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Original Article

Screening of flavonoids rich fractions of three Indian medicinal plants used for the management of liver diseases

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ABSTRACT

The decoctions of the *Butea monosperma* (Lam.) Taub., Fabaceae, *Bauhinia variegata* L., Fabaceae, and *Ocimum gratissimum* L., Lamiaceae, are traditionally used for the treatment of various types of hepatic disorder. Phytochemical studies have shown that total flavonoids from these plants were the major constituents of the picked out part of each plant. The present study was planned to investigate the hepatoprotective effect of flavonoid rich fractions of the *B. monosperma*, *B. variegata* and *O. gratissimum* against paracetamol induced liver damage. Flavonoid rich fractions were isolated by solvent fractionation from each plant. Each fraction was subjected to various qualitative chemical tests to findout the metabolites. Flavonoid fractions of each plant were subjected for pharmacological screening. The rats were monitored for change in liver morphology, biochemical parameters like serum glutamate pyruvate transaminase, serum glutamate oxaloacetate transaminase, alkaline phosphatase and total bilirubin for the groups receiving the flavonoid-rich fractions. All flavonoid rich fractions showed significant hepatoprotective activity. The histological studies supported the biochemical parameters. From the results of biochemical analysis and histopathological studies, it can be accomplished that in the ethyl acetate fraction of *O. gratissimum* showed highest hepatoprotective activity as compared to other fractions. The present study was the first evidence of flavonoid-rich fractions of each plant have a remarkable hepatoprotective effect. All fractions contain a potent hepatoprotective agent suggested to be a flavone, which may find clinical application in amelioration of paracetamol-induced liver damage.

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Introduction

Liver diseases remain one of the serious health problems (Baranisrinivasan et al., 2009). Modern medications have little role to alleviation of hepatic disease and the plant-based preparations which are chiefly available medicines employed for the treatment of liver disorders (Raju et al., 2008). The strength of these plant products must be established, thus as to identify newer medicaments acting against hepatic injury. In the absence of a reliable liver protective drug in the modern system of medication, a number of medicinal plants in Ayurveda are recommended for the treatment of liver disorders. Natural treatments from medicinal plants are thought to be efficacious and safe medicaments for hepatotoxicity.

Butea monosperma (Lam.) Taub. (BM) belongs to family Fabaceae is a medium size deciduous tree, found throughout India and traditionally used for the treatment of hepatopathy, ulcers, tumors, and diabetes (Kirtikar et al., 1999). The plant mainly contains flavones (quercetin) (Nadkarni, 1994; Gupta et al., 2013a), kinotannic acid and gallic acid.

Bauhinia variegata L. (BV) belongs to the family Fabaceae commonly known as Kachnar, is found to be beneficial in Ayurveda as a tonic to the liver and anti-inflammatory, healing activity, antioxidant activity (Bodakhe and Ram, 2007). It has been reported to contain quercetin, rutin, apigenin and apigenin 7-*O*-glucoside. Flavonoids and quercetin in particular are strong antioxidants and are known to regulate the activities of various enzyme systems due to their interaction with various biomolecules (Maldonado et al., 2003).

Ocimum gratissimum L. (OG) belongs to family Lamiaceae is an erect, multi-branched perennial shrub that grows up to a height of two meters with a tap root and many adventitious rootlets (Ramachandran et al., 1986). Essential oils obtained from *Ocimum* species showed various medicinal potentials in chemo-preventive, anti-carcinogenic, free radical scavenging, and radio-protective

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uses (Gupta et al., 2002; Onajobi, 1986; Prakash and Gupta, 2000). Additionally, OG leaf also revealed significant chemo-preventive effects on chemical-induced papilloma genesis by modulating metabolizing enzymes such as cytochrome P450, glutathione-*S*-transferase, and aryl hydrocarbon hydroxylase (Karthikeyan et al., 1999; Prashar et al., 1994). A recent study indicated that administered orally aqueous extract of OG leaf could oxidative and toxicant activity and enhance specific activities of hepatic antioxidant enzymes in rats (Ighodaro and Ebuehi, 2008). Notably, recent study also showed that the OG leaf aqueous extract (OGAE) may be important in protecting H9c2 cells from H_2O_2-induced cell death by inhibiting the mitochondrial dependent apoptosis pathway (Lee et al., 2010). Epicatechin, caffeic acid, rutin, gallic acid, quercetin, epigallocatechin gallate were identified as major components of phenolic acids and flavonoids in OGAE (Chiu et al., 2012; Grayer et al., 2000). Ursolic acid was determined in dichloromethane and ethyl acetate fractions of methanolic extract of *O. gratissimum* in previously published report (Gupta et al., 2013b).

The flavonoid, quercetin in acetone fraction of *B. monosperma*, ethyl acetate and *n*-butanol fractions of *B. variegata*; dichloromethane and ethyl acetate fractions of *O. gratissimum* were identified in previously published report (Gupta et al., 2013a).

Quercetin (flavonoid) and ursolic acid (triterpenic acid) are well known for its hepatoprotective effects in acute chemically induced liver injury and chronic liver fibrosis and cirrhosis (Janbaz et al., 2004).

Although these studies strongly implicated the medicinal effects of the above plants, there is no study for the beneficial effects of flavonoid rich fractions of these plants on paracetamol-induced hepatic injury. Therefore, in order to fully develop the medical plant resources and to justify the use of this preparation in traditional medicine for the treatment of liver complaint, the present study was designed to investigate the hepatoprotective effect of the flavonoid-rich fractions obtained from different parts of *B. monosperma*, *B. variegate* and *O. gratissimum* against paracetamol-induced liver injury *in vivo*.

Materials and methods

Animals

Albino Wistarmale rats (125–175 g) were used for determination of maximum tolerable dose (MTD) and evaluation of hepatoprotective activity. The animals were housed in polypropylene cages at 25 ± 1 °C with the relative humidity of 55 ± 5% under 12/12 h light/dark cycles. They were received standard chow and water during experimentation. The food was withdrawn on the day before the experiment, but free access of water was allowed.

A minimum of six animals was used in each group. Throughout the experiment, animals were treated according to the suggested international ethical guidelines for the maintenance of laboratory animals. The study protocol was approved by the Institutional Animal Ethics Committee, according to the regulation of the Committee for the Purpose of Control and Supervision of Experiments on Animals (MPC 1007: dated: 30/01/2010).

Plant materials

The fresh bark of *Butea monosperma* (Lam.) Taub., Fabaceae (BM), *Bauhinia variegate* L., Fabaceae (BV) and fresh leaves of *Ocimum gratissimum* L., Lamiaceae (OG) were collected from the campus of Maliba pharmacy college, Bardoli. Voucher specimen (No: MPC/13032010/01, 2 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.

Extraction and fractionation procedures

The dried and powdered material of each plant (500 g) was extracted with methanol at room temperature for three weeks with shaking and stirring. Combined methanolic extracts were evaporated to dryness under reduced pressure below 40 °C and then dissolved in distilled water and subjected to solvent–solvent fractionation.

B. monosperma: Methanolic extract obtained was fractionated with petroleum ether, benzene, chloroform and acetone in the order of their increasing polarity to obtain respective fractions (Sharma and Deshwal, 2011).
B. variegata: Methanolic extract was fractionated with hexane, ethyl acetate and *n*-butanol in the order of their increasing polarity to obtain respective fractions (Silva et al., 2008).
O. gratissimum: Methanolic extract was fractionated with hexane, dichloromethane and ethyl acetate in the order of their increasing polarity to obtain respective fractions (Chattopadhyay, 2003).

Each fraction was concentrated to dryness under reduced pressure and below (40–50 °C) on a rotary evaporator to give acetone fraction of *B. monosperma* [yield 9.4%, w/w], ethyl acetate fraction [yield 2.2%, w/w] and *n*-butanol fraction [yield 5.0%, w/w] of *B. variegate* and dichloromethane fraction [yield 4.2% w/w] and ethyl acetate fraction [yield 4.8%, w/w] of *O. gratissimum*, respectively.

Establishment of qualitative and quantitative phytoprofile of fractioned extracts

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. (Khandelwal, 2001).

Chemical test for flavonoids. Chemical tests were performed for flavonoids according to Macdonald (Macdonald et al., 2010).

Quantitative phytochemical analysis

Determination of total phenols. Each sample was mixed with 1 ml Folin-Ciocalteu reagent and 0.8 ml of 7.5% Na_2CO_3. The resultant mixture was evaluated 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 $r^2 = 0.992$ (Yuvaraj et al., 2011).

Determination of total flavonoids. Standard quercetin was used to create the calibration curve [0.04, 0.02, 0.0025 and 0.00125 mg/ml in 80% ethanol (v/v)]. The standard solutions and test samples (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 the blank. After incubation at room temperature for 30 min, the absorbance of the reaction mixture of each sample 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 $r^2 = 0.99020$ (Kosalec et al., 2004).

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