**Evaluation of Efficacy of Phalatrikadi Ghan Vati in Patients of Non-Alcoholic Fatty Liver Disease through Reverse Pharmacology Approach – Study Protocol**

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Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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**ABSTRACT**

**Background**: Non-alcoholic fatty liver disease (NAFLD), mostly diagnosed incidentally, is a rapidly emerging liver disorder. In absence of any specific treatment, current management focuses on the use of hepatoprotective agents in addition to lifestyle modification and prevention of metabolic
syndrome. Several Ayurveda agents have shown promising effects in patients over centuries of use. But this evidence needs to be assessed scientifically through reverse pharmacology approach. A polyingredient Ayurveda drug, Phalatrikadighanvati (PGV) has been selected for this study because of its long history of use and that its individual contents have shown positive results in liver disorders.

**Objective:** Evaluation of efficacy of Phalatrikadighanvati in patients of non alcoholic fatty liver disease (NAFLD) along with its pharmaceutical and analytical study.

**Materials and Methods:** The drug shall be pharmaceutically processed and analyzed as per pharmacopoeial standards. Present study has been designed as a randomized placebo controlled double blind clinical trial in two stages. The first stage shall be a pilot study to decide the best effective and safe dose in patients of NAFLD. The pilot study shall include two groups of 10 patients each in a dose of PGV 500mg and 1gm respectively twice a day for 12 weeks. After theselection of thebest dose, RCT will be conducted on that dose in the second stage. It shall be a Phase 2 trial with 60 patients divided equally in two groups. The patients in group one shall be given a dose as per the outcome of the pilot study twice a day and another group shall be administered placebo for a period of 12 weeks.

**Results:** Efficacy of Phalatrikadi ghan vati will evaluated in terms of subjective and objective parameters using paired and unpaired t-test.

**Conclusion:** PGV is expected to improve the diagnostic parameters in patients of NAFLD thus proving to be efficacious in managing NAFLD and act as a potent hepatoprotective agent.

**Keywords:** Ayurveda; non-alcoholic fatty liver disease; phalatrikadighanvati; reverse pharmacology.

1. INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a rapidly emerging disease with a prevalence of 6-35% worldwide [1]. Recent studies across different parts of India have stated the prevalence of NAFLD to vary from 9 to 35% [2]. It is a condition where excess fat gets accumulates in the liver cells in individuals having no significant history of alcohol consumption [3]. The spectrum of NAFLD ranges from mere steatosis without cirrhosis to non-alcoholic steatohepatitis (NASH) which may or may not be accompanied by cirrhosis. NASH is a progressive entity affecting about 5-7 percent of the general population and 30-40 percent of patients with raised liver enzymes. NAFLD is fast emerging as a main non-viral etiological cause of Hepatocellular carcinoma (HCC) [4].

NAFLD is considered as the hepatic expression of the metabolic syndrome that is most typically linked to obesity [5]. Moreover, the Indian diet comprising of high fat & carbohydrates accompanied with a sedentary lifestyle facilitates the pathology leading to metabolic syndrome and its expressions like NAFLD. Currently, there is no specific treatment for NAFLD. All the clinical protocols are based on providing hepatoprotective drugs to improve liver function apart from managing the concurrent symptoms of metabolic syndrome [6]. The liver has a unique capacity to compensate and perform its functions despite stress due to any physical, metabolic or dietary cause. It is only because of this reason that there may be minimal or even absent signs and symptoms of liver disease. As such NAFLD is most of the time detected incidentally during a routine health check-up or while investigating other symptoms.

Recent literature suggests the use of herbal or plant-based medicines in successfully managing NAFLD and improving liver function [7-8]. Ayurvedic medicine includes plants and minerals used either single or in polyingredient formulations. A fact that Ayurveda medicines are already in use for centuries, demands a different way of approach towards their scientific validation. Despite their use spanning over centuries, scientific evidence regarding their safety and efficacy needs to be documented, which cannot be done via conventional methodology. A novel process, termed Reverse Pharmacology (RP) can be widely employed for validation of therapeutic actions of Ayurveda drugs, which are already in use traditionally. It follows a bedside to bench side approach. The traditional treatment which is been used for centuries to manage patients is evaluated in a clinical setting, leading to its phytoanalysis in the laboratory. Starting with knowledge/data gained through experience, exploratory research, and applicable clinical/experimental studies, RP progresses to the isolation of active components (Fig. 1). Taking a cue from this process of RP,
the drug, PhalatrikadiKwath in the form of its Phalatrikadi Ghanvati (PGV, solidified aqueous extract), which is advised for management of liver disorders in the classical medical text of Chakradatta [9] has been chosen for the proposed study.

Only the first three stages of RP shall be conducted in this study, leaving the stage of isolation of active ingredients out of the scope. Stages of the Reverse Pharmacology approach have been divided in the following manner:

1. Selection of Herbal Remedy
2. Dose-escalating clinical trial – Pilot Study
3. Randomized Clinical Trial

1.1 Selection of Herbal Remedy (Stage-1)

The proposed drug under this study is currently being used by Ayurveda physicians for the management of yakrit rog like acute viral hepatitis. Some studies have also been published for its effective role in acute viral hepatitis. A case study on two patients has also been published for its effectiveness on patients of non-alcoholic fatty liver disease [10]. PhalatrikadiKwath is advised as a choice of drug in liver disorders of varied etiology including NAFLD. It is a combination of eight herbs (Table 1) that have individually been studied for their effect on the liver [6]. The individual drugs in this combination have shown a hepatoprotective effect in NAFLD [11-13]. Guduchi has experimentally shown to be therapeutically effective in the amelioration of obesity and associated hepatic dysfunction, protection of hepatic function and help to prevent fibrosis and stimulates regeneration of hepatic tissue along with protection from Hepatitis B & E surface antigen, which makes it a potential candidate for use in NAFLD [14-15]. Kalmegha has certain bioactive phytonutrients having an antioxidant and anti-inflammatory activity which ameliorate rich fat diet-induced steatohepatitis and liver injury [16-17].

PhalatrikadiKwatha is a combination of dried herbs, pulverized to make a coarse powder. This coarse powder is then given to a patient for preparation of Kashaya (decoction) at home. The preparation of Kashaya (decoction) requires following a certain set of principles like fixing the ratio of raw herbs to the amount of water, duration of heating, the quantum of heat to be used for heating, and most importantly the dose of a drug. Most of the time the patient is unaware of these guidelines. Moreover, in the era of globalization and fast-moving life, it does not seem feasible to sacredly prepare decoction every time. Modifying the dosage form based on ancient Ayurveda pharmaceutical principles for increasing patient compliance and making it easier to administer is the need of the hour. Thus, dried aqueous extract of PhalatrikadiKwatha has been used as a lead drug in the form of pills, that is, Phalatrikadi Ghanvati (PGV) for studying its effectiveness in non-alcoholic fatty liver disease.

2. AIM AND OBJECTIVES OF STUDY

Aim: Evaluation of efficacy of Phalatrikadicghanvati in patients of non alcoholic fatty liver disease (NAFLD) along with its pharmaceutical and analytical study.

| No | Contents | Botanical Name | Part used | Ratio |
|----|----------|----------------|-----------|-------|
| 1. | Amalaki  | EmblicaofficinalisGaertn. | Fruit | 1 part |
| 2. | Haritaki | Terminalia chebulaRetz. | Fruit | 1 part |
| 3. | Bibhitaki | Terminalia bellericaRoxb. | Fruit | 1 part |
| 4. | Vasa     | AdhatodavasicaNees. | Leaf     | 1 part |
| 5. | Guduci   | TinosporacordifoliaMiers | Stem | 1 part |
| 6. | Nimba    | AzadirachtaindicA. Juss. | Bark | 1 part |
| 7. | Kutaki   | PicrorrhizakurroaRoyale ex Benth. | Root | 1 part |
| 8. | Kalmegha | AndrographispuniculataNees. | Whole plant | 1 part |
| 9. | Water    |                |           | 64 parts |
Fig. 1. Stages of reverse pharmacology

2.1 Objectives

2.1.1 Primary objectives

1. Evaluation of efficacy of *Phalatrikadi-ghanvati* hepatic biochemical parameters in patients of non-alcoholic fatty liver disease (NAFLD).

2.1.2 Secondary objectives

1. To prepare *Phalatrikadi Ghanvati* described traditionally by preparing its water extract (PGV).
2. To assess the prepared formulation *Phalatrikadi GhanVati* (PGV) for its quality control parameters.

3. MATERIALS AND METHODS

3.1 Pharmaceutical Study

Three different batches of PGV shall be prepared to establish pharmaceutical standardization.

Pharmaceutical study will be done in following steps;

**Procurement of Raw materials:** All required raw materials will be procured from field and authentic reliable source.

**Authentication of Raw materials:** Raw drugs will be verified and authenticated by Department of Dravyaguna of MGAC & RC, Salod, Wardha.

Raw drug will be standardized as per A.P.I. specifications.

**Preparation of Phalatrikadi Ghanvati (PGV):** The contents of PGV shall be pharmacologically processed to prepare pills of the drug. (Fig. 2).

**Analytical study:** [18]: Analytical study of finished products, *Phalatrikadi Ghanvati* shall be conducted as per pharmacopoeial parameters.

**Organoleptic Characteristics:** Appearance, taste & colour.

**Physico-chemical parameters:** Loss on drying at 105°C, Total ash, Water soluble extractive, Alcohol Soluble extractive, Acid insoluble ash, Disintegration Time, Hardness, Identification TLC/HPTLC, Test for heavy/toxic metals – Lead/Mercury/Arsenic, Microbial Contamination.

4. METHODOLOGY

**Study design:** Randomized Placebo Controlled Double Blind Clinical Trial (Stage 2 & 3 of Reverse Pharmacology). The randomization will be done based on computerized generated table. A computer-generated random allocation software method shall be used to avoid bias in the study. The allocation sequences will be generated in advance, which shall then be sealed in consecutively numbered opaque envelopes. The packing of both the interventions will be kept very identical thereby both investigator and supervisor could not know about
intervention. The allocation sequence will be generated by 3rd person in the department by coding envelope method. Double-Blind means Researcher and Participants both will be blinded. The CONSORT 2010 guidelines shall be followed during the study.

**Study site:** Department of Rasa Shastra & Bhaishajyakalpana MGACHRC, Salod, (H) Wardha.

**Dose- escalating clinical trial (Stage 2 Reverse Pharmacology):** A pilot study shall be done to decide the best effective and safe dose in patients of NAFLD (Fig. 3). The pilot study shall include two groups of 10 patients of NAFLD each. The patients in group one shall be given a dose of PGV 500mg twice a day and another group shall be administered a dose of 1gm of PGV twice a day. The duration of the pilot study shall be 12 weeks. The participants in the pilot study shall be evaluated on the same parameters as the RCT. The results of the pilot study shall be evaluated statistically to determine the best effective dose. Any patient-reported side effects of the drug shall also be noted. After selection of the best effective dose, randomized placebo control double-blind clinical study will be conducted on that dose.
4.1 Randomized Clinical Trial (Stage 3 - Reverse pharmacology)

A randomised placebo-controlled double-blind clinical trial with the best effective dose of PGV shall be conducted in this stage. It shall be a Phase-2 trial with 60 patients divided equally in two groups. The patients in group one shall be given a dose as per the outcome of the Pilot study twice a day and the other group shall be administered Arogyavardhini vati (standard Ayurveda treatment) for a period of 12 weeks.

Screening: Patients presenting themselves with anorexia, nausea, vomiting, fatigue, pain in abdomen, and having abnormal liver function tests as an incidental finding shall be screened for inclusion in the study.

4.2 Participant’s Inclusion Criteria

- Subjects of either sex, age group 30-60 years, non-alcoholics, or history of alcohol intake less than 20g/day (history of alcohol intake was taken separately from the patients and close relatives).
- Clinical signs and symptoms suggesting NAFLD/YakṛṭRoga, that is, pain in the right upper quadrant/epigastric region of the abdomen, feeling of nausea, and vomiting, loss of appetite, burning sensation in the abdomen.
- Incidental finding during investigations for some other disease
- Ultrasonography (USG) abdomen suggestive of NAFLD
- Biochemical: Liver function tests showing raised alanine transaminases (ALT) or aspartate transaminases (AST) levels raised above the normal limits (40IU/L) up to 300 IU/L and with/without raised lipid profile and fasting/random blood glucose levels within normal limits.

4.3 Participant’s Exclusion Criteria

- Patients unwilling to participate in the study
- Patients with a history of alcohol intake exceeding 20 g/day (Alcohol consumption history shall be separately obtained from the patients and family)
- Patients testing positive for markers of other viral hepatitis

Criteria for discontinuing or modifying allocated interventions: Patients will be withdrawn from intervention if any harmful incidence, signs of drug allergy, or any problem will occur; the patient will be offered treatment free of cost till the disease subsides.

4.4 Assessment Criteria

Subjective criteria: After selection, each participant will be evaluated individually for the following sign and symptoms [parameters]: Udaraśūla (pain in the abdomen), utkleśa (feeling of nausea and vomiting), agnimandya (impaired digestion), klama (Fatigue), aruci (loss of appetite), sadana (malaise). These Ayurvedic parameters will be assessed by the gradation scale.

4.5 Objective Criteria

Anthropometric measurement: Weight, height ratio (body mass index [BMI]), Blood Pressure.

Haematological: Hb%, TLC, DLC, ESR (as an initial screening tool and to monitor any other effects of drug on haematological parameters)

Biochemical Tests: Direct bilirubin, Indirect bilirubin, Total bilirubin, ALT, AST, Alkaline phosphatase, AST/ALT Ratio, Serum Cholesterol, Triglycerides, LDL, HDL, VLDL, FBS/RBS

Radiological: USG abdomen.

Follow-up: Each participant will receive the respective treatment from day one for 12 weeks (84 days). A dose of 14 days will be given to patients initially. In-person follow up will be taken fortnightly to ensure patient compliance for taking medication. After completion of the treatment, each participant will be assessed on subjective and objective parameters. Individuals, who will not turn up for follow-up, will be dropped out from the clinical study. All investigations will be done before starting and end of the treatment.

5. OBSERVATION AND RESULTS

Observations will be noted and presented in the form of tables, charts, graphs and the data will be analysed with the application of suitable inferential statistics. Post-test assessment will be tabulated as under corresponding to the grades above, noted prior to treatment:

A. Full mitigation (Maximum Improvement in subjective & objective criteria): 75 – 100% relief
B. Moderate improvement: 50-74% relief
C. Mild improvement: 25 – 49% relief
D. Unsatisfactory: < 25% improvement from the pre-test condition

5.1 Methods of Statistical Analysis

The stage-3 of RP is a phase-2 clinical trial, hence a sample size of 30 in each group is considered for the study. Statistical analysis will be done by applying paired and unpaired t-test. The tool used for the statistical tests will be SPSS. Hypothesis testing will be done using the corresponding tests at a significance level of p=0.05 to validate the statistical significance of the sample population.

6. DISCUSSION

The treatment components as mentioned in the texts of Ayurveda are not fulfilled unless the Dravya(substance) is converted into a palatable and effective dosage form known as Bheshaj (pharmacologically active agent). In Charaka Samhita, it is mentioned that the Matrayukta Aushadha (optimum dosage) has Laghupakam (easily metabolised), Sukhaswadam (palatable), Vyadhinashana (therapeutic efficacy) properties. In Ayurveda, various drugs and preparations are mentioned to keep the body healthy and disease-free. Formulations described in Ayurveda treatise are of different varieties, innovative and compounded to increase the potentiality of the therapeutics [19]. A modified dosage form of Phalatrikadi Kwatha has been proposed for the current study. Processing of Phalatrikadi Kwatha as an aqueous extract in to Ghanavati (pills), shall be done pharmaceutically with regards to dosage modification. Since Ghanvati is processed as a water extract, it contains a high concentration of water-soluble extracts in comparison to the decoction form of the same drug, hence proposed for this study. This drug dosage modification has been strategically devised to make the drug easy to administer, palatable for the patient and to maintain uniformity in the dose of drug [20-21].

The individual drugs in PGV have shown hepatoprotective effect in NAFLD. Experimental studies have also shown, Guduchi to be therapeutically effective in the amelioration of obesity and associated hepatic dysfunction. It is also known to stimulate the regeneration of hepatic tissue [14-15]. Kalmegha has certain bioactive phytonutrients having antioxidant and anti-inflammatory activity which ameliorate rich fat diet-induced steatohepatitis and liver injury [16-17]. The combined actions of ingredients of PGV can help improve the hepatobiliary function, protect the loss of functional integrity of the hepatic cell membrane, protec hepatic parenchyma against toxins, promotes hepatocyte regeneration [22]. These actions of the drugs can control the progress of the disease and also cause a reversal in early stages of NAFLD.

7. CONCLUSION

Considering the prevalence and global distribution of NAFLD and its inconspicuous tendency to progress into cirrhosis and further hepatocellular carcinoma, if this study showed potential hepatoprotective action, this drug modification will surely improve patient compliance.PGV is expected to be efficacious in ameliorating the signs and symptoms of NAFLD and act as a potent hepatoprotective agent.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

The volunteers will be informed about the study protocol. Willing participants shall be randomly selected for different groups. Clinical research format will be prepared and validated. Informed written consent of each participant will be obtained prior to study.

ETHICAL APPROVAL AND TRIAL REGISTRATION

Ethics approval vide no MGACHRC/IEC/july-2021/321 dated 31.07.2021 has been taken from Institutional Ethics Committee of the study centre. The trial shall be registered in CTRI prospectively.

NOTE

The study highlights the efficacy of “Ayurveda” which is an ancient tradition, used in some parts...
of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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