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RESEARCH ARTICLE

IN VITRO ANTI-ULCER ACTIVITY OF DIFFERENT EXTRACTS OF *CISSUS QUADRANGULARIS*

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Abstract

Aim: The aim of this study was undertaken to assessin vitro H+-K+ ATPase inhibitory activity of different extracts of *Cissus quadrangularis Linn.*

Materials and Methods: Phytochemical and Phyto-analytical studies like total phenolic compound and total flavonoid contents from extract were quantified and H+-K+ ATPase inhibition assay was performed in presence of different concentrations(10,20,50 and 100 µg/ml) of standard (omeprazole),chloroform and methanolic extract.

Results: The extract has shown dose dependent significant (*P < 0.05) proton pump inhibitory activity in the goat gastric mucosal homogenate which was compared to standard Omeprazole.

Conclusions: Hence, from this study we have concluded that methanolic extract of *C.quadrangularis* has more inhibitory effect than chloroform extract.

Introduction:-

Peptic ulcer is a prevalent health problem with a huge rate of unwholesomeness and has become the centre of interest of experimental and clinical investigations, mostly due to its high prevalence in the global population.[1]In the pathogenesis of gastrointestinal disorders like duodenal ulcer andgastroesophageal reflux disease, gastric acid was found to be the primary and important factor.[2,3]The gastric H+/K+-ATPase pump is located in the gastric membrane vesicles and catalyzes the electro-neutral exchange of intracellular H+ and extracellular K+ coupled with the hydrolysis of cytoplasmic ATP, which is responsible for the acid secretion in the stomach.[4,5] Drugs which treat acid-related diseases act ultimately through inhibiting H+/K+-ATPase activity [3]

A variety of heterocyclic drugs have been described inliterature as the (PPIs) proton pump inhibitors (omeprazole, esomeprazole, lansoprazole and pantoprazole) act by binding to the H+/K+-ATPase and have been used as therapeutics for a long time[6,7] However, the adverse side effects of long-term usage of these drugs caused strong desire for researchers to develop newalternativemedicines.[8]

Gastric ulcer and duodenal ulceroccur in the stomach and duodenal ampulla and are the common ulcers that young males are easy to infect these diseases.[9]

It is of great importance to enhance early preventionand treatment of gastric ulcer. A large number of chemical compounds from medicinal herbs express antiulcer activity.[10]Cissus quadrangularis L. is commonly known as

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"bone setter" which is used as a common food item in India. Various diseases like piles, asthma, tumors,fractures of bones, irregular menses and ear and eye diseases are found to be treated.

Studies haverevealed that C. quadrangularis also possesses analgesic, fracture healing and anti-oxidant property. The stem is found to be having gastro protective activity.[11]

Various phytoconstituents such as quercetin, β-sitosterol, ketosteroid, α-amyrin and α-amyrone, oxo-steroid, onocer-7-ene-3α, 21β-diol and onocer-7-ene3β, 21α-diol, stilbene derivatives, are identified. It is also rich in β-carotene.[12,13] The extract of Cissus quadrangularis Linn. has been reported to be having antiulcer and cytoprotective activity previously by various experimental ulcer models.[14] Here attempt has been made to perform in-vitro anti-ulcer activity of methanolic and chloroform extract of leaves of Cissus quadrangularis.

Materials And Methods:-

Chemicals:

Chemicals Folin-Ciocalteu's phenol reagents, Tris-HCl, Aluminum trichloride, were purchased from Sigma Aldrich. Magnesium chloride, Potassium chloride, Methanol and ATP were purchased from Sisco Research Laboratories, India.

Collection and authentication of plant:

The plant material was collected from the surroundings of Ramakrishna Colony,Thimmapur, Karimnagar district, Telangana, India during the month of January 2019 and was identified and authenticated by P. V. Prasanna, Botanical Survey of India, Deccan Regional Centre, Attapur (V), Hyderguda (P.O), Hyderabad-500048, Telangana, India. A voucher specimen No. **BSI/DRC/2018 - 19/Tech./801** dated 22/01/2019 has been deposited for further reference.

Extraction of plant material:

1. The leaves of Cissus quadrangularis L. were shade dried for a week and powdered. Soxhlation extraction was done using Soxhlet apparatus for 100 g of the powdered drug with 95% of methanol. The extract was filtered with a muslin cloth and then filtered with a Whatman filter paper (Whatman No. 4) and concentrated at a rotary vacuum evaporator and was stored at 4°C for further use.
2. 100g of dried leaves of Cissus quadrangularis L. was extracted with chloroform by maceration. The extract/mixture thus obtained was subjected to evaporation on water bath until it becomes semisolid, then was stored in air tight container for further use.

Phytochemical analysis:

Test for alkaloids

Alkaloids are tested by the following reagents; each reagent or test has accuracy and specificity.

1. Dragendroff'sreagent test

The reagent is constituted with Potassium Bismuth Iodide (PBI). Alkaloids give reddish brown color with this reagent.

2. Hager's reagent test

This reagent constitutes of picric acid alkaloids give yellow precipitate with the Hager's reagent.

Tests for glycosides

1. Test-A

Dissolve the 200mg drug with Sulphuric acid, then add5% NaOH solution for neutralization, add Fehling's solution A&B to the above mixture. Red color is produced.

2. Test-B

Dissolve he 200mg of drug in sufficient amount of water. Add further water to dilute the solution. The solution is tested with Fehling's solution A&B. reducing sugar present in the drug produces red color.

- Compare the red color produced from the two tests of the drug
- If the color of the test-A is more intense than test-B; glycosides presence is confirmed.

Test for saponins
Foam test

- Place 1ml solution of drug in water in a semi-microtube and then it is shaken
- Later the formation of stable foam confirms the presence of saponin.

Test for carbohydrates
Molisch's test
Test solution along with a naphthol and then concentrated H_2SO_4 gives violet color which indicated the presence of carbohydrates.

Selivanoff's test
Test solution along with concentrated H_2SO_4, produces rose color which indicates the presence of carbohydrates.

Test for flavonoids
Shinoda test
A pinch of the dried extracts was dissolved in ethanol, mixed thoroughly and filtered. To the filtrate pieces of magnesium metal and concentrated hydrochloric acid were added and heated. Formation of magenta colour indicates the presence of flavonoid.

Alkaline reagent test
As the name suggest alkaline is used as a reagent for this test. Sodium hydroxide is added to the drug, yellow color is produced; if on addition of dilute acid then this color disappears then it confirms the presence of flavonoids

Test for tannins
FeCL3 test
Yellow color is produced with $FeCl_3$ in the case of hydrolysable tannins whereas condensed tannins produce green color.

Gelatin test
Precipitate is produced with gelatin which confirms the presence of tannins.

Phyto-analytical studies:
Determination of total phenolic compounds: [15]
According to Chun et al (2003) and few modifications in it by using Folin-Ciocalteu reagent and pyrocatechol as a standard phenolic compound. The total phenolic concentration dissolved in the extract was determined as µg of pyrocatechol equivalent by using an equation which was obtained from the graph of standard pyrocatechol:

Absorbance = 0.0057×total phenols [pyrocatechol equivalent (µg)] -0.0061

Assay for the total flavonoid content[16,17]
By using aluminum chloride colorimetric assay (Zhishen et al 1999, Zou et al 2004). The concentrations of total flavonoid compounds were determined and calculated. To 0.5 mL of test sample solution in methanol (5mg/100mL) 150 µl of 5% sodium nitrate and 2mL of distilled water were added. After few min., 150 µl of 10% aluminum chloride and 2mL of 1 M NaOH was added and left untouched for 15 min at room temperature. Absorbance was determined at 510 nm and total flavonoid contents was calculated as equivalents of quercetin from a quercetin calibration curve.

Absorbance = 0.0334 quercetin (µg) - 0.0002; $R^2 = 0.9996$

Assay of H^+-K^+ ATPase activity:
Extraction of parietal cells:
The sheep's stomach was obtained from the slaughter house in Karimnagar. Immediately after the slaughter it was brought to the lab and was washed with ice cold normal saline and the mucosal scraping was done and was homogenized in 200mM Tris HCl buffer of pH-7.4 and then centrifuged at 5000rpm for 10 min., further the supernatant was centrifuged for 20 min at 5000rpm.

The protein concentration was determined using BSA as a standard. The parietal cells extracted were further used for the study of H^+-K^+ ATPase activity.[18]

Determination of H^+-K^+ ATPase activity:

The H+K+ ATPase activity of the chloroform and methanolic extract was determined by using the method Reyes-Chilpaet al 2006, by some modifications. The parietal cell extract was preincubated with different concentration of the different (chloroform and methanol extract) test materials/standard (10,20,50 and 100 µg/ml) for 30min. Later 20mM tris-HCl (pH 7.4), 2mM magnesium chloride (MgCl2) and 2mM potassium chloride (KCl) were added and the reaction was started by the addition of 2mM adenosine-5'-triphosphate (ATP) and incubated for 30 mins at 27±5ºC and was terminated with the addition of 10% trichloroacetic acid.Finally centrifugation was done at 2000rpm for 10min. The amount of inorganic phosphorous released from adenosine-5'-triphosphate (ATP) was analyzed with spectral analysis at 640nm.[18]

Results:-

Phytochemical analysis:

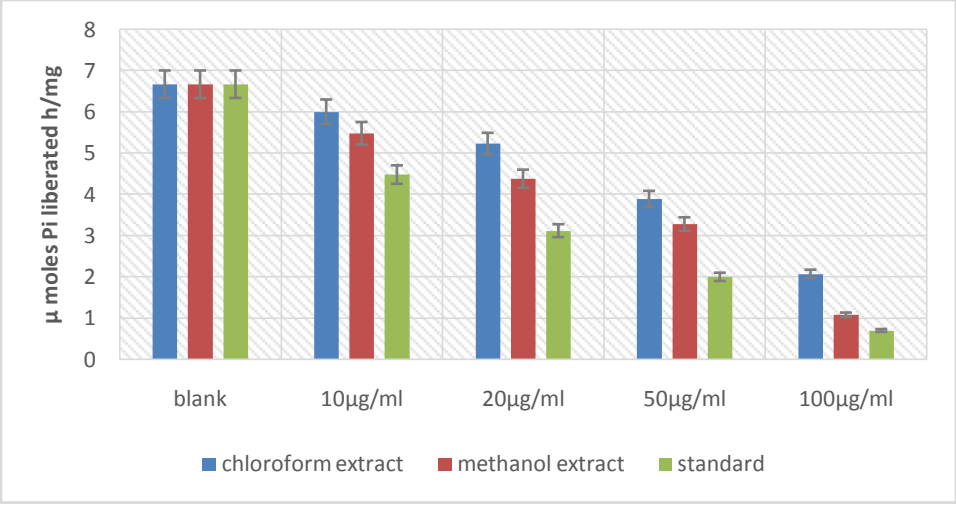
	Different Solvents	
Test	**Chloroform**	**Methanol**
Flavonoids	+	+
Alkaloids	+	+
Phenolic compounds	+	–
Tannins	–	+
Triterpenoids	+	+
Carbohydrates	–	–
Steroids	–	–
Saponins	+	+
Glycosides	+	–

Phyto-analytical studies:

It was performed for the methanolic extract and the total amount of the phenolic compound present in the extract was found to be 709.2 ± 2.32 mg PE (pyrocatechol equivalent)/100g. By using the standard curve of quercetin (R^2 = 0.9996), the total flavonoid content of the extract was found to be 162.9 ± 1082 mg QE (Quercetin equivalent)/100g. The total alkaloidal composition in the extract was found to be 15.84 mg/kg dry basis.

H^+-K^+ ATPase activity:

The H+-K+ATPase inhibition activity of chloroform and methanolic extract of *Cissus quadrangularis* at various concentration (10,20,50 and 100 µg/ml) were compared with Omeprazole as standard. The extract has shown significant (*P<0.05) proton pump inhibitory activity in the goat gastric mucosal homogenate in a dose dependent manner in which methanolic extract has shown better activity than chloroform extract.



Effect of the chloroform and methanolic extract of *C. quadrangularis*and omeprazole on H+-K+ ATPase activity

Conclusion:-

H+-K+ ATPase is an enzyme on apical secretory membrane of partial cell. Several drugs like PPIs, H2 blockers etc., are available for ulcer treatment but as they induce side effects and interactions, there is a need to produce herbal drugs with modern technology for effective and lesser side effect drugs. In the present research, dose-dependent inhibition of enzyme by extracts and omeprazole was observed. Methanolic extract has shown good inhibition than the chloroform extract and were significantly (*P<0.05) able to inhibit enzyme H+-K+ ATPase. The active compounds responsible for the gastro-protective action should be further studied for the safer drugs.

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