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Original article

# Design and evaluation of herbal hepatoprotective formulation against paracetamol induced liver toxicity

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ABSTRACT

*Aim:* To isolate and identify the quercetin from polyherbal hepatoprotective formulation. Polyherbal formulations were developed by using five bioactive fractionated extracts of *Butea monosperma*, *Bauhinia variegata* and *Ocimum gratissimum* for treatment of liver disorders by exploiting the knowledge of traditional system of medicine and evaluated for hepatoprotective activity using acute liver toxicity model of paracetamol induced liver damage in rats.

*Methods:* Major active fractions were isolated by solvent fractionation and quantified by HPTLC method. Two polyherbal tablet formulations were developed by the wet granulation method using microcrystalline cellulose, aerosil and other excipients and subjected for physicochemical evaluation to assess physical stability followed by pharmacological screening. The prepared tablets were finally subjected to stability testing to assess its shelf-life. The rats were monitored for change in liver morphology, biochemical parameters like serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP) and total bilirubin for polyherbal tablet formulation at 50 mg/kg and polyherbal tablet formulation at 100 mg/kg.

*Results:* Active principle was isolated, quantified by HPTLC and characterized with IR. Both formulations showed significant hepatoprotective activity. The histological studies were also support the biochemical parameters. From the results of biochemical analysis and histopathological studies, it can be accomplished that polyherbal tablet formulation at 100 mg/kg can be effectively formulated into a suitable dosage form with added benefit of no side effects for control and cure of chronic ailments like liver disorders. A comparative histopathological study of liver exhibited almost normal architecture as compared to toxicant group.

*Conclusion:* Biochemical marker showed improved results for polyherbal tablet formulation at 100 mg/kg. Polyherbal tablet formulation contains a potent hepatoprotective agent suggested to be a flavone concentrated in polyherbal formulation which may find clinical application in amelioration of paracetamol induced liver damage.



## 1. Introduction

In the last few decades there has been an exponential growth in the field of herbal medicine. It is getting popularized in developing and developed countries owing to its natural origin and lesser side effects.[1] Herbal remedies provide rational means for the treatment of many internal diseases which are considered to be stubborn and incurable in other system of medicines. It lays a great deal of emphasis upon the maintenance of positive health of an individual. It thus aims at both the prevention and cure of diseases.[2] An ancient system of traditional medicines like Siddha, Ayurveda, Chinese and Japanese have been adopted for the diagnosis, prevention and treatment of liver disorders. This attempts to prove scientific insight behind the traditional adaption. Less toxicity, better therapeutic effect, good patient compliance and cost effectiveness are the reasons for choosing drug from natural origin.[3]

Ayurvedic and herbal medicinal products contain a combination of number of chemical compounds that may give the anticipated activity in combination.[4]

The development of a stable polyherbal formulation is a challenging task because of the large number of varied chemical

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compounds present in the different medicinal plants. Hence, the entire herbal drug or herbal drug preparation is regarded as active drug substance, regardless of whether constituents with defined therapeutic activity are known and unfortunately, the quality of a majority of them remains uncontrolled. All these issues have been acknowledged in the draft of the Strategic Plan for Regional Traditional Medicine of the World Health Organization.[5]

A great deal of research has been carried out to evaluate scientific basis for the claimed hepatoprotective activity of herbal agents as in the form of polyherbal formulation. *Butea monosperma* (Fabaceae) is commonly known as *palas* and is beneficial in conditions of liver disease and inflammation.[6] The plant mainly contains flavones (quercetin),[7] kino-tannic acid, and gallic acid.[8]

*Bauhinia variegata* (Leguminosae) commonly known as Kachnar, is found to be beneficial in Ayurveda as tonic to the liver and anti-inflammatory, healing activity, antioxidant activity.[9] *B. variegata* has been reported to contain quercetin, rutin, apigenin and apigenin 7-O-glucoside. Flavonoids and quercetin in particular are potent antioxidants and are known to modulate the activities of various enzyme systems due to their interaction with various biomolecules.[10]

*Ocimum gratissimum* Linn. (Lamiaceae) is known traditionally for its effect in liver diseases, inflammation and antibacterial activity.[11] The active constituents, flavonoids, quercetin 3-O-glucoside, rutin, kaempferol 3-O-rutinoside and vicenin-2 were identified.[12]

Quercetin (flavonoid) and ursolic acid (triterpenic acid), well known for its hepatoprotective effects in both acute chemically induced liver injury and chronic liver fibrosis and cirrhosis.[13]

Ursolic acid was previously determined in dichloromethane and ethyl acetate fractions of methanolic extract of *O. gratissimum* and in developed herbal hepatoprotective tablet in my previous article.[8]

The present study was designed to develop polyherbal formulation taking advantage of ayurvedic principles, where herbal ingredients present in plants are known to have specific activity in modulation of liver disease condition so the present study was undertaken to evaluate the hepatoprotective effect of developed polyherbal formulation in which acute hepatotoxicity was induced by paracetamol treatment.

## 2. Material and methods

### 2.1. Material

The fresh bark of *B. monosperma*, *B. variegata* and fresh leaves of *O. gratissimum* were collected from Maliba Pharmacy College Campus. Voucher specimen (No: MPC/13032010/01, 02 and 03) has been deposited in the Department of Bioscience, Veer Narmad South Gujarat University, Surat, India. Rats were used for hepatoprotective study, with prior approval from the Institutional Animal Ethical Committee (Registration No. 717/02/a/CPCSEA/30 Jan 2010) of Maliba Pharmacy College, Uka Tarsadia University.

### 2.2. Extraction

All the three crude drugs were extracted with alcohol and then alcoholic extract of each plant was subjected to solvent fractionation.

***B. monosperma***: Ethanolic extract obtained was fractionated with petroleum ether, benzene, chloroform and acetone (AcO) in the order of increasing polarity to obtain respective fractions.[14]

***B. variegata***: Methanolic extract was fractionated with hexane, ethyl acetate (EtOAc) and n-butanol (n-ButOH) in the order of their increasing polarity to obtain respective fractions.[15]

***O. gratissimum***: Methanolic extract of *O. gratissimum* was fractionated with hexane, dichloromethane(DCM), ethyl acetate (EtOAc) and methanol in the order of their increasing polarity to obtain respective fractions.[16]

### 2.3. Establishment of qualitative phytoprofile of fractionated extracts

#### 2.3.1. Qualitative phytochemical analysis

Each fraction was subjected to various qualitative chemical tests using reported methods to determine the presence or absence of metabolites viz., alkaloids, tannins, flavonoid, steroid, terpenoids and phenolic compounds etc.[17]

##### 2.3.1.1. Chemical test for flavonoids in each fraction

i. Dilute ammonia solution 5 mL was added to a portion of each extract fraction followed by addition of concentrated $H_2SO_4$ a yellow coloration observed indicated the presence of flavonoids.

ii. The yellow coloration disappeared on standing. Few drops of 1% aluminum solution were added to a portion of each fraction. A yellow coloration was observed indicating the presence of flavonoid.[18]

### 2.4. Qualitative phytochemical analysis

#### 2.4.1. Determination of total phenols

Acetone fraction of *B. monosperma*, ethyl acetate and n-butanol fractions of *B. variegata* and dichloromethane and ethyl acetate fractions of *O. gratissimum* (100 μL) of each fraction were mixed with 1 mL Folin–Ciocalteu reagent and 0.8 mL of 7.5% $Na_2CO_3$. The resultant mixture of each fraction was measured at 765 nm after 2 h at room temperature. The mean of three readings was used and the total phenolic content was expressed in milligram of gallic acid equivalents/1 g extract. The coefficient of determination was found to be $r^2 = 0.992$.[19]

#### 2.4.2. Determination of total flavonoids

Standard quercetin was used to make the calibration curve [0.04, 0.02, 0.0025 and 0.00125 mg/mL in 80% ethanol (v/v)]. The standard solutions and acetone fraction of *B. monosperma*, ethyl acetate and n-butanol fractions of *B. variegata* and dichloromethane and ethyl acetate fractions of *O. gratissimum* (0.5 mL) of each fraction was mixed with 1.5 mL of 95% ethanol (v/v), 0.1 mL of 10% aluminum chloride (w/v), 0.1 mL of 1 mol/L sodium acetate and 2.8 mL water. The volume of 10% aluminum chloride was substituted by the same volume of distilled water in blank. After incubation at room temperature for 30 min, the absorbance of the reaction mixture of each fraction and standard solution was measured at 415 nm. The mean of three readings was used and the total flavonoid content was expressed in milligram of quercetin equivalents/1 g extract. The coefficient of determination was $r^2 = 0.990$.[20]

### 2.5. Preparation of formulations

Polyherbal tablet formulations (PTF) contain the crude raw materials of *B. monosperma*, *B. variegata* and *O. gratissimum* prepared by wet granulation method using suitable excipients like microcrystalline cellulose, starch, crospovidone, aerosil and magnesium stearate.[21] The composition of tablet formulations is shown in Table 1.