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Free Radical Scavenging and Analgesic Activities of *Cucumis sativus* L. Fruit Extract

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ABSTRACT

The aqueous fruit extract of *Cucumis sativus* L. was screened for free radical scavenging and analgesic activities. The extract was subjected to *in vitro* antioxidant studies at 250 and 500 µg/ml and analgesic study at the doses 250 and 500 mg/kg, respectively. The free radical scavenging was compared with ascorbic acid, BHA (Butylated hydroxyl anisole), whereas, the analgesic effect was compared with Diclofenac sodium (50 mg/kg). The *C. sativus* fruit extract showed maximum antioxidant and analgesic effect at 500 µg/ml and 500 mg/kg, respectively. The presence of flavonoids and tannins in the extract as evidenced by preliminary phytochemical screening suggests that these compounds might be responsible for free radical scavenging and analgesic effects.

Key words: Analgesic, antioxidant, aqueous extract, *Cucumis sativus*

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INTRODUCTION

Antioxidants are the agents that can interfere with the oxidation process by various mechanisms, such as, reacting with free radicals, chelating free catalytic metals, and acting as oxygen scavengers.[1,2] Free radicals, with unpaired electrons, are produced in normal or pathological cell metabolism and reactive oxygen species (ROS) react easily with the free radicals to convert them into radicals. Reactive oxygen species (ROS) are highly reactive molecules, that include the superoxide anion radicals (O_2*−), hydroxyl radicals (*OH), and hydrogen peroxide (H_2O_2) and peroxyl radicals (ROO*).[3-6] The ROS species, as a result, generate metabolic products that attack lipids in cell membranes or DNA. Lipid peroxidation takes place in the cell membranes or the DNA involves a series of free radical chain reaction processes and is associated with several types of biological damage — DNA damage, carcinogenesis, and cellular degeneration related to aging. Cell damages are protected by their endogenous scavenging systems or by other substances.[7] Presently, the use of synthetic antioxidants has been criticized. It is usually implied that regular consumption of natural antioxidants from vegetables, fruit, tea, and herbs may contribute to a shift in balance toward an ample antioxidant status.[3] The interest in natural antioxidants, especially phytochemicals has greatly increased in recent years.[8] Many phytochemicals including phenolics, flavonoids, tannins, proanthocyanidins, and various herbal extracts have been reported as antioxidants.[9,10] Pain represents the symptom for several diseases. Analgesics only relieve pain as a symptom, having no effect on its cause.[11]

Cucumis sativus L. belonging to Cucurbitaceae family is commonly known as Cucumber (English), Khira (Hindi), Sakusa (Sanskrit). It is found wildly in the Himalayan

regions and also cultivated throughout India. Traditionally, this plant is used for headaches; the seeds are cooling and diuretic, the fruit juice of this plant is used as a nutritive and as a demulcent in anti-acne lotions. The fruits contain an enzyme, erepsin, Vitamin B_1 and C, ascorbic acid, proteolytic enzyme, rutin, oxidase, succinic and maleic dehydrogenases, and so on. The seeds contain α- and β-amyrin, sitosterols and cucurbitasides, whereas, the leaves contain free cucurbitasides B and C and ferredoxin. Based on its traditional use and phytoconstituents, the fruit of the plant was selected and screened for free radical scavenging and analgesic activities using *in-vitro* and *in-vivo* models, respectively.[12,13]

MATERIAL AND METHODS

Collection of plant materials

The fruits of *C. sativus* were collected from the local vegetable market in Kurukshetra, in November, 2008. The plant was authenticated by Dr. B.D. Vasisht, Botany Department, Kurukshetra University, Kurukshetra (Haryana, India). A voucher specimen (No. KUK/IPS/CS-1/2009) of the plant has been deposited in the Institute Of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra.

Extraction

Fresh fruits of *C. sativus* were cleaned, cut into small pieces, and macerated with water. The extract was filtered and distilled in a water bath. The extract was solidified under reduced pressure in a rotary evaporator. The yield of the extract was 8.4% w/w of fresh drug.

Preliminary phytochemical screening

Various phytochemical methods[14,15] were used to screen the aqueous extract of *C. sativus* fruit.

Antioxidant screening

The DPPH-free radical scavenging activity

Measurement of the free-radical scavenging activity of the *C. sativus* fruit extract was done by decreasing the absorbance of methanol solution of DPPH (2, 2-diphenyl-1-picryl-hydrazyl).[16] A stock solution of DPPH was prepared by dissolving 33 mg DPPH in 1 L methanol, and 5 ml of this stock solution was added to 1 ml of CS fruit extract solution at different concentrations (250 and 500 μg/ml). After 30 minutes, the absorbance was measured at 517 nm and compared with the standards of the same concentrations. The scavenging activity was calculated as the percentage of inhibition, using the following formula:

$$\% \text{ Antiradical activity} = \frac{\text{Control Absorbance} - \text{Sample}}{\text{Control Absorbance}} \times 100 \text{ Absorbance}$$

Nitric oxide scavenging activity

The nitric oxide scavenging activity was measured spectrophotometrically.[17] Sodium nitroprusside (5 mM) was prepared in phosphate buffered saline and mixed with different concentrations of the extract (250 and500 μg/ml)) prepared in distilled water and incubated at 25°C for 30 min. A control was taken without the test compound but with an equivalent amount of distilled water. Then 1.5 ml of the incubated solution was diluted with 1.5 ml of Griess reagent (1% sulfanilamide, 2% phosphoric acid, and 0.1% N-1-naphthylethylenediamine dihydrochloride). The absorbance was measured at 546 nm and the percentage scavenging activity was calculated with reference to the standard.

Animals

Albino mice (25–30 g) of either sex were selected for the experimental study. They were obtained from Haryana Agriculture University, Hisar, Haryana, India. The animals were maintained under controlled conditions of temperature (21.5 ± 2°C), humidity (60 ± 1%), and a 12-hour light / dark cycle; and were allowed free access to food (standard pellet diet) and water *ad libitum*. Albino mice (25–30 g) were divided into four groups each containing six mice. The animals were deprived of food for six hours before the commencement of the experiment, but allowed free access to water.

Analgesic screening

Tail immersion test

The analgesic effect of *C. sativus* fruit extract was determined as per the reported method.[18] The tails of all the mice were marked 2 cm from the tip. The tails were immersed up to the mark in warm water kept constant at 55°C. The reaction time was determined. It was the time taken by the mice to deflect their tails. The first reading was discarded and the mean of the next three readings was recorded as the reaction time. The reaction time was recorded before and 0, 30, 60, and 90 minutes after administration of the drugs.

Acetic Acid-Induced Writhing Test

The analgesic activity of the *C. sativus* fruit extract was studied using the acetic acid-induced writhing model in mice. The extract doses and the vehicle were given orally 30 minutes before intraperitoneal administration of 0.7% acetic acid, and