

***In-Vitro* the Anti-Protozoal Activity of Onions Extract (*Allium Cepa*) and Metronidazole in *Entamoebagingivalis* Which Cultured in Tysgm-9 Medium**

Raflaa S.H. Hussian*¹, Sura I.A. Jabuk¹, Maani Seher Abid Al-Khafaji², Shaymaa O.H. AL-Mamoori¹, Anmar M.AL mahmory¹, Zainab Amran¹ and Zeana Sh. Al-hindi³

¹ College of Science, University of Babylon/ Iraq.

³College of Medicine, University of Babylon/ Iraq.

³College of Science for Women, University of Babylon/ Iraq.

***Corresponding Author: Raflaa S.H. Hussian**

Abstract

Entamoebagingivalis (*E. gingivalis*) is an amoebae that is present in the human oral cavity associated with poor oral hygiene and advanced periodontal disease. The aim of the present study is to confirm antiprotozoal activity of Onions extract and metronidazole in *E. gingivalis* which cultured in TYSGM-9 medium as well as determinethe frequency of this among patients with periodontitisdisease. The study included 46 sample collected from patient with age (11-20) years, result show infection with this parasite 56.5 %. These samples are cultured in TYSGM-9 media, and then treated with Onions extract (*Allium cepa*) and metronidazole which show inhibited growth of parasite at 100µg/ml.

Keywords: *Entamoebagingivalis*, *Allium cepa*, *Metronidazole*.

Introduction

Periodontal disease is a disease, or more likely a number of diseases of the periodontal tissues that results in attachment loss and destruction of alveolar bone, about 5% to 20% of people are affected by intense periodontitis. [1, 2] *Entamoebagingivalis* human buccal protozoa, they are live in dental tartar in the necrotic mucosa of the cells and the gingival fringes of the gum [3].

The oral cavity is suitable for invasion of many microorganism, the protozoan *Entamoebagingivalis* recognized eukaryotic representative, Although these forms are not generally associated with pathogenesis, their presence in the oral cavity is taken as a sign of poor dental hygiene [4, 5, 6].

Entamoebagingivalis, is morphologically similar to *E. histolytica*, it has no cyst stage in its life cycle. It might be mistaken for *E. histolytica* from pulmonary abscess [7]. The exact method of transmission of this organism is not known, but mostprobably, it

may be transmitted through droplet spray from the infected mouth, from kissing, or from contaminated cups, dishes or eating utensils [8,9]. Metronidazole, a molecule previously demonstrated to be active against anaerobic bacteria and parasites [10]. Several studies indicated to the effectiveness of this antibiotic against *E. gingivalis* [11,12].

Onion is one food ingredients widely used in our gastronomy. Moreover, onion extract has been recently reported to be effective in cardiovascular disease, because of their hypocholesterolemic, hypolipidemic, anti-hypertensive, anti-diabetic, antithrombotic and anti-hyperhomocysteinemia effects, and to possess many other biological activities including antimicrobial, antioxidant, ant carcinogenic, ant mutagenic, antiasthma tic, immunomodulatory and prebiotic activities[13]. Numerous studies have proven the importance of the onion extracts as a promising treatment against bacterial and fungal and parasitic diseases [14-16].

Materials and Methods

Sample Collection

30 samples collected from dental patients in Private clinic to detect *Entamoeba gingivalis*.

Examine Parasite under Microscope

By using sterile cotton swap and examined directly under microscope to found the parasite. [17]

Used Culture

In current study used TYSGM-9 (Trypticase-Yeast Extract-Serum-Gastric Mucin) medium to incubate *E. gingivalis* at 37°C for 48-72 hours. This media preparation from Potassium phosphate dibasic 2.8 gm, Potassium phosphate monobasic 0.4 gm, Sodium chloride 7.5 gm, Casein digest peptone 2.0gm, Yeast extract (BBL) 1.0 gm, distilled water 970 ml. then incubated parasite at 37°C for 48-72 hours [18].

Used Extract of Onion (*Allium Cepa*)

Onion was drying at room temperature for several days then crush it to get powder, take (50gm) from it to mix with 100ml ethanol (99.9%) in an electric blender for 30 min. This suspension was filtered. Then methanol was removed in a rotary

evaporator to produce a dry powder. To obtain concentrations 12.5µg/ml, 25µg/ml, 50µg/ml and 100µg/ml, powder was dissolved in ethanol [19].

Metronidazole

Bought dissolved Metronidazole 500mg/ml from pharmacy, which prepare from it concentrations 12.5µg/ml, 25µg/ml, 50µg/ml and 100 µg/ml.

Spectrophotometer have been Used at 540 nm

Results

46 samples were collected from patients aged 11 to 20 years; the prevalence of *E. gingivalis* in our study was (56.5%). All samples were positive when cultured in TYSGM-9. In present study was investigated in vitro effect of Ethanol Extract of *Allium cepa* on the growth and motility of *E. gingivalis* is compared to the standard drug metronidazole with significant differences at the 0.05 level. Growth of parasite show in figure 1 and 2. Take drop of broth and calculate dead *E. gingivalis* / 100, which show 53% of *E. gingivalis* is killed with ethanol extract of Garlic and 85% of this parasite killed with metronidazole.

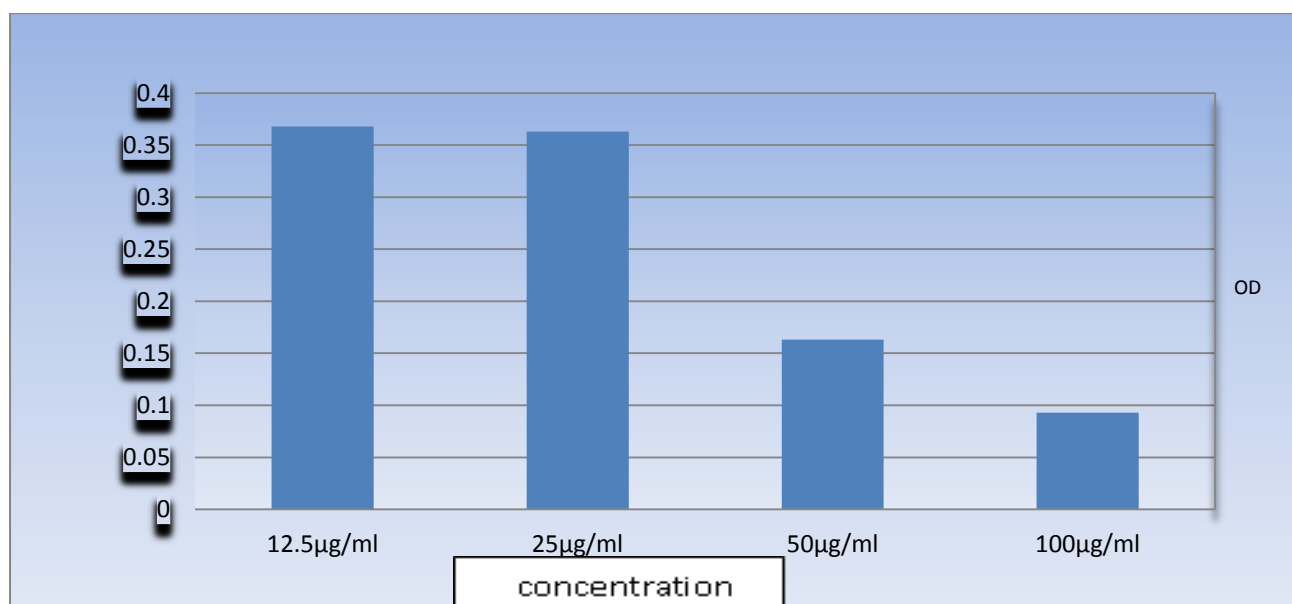


Figure 1: The effect of alcoholic extract of *Allium cepa* on *Entamoeba gingivalis*

Discussion

This study showed that people who suffer from inflammation in periodontal are most susceptible to parasites especially *Entamoeba gingivalis*. Prevalence of this parasite in our study reached to 56.5% among

patient with age (11-20) years. This finding was compatible with another study in Babylon city which show that the rate of infection with *E. gingivalis* was 53% [20] while was higher than another published reports which mostly ranged from 11.3 – 42.9 % [17,21,22,23].

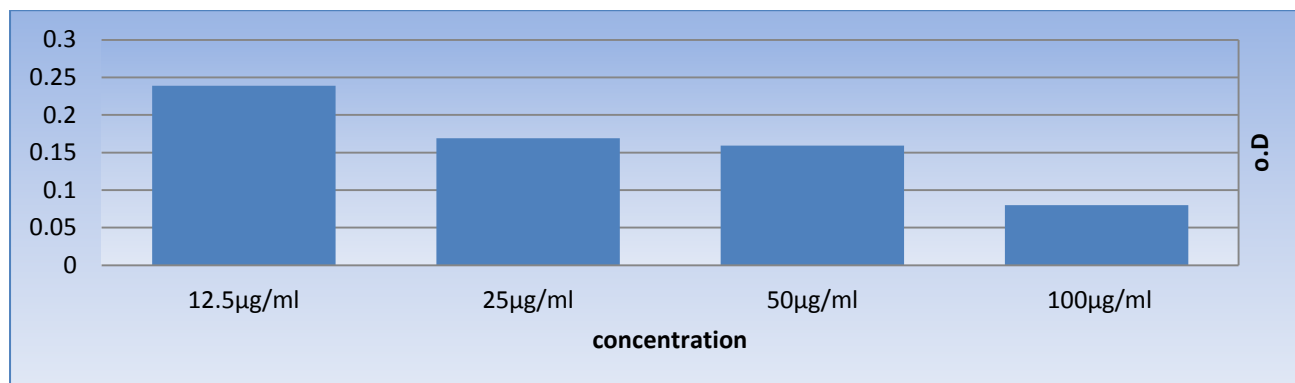


Figure 2: The effect of metronidazole on *Entamoebagingivalis*

The high prevalence rate of *Entamoebagingivalis* was related to mouth hygiene habit, periodontal tissue condition, gum bleeding, degree of decayed and loose teeth [21]. It has been noted in publications that the increased prevalence of *E. gingivalis* connected with diseases of the oral cavity, and in particular with periodontal diseases [24]. Furthermore [25] reported that parasitic infections are relatively common among patients with periodontal diseases. We investigated in vitro the activity of alcoholic extract of *Allium cepa* against *E. gingivalis* (Figure 1). After cultured parasite and deal with extract of onion, noted growth of

parasite was inhibition in 100 µg/ml (0.093) in compare with metronidazole 100 µg/ml (0.080) (figure 1). Onions is a one of the most important medicinal plants used in the treatment of many bacterial and parasitic diseases, the study of [26] showed antibacterial activity against multidrug resistant *Pseudomonas aeruginosa*, *Salmonella typhi*, *A. aerogenosa* and *P. vulgaricus*. Metronidazole was killed *E. gingivalis* (figure 2) and this result was similar to another result in other research which insures Metronidazole killed the parasite [27,28]. While other study proves metronidazole treat amoebiasis [29].

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