MEDICAL ASSESSMENT OF TRADITIONAL LIVER PROTECTIVE FORMULATION BY CLINICAL TRIAL

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ABSTRACT:

Background: Hepatitis is one disease which has been demonstrated itself as expletive for years together. Even during the present day, we could not find an excellent western medicine for management and comprehensive therapy for hepatitis. This work targets to prepare a product as a remedy for hepatitis through coalescing different herbals to treat hepatitis.

Methods: Selection of crude drugs for the formulation was based on the traditional and ethno-medical literatures. the drugs selected for our formulation are ajowan, cardamom, cinnamon, clove, coriander, cumin, ginger and nutmeg. Further, the selected crude drugs had been collected and further subjected to different Pharmacognostical studies and Phytochemical evaluations, to analyze it quality and purity. Then, the liver protective product had been prepared & its safety was adjudged by toxicological evaluation. Finally at the outset, the efficacy was evaluated by pharmacological evaluation and clinical trials of the liver protective formulation was carried out at Ayushkaram Ayurveda Hospital and Research Center, Sulur, Coimbatore subsequently after receiving the approval of Human Ethical Clearance issued by IEC, IAD, Kasaragod, Kerala and prospective registration of trial at Clinical Trial Registry of India. **Results**: The present investigation has been performed with 30 numbers of patients. Each and every patient were administered at a quantity of 60ml thrice a day immediately one hour after ingesting food for three consecutive days. The Liver protective formulation showed substantial recovery in pyrexia by 36% ($P<0.015$), recovery in fatigue by 32% ($P<0.025$), recovery in yellow staining of sclera by 33% ($P<0.025$), and recovery in yellow staining of urine by 27% ($P<0.010$). This also has relief in the yellow staining of nail and skin by 51% with a P value of 0.001, and found statistically significant.

Conclusion: Liver protective formulation has significantly demonstrated its effectiveness in managing the hepatitis.

Keywords: *Ajowan, Cardamom, Cinnamon, Coriander, Cumin, Clove*, Liver protective.

INTRODUCTION:

Moral healthiness is the origin of good actions, gaining of prosperity, gratification of needs and the ultimate emancipation. Illnesses abolish well-being and life of a human. It turned out to be a excessive hurdle in lively means of ones life. Artham, Dharmam, Kamam & ultimate Moksham remain conceivable first when Arogyam is existing in one's lifecycle. Arogyam signifies wellbeing and wellbeing represents the equilibrium of dhatu in the lack of illness. Therefore, Arogyam is the eventual motive for quality in attaining all these four objectives in life. Kaamala disease (hepatitis) is a rising universal issue. Ayurveda defines two focal clusters of illnesses viz. Sahaja and Jataya. A distinct reference of Kaamala (yellow coloration) is justified as it fit into this group. "hepatitis" is the chief indication of Kaamala (hepatitis) which is also experiential in Kaamala (hepatitis). In Ayurvedhic therapy and texts, well-known Ayurvedic specialists & surgical physicians A. Charak & A. Sushruta deceptively documented disorders similar to hepatitis, it is most comparable to hepatitis in contemporary medicine. The disease Kaamala is linked to the liver. The hepatic functions as important organ in nourishing metabolic homeostasis. The progress of being clinically significant hepatic infection are being escorted by means of numerous appearances of metabolic complaints [1]. Recently, there has been a focus on herbal research worldwide and a lot of evidence has accumulated showing the enormous prospective of medicinal and aromatic floras are used in several traditional systems. Approximately around 10,000 medicinal and aromatic floras have been investigated in the past four to five years. The reason for this review was to collect data from research activities of the last four to five years, ie. 1993-1998, using current scientific methodologies and pioneering scientific tackles [2].

The human liver plays a dynamic part in the metabolism & removing numerous exo and endogenous mixtures. As an outcome of constant contribution, it is vulnerable to toxic destruction produced by certain compounds, and conceivable hepatocellular injury interrupts the one’s body metabolism. Currently, there has been abundant interest in discovering natural therapies for hepatic diseases produced by toxins, that could be alcohol & the hepatitis virus [3].

Hepatitis is known to disturb the liver. Likewise, when rats are administered with Carbon tetrachloride, the liver is damaged nor injured. Herbal therapy is known to be worthy in the management of virus-related hepatitis and poisonous hepatitis were evaluated in a quantifiable model of Carbon tetrachloride hepatic injury [4]. Maximum of the population of our nation survives in unsanitary and unhygienic environments where the probability of hepatic contamination will be very high [5].

THE IMPORTANCE OF TRADITIONAL LIVER PROTECTIVE PRODUCT

1. The effectiveness of the drug or the formulation in viral hepatitis and hepatic damage caused by the chemicals which are hepatotoxic, is through oxidative or supplementary mechanisms necessity be verified.
2. Investigation to recognize the merits and the demerits of liver protective of multi-herbal

preparations could be evaluated.

3. Operative control of hepatitis with or without adverse effects with polyherbal preparation. The investigational Polyherbal formulation, which is a combination of ancient therapy, was chosen for this research. The core of this formulation is ajowan, cardamom, cinnamon, clove, coriander, cumin, ginger and nutmeg. This herbal formulation has a liver protective and purgative potential, hence it could possess to enhance the consequence in the treatment of Kaamala (hepatitis).

Ayurvedic physicians in Tamil Nadu have traditionally used the experimental drug Polyherbal formulation, although the drug has been shown to be effective in the treatment of hepatitis, the effectiveness also has to be demonstrated clinically. The drugs used in the polyherbal formulation was identified & included in this research is to evaluate its effectiveness in Kaamala (hepatitis). Although this blend has not been evidenced by direct reference in the conventional manuscripts, the specific crud drugs are recognized to relieve Kaamala (hepatitis) properties. Considering the above facts, the experimental polyherbal formulation was chosen for this study. The diagnosis of the Kaamala (hepatitis) were detected by the signs & indications defined in the classical and contemporary laboratory studies. In this research, we studied 30 Kaamala (hepatitis) patients by administering a polyherbal formulation for three days at a quantity of 3.3 g 3 times a day prior to meals along with red tender coconut water as vehicle. Outcomes were divided into 5 groups: 1. completely relief, 2. noticeable development, 3. reasonable development, 4. complete development, 5. no change.

MATERIALS AND METHODS:

The herbal components involved in polyherbal formulations like Ajowan, Cardamom, Cinnamon, Clove, Coriander, Cumin, Ginger and Nutmeg were procured from RK Tharagar agencies, CBE and subjected to physical, chemical, microbiological and microbiological studies.[4], Pesticide, Heavy Metal and Toxicological Evaluations in Pharmacognosy Department, Karpagam College of Pharmacy and confirmed to be harmless, unaffected and pure. These raw herbs have been indelicately ground, assorted, made boiling with red tender coconut water, finally filtered using filter. This study was permitted by the I E C of the I A D, Kerala and is finally recorded in the C T R I with number: 05/002701. Polyherbal preparation was tested for efficacy in 30 liver disease patients at Ayushkaram Ayurvedha Hospital, Sulur, Coimbatore. Patients were divided according to age and gender in Table No. 3. The polyherbal preparation was administered orally three times a day (3.3g each time) for 3 consecutive days. The Polyherbal Formulation were screened for 8 subjective parameters such as pyrexia, fatigue, yellow discoloration of sclera, urine, nail, skin, anorexia and nausea along with 7 objective comparative pathological parameters such as serum glutamic-oxaloacetic transaminase, serum glutamic pyruvic transaminase, bilirubin, serum alkaline phosphatase, cholesterol, albumin and total protein were monitored during treatment.

Serum glutamic-oxaloacetic transaminase, serum glutamic pyruvic transaminase, serum alkaline phosphatase levels in IU/l, total bilirubin, total cholesterol mg/dl and total proteins,

albumin in grams/dl. Materials and reagents used in clinical trials were collected from Span Diagnostic Ltd, Shivam Surgical Ahmedabad and the serum glutamic-oxaloacetic transaminase, serum glutamic pyruvic transaminase, bilirubin, serum alkaline phosphatase, cholesterol, albumin and total protein parameters had been estimated.

STATISTICAL ANALYSIS

Biochemical evaluation results serum glutamic-oxaloacetic transaminase, serum glutamic pyruvic transaminase, bilirubin, serum alkaline phosphatase, cholesterol, albumin and total protein were presented using Mean, Standard Error of Mean (SEM) and Standard Deviation (SD) and Median. The between-group difference for each parameter was analyzed separately to determine a significant P-value. P-value analysis of variance was performed using Graph Pad statistical software.

RESULTS AND DISCUSSION

Age: Maximum numbers of affected population had been obtained in the age of 25-27 yrs (40.00%) of affected population, in the age of 21-24 yrs (30.00%) of affected population, in the group of 28-30 yrs (23.33%) of affected population, in the age of 18-21 yrs (6.70%) of affected population, and minimum number of patients was obtained in the group of 18-20 yrs. i.e. 6.70% of affected population. This finding clearly shows that the age group of 25-27 yrs. is mostly affected shown in Table 2.

Sex: Male patients i.e. 80.00% exceeded the female patients who were 20.00%. This may be due to the demographic facts shown in Table 2.

Effect of PHF on main symptoms of 30 patients of Kaamala (Jaundice) after treatment: Polyherbal formulation provided significant relief ($p<0.025$) in Pyrexia (Fever) by 36%, In Fatigue, Yellow staining of Sclera relief was noticed at 32%, 33% respectively, which was significant with $p<0.025$, in Yellow staining of Urine it provided significant relief ($p<0.010$) by 27%, in Nausea (Vomiting sensation), Anorexia (Loss of appetite) ,Yellow staining of Nail, Skin it provided highly significant relief ($p<0.001$) by 51% each shown in Table 3.

Effect of PHF on main symptoms of 30 patients of Kaamala (Jaundice) after follow-up: The Polyherbal Formulation provided substantial reprieve in pyrexia by 36% ($P<0.015$), fatigue and yellow staining of sclera by 32 & 33% respectively ($P<0.025$), and yellow staining of urine by 27% ($P<0.010$). This also has reduced the yellow staining of nail and yellow discoloration of skin by 51% with a P value of 0.001, which is statistically significant shown in Table 3.

The mean standards of serum glutamic pyruvic transaminase for PHF were Preliminary 912.23 Pre-Therapy and 801.91, 612.32, 146.95, 27.41 on the 3rd day, 5th day, 14th day and 21st day Post Therapy respectively. The P value is < 0.0001, which is considered

as highly significant shown in Table 4 and Graph 1

The mean standards of serum glutamic-oxaloacetic transaminase for PHF were Preliminary 941.11 Pre-Therapy and 816.86, 697.08, 151.13, 32.79 on the 3rd day, 5th day, 14th day and 21st day Post Therapy respectively. The P value is < 0.0001, which is considered as highly significant shown in Table 4 and Graph 2.

The mean standards of serum alkaline phosphatase for PHF Preliminary 1236.26 Pre-Therapy and 1123.55, 696.69, 149.66, 63.65 on the 3rd day, 5th day, 14th day and 21st day Post Therapy respectively. The P value is < 0.0001, which is considered as highly significant shown in Tab 4 & Graph 3.

The mean standards of bilirubin for PHF Preliminary 5.51 Pre-Therapy and 4.56, 2.87, 1.87, 0.83 on the 3rd day, 5th day, 14th day and 21st day Post Therapy respectively. The P value is < 0.0001, which is considered as highly significant shown in Table 4 and Graph 4.

The mean standards of Cholesterol for PHF Preliminary 354.17 Pre-Therapy and 299.35, 257.15, 218.92, 172.12 on the 3rd day, 5th day, 14th day and 21st day Post Therapy respectively. The P value is < 0.0001, which is considered as highly significant shown in Tab 4 & Graph 5.

The mean standards of Protein for PHF Preliminary 3.96 Pre-Therapy, 4.74, 5.91, 6.33, and 6.91 on the 3rd day, 5th day, 14th day and 21st day Post Therapy respectively. The P value is < 0.0001, which is considered as highly significant shown in Table 4 and Graph 6.

The mean standards of Albumin for PHF Preliminary 2.16 Pre-Therapy, and 2.81, 3.40, 4.10, 4.66 on the 3rd day, 5th day, 14th day and 21st day Post Therapy respectively. The P value is < 0.0001, which is considered as highly significant shown in Table 4 and Graph 7.

Figure 1: Crude drugs used in Polyherbal formulation

Table 1: Crude drugs used in Polyherbal formulation

NAME OF THE DRUG	BOTANICAL NAME	FAMILY	PARTS USED	CHEMICAL CONSTITUENTS	TRADITIONAL USES
Ajowan	***Trachyspermum ammi***	Apiaceae	Fruit	Volatile oil	Relieve indigestion, bloating and gas [6].
Cardamom	***Elettaria cardamomum***	Zingiberaceae	Seed	Volatile oil	Control of asthma, teeth and gum infections [7].
Cinnamon	***Cinnamomum zeylanicum***	Lauraceae	Bark	Volatile oil	Used as to treat dental problems, Treats sore throats, indigestion and abdominal cramps [8].

Coriander	***Coriandrum sativum***	Umbelliferae	Fruit	Volatile oil	Used as a culinary spice and to prevent food poisoning [9].
Cumin	***Cuminum cyminum***	Umbelliferae	Fruit	Volatile oil	Used to treat hypolipidemia, cancer, and diabetes [10].
Clove	***Syzygium aromaticum***	Myrtaceae	Flower bud	Volatile oil	Cure nausea, vomiting, liver disorders [11].
Ginger	***Zingiber officinalis***	Zingiberaceae	Rhizomes	Volatile oil	Used to treat vomiting, pain, cold symptoms and food flavoring agent [12].
Nutmeg	***Myristica fragrans***	Myristicaceae	Seed	Volatile oil	Therapy in anxiety, nausea and rheumatism [13]

Table 2: Data of spreading of affected population with respect to Age & Sex

Polyherbal formulation				
Age group	**No. of Affected Population (30)**	**Gender**	**Affected Population / Gender**	**Percentage of Affected Population**
18 years to 21 years	**Two**	Male	Two	Hundred
		Female	Nil	Nil
22 years to 24 years	**Nine**	Male	Six	Sixty-Seven
		Female	Three	Thirty-Three
25 years to 27 years	**Twelve**	Male	Ten	Eighty-Three
		Female	Two	Seventeen
28 years to 30 years	**Seven**	Male	Six	Eighty-Six
		Female	One	Fourteen

Table 3: Details of Subjective Parameters observed for Polyherbal formulation

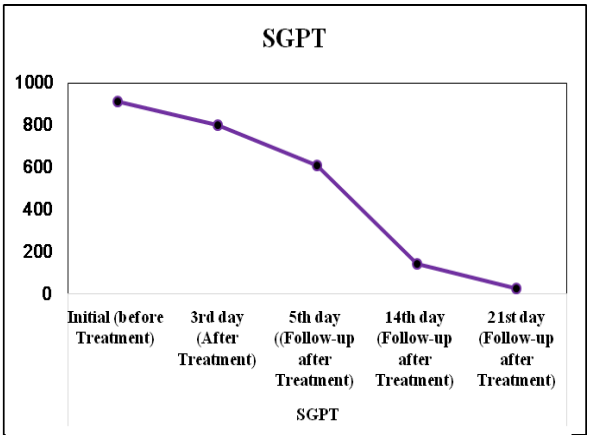
SIGNS & SYMPTOMS	GRADING					AT – BT (n)	Ist FU - BT (n^1)	2nd FU - BT (n^2)	3nd FU – BT (n^3)
	Before treatment 0th day (BT)	After treatment 3rd day (AT)	First following 05th day (AFU)	Second following 14st day (AFU1)	Third following 21st day (AFU1)				
1. Yellow discoloration of Skin	3	3	2	1	0	0	- 1	- 2	- 3
2.Yellow discoloration of Sclera	3	2	2	2	1	- 1	- 1	- 1	- 2
3.Yellow discoloration of Urine	3	2	1	0	0	- 1	- 2	- 3	- 3
4.Yellow discoloration of Nail	3	2	2	2	1	- 1	- 1	- 1	- 2
5.Anorexia (Loss of appetite)	2	2	1	1	0	0	- 1	- 1	- 2
6.Nausea (Vomiting sensation)	3	3	2	1	0	0	- 1	- 2	- 3
7.Pyrexia / Fever	3	2	2	2	1	- 1	- 1	- 1	- 2
8.Fatigue	2	2	1	1	0	0	- 1	- 1	- 2

Table 4: The responses of biochemical parameters of Polyherbal formulation

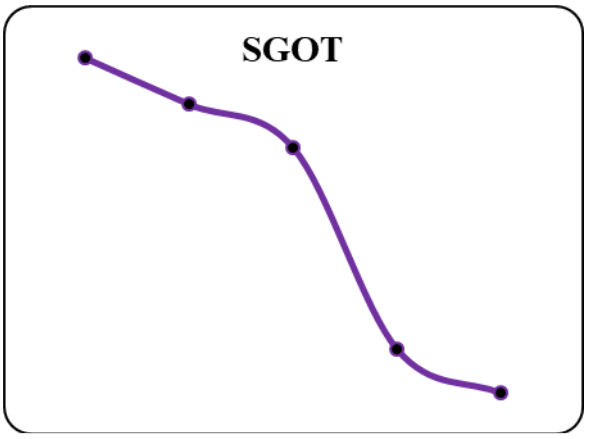
Biochemical Parameters	Duration of therapy	Mean ±SD	SEM	Median
SGPT	Preliminary (Pre-Therapy)	912.23±63.23	10.99	933.84
	3rd day (Post Therapy)	801.91±42.51	9.36	827.34*
	5th day ((Complement Post Therapy)	612.32±32.92	7.25	628.01**
	14th day (Complement Post Therapy)	146.95±15.05	4.75	145.28**
	21st day (Complement Post Therapy)	27.41±2.90	1.51	29.93**
SGOT	Preliminary (Pre-Therapy)	941.11±64.19	11.72	964.10
	3rd day (Post Therapy)	816.86±55.37	10.66	842.75*
	5th day ((Complement Post Therapy)	697.08±41.13	8.82	638.09**
	14th day (Complement Post Therapy)	151.13±31.95	5.83	146.08**
	21st day (Complement Post Therapy)	32.79±3.98	0.73	31.38**
Serum ALP	Preliminary (Pre-Therapy)	1236.26±130.55	23.84	1254.89
	3rd day (Post Therapy)	1123.55±115.31	21.43	1157.29*
	5th day ((Complement Post Therapy)	696.69±95.00	17.35	729.04**
	14th day (Complement Post Therapy)	149.66±36.89	5.64	144.51**
	21st day (Complement Post Therapy)	63.65±21.38	3.90	65.39**
Total Bilirubin	Preliminary (Pre-Therapy)	5.51±1.63	0.29	5.58
	3rd day (Post Therapy)	4.56±1.47	0.27	4.92*
	5th day ((Complement Post Therapy)	2.87±0.97	0.18	3.26**
	14th day (Complement Post Therapy)	1.87±0.53	0.09	1.93**
	21st day (Complement Post Therapy)	0.83±0.23	0.04	0.93**
Total Cholesterol	Preliminary (Pre-Therapy)	354.17±58.27	10.64	378.15
	3rd day (Post Therapy)	299.35±48.24	8.81	316.43*
	5th day ((Complement Post Therapy)	257.15±41.21	7.52	272.55**
	14th day (Complement Post Therapy)	218.92±27.96	5.11	224.42**
	21st day (Complement Post Therapy)	172.12±16.77	3.06	176.72**
Total Protein	Preliminary (Pre-Therapy)	3.96±0.81	0.15	3.77
	3rd day (Post Therapy)	4.74±0.73	0.13	4.55*
	5th day ((Complement Post Therapy)	5.91±0.69	0.12	5.69**
	14th day (Complement Post Therapy)	6.33±0.55	0.09	6.28**
	21st day (Complement Post Therapy)	6.91±0.38	0.07	6.98**
Albumin	Preliminary (Pre-Therapy)	2.16±0.60	0.11	2.12
	3rd day (Post Therapy)	2.81±0.55	0.10	2.84*
	5th day ((Complement Post Therapy)	3.40±0.46	0.08	3.29**
	14th day (Complement Post Therapy)	4.10±0.41	0.07	4.01**
	21st day (Complement Post Therapy)	4.66±0.46	0.08	4.66**

The Statistical values = (**SEM**) Standard Error of Mean, (**SD**) Standard Deviation

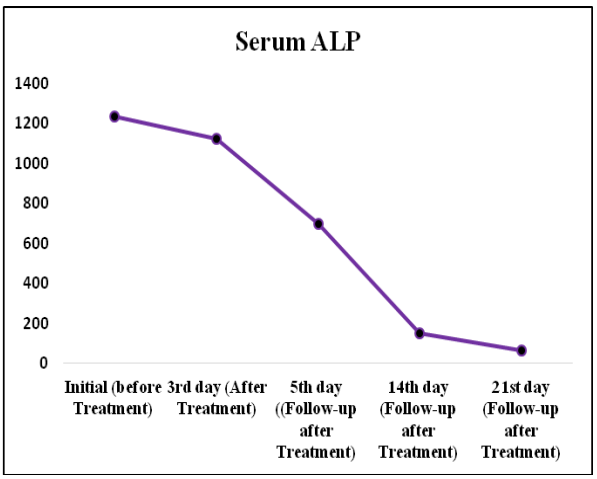
***p value** was < 0.01 initial Vs 3rd day; ****p value** was < 0.0001 initial Vs 5th day, 14th day and 21stday.



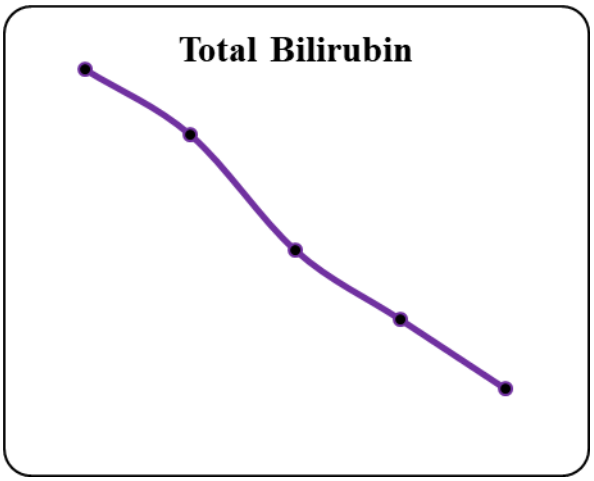
Graph 1: Biochemical parameter (SGPT)



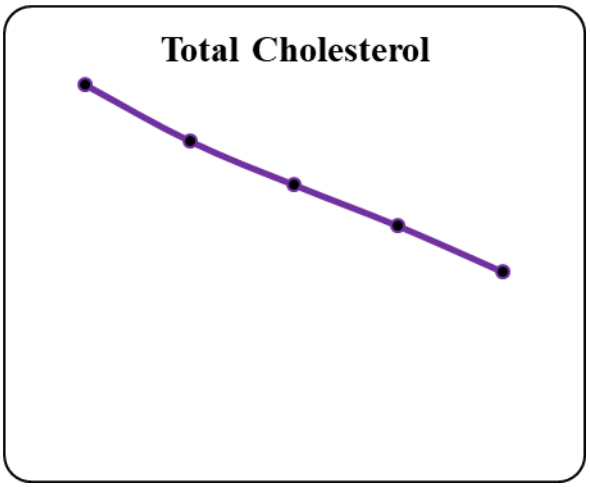
Graph 2: Biochemical parameter (SGOT)



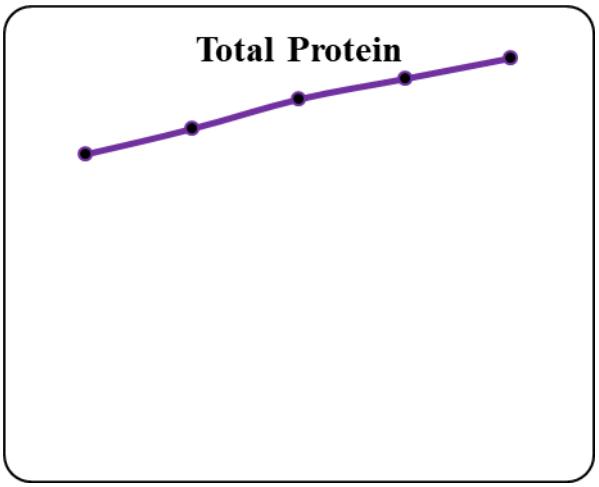
Graph 3: Biochemical parameter (Serum ALP)



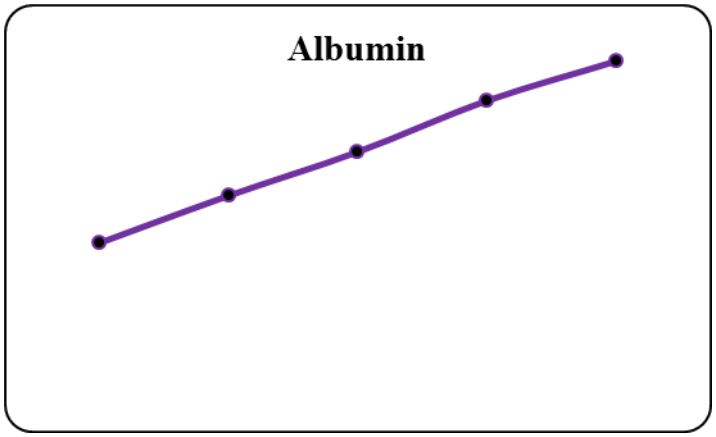
Graph 4: Biochemical parameter (Total Bilirubin)



Graph 5: Biochemical parameter (Total Cholesterol)



Graph 6: Biochemical parameter (Total Protein)



Graph 7: Biochemical parameter (Albumin)

CONCLUSION:

Polyherbal formulation provided **extremely substantial respite** ($p<0.001$) in all indications of kaamala (hepatitis) by 50.00%., 65.00%, 55.00%, 57.89%, 60.00%, 60.00%, 61.53% & 57.89% respectively for Yellow staining of skin, Yellow staining of Sclera, Yellow staining of Urine, Yellow staining of Nail, Anorexia (Loss of appetite), Nausea (Vomiting sensation), Pyrexia (Fever) & Fatigue.

Treatment with **polyherbal formulation significantly reduces** serum glutamic-oxaloacetic transaminase, serum glutamic pyruvic transaminase, bilirubin, serum alkaline phosphatase, and cholesterol and **significantly improves** albumin and total protein profile in **patients having hepatic injury.** Polyherbal formulation showed potent hepatoprotective **effects** and **immediate recovery** of serum glutamic-oxaloacetic transaminase, serum glutamic pyruvic transaminase, bilirubin, serum alkaline phosphatase, and cholesterol**.** Polyherbal formulation **can help treat** liver damage by **reducing** serum glutamic-oxaloacetic transaminase, serum glutamic pyruvic transaminase, bilirubin, serum alkaline phosphatase, and cholesterol and **increasing** albumin and total protein profile **to improve** the **lifestyle** of such patients.

REFERENCES:

1. Madhu Singh et al: Conceptual Study of Kamala (Jaundice), IAMJ:May–June 2013; 1(3).
2. Dahanakar S. R., Kulkarani R.A., Pharmacology of medicinal plants and natural products,Ind. J. Pharmacol, 2000; 32(4): S81 - S118.
3. Jitendra S. Patel, Hemangi J. Patel, K. N. Patel, A K Seth1, Keyur D. Patel : Comparative clinical studies of eclipta prostrata with marketed polyherbal formulation liver care churna for liver disease. An international journal of pharm. sciences, Vol-2, Issue-2.
4. Chandra T, Sadique J: A new recipe for liver injury. Ancient Sci Life 1987; 7(2): 99-103.

5. Yadav, An Etiological Study (Nidan) of Kamala with Special Reference to Jaundice, J Homeop Ayurv Med, S1 2014.
6. Mohammed M. Zarshenas, Mahmoodreza Moein, Soliman Mohammed Samani and Peyman Petramfar. An Overview on Ajwain (Trachyspermum ammi) Pharmacological Effects; Modern and Traditional. Journal of Natural Remedies. 2014 March; 14(1):98-105
7. Ashokkumar K, Murugan M, Dhanya MK, Warkentin TD. Botany, traditional uses, phytochemistry and biological activities of cardamom [Elettaria cardamomum (L.) Maton] – A critical review. J Ethnopharmacol. 2020 Jan; 246:112244
8. Pasupuleti Visweswara Rao and Siew Hua Gan. Cinnamon: A Multifaceted Medicinal Plant. Evidence Based Complementary Alternative Medicine. 2014 Apr.
9. Najila Gooda Sahib, Anwarul Hassan Gilani, Azizah Abdul Hamid, Nazamid Saari and Khalid M Alkharfy. Coriander (Coriandrum sativum L.): A Potential Source of High-Value Components for Functional Foods and Nutraceuticals- A Review. Phytotherapy Research. 2013 Oct; 27(10): 1439-56.
10. Sami Mnif, Sai Aifa. Cumin (Cuminum cyminum L.) from traditional uses to potential biomedical applications. Chem Biodivers. 2015 May; 12(5):733-42.
11. Gaber El-Saber Batiha , Luay M. Alkazmi , Lamiaa G. Wasef , Amany Magdy Beshbishy, Eman H. Nadwa and Eman K. Rashwan. Syzygium aromaticum L. (Myrtaceae): Traditional Uses, Bioactive Chemical Constituents, Pharmacological and Toxicological Activities. Biomolecules. 2020 January; 10: 202.
12. Shahrajabian, M. H., Sun, W., & Cheng, Q. (2019). Pharmacological Uses and Health Benefits of Ginger (Zingiber officinale) in Traditional Asian and Ancient Chinese Medicine, and Modern Practice. Notulae Scientia Biologicae. 2019; 11(3): 309-319.
13. Kaliyaperumal Ashokkumar, Jesus Simal-Gandara, Muthusamy Murugan, Mannananil Krishnankutty Dhanya, Arjun Pandian. Nutmeg (Myristica fragrans Houtt.) essential oil: A review on its composition, biological, and pharmacological activities. Phytotherapy Research. 2022; 36: 2839–2851.