Research Article

Assessment of anthelmintic and *in vitro* H^+-K^+ ATPase inhibitory potential of hydroalcohalic extract of *Achyranthes aspera* Linn.

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Abstract

Objective: Objective of this work was to evaluate the *in vitro* anthelmintic and H^+-K^+ ATPase inhibitory possible of Hydroalcohalic (70:30) extract of *Achyranthes aspera* Linn. aerial part. **Materials and Methods:** The total flavonoid content, phenol content, anthelmintic activity and H^+-K^+ ATPase inhibition assay was performed in presence of different concentrations of hydroalcohalic (70:30) extract. **Results:** The hydroalcohalic (70:30) extract of *Achyranthes aspera* Linn showed considerable ($*P < 0.05$) anthelmintic and 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 the hydroalcohalic (70:30) extract of *Achyranthes aspera* were found to suppress sheep mucosal H+K+ ATPase activity *in-vitro*. So, further study is needed to confirm the gastro protective property of *Achyranthes aspera* aerial part.

Keywords: *Achyranthes aspera* Linn, H+ K+ ATPase inhibition, anthelmintic, Folin-Ciocalteau, Albendazole

Introduction

Gastro-duodenal ulcers are one of the most average problems faced by public in worldwide. The Hyperchlorhydria is common gastric disease and it is characterized by uncontrolled hyper secretion 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 have some side effects like, allergic reaction, arrhythmia, gynecomastia etc. (Schöll et al., 2005). The 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. It contains several phytochemical constituents belonging to terpenoids category. *Achyranthes aspera* 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 *Achyranthes aspera* are well established. The root is created with bitter, stomachic, antidiarrhoel and anthelmintic properties, mainly triterpenoid, saponins possessing oleanolic acid as aglycone viz; A, B, C, D. Other such as Ecdysterone an insect molting hormone long chain alcohols viz., 17-penta triacontanol, 27-cyclohexylheptacosan-7-ol, 16-hydroxy-26-methylheptacosan-2-one and 36, 47-dihydroxyhen-pentacontan-4-one; a water soluble base, betaine. *Achyranthes aspera* leaves reported to have antimicrobial, anti-asthmatic property so mostly used in the treatment of skin and teeth disorder, hence further study is needed to confirm the gastro protective property of *Achyranthes aspera* aerial part (Bajaj et al., 2012; Gadekar et al., 2010; Jainu et al., 2003; Bhati et al., 2014; Shukla et al., 2016).

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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

The aerial part of *Achyranthes aspera* were collected from the herbal garden of Guru Ramdas Khalsa Institute of Science and Technology (Pharmacy), Jabalpur, District of Madhya Pradesh, India. The plants aerial parts were cleaned well and dried under shed at room temperature for extraction.

Extraction of plant material

Plant material aerial part of *Achyranthes aspera* (aerial parts, 1 Kg) was weighed and packed with Petroleum Ether in air tight container for maceration. After 15 days solvent was filtered under vacuum. Marc was dried under shade and further packed with Ethanol: Water (70:30) solvent system for fifteen days with regular shaking. Solvent was filtered and evaporated in rotary vacuum evaporator at 40°C, then after *Achyranthes aspera* Extract (AAE) was packed in air tight container and kept in cool place for further studies (Shukla et al., 2012).

Phytochemical analysis

The phytochemical analysis of the plant was carried out by the standard methods. Following chemical constituents were present in extract Carbohydrate, cardiac glycoside, flavonoid, alkaloids, taninins, saponin, alkaloids, tannin and phenolic component (Gupta et al., 2015, Shukla et al., Garg et al., 2016, 2016 and 2017).

Phytoanalytical studies

Determination of total phenolic compounds: Total soluble phenolic compounds 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 µg/ml 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 Hydroalcohalic (70:30) extracts of *Achyranthes aspera* was found 91.5 µg/ml of extracts respectively to Gallic acid (The results are expressed in terms of Gallic acid which was used as Standard) (Sweta et al., 2018; Charde et al., 2012; Shukla et al.,2014).

Total flavonoid content

The spectrophotometer assay for the quantitative determination of flavonoid content was carried out as described by Zhishen 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 75µL 5% $NaNO_2$. After 6 min, 75µL 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). $Y = 0.000x + 0.022$, $R^2 = 0.915$. Using the above equation the total flavonoids in the ethanol-distilled water (70:30) extracts of *Achyranthes aspera* were found to be 490µg/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).

Anthelmintic Activity

For the study of anthelmintic activity Indian adult earthworms (Pheretimapostuma) were taken. The earthworm which was used for study has similarity in both physiologically and anatomically to the parasites of the intestinal roundworm of the human beings, hence it is suitable to study anthelmintic activity. The earthworms can be categorized into individual groups (each group having three organisms) for each cure at different concentrations. Albendazole the standard drug at three different concentrations of 15, 20 and 25mg/ml with ethanol was made. The hydroalcoholic extract of (AAE) were taken in different concentration of 15, 20 and 25mg/ml as test. To each Petri dish three earthworms were taken. Movements of Earthworms were examined to each Petri Dish. The time taken for the worm to lose its movement was considered for paralysis time and the time taken to drop its motility even in the occurrence of outside stimulus (when dipped in warm

water at 55ºC) and faded body color was measured for mortality time. Death time and paralysis time of each earthworm in the group was recorded (Gupta et al., 2015; Tiwari et al., 2016; Nayak et al., 2012; Ramchandra et al., 2013).

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 Hydroalcohalic (70:30) aerial part extracts of *Achyranthes aspera*. 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 min 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; Shukla et al., 2014).

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 ethanol-distilled water (70:30) aerial part extracts of *Achyranthes aspera*. Hydroalcohalic extract showed abundant presence of alkaloids, terpenoids, saponins, tannins, and phenols **(**Shukla 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 hydroalcohalic extract was found to be 91.5µg/ml. By using the standard curve of quercetin (R^2 = 0.9998), the total flavonoid content of the extract was found to be 490 µg/ml respectively to QE (Quercetin equivalent)/100g (Ricardo et al., 2006and Zhishen J et al., 1999).

Assay of H+ K+ ATPase activity

The hydroalcoholic 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 Hydroalcohalic (70:30) aerial part extracts of *Achyranthes aspera*. Potently reduced the hydrolysis of ATP by the goat gastric ATPase with IC_{50} of 25µg/mL. Omeprazole (10-70µg/mL) used as positive control reduced H^+-K^+ ATPase activity with an IC_{50} = 29.5µg/mL (Table 1). H+K+ ATPase inhibitory activity of various fractions of hydroalcoholic extract (70:30) aerial part extracts of *Achyranthes aspera* % inhibition.

Table 1 showed the results of effect of the hydroalcohalic extract of *Achyranthes aspera* and omeprazole on H+-K+ ATPase activity: 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).

Table 1. Anthelmintic activity of the *Achyranthes aspera* aerial part of hydroalcohalic extract

Treatment	Groups	Concentration (mg/ml)	Time of Paralysis (min.)	Time of Death (min.)
Albendazole	I	15	42.4±0.47	50.8±0.16
		20	38.7±0.36	52.2±0.58
		25	26.4±0.43	33.5±0.38
Acyrantrhes aspera hydroalcohalic extract	II	15	36.1±0.94	44.7±0.16
		20	31.2±0.39	41.1±0.62
		25	21.5±0.45	29.1±0.12

Table 2. *H+K+ ATPase* inhibitory activity of *Achyranthes aspera* aerial part of hydroalcohalic extract

Plant extract	10	20	30	40	50	60	70
Hydroalcohalic (70:30)	16.3±1.86	26.1± 2.0	27.9 ± 7.1	29.1± 3.0	36.9± 3.8	37 ± 6.4	41± 4.0
Omeprazole	19 ± 7.01	26.4±2.79	30.11±4.26	34.0±5.33	37.6±3.91	45.5±7.40	48±6.52

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.

Discussion

Preliminary phytochemical screening of aqueous and hydroalcohalic extract of *Achyranthes aspera* aerial part showed the presence of flavonoid, glycosides and tannins. Chemically tannins are poly phenolic compound. Total phenolic and flavonoid contents of hydroalcoholic extract was found to be 91.5 mcg/ml of extracts respectively to Gallic acid and flavonoid, 490µg/ml respectively to rutin equivalent. Some synthetic phenolic anthelmintic agents such as niclosamide, oxyclozanide and bithionol are shown to interfere with energy generation in anthelmints parasites by uncoupling oxidative phosphoryation. It is possible due to presence of tannins. It contained tannins in the extract of *Achyranthes aspera* may produce similar effects. Another possible anthelmintic effect of tannins is that they can bind to free protein in the gastrointestinal tract of host animals. From the result, it can be concluded that the hydroalcoholic extract of *Achyranthes aspera* showed significant anthelmintic activity and comparable with standard drug. 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 *Achyranthes aspera* aerial part, 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 hydroalcohalic (70:30) 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 *Achyranthes aspera*. The result is shown in table 2. The hydroalcohalic 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, In-vitro study revealed that hydroalcohalic extract of *Achyranthes aspera* acted as potential 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 hydroalcohalic extract (70:30) aerial part extracts of *Achyranthes aspera*. Plants possess potent anthelmintic and H+K+ ATPase inhibitory activity *In-vitro*. 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 gastro protective activity.

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