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THE H^+/K^+ ATPase INHIBITORY ACTIVITY OF ETHANOLIC EXTRACT OF *DIOSPYROS PANICULATA Dalz* BARK

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ABSTRACT

The Diospyros Paniculata is a folkforic medicine which has been used in Ayurveda and Siddha system of medicines. It has been commonly used in the treatment of ulcer, burns, poisoning etc. The present study is conducted to evaluate the In vitro H^+/K^+ ATPase inhibitory activity of ethanolic extract of Diospyros Paniculata (EEDP). H^+/K^+ ATPase inhibition assay was performed by using different concentration of EEDP ranging from 10-50µg/ml and % inhibition calculated which is compared with the standard drug lansoprazole. The results obtained indicate that the EEDP has significant In vitro H^+/K^+ ATPase inhibitory activity ($P<0.001$).IC 50 value of the EEDP is 26µg/ml and the IC 50 value of Standard drug lansoprazole is 18µg/ml. The ethanolic extract of Diospyros Paniculata is having good H^+/K^+ ATPase inhibitory action.

KEYWORDS: Diospyros Paniculata, In vitro, H^+/K^+ ATPase.

INTRODUCTION

Acid-related disorders are highly prevalent in the developed world, which have a significant impact on patient quality of life and cause heavy burden on health care systems.[1] Gastric acid was identified as an important pathogenic factor for most prevalent gastrointestinal disorders such as duodenal ulcer and gastro esophageal reflux disease.[2,3] Hyperchlorhydria is a condition characterized by uncontrolled hyper secretion of hydrochloric acid from parietal cells of gastric mucosa through proton pump.[4] A large number of therapeutic interventions are available for treatment of gastric ulcers, such as proton pump inhibitors,

anticholinergics, histamine H2 receptor antagonist, antacids and anticholinergics. These drugs suffer from side effects including, allergic reaction, arrhythmia, gynecomastia etc.[5,6]

Natural remedies were used in the treatment of gastric ulcers from earlier times .One of the such commonly used folk medicine is Diospyros Paniculata. *Diospyros paniculata* is a moderate sized handsome tree attaining a height of 50 ft and a diameter of 1.25 m. The fruits are green and ovoid, about 1 in long. The wood is whitish grey, occasionally with narrow stripes of black.

This plant does not yield black heartwood. Bark is soft and moderately heavy (wt. 46 lb /cu ft).

Leaves of the tree are used as fish poison; dried and powdered fruits are applied to heal burns;

Decoction of the fruit is used in gonorrhea, biliousness and blood poisoning; powdered stem bark is used for rheumatism and ulcer.[7] In Indian system of traditional medicines like Ayurveda and Unanni, various *Diospyros* species are used medicinally to cure fever, diabetes, snake bite, diarrhea, biliousness, ulcer etc.[8]

Since the In vitro H^+/K^+ ATP ase inhibitory activity of the Diospyros Paniculata had not yet reported scientifically the present study is conducted to evaluate the In vitro inhibitory activity of Diospyros Paniculata on H^+/K^+ ATPase.

MATERIALS AND METHODS

PLANT COLLECTION AND AUTHENTICATION

Diospyros Paniculata was collected from Gootrical Forest range, sabarimala, Pathanamthitta district, kerala in October 20,2015 and it was authenticated by Mr.M V Krishna raj M.Sc., B.Ed., Ph.D Assistant Professor, Department of Botany , Baselius College Kottayam.

PREPERATION OF THE ETHANOLIC EXTRACT OF BARK OF DIOSPYROS PANICULATA

Fresh bark of Diospyros Paniculata was collected and washed thoroughly with distilled water and dried in open air at shade. Later the dried bark were chopped into small pieces and the material were properly packed and kept in soxhlet extractor and is made to undergo successive soxhlet extraction using ethanol as solvent. After 48hrs the extract was collected

and it is air dried to remove the solvent. The extract collected is properly packed and kept for further studies.

IN VITRO METHOD

H^+/K^+ ATP ase INHIBITION[9]

Preparation of H^+/K^+-ATPase

Gastric membrane containing H^+, K^+-ATPase was prepared from mucosal stomach scrapings of sheep and was homogenized in 20 mM Tris–HCl buffer (pH 7.4). The homogenate was centrifuged for 20 min at 15,000 rpm and the resulting supernatant was used to determine the H^+, K^+-ATPase activity and its inhibition.

Estimation of Protein[10]

The protein content of the supernatant was determined by Lowry's Method using bovine serum albumin as a standard. Different dilutions of BSA solutions were prepared by mixing stock BSA solution (1g/ ml) and water in the test tube. The final volume in each of the test tubes was 5 ml. The BSA range is 0.05 to 1 g/ ml. From these different dilutions, 100 µl protein solution was pipetted out to different test tubes and added 2 ml of alkaline copper sulphate reagent (analytical reagent). The solutions were mixed well and incubated at room temperature for 10 minutes. Then added 200 µl of reagent Folin Ciocalteau solution (reagent solutions) to each tube and incubated for 30 min. The colorimeter was set at zero with blank and the optical density (measure the absorbance) was recorded at 660 nm. The absorbance was plotted against protein concentration to get a standard calibration curve. The absorbance of sample was measured and determined the concentration of the sample using the standard curve. The concentration of the protein was adjusted to a final concentration of 3 mg/ml.

H^+/K^+ ATPase Assay[9]

The enzyme extract containing 100 µl (300 µg) proteins was taken for testing the activity of H^+/K^+ -ATPase. Reaction was carried out in 16mM Tris buffer (pH 6.5). The reaction was initiated by adding substrate (2mM ATP, 2mM $MgCl_2$ and 10mM KCl), made up to 2 ml and incubated for 30 min at 37°C. The reaction was stopped by the addition (1 ml) of an assay mixture containing 4.5% ammonium molybdate and 60% perchloric acid. Phosphomolybdate formed was measured spectrophotometrically at 400 nm.

Inhibition of H^+/K^+-ATPase *in vitro*.[9]

The enzyme extract containing 100 µl of protein was taken for testing the activity of H^+, K^+-ATPase in the presence of different concentrations (10–50 µl) of each sample fraction. Plant extracts were incubated with H^+, K^+-ATPase for 30 min. Subsequently, reaction was carried out as described above. The results were expressed as percent inhibition of enzymatic activity at each concentration.Lansoprazole was employed as a standard anti-ulcer drug.

The inhibition was calculated from the following equation.

$$\text{Inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

STATICAL ANALYSIS

The data were expressed as mean ± SEM. Experiments were always performed in triplicates. Statistical comparison was performed using analysis of variance (ANOVA) followed by Bonferroni's test and P<0.001*** considered as significant.

RESULTS

1. EXTRACTION

The ethanolic extract of diospyros paniculata (Bark) is prepared.

2. IN VITRO METHOD

2.1 H^+/K^+ ATP ase INHIBITION

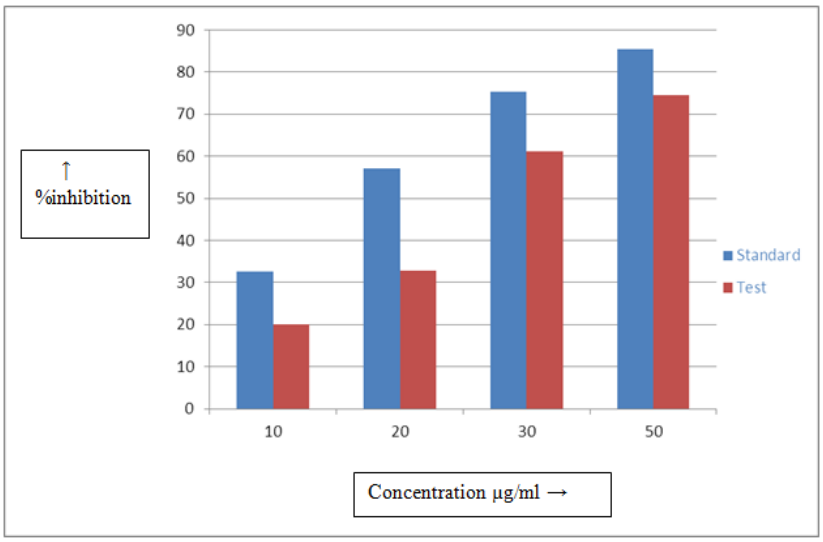
The H^+/K^+ ATP ase activity of Ethanolic extract of Diospyros Paniculata(EEDP) is shown in the table no 1.The different concentrations of standard drug lansoprazole and EEDP is taken for the experiment ranging from (10-50µg/ml).It was found that EEDP showed significant H^+/K^+ ATP ase inhibitory action ranging from 20-74% as compared to lansoprazole which is having inhibitory action ranging from32-85%.The result obtained show significant activity with P<0.001*** and the IC 50 value of standard is 18µg/ml as compared to EEDP is 26µg/ml.

Table No: 1 H^+/K^+ ATP ase Inhibitory action of Ethanolic Extract of Diospyros Paniculata

SAMPLE	CONCENTRATION µg/ml	ABSORBANCE	% INHIBITION
Lansoprazole (Standard)	10	0.474 ± 0.0003***	32.58%
	20	0.304 ± 0.0003***	57.11%
	30	0.177 ± 0.0003***	75.37%

	50	0.106 ± 0.0003***	85.37%
Ethanolic extract of diospyros paniculata (EEDP) Test compound	10	0.560 ± 0.0003***	20%
	20	0.470 ± 0.0003***	32.8%
	30	0.274 ± 0.0003***	61.2%
	50	0.178 ± 0.0003***	74.42%

The data were expressed as mean ± SEM. Statistical comparison was performed using analysis of variance (ANOVA) followed by Bonferroni's test and P<0.001*** considered as significant.



Graph 1, Percentage inhibitory action of EEDP and Standard drug

DISCUSSION

The results obtained in the study clearly indicate that the Ethanolic extract of Diospyros Paniculata is having significant H^+/K^+ ATP ase inhibitory activity. Since the powdered bark has been used as a folkforic medicine in the treatment of ulcer it indicate that the Diospyros Paniculata is having Anti ulcer activity. The phytochemical evaluations performed on the Diospyros Paniculata indicate that it composed of phytochemicals like flavanoids, alkaloids, saponins etc.[11,12,13] which are having Anti ulcer activity whose Anti ulcerogenic activity are scientifically proved and the presence of these phytochemicals may be the contributing to Anti ulcer activity of Diospyros Paniculata.

CONCLUSION

The results obtained in the In vitro study indicate the the EEDP is having significant H^+/K^+ ATP ase inhibitory activity along with this the phytochemical test performed also show the presence of Alkaloids, Flavanoids, saponins etc and these may be responsible for the Anti ulcerogenic activity of the Diospyros Paniculata. Even though further extraction of the active

constituents and further studies are needed to confirm the exact mechanism of action by which Diospyros Paniculata show H^+/K^+ ATPase inhibitory action.

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