In vitro H^+-K^+ ATPase inhibitory potential of methanolic extract of *Cissus quadrangularis* Linn.

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ABSTRACT

Objective: This study was undertaken to study *in vitro* H^+-K^+ ATPase inhibitory potential of methanolic extract of *Cissus quadrangularis* Linn. **Materials and Methods:** Total phenolic and flavonoid contents from extract was quantified and H^+-K^+ ATPase inhibition assay was performed in presence of different concentrations of standard (omeprazole) and methanol extract. **Results:** Extract showed significant ($^*P < 0.05$) proton pump inhibitory activity in the goat gastric mucosal homogenate which was comparable to standard. **Conclusions:** These findings showed that methanolic extract of *C. quadrangularis* Linn. is potent inhibitor of proton pump.

Key words: *Cissus quadrangularis* Linn., H+-K+ ATPase inhibition assay, *in vitro*

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INTRODUCTION

Gastroduodenal ulcers are one of the most common problems faced by populace worldwide. Hyperchlorhydria is a condition characterized by uncontrolled hypersecretion of hydrochloric acid from parietal cells of gastric mucosa through proton pump.[1] A large number of therapeutic interventions are available for treatment of gastric ulcers, such as proton pump inhibitors, anticholinergics, histamine H_2 receptor antagonist, antacids and anticholinergics. These drugs suffer from side effects including, allergic reaction, arrythmia, gynecomastia, etc.[2,3]

Nature serves to be a rich repository of medicinal plants[4-6] and macrofungi,[7] from time immortal man is using herbs for health benefits. A large number of chemical compounds from medicinal herbs express antiulcer activity.[8,9] Many plants in folk medicine are known for their antiulcer potential. *Cissus quadrangularis* Linn. (Vitaceae) commonly known as 'bone setter', is frequently used as a common food item in India.[10] The stout fleshy quandrangular stem of *C. quadrangularis* Linn. is an edible plant found throughout the hotter parts of India, Malaya, West Africa, and Sri Lanka.[11] The stem is used for the treatment of eye and ear diseases, irregular menstruation, asthma, piles, and tumors, fractures of bones, wounds, and scurvy.[12]

A large number of phytoconstituents such as α-amyrin and α-amyrone, β-sitosterol, ketosteroid, oxo-steroid, onocer-7-ene-3α, 21β-diol and onocer-7-ene3β, 21α-diol, stilbene derivatives, and quercitin are found in it,[13] herb is also rich in β-carotene.[14] The extract of *C. quadrangularis* Linn. was reported to show antiulcer and cytoprotective property in various experimental ulcer models,[15,16] but still its activity on enzyme H^+-K^+ ATPase remains to be unknown. On the basis of these observations, the aim of the study was to determine the activity of plant extract on enzyme H^+-K^+-ATPase.

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MATERIALS AND METHODS

Chemicals

Folin-Ciocalteu's phenol reagent, aluminum trichloride, Tris-HCl were purchased from Sigma, Germany. $MgCl_2$, KCl, methanol, and ATP were purchased from Loba Chemie, India.

Plant material

The tubers of *C. quadrangularis* Linn were purchased from a local market of Jabalpur and identified and authenticated by Dr. A.B. Tiwari, Senior Scientist and Botanist, Department of Crop and Physiology, Jawaharlal Nehru Agricultural University, Jabalpur.

Extraction of plant material

The tubers of *C. quadrangularis* Linn. were dried for a week in shade and powdered. A 100 g of the powdered drug was extracted with 95% of methanol in asoxhlet apparatus. The extract was filtered using a muslin cloth and then with a filter paper (Whatmann No. 4) and concentrated at a rotary vacuum evaporator. The dried extract was stored at 4°C until use.

Phytochemical analysis

The phytochemical analysis of the plant was carried out by the standard methods.[17,18]

Phytoanalytical studies

Determination of total phenolic compounds

Total soluble phenolic compounds in the HEE were determined with the Folin-Ciocalteu reagent according to the method of Slinkard *et al.*,[19] using pyrocatechol as a standard phenolic compound. The total concentration of phenolic compounds in the extract determined as micrograms of pyrocatechol equivalent by using an equation that was obtained from a standard pyrocatechol graph:

Absorbance = 0.0054×total phenols [pyrocatechol equivalent (µg)] -0.0058.

Assay for the total flavonoid content

The total flavonoid content was determined using the method given elsewhere.[20,21] The concentrations of flavonoid compounds were calculated according to the following equation that was obtained from the standard quercetin graph:

Absorbance = 0.0338 quercetin (µg) - 0.0002; R^2 = 0.9998

Assay of H^+-K^+ ATPase activity

Proton potassium ATPase was prepared from mucosal scrapings of goat by the method reported by Cheon *et al.*, with necessary modifications. [22] Stomach from freshly slaughtered goats was washed gently with tap water. The mucosal layer of fundus was scrapped and homogenized in ice-cold phosphate buffer, pH 7.4. The homogenate was centrifuged for 20 min at 18,000 rpm. The supernatant so obtained was recentrifuged for 60 min at 100,000 rpm. The pellet was resuspended in homogenisation buffer. Ficoll-sucrose discontinuous density gradient centrifugation was utilized to prepare H+K+ATPase. Protein was determined by the method of Lowry *et al.*[23]

Assay of H^+-K^+-ATPase

Different concentrations of the extract 10-50 µg/ml were incubated in the reaction mixture (40 mM Tris-HCl buffer, pH 7.4, containing 2 mM $MgC1_2$ and 10 µg membrane protein) to make a volume of 1 ml. Then, 2 mM ATP Tris salt was utilized to start the reaction, this preparation was incubated for 20 min for 37°C. The reaction was terminated by adding 1 ml of ice-cold trichloroacetic acid (10% v/v). The H^+-K^+ ATPase activity was assayed [24] in the presence and the absence of different doses of the extract and omeprazole. The amount of inorganic phosphate released from ATP was determined spectrophotometrically at 400 nm.

Statistical analysis

The results are expressed as mean±standard error of mean. Experiments were always performed in triplicates. Statistical comparison was performed using analysis of variance (ANOVA) followed by Bonferroni's test (*P<0.05).

RESULTS

Phytochemical analysis

The results of the preliminary phytochemical analysis extract of *C. quadrangularis* Linn. showed abundant presence of alkaloids, terpenoids, saponins, tannins, and phenols.

Phytoanalytical studies

Screening of phenolic compounds with NaOH and $FeCl_3$ revealed their presence and quantification was done. The total amount of the phenolic content present in the extract was found to be 715.2 ± 2.57 mg PE (pyrocatechol equivalent)/100g. By using the standard curve of quercetin (R^2 = 0.9998), the total flavonoid content of the extract was found to be 169.2 ± 1.97 mg QE (Quercetin equivalent)/100g. The total alkaloidal content in the extract was found to be 17.2 mg/kg dry basis.

H^+-K^+ ATPase activity

The extract showed significant (*P<0.05) proton pump inhibitory activity in the goat gastric mucosal homogenate. The inhibitory activity was concentration dependent, and the results were comparable to standard drug omeprazole. *In vitro*, the methanolic extract of *C. quadrangularis* Linn. potently reduced the hydrolysis of ATP by the goat gastric ATPase with IC_{50} of 38 µg/mL. Omeprazole (10-50µg/mL) used as positive control reduced H^+-K^+ ATPase activity with an IC_{50} = 26 µg/mL [Figure 1].

DISCUSSION

A large number of phytochemicals such as tannins,

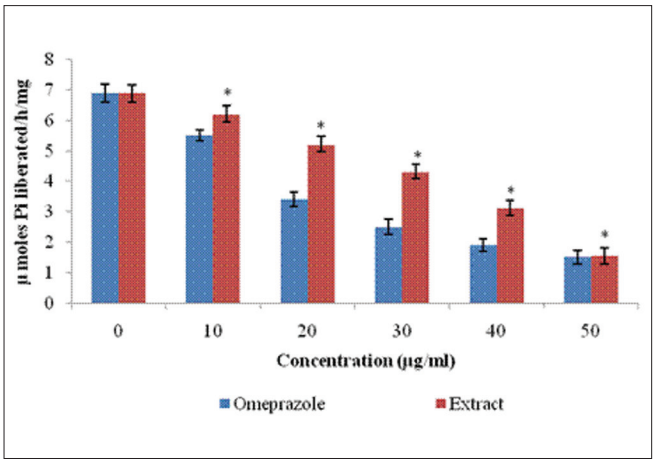

Figure 1: Effect of the ethanol extract of *C. quadrangularis* and omeprazole on H+-K+ ATPase activity: H+-K+ ATPase activity was measured with 10-50 µg/mL of the extract and omeprazole. Experiments were always performed in triplicates. The results are expressed as mean ± standard error of mean. Statistical comparison was performed using analysis of variance (ANOVA) followed by the Bonferroni's test (*P< 0.05)

flavonoids, tannins, and triterpenes from plants have previously demonstrated potential antiulcerogenic activity.[25,26] Catechin and epicatechin are reported to be potent non-competitive inhibitors of H^+-K^+-ATPase.[27] Plant polyphenols and flavonoids are used in treatment of gastric ulcers.[27] Flavonoids are capable of defending gastric damage. Flavonoids are excellent antioxidant; some of them are capable of enhancing mucosal content of prostaglandins. Apart from this, they preserve capillary integrity and restore normal function of mucus membrane.[28]

Quantitative estimation on the plant extract showed the presence of phytoconstituents such as phenolics and flavonoids. In this study, the possible mechanism of protection to gastric ulcer was evaluated. H^+-K^+ ATPase is a key enzyme in inducing acidity; in this study, the ability of methanolic extract to inhibit H^+-K^+ ATPase *in vitro* isolated from goat stomach was studied. *In vitro* studies are considered necessary in order to evaluate the potential of phytochemicals to enter in the cell and additionally to exemplify their interaction with the gastric ATPase. Enzyme H^+-K^+ ATPase is an important enzyme system located on apical secretory membrane of partial cell. In this study, dose-dependent inhibition of enzyme by omeprazole and extract was observed, suggesting that the *C. quadrangularis* Linn. extract was significantly (*P<0.05) able to inhibit enzyme H^+-K^+ ATPase, responsible for the secretion of acid and effect was comparable to omeprazole.

Therefore, it could be concluded that the inactivation of H^+-K^+ATPase is the major gastroprotective mechanisms of action of *C. quadrangularis* Linn., which indicates its protective role against inhibiting gastric proton pump and opens a door for isolation and characterization of active compounds responsible for it.

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