

The Effect of Red Rose Extract on Helicobacter Pylori Eradication: A Randomized Controlled Clinical Trial

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Abstract

Background

Helicobacter pylori (*H. pylori*) is believed to be the most common bacterial infection worldwide. Herein, we aimed to investigate the effect of red rose extract on *H. pylori* eradication in 9 to 15 year-old children.

Materials and Methods

In this randomized controlled clinical trial, by the convenience sampling method 332 children were screened for *H. pylori* infection. Their stool samples were studied by the *H. pylori* Antigen Enzyme Immunoassay (EIA) Test Kit. Positive cases were divided into two groups and received either red rose extract or placebo for 14 days. The stool exam was repeated once again at treatment termination. Data were analyzed using SPSS software (version 21.0).

Results

In total 17.8% (n=59) of the 332 cases were positive for Helicobacter pylori stool antigen (HpSA), and 56 completed the study. The mean HpSA titer remained stable in the red rose extract treated group but it increased in the placebo group following intervention; however, the difference was statistically insignificant (p=0.57). Moreover, the intervention resulted in no difference in the eradication rate between the two groups (p=0.57).

Conclusion

Red rose extract did not reduce HpSA titer after intervention, so it cannot be solely considered as an alternative for *H. pylori* eradication. Further studies with a different dose and duration or with combined regimens are recommended.

Key Words: Children, Helicobacter pylori, Red rose extract.

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1- INTRODUCTION

In general, around 50% of the world population is affected by Helicobacter pylori (*H. pylori*) infection, among which 70% of the cases are asymptomatic (1). *H. pylori* has a prevalence of 25% in the developed world which goes up to 66% in developing nations (2, 3). *H. pylori* is a major risk factor for peptic ulcer disease and gastric malignancies such as mucosa-associated lymphoid tissue lymphoma and gastric adenocarcinoma (4). In children, it can cause abdominal pain, vomiting, less commonly iron-deficiency anemia and rarely autoimmune thrombocytopenia (5). Diarrhea, heartburn and morning hunger are its other reported symptoms (6). WHO has classified *H. pylori* as a Class I carcinogen (5); therefore, *H. pylori* eradication in childhood will not only result in peptic symptoms relief, but could also prevent long-term complications such as cancer (7).

H. pylori infection can be diagnosed by invasive techniques such as endoscopy and biopsy or noninvasive methods such as culture, rapid urease test (RUT), histology or polymerase chain reaction (PCR). The non-invasive approaches are based on detecting the infection in breath by urea breath test, in blood by serologic tests and in stool by *H. pylori* stool antigen (HpSA) testing. The latter test has a sensitivity of 94% and specificity of 98% (6). In a study conducted in Iran the sensitivity and specificity of HpSA testing for *H. pylori* diagnosis was reported 85% and 93%, respectively (3); the same figures were 96.3% and 95.1% in a Mexican study (7). On the other hand, as the successful treatment of *H. pylori* is a multifactorial task with regards to antibiotic resistance, patient compliance, mucosal drug concentration and the cost and side effects, its treatment is still a major challenge (8). Treatment in children, similar to adults, is based on the administration of proton-pump inhibitors (PPI) plus two antibiotics

for 10-14 days according to the microbial resistance in that area (9). Antibiotics (ABs) overuse and accumulation of point mutations in the *H. pylori* DNA have been regarded as the main causes of increased antibiotic resistance in the last decade (10). Therefore, special attention is required regarding the regional resistance of *H. pylori* to antibiotics in choosing the appropriate treatment regimen. Nevertheless, today treatment failure is experienced in 20% of the treated cases; this rate is even higher among children. The most common cause for this undesired event is poor compliance and AB resistance (11). Therefore, today many research programs have focused on the discovery of new molecules which are effective in the treatment of *H. pylori* infection (8); the development of a novel alternative treatment regimen which is safe, cheap, has fewer adverse effects and results in better compliance among patients is highly anticipated for *H. pylori* eradication. On the other hand, ABs can interfere with the normal gut microflora resulting in the dominance of pathogenic bacteria.

In recent years, a number of studies have recommended Phytomedicine to have complementary function in *H. pylori* treatment via its prevention through the use of safe, inexpensive and non-toxic anti-*H. pylori* formulations from medicinal plants (8). *Rosa Damascenais*, one of the most important species of the Rosaceae family which has been considered in the treatment of *H. pylori* in animal studies (12). In addition to its applications in the perfume industry, pharmacology and industrial products, in ancient medicine it was used for abdominal pain and chest pain and for increasing the contractibility of the heart muscle (13). It has shown positive effects in the treatment of digestive disorders and has anti-HIV, anti-bacterial, antioxidant, hypnotic, anti-diabetic and sedative effects (14, 15). In

this study we aimed to investigate the effect of *Rosa Damascena* on *H. pylori* eradication in 9 to 15-year-old children in the city of Birjand, Iran.

2- MATERIALS AND METHODS

2-1. Trial design

In this randomized controlled clinical trial 332 children aged 9-15 years selected from 8 schools of Birjand (Iran) were enrolled from March 2015 to 2016. The city of Birjand was initially divided into 4 socioeconomic zones which were considered as statistical classes; the study population was selected from the schools of each zone based on the convenience sampling method. Each time 40 to 80 samples were tested along with 2 controls and 6 calibrators. In order to increase the test's accuracy in each series, 3 samples were prepared twice, tested and the results were compared. An antigen titer ≥ 3 $\mu\text{g/mL}$ was considered as positive.

2-2. Participants

All cases who were not willing to cooperate, had clear gastrointestinal symptoms requiring treatment, had used ABs in the last month, were using PPI or H_2 -blockers and had immunodeficiency or diarrhea at sampling, were excluded in the initial phase of the study. Children with any type of food or drug allergy and AB consumption due to any other disease during the intervention course were also excluded from the study. In the initial study phase 59 of the 332 samples resulted as positive for HpSA. Three cases were excluded due to AB consumption and therefore 56 entered the 2nd study phase. They were then divided into the red rose extract treated group and placebo group with 1:1 block randomization.

2-3. Ethics

In this study, all researchers were committed to the Helsinki Statement. The study protocol was fully approved by the

Research Ethics Committee of Birjand University of Medical Sciences and a written informed consent was obtained from each child or their parent/guardian prior to study entrance. Also, it was registered in Iranian Registry of Clinical Trials (IRCT2016052328012N1).

2-4. Intervention

The prescribed drug and placebo were given to the patients in a double blind manner.

2-5. Preparation of red rose extract capsules

The flowers of *Rosa Damascena* were collected from Kashan area (Isfahan Province, Iran), in spring and were identified by the botanists of Pharmacognosy Research Center, School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences (Iran). The flowers were dried in shadow and ground into a fine powder using a grinder. The aqueous extract was prepared by macerating the powder in distilled water (120 g/L) for 30 min at 90 °C. The extract obtained was filtered through a paper filter and dried; the yield of extraction was 30% w/w of the dried flower (1 g extract = 3.3 g dried herb). The red rose extract capsules were manufactured by the Phytopathology Research Center of Jundishapur University, Ahvaz, Iran. The placebo capsules were matched in shape, color and size with the red rose capsules and placed in similar 28-capsule packets with a certain code. The patients were asked to take 2 capsules daily for 14 days. In addition to the weekly follow-up by telephone, the parents were free to contact the researchers at any time. In the weekly follow-up the timing and amount of drug consumed, reasons for not taking the drug, the observed outcomes in the child and type of consumption was recorded in the questionnaire. The participants were once again visited at the end of the 2-week period and another stool sample was taken

in two consecutive days. Drug consumption over 80% was regarded as acceptable. The study protocol was described to all cases and a designed questionnaire including demographic data was completed for each case. To study H. pylori infection, the level of HpSA was measured in their stool. Each visit a 1-2 g stool sample was collected from each case in a sterile and dry sampling dish at 7 AM. The sample was delivered to the lab in under 2h at -4 °C; it was stored at -20 °C up to the time of analysis. HpSA level was measured by the ELISA sandwich method and the IgM H. pylori kit (manufactured by IBL, Germany). The two main studied outcome measures were the changes in the HpSA titer and the H. pylori eradication rate.

2-6. Statistical analysis

The collected data were then analyzed by the SPSS software ver. 21. Chi-square test was used for qualitative variables. T test and Mann-Whitney test were used for intergroup comparisons while paired sample t test and Wilcoxon's test were used for before-after comparisons in each group. The significance level was set at P<0.05.

3- RESULTS

In total 332 children were studied, among which 59 (17.8%) had a positive HpSA test. Three cases were excluded due to non-compliance or a simultaneous disease requiring medication. Therefore, 56 children completed the study; 28 in each of the red rose extract and placebo

groups. Moreover, 4 cases in the placebo group and 5 cases in the red rose extract group were excluded due to not taking the medication regularly. The final analyses were performed on the remaining 47 subjects; 23 cases and 24 controls. The patients' mean age in the red rose extract and placebo groups were 13.33±1.47 and 13.04±1.61 years, respectively (P=0.52). Regarding a family history of digestive disorders, 57.1% and 36.4% of the cases in the red rose extract treated and placebo groups had such a history, yet no significant difference was demonstrated between the two groups (P=0.77). The mean HpSA titer before the intervention was slightly higher in the placebo group, but statistically insignificant (P=0.68; **Table.1**). After the intervention 22 (95.7%) cases and 18 (81.8%) controls had a positive HpSA test, but no significant difference was observed between the two groups (P=0.19). T-test showed an increase in the mean HpSA titer in the placebo group after the intervention whereas it remained stable in the red rose extract treated group (26.10±29.50 vs. 18.10±18.83); indicating no meaningful difference (P=0.28). The mean change in HpSA titer before and after intervention was higher in the placebo group, still demonstrating no significant difference (P=0.57). In addition, no significant difference was seen in HpSA titer change before and after the intervention in either group (**Table.1**). In general, the intervention resulted in no difference in the eradication rate between the two groups.)

Table-1: Comparing the mean H. pylori fecal antigen level before and after the intervention between the two groups.

Group	Stage	Mean	Standard deviation	t-test	df	P-value
Red Rose Extract Group	Before Intervention	18.01	15.38	0.02	22	0.99
	After Intervention	18.10	18.83			
Placebo group	Before intervention	21.53	29.23	0.82	21	0.42
	After intervention	26.10	29.50			

df: Degree of freedom.

4- DISCUSSION

The higher prevalence of *H. pylori* infection in the aforementioned studies may be due to conducting the current study on asymptomatic children whereas in such studies all the cases visiting the clinic, regardless of AB consumption or just having clinical symptoms, were studied. Moreover, the age range also differs between these studies. In this study we aimed to investigate the effect of *Rosa Damascena* on *H. pylori* eradication in 9 to 15-year-old children in the city of Birjand, Iran. In our study the prevalence of *H. pylori* infection increased by age ($p < 0.05$) which was similar to the findings of Ahanjan et al., and Saket et al.'s studies (17, 13). In Soltani et al.'s study (2013) performed in the western parts of Iran, a significant correlation was reported between aging and *H. pylori* infection (3). However, the findings of Ghanei et al. (2009), and Namakin et al.'s studies (2014) revealed no such correlation (15, 16).

Despite numerous studies, *H. pylori* infection is still treated by routine clinical prescriptions as triple or quadruple antibiotic therapy and its optimal treatment is not yet fully recognized (18). The prevalence of antibiotic resistance to various antimicrobials is highly dependent on the consumption of antibiotics in those areas, varying in different geographical regions (19). In a systematic review of literature on *H. pylori* antibiotic resistance carried out in Iran between 1997 and 2013, *H. pylori* resistance to various antibiotics was as follows: metronidazole 61.6%, clarithromycin 22.4%, furazolidone 21.6%, amoxicillin 16.0%, tetracycline 12.2%, ciprofloxacin 21.0% and levofloxacin 5.3% (20). Given the vast AB resistance in various diseases, in recent decades, special focus has been targeted at medicinal plants and herbal medicine by traditional and complementary medicine specialists for diseases' treatment and infection eradication. Several researches

also have been performed on different medicinal plant extracts containing polyphenol and sulforafan active substances in *H. pylori* infection. The antibacterial effects of red rose extract have been approved in animal models and therefore further human studies are anticipated. Some bioactive compounds from medicinal plants with anti-*H. pylori* activity mentioned in various literature include carvacrol, polyphenoliccatechins, tannins, cinnamaldehyde, eugenol, quercetin, licoricidin, licoisoflavone B, Berberine, sanguinarine, chelerythrine, protopine, β -hydrastine, mastic and plumbaginprotocatechuic acid (8). In the study by Ulusoy et al., in 2009 on the antibacterial effects of red rose extract and its components on *E. coli*, *S. aeruginosa*, *S. aureus* and *C. violaceum*, a stronger effect was observed on *C. violaceum* and *E. coli*, respectively (21). In another study by Zaidi et al., in 2012, red rose extract showed anti-inflammatory and antibacterial effects due to the inhibitory effect of this extract on IL-8 secretion (22).

These results are not exactly consistent with our findings; however, in our study the HpSA titer showed a rise in the placebo group after the intervention whereas no such increase was observed in the red rose extract treated group, rather it remained constant; this difference could be described by the inhibitory effect of red rose extract. However, it did not significantly affect the eradication rate or the decrease in the HpSA titer. Rosanol, a substance with a 1% concentration in red rose extract has shown beneficiary effects invitro such as elimination of inflammatory cells infiltration in peptic ulcer in rats. The rose oil and geraniol with 2mg/L concentration resulted in *H. pylori* growth inhibition as 85.7% and 92%, respectively. Accordingly, Rosanol was introduced as a substance with inhibitory effects on *H. pylori* growth besides its anti-ulcer, spasmolytic and antibacterial effects

(12). In a recent study the white rose (*Rosa hybrida*) petals extracted with ethanol or butanol resulted in inhibited growth of various fungi and bacteria besides effective killing of *H. pylori* and the elimination of bacteria from the mouse stomach (23). In the study by Horvath et al., carotenoid extracts of Rose hips (*Rosa canina* L.) revealed anti *H. pylori* activity in vitro (24). Nevertheless, the different method of the red rose extract preparation used in our study may have resulted in the reduction or elimination of this active substance.

On the other hand, several factors affect the increase in HpSA titer after the intervention in comparison to that before the intervention as follows: time duration between stool sample collection till stool examination, type of kit used at each time point, the lab and operator, other medications used during the study period, daily diet before and after the intervention, duration of drug consumption and the accompanying diseases during the study course. We considered all such factors in our study and all lab tests were done in the same lab by the same personnel.

In the present study, red rose extract was not effective on *H. pylori* colonization and infection eradication; however, it did stabilize the HpSA titer in the red rose extract treated group whereas a rise was observed in the placebo group. As the mode of action, potential cytotoxicity and benefits of herbal medicine are complex, incomplete and confusing (25), conducting precise clinical trials on promising herbal products is highly recommended in future investigations. Nevertheless, due to the variety of red rose species and also its extractable active substances, different extracting methods should be considered in future studies under the supervision of traditional medicine specialists along with a larger population and various durations of drug consumption.

5- CONCLUSION

Although the mean HpSA titer remained stable in the red rose extract treated group, it did not reduce *H. pylori* stool antigen titer after the intervention and therefore is not considered adequate for its eradication. Further studies with a different dose and duration or with combined regimens are recommended.

6- CONTRIBUTORS' STATEMENT

K.N. conceived the presented idea. H.M. developed the theory and performed the computations. M.H.M. and H.M. verified the analytical methods. K.N. encouraged M.H.M. to investigate laboratory tests and supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

7- CONFLICT OF INTEREST: None.

8- ACKNOWLEDGEMENTS

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