Traditional Herbal Medicine Against Caga and Vaca Toxin Genes-Producing Drug Resistant Helicobacter Pylori

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Abstract— Pan-Drug Resistant (PDR), Helicobacter pylori remains an unparalleled challenge to public health worldwide and this is largely due to the presence of the A-associated cytotoxin type A (CagA) and release of cytotoxin A (VacA). On the other hand, plant plants such as Syzygium aromatic contains various types of secondary metabolites, which can be used to fight H.pylori germs. To our knowledge, this is the first biomedical energy report of Aromatic extract against the cytotoxin-associated genes that produce PDR H. pylori. In this study, out of 45 gastral antral biopsy examples of patients with dyspeptic, 20 cases were confirmed as H. pylori. Eight (40%) out of 20 constipation was PDR H. pylori while other complications were Multi-Drug Resistant (MDR) types. Genotypic analysis of PDR H. pylori viruses showed that the genes of cagA and vacA were found in 75% and 87.5%, respectively and m2s2 was the most common subtype of the vacA gene. S. aromatic showed very high anti-H. pylori compared to Cinnamomum zeylanicum and Tymus vul- garis. Eugenol was the largest phenolic compound (28.14%) found in the methanolic extract of S. aromaticum. Clearly, the results of the toxicity test confirmed the safety of S. aromaticum of use. Therefore, these results suggest that S.Aromaticum may be a new and effective antimicrobial agent unable to fight the cytotoxin genes that produce the drug H. pylori. Moreover, these the findings provide a scientific basis for the development of antimicrobial agents from traditional remedies anti-inflammatory drugs against gastric ulcer.

I. INTRODUCTION

Currently, there is a shortage of effective antimicrobials, too equipment is still being developed to treatb coronavirus 2019 infection (COVID-19). China used Chinese and Western combined drug for treating patients with COVID-19. As a fourth version Guide (Diagnosis and Treatment Protocol of Novel Corona- the virus is pneumonia issued by the National Health Commission and the State Chinese Traditional Medicine Management) 1 clearly identified use that blinds of antimicrobials should be avoided. It is commonly believed in traditional Chinese medicine that COVID-19 is a pandemic considering its epidemic as well infectious environment. Included time and event geo-graphical environment, Infection with Helicobacter pylori (H. pylori) is a well-known risk factor with human gastritis, stomach cancer and associated with inflammation diseases.1e3 Stomach cancer is an example of the creation caused by inflammation cancer, known as the third most common cause of cancer-related deaths worldwide.4 H. pylori formerly chris- tened as a class 1 carcinogen by the World Health Organization (WHO), identified the formation of an epidemic that has already emerged as a system the cause of gastric carcinoma.5 Up to 50% of the world's population H. pylori ports in the upper intestine and highly infected people have symptoms.6 On the other hand, the increase in H. pylori infection is growing at an alarming rate in the development of the world tries as Egypt and India. The characteristics of bacterial virulence are often important factors in H.pyloriehost interaction of

pathogenesis, in which the risk of lesions is higher for more severe diseases.9 Between 5 and 10% H. pylori's 1600. The genes are thought to be related to H. pylori, are related to cytotoxin (CagA) and cytotoxin release (VacA) are considered to be the best- defined the adjectives of violence in H. pylori.

II. MATERIALS AND METHOD

- Processing of gastric biopsy samples

Biopsy specimens were collected in 45 dyspeptic patients who studied at the Endoscopic Unit of Tanta University Hospital, after written consent from these informed patients final results. Two abdominal biopsies were taken from the antrum for each patient. Patients taking antiretroviral drugs, pompon proton inhibitor, and / or bismuth salt two weeks before endoscopy released. The first biopsy was inserted directly into the sterile tube containing 1 ml of Phosphate Buffer Saline (PBS) solution or more up to 1 ml Tryptic Soy Broth (TSB) as a means of transportation, then transferred to Microbiology, Faculty of Science, and Tanta**.** The University also explored culture as described earlier a second biopsy was used for histopathology examination.

- Separation and subdivision of H. pylori

Phenotypic Definition

Processed specimens are less than 1 h. Each biopsy the sample from each patient was separated separately within it transport to the mill (mud) mill using sterile pestle and immediately injected into the Columbia Blood Agar (CBA; Oxoid, England) plates are added with 5% melted sheep blood, including, trimethoprim (5 mg / l), cefsulodin (5 mg / l), van- comycin (10 mg / l), and amphotericin B (5 mg / l). Plates were not included rated 37. C in anaerobic pot 3e10 days less microaerophilic conditions (5% O2, 10% CO2 and 85% N2) through Campygen kits (Oxoid, Basingstoke, UK). Small pieces from each a milled biopsy sample was inserted separately from Christensen Urea Broth (CUB) for quick urease test. Bacterial morphology was tested by Gram staining to confirm the presence of Gram-negative rod-shaped bacteria as well normal colonial morphology (small circular colonies) as shown in Additional data;. Bacterial detection was obtained from. The microaerophilic conditions are confirmed as phenotypically as H. pylori on the basis of positive reaction of urease, catalase and oxidase testing.39 In this study 20 of the 45 biopsies implanted in the abdomen were right on H. pylori and were designated as HP-1 to HP-20.

Definition of cells

Total genomic DNA was extracted from biopsy homogenates using Quick-DNA ™ Mini prep kit (Zymo Research, USA) in accordance with the manufacturer's guidelines. Methods used for determines the concentration of genomic DNA virus, purification, PCR amplification, and DNA sequencing were actually built according to previous reports .40e43 Genotypic characteriza- H. pylori performed PCR analysis for multiple pathogens Genetics including cagA, vacA and 16S rRNA. Primers & PCR ampli- The design conditions used in this study are given in PCR products are guaranteed size and purity in 2% agarose gel run with 1 TAE buffer and stained with ethidium bromide.

Histopathologic examination

Histopathology was performed to confirm infection with H. pylori. Abdominal biopsies used for histopathology were present delivered with neutral formalin (10%) for at least 24 h to Histopathology Laboratory, Faculty of Medicine, Tanta Univer- unity. These biopsies were analyzed.44 Stages of biopsy contaminated with a changed Giemsa color to find that ence kaH. pylori and pathological mutation testing gastric mucosa.

III. TESTS FOR ANTIBIOTIC RESISTANCE

In the current study sections of antibiotics of different classes (Oxoid, In England) it was used to detect the in vitro sensitivity of H. Pylori constructive clinical issues in ten of the most widely used anti-viral agents in the treatment of H. pylori the tendency of bacteria to antibiotics are made in the form of disk diffusion. Vated plates are placed under microaerophilic conditions 72 h to 37 C. The block area was interpreted based on Clinical and Laboratory Standards Institute (CLSI) .45 Twenty H. pylori positive clinical issues, eight are shown as PDR types.

- Planting materials

Three herbs are Clove (Syyyomgium aromaticum L.) Cinnamon (Cinnamomum zeylanicum Nees.) And Thyme (Thymus Vulgaris L) was used in this study and they are selected based on research in their traditional use wood, and its use in popular foods especially in Egypt. The plants were purchased at a local market in Tanta, Egypt. Botanical identification of plant samples was performed at the site of Herbarium, Department of Plants, Faculty of Science, Tanta Univer- sity, and Egypt.

- Remove preparations and antibacterial activity

The dried parts of the selected plants were ground into a powder using a blender. The extraction process is done as before mentioned.27 In short, methanol and ethanol have been used as organic extraction solvents. Five grams of herbal powder each. The plant used in this study was immersed in 40 ml of 3e4 solvent days. Keep the output filtered and focused on the rotating machine vapor at 35 C. Remaining water removed by vac-uum pump. Unused pieces of weight were suspended in dimethyl sulfoxide (DMSO) goes to the final filter of 50 mg / ml and stored in the refrigerator. Since DMSO has no antimicrobial activity, it was used as a negative control. Preparation of aqueous output, the same amount of planting material was distilled water. Types of PDR H. pylori tested by suscepti- the art of different extraction of selected plants makes good use of agar method of distribution.46 In short, 100 ml of new H culture. Pylori (106 CFU / ml) was applied to the face with sterile cigarettes Muller-Hinton blood agar (MHBA). Plates raised for thirst 10 min and springs with a diameter of 9 mm were made in the MHBA area using a coork borer. A fixed dose of 100 ml per extract in 100 mg / ml was added to the sources. DiMethyl SulfOxide (DMSO) was regarded as poor control. Tests determined on three times the results are presented as descriptive ± SD. Meaning calculates the area of the width of the barriers and is written in millimeters.

- Description of Syzygium aromaticum extraction

The methanol extract of S.Aromaticum was made by Gas Chromatography-Mass Spectrometry (GC-MS) Analysis using GC- MS Model Claus 580 / 560S, Perkin Elmer Company. GC status tions used according to Safrudin et al. 47 Name, molecular weight and integrated nature of S.aromaticum extraction identified based on the National Institute of Standard also Digital database database (NIST) data. Fourier Transform InfraRed (FT-IR) analysis was performed determining the functional groups of the element S. aromaticum extract using FT / IR spectrophotometer Perkin-Elmer 1430. Samples were prepared21 and scanned within transmittance range 4000-400 cm.

- Cytotoxicity testing

Cytotoxicity testing was used to determine treatment focus- tion has no toxic effect on normal cells. Border Blood Mononuclear Cells (PBMCs) are selected as normal cells modeling for this experiment. Potential cytotoxicity of the selected S. aromaticum methanol extract was made48 using a different method concentration of this plant extract (100e1.5 mg / ml). Cell functioning is listed as follows [cell functionality% ¼ (managing controlled cells cells) / controlled cells x 100]. This experiment was decided three times- gates and outputs are introduced as a word meaning ± SD.

- Statistical analysis

In this study, PC-ORD for windows (ver.5) was used in two waysposition Analysis of the set of positions using Sorensen methods of distance and beta (0.025) for group communication. Data collected, table tables analyzed statistics using Minitab 17.1.0.0 windows (Minitab Inc., 2013, Pennsylvania, USA). All tests were two on the sides. P-value <0.05 is considered significant. Data familiarity tested using the Shapiro-Wilk test. India-a pendent t-test was used, and a chi-square test comparison of between two or more categories of category details. One way or two how the ANOVA test was used to compare between more than two groups.

IV. RESULTS AND DISCUSSION

- Personality characteristics and clinics of patients

The current study was performed on 45 dyspeptic patients at the clinic it is expected that there will be an infection caused by H. The mean age of patients with positive H. pylori was 53 years, i.e. was older than

those with pneumonia H. pylori but with insignificant statistical differences. Most patients were present men (31/45; 68.9%) and from rural areas (36/45; 80%) with an unimportant mathematical organization of a particular gender or residence with good H shape. pylori Or 40% of H patients. constructive pylori smoked but insignificant effect, so there is no relationship between smoking and infection in H. pylori. Endoscopic diagnosis was different in patients the majority (65%) of patients with positive H. pylori showed peptic ulcer (duodenal or gastric), inflammatory mucosa was present in 20% of cases, with insignificant peptic congestion a wound or swelling with a positive H-shape. pylori. In addition, 20 (44.4%) of gastral antral biopsy samples were obtained the tradition of H. Pylori. Antrum is a abdominal biopsy site used by Most endoscopists have great clarity and sensitivity (up to 90%). Gastric Antral biopsy specimens were recorded as such very sensitive to H. pylori compared to corpus males.

- Histopathologic examination

Positive H. pylori biopsies gastric showed pathological changes in the gastric mucosa and indicate the presence of H. pylori is colonized in the light of the gastrointestinal tract, chronic inflammatory penetration into lamina propria. Use On the other hand, the negative H. pylori biopsies gastric showed normal hu-gastrointestinal tract and the absence of H.pylori bacteria from lumen of the gastric mucosa Advertisement for histopathology- the advantages include its ability to ensure infection with H. pylori in high definition and can explain the level of inflammation. In this study H. Pylori was determined by histopa- thology in 44.4% of the total cases studied. The inflamed mucosa was present the most prominent picture of the history of endoscopic biopsy in both positive and negative H. pylori patients, non-essential joining a particular group of them.

- Tests for antibiotic availability

Integrated combination of pharmaceutical production H. pylori presented revealed that the green color collections, contained eight species; HP-1, HP-5, HP-8, HP-10, HP-12, HP-14, HP-15 and HP- 16 showed 100% resistance to antibiotics tested and available proved to be PDR issues, while the remaining 12 species (red colour clusters) are classified as MDR variants .High level of antimicrobial resistance among distant species H Egyptian dyspeptic patients are considered important findings in this study, in which the highest resistance of clinics was 20 H. pylori types recorded by amoxicillin (AX) and ampicillin (AM) have been found 100%, followed by metronidazole (MTZ; 95%), clarithromycin (CLR; 90%) and erythromycin (ERY; 90%.

- Anti-H. pylori activity of various plant extract

In current research, ethanol, methanol and powerful ads fo S. aromaticum, C. zeylanicum and T. Vulgaris tested in vitro for their preventive work against PDR H. pylori problems using agar distribution method. Results are displayed that all the extracts of the tested plants showed anti-H. pylori and function the diameter of the blocking area between zero and 25 ± 0.57 mm while, the methanol extraction of these tested plants shows consideration- Work against H. pylori is compared to ethanol and aqueous issued. In addition, DMSO does not have antimicrobial activity Castillo-Juarez et al.

- Definition of Syzygium aromaticum methanol extracted from GC-MS and FT-IR analysis

Seven parts found in S. aromaticum methanol extracted GC-MS analysis is provided and Eugenol ($C_{10}H_{12}O_2$) is the largest phenolic compound present in meth- extraction of anol of clove (28.14), followed by eugenol acetate (12.43%) and 4-hydroxy-4-methyl-2-pentanone (5%) High level of eugenol in clove is responsible for its strong biological activity tivities.71 Several studies reported antifungal, antibacterial and anti-inflammatory activities of eugenol, which also had anti-inflammatory properties. The work of H. Pylori.72,73 GC-MS data showed eugenol as the largest a portion of clove methanol extracted with molecular ion peak 164 m / z .

- Cytotoxicity testing

Cytotoxicity effects showed that clove The use of methanol extraction was safe even to the maximum concentration (100 mg / ml) tested in PBMCs. Obviously, the level of inhibition increases with increasing concentration of S.Aromaticum discharge was tested, while high concentration of clove

extraction did not reach the IC50 test, and showed the prevention rate is 46.73%. These results are consistent Hamad et al. 50 studied the cytotoxicity of different herbs released and reported that up to a high concentration (20 mg / ml), emissions did not reach IC50 and show notion rate of 22.51%.

CONCLUSION

To the best of our knowledge, this study could begin to be investigate the biomedical potential of S.aromaticum extract against the cytotoxin-associated genes that produce drug resistance H. pylori. This study has shown that the methanol release of S. aromaticum has been shown to be promising for biomedical use program fields, because it is combined with the best and most important antiac- tone function against PDR H. pylori. Efficiency of S. aromaticum may be due to the presence of eugenol as a major phenolic it was his. Therefore, S. aromaticum methanolic extract can be It is recommended to treat cytotoxin-associated gene production PDR, which has the potential to increase the effectiveness of combat drug-resistant germs. This course can serve as a fruitful platform for check out the novel output as a new limited lead structure Medications against H. pylori. Moreover, continuously studies are needed to develop intestinal protective equipment the aroma of S. aromatic.

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