# Estimation The Effect of two Types of Honey "Natural and Artificial" on The Viability of *Escherichia coli in vitro* and *in vivo*

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## Abstract

In pre-Ancient Egyptian times more than five millennia ago used honey, the beneficial properties of honey have been explored in modern times. The present study were aimed to examine the effect of two types of honey ( Anchusa natural) and artificial in deferent concentration effect on *Escherichia coli,* The bioassay applied for determining of antimicrobial effect the well-agar diffusion method. The present study showed different honey concentrations (100, 75, 50, 25, 12,5 and 6,25 % v/v) against *Escherichia coli* with zones of inhibition ranges from 26 in 100% v/v to 6.5 mm in 25% v/v concentration and 0.5 mm for honey artificial, as in vitro examination. The differences between two types honey results were significant with Anchusa honey in compered with artificial honey. The BALB/c mouse model using for detect of shortens the duration of bacterial diarrhea by natural honey as in vivo colonization of *Escherichia coli*. The results obtained for the antibacterial characterization of honey when used as (100% v/v concentration – 0.1cc dose -4 times/daily) were disappear clinical signs of acute diarrhea within two to three days. The natural honey showed significant $P< 0.05$ effect to kill of *E.coli* bacteria compared with the control group and with artificial honey.

## تقدير تأثير نوعين من العسل "الطبيعي والاصطناعي" على حيوية ألأشريشيا القولونية خارج وداخل جسم الكائن ألحي

**الخلاصة**

في ألحضاره المصرية القديمة ومنذ أكثر من خمسة آلاف عام استخدم العسل، وقد تم اكتشاف الخصائص المفيده للعسل في العصر الحديث. تهدف هذه الدراسة إلى دراسة تأثير نوعين من العسل (لسان الثور الطبيعي) والمصنع على جدوى البكتريا القولونية، وألتجارب ألمختبريه داخل وخارج جسم ألكائن الحي. أظهرت هذه الدراسة تأثير تركيزات مختلفة للعسل (١٠٠، ٧٥، ٥٠، ٢٥، ١٢،٥ و ٦،٢٥٪ ت / ت) ضد ألأشريشيا القولونية مع مناطق تثبيط يتراوح من ٢٦ في ١٠٠٪ ت / ت إلى ٦.٥ ملم في ٢٥٪ ت / تركيز الخامس و ٠.٥ مم للعسل ألمصنع، كفحص مختبري باستخدام طريقة أنتشار ألأكار. أظهرت ألنتائج اختلاف كبير بين العسل ألطبيعي مقارنة مع العسل المصنع. أما نتائج التجارب داخل جسم ألكائن ألحي ألتي أستخدم فيه ألفأر كحيوان مختبري للكشف عن تقصير مدة الإسهال البكتيري المصاحب لبكتريا ألأشريشيا القولونية. وكانت (جرعة ٠.١-٤ مرة / يوميا ١٠٠٪ تركيز ت / ت) حيث تختفي ألعلامات ألسريرية من الإسهال الحاد في غضون يومين إلى ثلاثة أيام ،بينت النتائج وجود فروقات معنويه < ٠.٠٥ كمضاد للبكتيريا القولونية مقارنة مع مجموعة التحكم من جهه ومع العسل المصنع من جهه أخرى.

## Introduction

*Escherichia coli*, is a zoonotic enteric pathogen that causes human gastrointestinal illnesses, commonly found in the gut of endotherms (warm blooded organisms) [1]. Pathogenic *E. coli* strains are categorized into pathotypes. Six pathotypes which are associated with diarrhea and collectively are referred to as diarrheagenic *E. coli* [2] Entero-pathogenic *E. coli* (EPEC) is recognized as an important intestinal pathogen that frequently causes acute and persistent diarrhea in humans and animals [3]. Rotavirus was the most common enteric pathogen, detected in 27% of patients, followed by *Shigella* (12%), diarrhoeagenic *Escherichia coli* (8%), enteric adenovirus (8%), *Salmonella*

(4%), *Campylobacter jejuni* (3%) and *Plesiomonas shigelloides* (2%). Mixed bacterial/viral infections were detected in 6% of cases. Parasites, mostly protozoa, were detected in 14% of children [4].

Patients start off with *E. coli* infection symptoms- severe abdominal cramps, diarrhea which eventually becomes bloody, and nausea and vomiting (sometimes). While the most of patients make a full recovery within a week to 10 days [5]. Development of resistance against antibiotics and side effects of the drugs lead to search of alternative treatment. Among others, plant-based therapy including the combination with antibiotics or the usage of honey might be one option [6]. The high sugar concentration, hydrogen peroxide, and the low pH are well-known antibacterial factors in honey and more recently, methylglyoxal and the antimicrobial peptide bee defensin-1 were identified as important antibacterial compounds in honey [7]. Cold and flu symptoms, such as coughs, sore throats, and congestion are also treated with honey [8] and shortens the duration of bacterial diarrhea when used honey in oral rehydration solution in children and infants with gastroenteritis [9], natural honey kills bacteria three times more effectively" than an artificial honey solution of the same thickness and sugar concentration [10].

Our study was aimed to determine the antimicrobial activity of two types of honey *in vitro* and its effects *in vivo* regarding with shortens the duration of bacterial diarrhea by using specific dose of *E. coli* bacteria.

## Material and Methods

### 1- Honey sample

Anchusa honey was brought from specific farm near Hilla city, the source of feeding the bees was flowers of *Borago officinalis*, Whereas the artificial honey was brought from the market.

### 2- Bacterial sample

*E. coli* was isolated from children under two years old, with bloody-diarrhea, All samples collected from lab of diagnosis Microbiology (Al- Hashmiya hospital). Tests was prepared according to [11].

### 3- Antibacterial assay

Nutrient agar plates were flooded with test organism already activated in sterile nutrient broth by culturing at 37°C for 24 h. The well- agar diffusion method [12] against *E. coli*. The plates were drained and allowed to dry at 37°C for 30 min before wells of 3 mm in diameter were punched using a sterile cork borer at different sites on the plates. Different dilutions (100- 75- 50- 25- 12.5and 6.25% v/v) of a particular honey sample in sterile distilled water (v\v) were separately placed in the different punched wells and the plates were incubated at 37°C for 24 h. The diameter of the zones of inhibition was measured and recorded to anchusa natural and artificial honey as in vitro examination.

To detect of shortens the duration of bacterial diarrhea with *E. coli* infection, twelve adult healthy mice aged 3 to 4 months were selected. All mice had negative fecal bacteriological culture for *E. coli*. They were reared in separate cages in the animal house of veterinary college, University of Al-Qasim Green. The animals were divided equally into two groups (six for each group).

The infective dose (ID) was estimated by using eight fold dilution for counting bacteria by viable bacterial plate count method [13]. Estimated by choosing the group of mice which have no mortality but all the mice showed specific clinical signs for *E.coli*.

The first group (experimental) was drenched at a dose of 0.1 ml containing $1x10^5$ CFU/ml [14]. After appear the clinical signs were drenched Anchusa honey 0.1 ml/four times daily [15] until disappear the clinical signs. The second group (control) was drenched 0.1 ml containing $1x10^5$ CFU/ml. The duration of bacterial diarrhea was counting by day.

Stool samples were collected from the experimental and control groups after two to three days of drenching. These were

performed culturing the stool samples in different culture restricted media including MacConkey, eosin methylene blue media and incubated at (37)˚C throw (24) hr then examined bacteriologically.

**Statistical analysis**

The data were analyzed statistically, using the least significance differences test (LSD), T test and analysis of variance (ANOVA) at the P value level of 0.05 [16].

## Results and Discussion

The agar well-diffusion assay showed a significant increase in zones of inhibition in the plats experimental with Anchusa honey compared to the artificial honey, the diameter of zones of inhibition (mm) was increased after 24 hours, and appeared high antibacterial activity between the different concentration. All data that showed the correlation between bacterial activity and honey concentration described in table (1).

**Table 1:** Diameter of zones of inhibition (mm) of different concentration of Anchusa honey and Artificial honey by agar well-diffusion assay

| **Concent. Anchusa honey %** | 100 % | 75 % | 50 % | 25 % | 12.5 % | 6.25 % | **LSD P valu < 0.05** | Control group |
|---|---|---|---|---|---|---|---|---|
| **Inhibition zone (mm)** | 26 | 21 | 11 | 6.5 | 0.5 | 0.5 | 1.02 | 0.5 |
| **Artificial honey** | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0. 03 | 0.5 |

As well as the present study demonstrated that no significant correlation between bacterial activity and honey concentrations less than 1%, with minimal inhibition at 5%.This result is in agreement with [17] who reported that different honey concentrations against *E. coli* (ATCC 25922), show that 91% of samples have counts equal or lower than 1,0 x 10 (1) CFU/g. Other studies showed that the highest activity of Coniferous and Thyme honeys showed the highest activity with an average minimum dilution of 17.4 and 19.2% (w/v) followed by citrus and polyfloral honeys with 20.8 and 23.8% respectively [18].

**Clinical signs and bacterial isolation after drenching**

All mice were drenched of pathogenic *E.coli* with $ID_{50}$ ($1\times10^5$ CFU/ml) the experimental group exhibited the clinical signs of rest listlessness, weakness, diarrhea (semisolid or like pellet feces), rough coat, and hunched posture. This is mentioned by [19] the proximal colon of infected mice contained semisolid stool, with stool pellets forming only in the distal colon. Then returned to normalcy within two (4 mice) –three (2 mice) days (Figure 1). Indicated that the values were significantly high in the experimental group compared to the control group. The control group also exhibited the clinical signs after drenching: illness and depression with diarrhea continue for seven days. Bacterial isolation of stool shows the heavy spread of *E. coli* in control animals after two to three days of drenching with little spread of microorganism in experimental animals.

**Figure 1:** The duration of bacterial diarrhea of experimental and control groups

The raw, organic honey is loaded with vitamins, minerals, and enzymes showed protection role of the body from bacteria and boost the immune system [20]. Other research have been studied the effect of for type honey was more active against Gram-negative bacterial strains than the Gram-positive bacterial strains. In this investigation, shortens the duration of diarrhea showed significantly higher value in experimental animals. This result is in agreement with the many studies of [21, 22] who investigate the natural honey samples used were effective in inhibiting the growth of diarrhea-causing bacteria.

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