkatanarayanan R, Anti-hepatotoxic activity of polyherbal formulation in Carbon tetrachloride induced toxicity in rats, RJPBCS, 1, 2010, 341.
- Schmidt E, Schmidt FW, Mohr J, Otto P, Vido I, Wrogemann K, Herfarth C, Liver morphology and enzyme release further studies in the isolated perfused rat liver, In Keppler, D. (Edn), Pathogenesis and Mechanism of liver cell necrosis, Medical and Technical, Lancaster, 1975, 47.
- Sallic R, Shimna Thasnim PS, Mohammed Basil E, Drugs and the liver biopharmaceutical drug disposition, Research Journal of Pharmaceutical, Biological and Chemical sciences, 12, 1991, 251-259.

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