Hepatoprotective and Free Radical Scavenging Activities of Extracts and a Major Compound Isolated from the Leaves of *Cineraria abyssinica* Sch. Bip. exA. Rich.

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ABSTRACT

In Ethiopian traditional medicine the aqueous decoction of the leaves of *Cineraria abyssinica* Sch. Bip. exA. Rich (Asteraceae) is used for the treatment of various ailments including liver diseases, however, to date, there appears to have been no scientific report on the phytochemistry and claimed hepatoprotective activity of the plant. The main purpose of this study was, therefore, to carry out hepatoprotective and antioxidant activities of the leaf extracts of *C. abyssinica*. Hepatoprotective activities of the aqueous and 80% methanolic extracts as well as the methanol fraction of the leaves of *C. abyssinica* were investigated against carbon tetrachloride-induced liver damage in rats. Intraperitoneal administration of 2 ml/kg of CCl_4 (50% in liquid paraffin) significantly ($p < 0.001$) raised the plasma levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the toxin group compared with the values in the control group. Pretreatment of rats with 200 mg/kg of the aqueous, 80% methanol extracts and the methanol fraction reduced the toxin-induced rise in plasma ALP (65%, 75.4%, 85%), ALT (46.1%, 42.3%, 75%), and AST (58%, 98%, 79%), respectively. The standard drug, silymarin (100 mg/kg) reduced serum ALP (88%), ALT (92%), and AST (87.3%). Bioactivity-guided fractionation of the methanol fraction resulted in the isolation of the flavonol glycoside rutin, whose structure was assigned on the basis of spectroscopic methods. The results of biochemical analysis were further verified by histopatholgical examination of the liver, which showed improved architecture, absence of necrosis and a decrease in inflammation, compared with the findings in the toxin group of animals. Both the extracts and rutin showed potent 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activities. Acute toxicity studies showed that the total extracts of the plant are nontoxic up to a dose of 3 g/kg. The present study revealed for the first time the presence of a hepatoprotective and antioxidant phytochemical in the leaves of *C. abyssinica* that scientifically validates the traditional use of the plant and its potential for the treatment of liver disorders.

Key words: *Cineraria abyssinica,* Asteraceae, hepatoprotective, rutin, free radical scavenging

INTRODUCTION

Liver disease is one of the major causes of morbidity and mortality in public, affecting humans of all ages throughout the world. Despite the great stride in allopathic medicine, modern drugs available for liver diseases have so many limitations. They are limited in number, they do not provide a complete cure and they are unaffordable to most people in the developing countries. This situation stresses the importance of worldwide public–private partnerships to enhance the research enterprise, bring new agents to market in a more cost-effective fashion, and provide effective therapies to suffering patients at costs that are within their reach.[1-3]

Traditional medicines continue to provide front-line pharmacotherapy for many millions of people worldwide. In the absence of safe and reliable antihepatotoxic modern drugs, several medicinal plants have been used worldwide in various traditional herbal recipes for the prevention and

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treatment of liver disease. In recent years there has been a growing focus to follow systematic research methodology and to scientifically evaluate the basis for traditional herbal medicines which are claimed to possess hepatoprotective activity.[1,4]

Cineraria abyssinica Sch. Bip. exA. Rich (Asteraceae) commonly known by its vernacular name '*Etsemefirh*', is an erect or scrambling, annual or perennial herb that can grow up to 20-100 cm high. It has repeatedly branched stem, with alternate, simple to lyrately pinnatified petiolate leaves and radiate capitula with yellow florets. It extends from Ethiopia into Yemen and Saudi Arabia.[5] Based on the information provided by the traditional community from Harar, eastern part of Ethiopia, the aqueous decoction of the leaves of *C. abyssinica* is employed for the treatment of various ailments such as hypertension, cancer, diabetes, diarrhea, kidney and liver diseases. However, despite its wider use in traditional medicine, there are no prior reports on the phytochemistry and pharmacological effects of this plant. The present research was therefore, undertaken to examine the possible hepatoprotective action of the plant using *in vivo* CCl_4-induced hepatotoxicity test in rats and to examine its *in vitro* DPPH free radical scavenging effect.

MATERIALS AND METHODS

Plant material

The leaves of *C. abyssinica* were collected from and around the town of Harar in the Harari People Region, 525 km East of Addis Ababa, Ethiopia in September 2008. The plant was authenticated by Ato Melaku Wondafrash of the National Herbarium, Addis Ababa University, where a voucher specimen has been deposited (Collection number, B 01).

Animals

Wistar albino male rats (200-250 g) and mice (25-30 g) obtained from the Ethiopian Health and Nutrition Research Institute (EHNRI) animal house were used for the experiments. The animals were housed under standard laboratory conditions and were fed commercial rat feed and tap water *ad libitum*. The animals were fasted overnight with free access to water and acclimatized for one week in the new environment before experiments were carried out. All animal experiments were conducted in accordance with the internationally accepted laboratory animal use, care and guideline[6] and approved by the Institutional Review Board of the School of Pharmacy, Addis Ababa university.

Chemicals and instruments

All the chemicals and reagents used for the experiments were analytical grade. Ultraviolet (UV) spectra were run on a Shimadzu UV-1800 spectrophotometer. Infra red (IR) spectra were taken on a Shimadzu IR Prestige-21 spectrophotometer in KBr pellets. ^{1}H NMR and ^{13}C NMR spectra were recorded in DMSO-d_6 using a Bruker A-400 spectrometer with TMS as an internal standard. Electro spray mass spectra were obtained with LCQ Deca XP, ESI, negative mode spectrometer.

Preparation of crude extracts

A hydroalcoholic extract of *C. abyssinica* was prepared by macerating 300 g of the powdered shade-dried leaves with 80% methanol (3x, each for 72 h) with occasional shaking. The combined filtrates were then dried in a rotary vacuum evaporator at a temperature not exceeding 40°C. Aqueous extract was prepared by boiling the plant material for 30 min followed by cooling, filtering and lyophilizing of the extract.

Preparation of solvent fractions and isolation of a compound

The air-dried powdered leaves of *C. abyssinica* (300 g) were successively extracted in a Soxhlet apparatus using solvents of increasing polarity, starting from chloroform then acetone and methanol. The solvents were removed using a rotary vacuum evaporator at a temperature not exceeding 40°C. The most active methanol fraction was subjected to silica gel preparative thin layer chromatography (PTLC) using butanol: acetic acid: water (4:1:5, upper phase) as a mobile phase. The yellowish powder obtained was further purified by LH-20 column chromatography using methanol as solvent and the purity of the eluate was checked by analytical TLC.

Identification of the isolated compound

The isolated compound was identified as rutin by comparison of its spectral data (^{1}H and ^{13}C-NMR) with those reported in the literature.[7,8] Furthermore, comparison of the ESI-mass spectra of the isolated compound was found to be superimposable on those of standard rutin.

Acute toxicity tests

Acute toxicity studies were carried out on the aqueous and 80% methanolic leaf extracts of *C. abyssinica* according to Daisya *et al.*[9] Normal healthy male mice fasted for 12 h were randomly divided into drug-treated 'test' groups and vehicle-treated 'control' group, of 6 mice per group. Each of the extracts (0.5, 2.0 and 3.0 g) suspended in 1% carboxyl methyl cellulose (CMC) was separately administered orally to the mice in each of the test groups. The mice in the control group were treated with vehicle alone (1% CMC). Two h after treatment, the mice in both the test and control groups were given free access to food and water, and behavioral changes were observed over a period of 24 h. Mortality, if any, caused by the extract within this period of time was also observed.