Short Communication

In vitro H^+ -K^+ ATPase inhibitory potential of methanolic extract of *Carissa carandas* Linn. leaves

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Abstract

Objective: This study was taken to study *in vitro* H^+ -K^+ ATPase inhibitory possible of methanolic acetone extract of *Carissa carandas* Linn. Leave. **Materials and Methods:** Total phenolic and flavonoid contents from extract were quantified and H^+ -K^+ ATPase inhibition assay was perform in presence of different concentrations of standard (omeprazole) and methanol extract. **Results:** Extract showed considerable ($P < 0.05$) proton pump inhibitory activity in the goat gastric mucosal homogenate which was equivalent to standard. **Conclusions:** The activities were found to be dose dependent and this study indicates that both methanol-acetone extract of *Carissa carandas* were found to suppress sheep mucosal H+K+ ATPase activity *in vitro*, So, further study is needed to confirm the gastroprotective property of *Carissa carandas* leaves.

Key words: *Carisa carandas*, H+ K+ ATPase inhibition assay, *in vitro*

Introduction

Gastro duodenal ulcers are one of the most average problems faced by public in worldwide. Hyperchlorhydria is a condition characterized by uncontrolled hypersecretion of hydrochloric acid from parietal cells of gastric mucosa through proton pump (Shen et al., 2002). 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 give side effects like, allergic reaction, arrythmia, gynecomastia etc. (Schöll et al., 2005). Natural herbs serves to be a rich repository of medicinal plant and from time immortal man is using herbs for health benefits (Sivarajan et al.,1994). A large number of chemical compounds from natural herbs have fast antiulcer activity (Sen et al., 2009). Many herbal plants used as folk medicine, for their antiulcer potential. (Review arti cle2013) It contains several phytochemical constituents belonging to terpenoids category. *C. carandas* is the best known member of the genus, as it has been used as a traditional medicinal plant over thousands of years in the Ayurvedic system of medicine as it is practiced on Indian subcontinent. Thus traditional uses of *C. carandas* are well established. The root is created with bitter, stomachic, antidiarrhoel and anthelmintic properties, while its leaves are prescribed in remittent fever, soreness and syphilitic pain of the mouth. A tincture of fruits is used in infections and skin infections and a decoction of wood is employed as a tonic to strengthen the tendons of slim patient. The unripe fruit is used medicinally as an astringent. The ripe fruit is taken as a source of vitamin c and remedy for biliousness. A higher gross heat value of this species indicates its higher potential to be used as good fuel source., the aim of the study was to determine the activity of plant extract on enzyme H^+ -K^+ -ATPase (Gadekar et al.,2010 and Jainu et al., 2003).

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Material and Methods

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

Plant material

Plant material was collected locally from Bhopal.

Herbarium was prepared and submitted at botany department of Safia College, Bhopal for authentication. The plant was authenticated by Dr. Ziaul Hasan, Department of Botany, Safia Science College, and Bhopal. A voucher specimen no.256 /Bot/Safia/2011 of the plant.

Extraction of plant material

Plant material leaves (1 Kg) was weighed and packed with Petroleum Ether (40:60) in air tight container for maceration. Sample was regularly shaked in between. After 15 days solvent was filtered under vacuum. Marc was dried under shade and further packed with Methanol: Acetone (70: 30) solvent system for fifteen days with regular shaking. Solvent was filtered and evaporated in rotary vacuum evaporator at 40oC. Extract (CCE) was packed in air tight container and kept in cool place for further studies (Tiwari et al., 2016)

Phytochemical analysis

The phytochemical analysis of the plant was carried out by the standard methods. Following chemical constituents have present in extract Carbohydrate, cardiac glycoside, flavonoid, alkaloids, taninins, saponin, alkaloids, tannin and phenolic component (Gupta et al., 2015).

Phytoanalytical studies

Determination of total phenolic compounds

Total soluble phenolics in the extracts were determined with Folin- Ciocalteau reagent according to the method of Slinkard and Singleton using Gallic acid as a standard phenolic compound. 1.0 ml of extract solution containing 1.0 g extract in a volumetric flask was diluted with 46 ml of distilled water. 1.0 ml of Folin-Ciocalteau reagent was added and the content of the flask mixed thoroughly. 3 min later 3.0 ml of 2 % sodium carbonate was added and the mixture was allowed to stand for 2 h with intermittent shaking. The absorbance of the blue color that developed was read at 760 nm. The concentration of total phenols was expressed as mg/g of dry extract. The concentration of total phenolic compounds in the extract was determined as mg of Gallic acid equivalent using an equation obtained from the standard Gallic acid graph. $Y = 0.002x + 0.037$, $R^2 = 0.992$. Using the above equation the total phenol in the methanol-Acetone (70:30) leaves extracts of *Carissa carandas* was found 181.5 mcg/ml of extracts respectively to Gallic acid (The results are expressed in terms of Gallic acid which was used as Standard) (Shukla et al., 2014).

Total flavonoid content

The spectrophotometer assay for the quantitative determination of flavonoid content was carried out as described by Wang et al. with minor modifications using rutin as a standard. Briefly, extracts or standard solutions (0.25 mL) were mixed with 1.25 mL distilled water and 75 µL 5% $NaNO_2$. After 6 min, 75 µL of 10% AlCl3 was added. After another 5 min, 0.5 mL of 1 M NaOH was added to the mixture. Immediately, the absorbance of the mixture was determined at 510 nm versus prepared water blank. Total flavonoids content was expressed as mg rutin equivalents (RE). $Y = 0.000x + 0.022$, $R^2 = 0.926$. Using the above equation the total flavonoids in the methanol-Acetone (70:30) leaves extracts of *Carissa carandas* were found to be 900 µg/ml respectively to rutin equivalent (Zhishen et al., 1999). The results are expressed in terms of Rutin which was used as standard.

Preparation of Parietal cells

Proton potassium ATPase was prepared from mucosal scrapings of sheep stomach obtained from slaughter house and then homogenized in 200mM Tris-HCl buffer, pH 7.4, centrifuged for 10 mins at 5000xg. The resulting supernatant was subsequently centrifuged at 5000xg for 20 min. The protein concentration in the supernatant was determined with bovine serum albumin as standard. The parietal cell extract was then employed to determine H+K+ ATPase activity (Ricardo et al., 2006).

Determination of H+K+ ATPase

The H+K+ ATPase activity in the presence of different concentrations of test extracts and omeprazole was assayed by the method of Reyes- Chilpa et al 2006. The enzyme source was preincubated with different concentration of the test material (10-70µg) for 30min. The assay was conducted in a mixture contained an aliquot of methanol-Acetone (70:30) leaves extracts of *Carissa carandas* Linn. extract treated enzyme in 20mM tris-HCl, pH 7.4, 2mM magnesium chloride (MgCl2) and 2mM potassium chloride (KCl). The reaction was started with the addition of 2mM adenosine-5'-triphosphate (ATP) and incubated for 30 mins at 30ºC and terminated by the addition of 10% trichloroacetic acid followed by centrifugation at 2000xg. The amount of inorganic phosphorous released from adenosine-5'-triphosphate (ATP) was determined spectrophotometrically at 640nm. The enzyme source was also treated similarly with the standard drug omeprazole and the enzyme activity was measured (Reyes-Chilpa et al., 2006).

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 methanol-Acetone (70:30) leaves extracts of *Carissa carandas* Linn. Showed abundant presence of alkaloids, terpenoids, saponins, tannins, and phenols (Bhati et al., 2014).

Phytochemical 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 (Ricardo et al., 2006; Zhishen et al., 1999).

Assay of H+ K+ ATPase activity

The methanol acetone extract showed important (*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 methanol-Acetone (70:30) leaves extracts of *Carissa carandas* Linn. Potently reduced the hydrolysis of ATP by the goat gastric ATPase with IC_{50} of 25 µg/mL. Omeprazole (10-70µg/mL) used as positive control reduced H^+-K^+ ATPase activity with an IC_{50} = 29.5 µg/mL (Table 1).

H+-K+ ATPase activity was measured with 10-70 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 (Reyes-Chilpa et al., 2006).

Discussion

A great number of phytochemicals constituents like as tannins, flavonoids, tannins, and triterpenes from herbs have previously confirmed potential antiulcerogenic activity. Catechin and epicatechin are investigated to be effective non-competitive inhibitors of H^+-K^+-ATPase. Herbal polyphenols and flavonoids are used in management of gastric ulcers. Flavonoids are able of protecting gastric damage. Flavonoids are brilliant antioxidant; a number of of them are capable of enhancing mucosal content of prostaglandins. Separately from this, they preserve capillary integrity and repair normal function of mucus membrane. Quantitative evaluation on the herbal extract showed the presence of phytoconstituents like as phenolics and flavonoids. (Bhati et al., 2014) Reported hepatoprotective activity of *Carissa carandas* Linn leave hence in this study, the feasible mechanism of protection to gastric ulcer was evaluated. H^+-K^+ ATPase are a key enzyme in suggest acidity; the ability of methanolic acetone extract could 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 demonstrate their interaction with the gastric ATPase. Enzyme H^+-K^+ ATPase are a significant enzyme system located on apical secretory membrane of partial cell. In this study, dose-dependent inhibition of enzyme by omeprazole and herbal extract was observed, signifying that the *Carissa carandas* Linn. Extract was significantly (*P<0.05) able to inhibit enzyme H^+-K^+ ATPase, accountable for the secretion of acid and effect was comparable to omeprazole. Therefore, Invitro study revealed that extract of *Carissa carandas* acted as potent ulcer reducing agents and the effect was comparable to that of standard drug omeprazole. The ulcer score was reduced significantly in all the experimental ulcer models studied. However the detailed study on the level of prostaglandins and gastric mucin in drug treated animals is essential to confirm the antiulcer property.

Conclusion

It can be concluded that the methanol-Acetone (70:30) leaves extracts of *Carissa carandas* Linn. plant possess potent H+K+ ATPase inhibitory activity invitro. The test drugs may probably influence the antiulcer property by preventing the formation and the unsafe action of toxic oxygen free radicals on gastric mucosa. The H+K+ ATPase inhibitory activity may also be accounted for gastroprotective activity.

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Table 1. H+K+ ATPase inhibitory activity of various fractions of methanol-Acetone (70:30) leaves extracts of *Carissa carandas* Linn. (%inhibition)

Plant extracts	10	20	30	40	50	60	70
Methanol Acetone	16.8±1.68	24.9± 2.5	26.4 ± 2.7	27.6± 2.8	34.9± 3.5	35 ± 3.6	40± 4.0
Omeprazole	20 ± 2.0$^{\$}$	26.2±2.7$^{\$}$	30.13±3.2$^{\$}$	34.7±3.5$^{\$}$	37.5±3.8$^{\$}$	45.4±4.6$^{\$}$	48±4.9$^{\$}$

Values are expressed as mean ± SD for six individual experiments. Statistically significant difference is expressed as $p<0.001, #p<0.01, *p<0.05.

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