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SYNERGISTIC EFFECT OF HONEY WITH FOOD ADDITIVES AS ANTIMICROBIALS AND ON WEIGHT LOSS AND VARIOUS PHYSIOLOGICAL STUDIES IN MICE

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ABSTRACT: The preliminary phytochemical evaluation and effect of Honey on Hematological parameters were investigated. Preliminary phytochemical screening was carried out to determine the presence of various bioactive constituents through different Tests. The Antimicrobial study of different Honey in combination with synergistics was carried out on selective microorganisms through well diffusion method. The effect of Honey on hemoglobin, WBC, Blood Sugar and weight loss was studied on swiss albino male mice under controlled conditions for 21 days. The Highest percentage of coumarins (+++), Resins (+++) were found in all the types of tested Honey among the different phytochemicals Screened. However the absence of carboxylic carbon and tannins were noticed during the study. The antimicrobial studies along with different synergistics shown significant results like Natural Omani Honey exerted 36mm zone of inhibition against *S.aureus*, while Turkish honey found to be more antimicrobial against *P. aeruginosa* (40mm). In the present study on Hematological and physiological parameters it was observed that increase in WBC % with greatest significance, among all the tested Honey with Natural Turkish Honey we found more increase of WBC % upto 30%. These findings provides the information about the traditional use of Honey along with various food additives triggers more beneficial in treating various human ailments like controlling from microbial diseases, improving WBC to increase immunity etc. further evaluation needs further evaluation and identification active principles are required for pharmacological preparations.

INTRODUCTION: Honey is a mixture of sugars prepared by honey bees from the natural sugar solutions called nectar obtained from flowers or other plant secretions. Traditional honey is good source of energy produce by *Apis mellifera* (honeybees), it also has antioxidant and antimicrobial properties.

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It is complex mixture of sugar concentrated solution that also contain some of carbohydrate, aromatic substance, water, minerals, waxes, trace of protein, amino acids and other substances. Honey bees transform nectar into honey by a process of regurgitation and store it as a primary food source in wax honeycombs inside the beehive.

It has attractive chemical properties for baking and distinctive flavor that makes some people prefer it over sugar and other sweeteners. The physical properties of honey such as high density, high viscosity and moisture absorbance from air and immunity from spillage are due to concentrated solution of sugar [1].

The phytochemicals produced from the medicinal herbs are curative constituents for several diseases. Natural products derived from higher plants may form the source to search for novel drugs based on their new modes of pharmacological action. This action may be due to the presence of carbohydrates, carbohydrate derivatives, gums, mucilages, pectin's, various forms of glycosides, tannins, phenolic compounds, lipids, fixed and volatile oils, resins, alkaloids etc. The significance of phytochemical analysis and its importance for the establishment of alternative medicine was expressed [2]. Honey has also been found to be effective in treating bacterial gastroenteritis in infants [3]. Different aspects of the antibacterial properties of honey have recently been extensively reviewed. Some workers believe that hydrogen peroxide is the main antibacterial agent in honey.

However, other authors found that the non-peroxide activity is more important. The argument for the later is that in ripe honey glucose oxidase is inactive and honey contains only small amount of peroxide, not sufficient to inhibit bacterial growth. However, when eaten or diluted, peroxide can be produced causing an antibacterial action [4]. Honey is at the base of many traditional remedies and herbal formulas in many ancient cultures. The Arabs consider honey as therapeutic and the Arabic name for bee is inhalant, which means a gift of Allah. No doubt honey is an integral part of Arabic cuisine and remedies.

In Oman, bee-keeping has been practiced since ancient times [5]. Oman has a varied landscape that provides the honey bee with the nutrients it requires. Omani bee-keepers have developed great skills in obtaining honey and propagating bee colonies in a sustainable manner. Bee-keeping is one of the main occupations in Rustaq Governorate of Oman. Omani Honey has many health benefits. First of all, it prevents cancer and heart disease as it contains flavonoids; antioxidants which help to reduce the risk of some cancers and heart disease. It also reduces ulcers and other gastrointestinal disorders [6, 7]. Due to the above said importance for Honey the present research is aimed to investigate phytochemical evaluation, antimicrobial role along with various food additives and role of honey on physiological Parameters. Omani, Turkish natural honey with commercial honey.

2. MATERIALS AND METHOD: Materials Nutrient agar, PDA, chloroform, ethanol, blood glucose meter (One touch Select-AW06505403A), Omani Honey, Turkish Honey, commercial Honey, Lemon (India), turmeric powder, Ginger powder, Swiss albino rat, microorganisms: *E coli*, *staphylococcus aureus*, *pseudomonas*, *Rhizopus* and *Penicillium*.

2.2. Method:

2.1 Phytochemical screening: Phytochemical screening was carried out according to [2]. Different types of Honey used to evaluate phytoconstituents were qualitatively tested for the presence of various biologically active substances using standard methods given by [8] and the extractive values were calculated by standard protocols. The concentrated pure Honey is dissolved in a small volume of DMSO initially and then final volume was made up to the required concentrations (1g / 100 ml, w/v), and these preparations are used for testing the following phytochemicals ; Coumarins, Flavonoids, Phenols, Resins, Saponins, Tannins, Fixed oils, Glycosides, Carboxylic acids, Steroids, Protein, Carbohydrates and Quinones.

2.2. Anti Microbial effect of different types of Honey alone and in combination with Lemon, Turmeric and Zinger:

Test organisms: Test organisms namely bacteria-*E.coli* (ATCC 25922), *S.aureus* (ATCC 25932) and *P.auriginosa* (ATCC 27853) were collected from microbiology lab, Higher college of Technology, Alkhuwair, Muscat, Sultanate of Oman, which are maintained overnight respectively on nutrient broth.

Test medium

In vitro investigation of antimicrobial study was carried out using nutrient agar medium. Antimicrobial to study the effect of Different Honey with different synergistic antibacterial and respectively.

***In-vitro* growth inhibition study:** The antimicrobial effects of the different Honey alone and in combination with different synergistics were tested using the agar well diffusion method. After 30 min of initial drying, agar plates were inoculated with 200µL of respective microorganism suspension at a density of 107 $CFUmL^{-1}$ and spread

on the surface uniformly and allowed to dry. Subsequently, four equidistant wells, 9 mm in diameter each, were punched into the inoculated medium with sterile cork borer and loaded with 50ul of test samples (NOH, NTH, CH, Lemon, turmeric and Zinger), incubated at 37°C. Cultures were incubated at 37°C and fungi at 25°C. Inhibition zones in mm around the disks were measured after 48h of cultivation. As positive controls for bacteria, Amoxicillin was used. All the experiments were repeated twice, including a control with Amoxicillin. The mean incubation, the zones of growth inhibition of the bacteria, around the disks were measured. The mean values of three trials and standard deviations were calculated.

2.3. Physiological effect of Honey on mice: Mice were selected (all females) and put in different cages (4cages per each cage three mice), from HCT animal house. The four cages were labeled as control, Omani honey, turkish honey, commercial honey, feeding carried out twice a week with one ml of the respective samples orally. The individual mouse of the cage was marked with different color by using marker on the tail. The mice were weighed, blood sugar, Hemoglobin and WBC count was studied prior to the treatment and treatment was continued for 21days. After treatment duration the mice's were weighed, Hb, WBC and blood sugar level was evaluated.

3. RESULTS AND DISCUSSION:

3.1. Phytochemical analysis of honey:

Preliminary phytochemical screening of different unprocessed and processed Honey showed the significant result that presence of most of the phytoconstituents which are Tested, shown in **Table 1**. Except caboxylic carbon and tannins remaining all the phyconstituents are found in the tested Honey samples. These phytochemicals are responsible for the treatment of various human ailments as recommended by traditional healers, therefore Honey has been used as natural remedy for most of the common diseases. The highest Percentage of coumarins (+++), Resins(+++) and Proteins are found in all the Honey samples while comparatively less percentage were noticed with commercial processed Honey(++). Flavonoids (+), Quinones (+) and glycosides (+) were found in less percentage in all the tested samples. These bioactive substances present in Honey are known to possess various therapeutic properties. Saponins found in the Honey have been reported as good source of antimicrobial by inhibiting the cell wall [9]. Another class of phytochemical Flavonoids help in healing of wounds and also treatment of skin diseases due to their ability to neutralize the acidity of wounds and also in inflammation[10]. Dietary saponins reduce plasma cholesterol level in primate, thus having the potential to lower the risk of coronary heart diseases in humans [11]. Treatment of heart diseases could be because of flavonoid, saponins and glycosides which stimulate heart, especially saponin that remain within gastrointestinal tract. Some interact directly with dietary cholesterol producing an insoluble complex which prevents the cholesterol from being absorbed.

The presence of alkaloid indicates that honey can be used as basic medicinal agents for their analgesic, antispasmodic and bactericidal effects [12, 13]. Glycosides found in Honey also contain health benefits in lowering the cholesterol and blood pressure according to many reports [14]. Apart, Honey containing carbohydrates known to be reducing sugars are of great value to living organisms since they are primary metabolites and are directly involved in their growth, development and reproduction.

TABLE 1: PHYTOCHEMICAL SCREENING OF HONEY

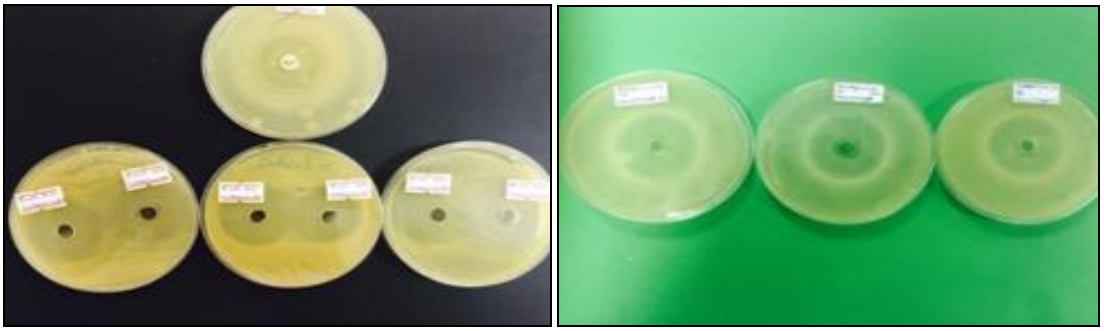
Name of the test	Natural Omani honey	Natural Turkish honey	Commercial honey
Coumarins	+++	++	++
Flavonoids	+	+	+
Phenols	++	++	++
Resins	+++	+++	++
Saponins	++	+	-
Tannins	-	-	-
Fixed oils	++	++	++
Glycosides	+	+	+
Carboxylic carbon	-	–	-
Steroids	++	+	++
Protein	+++	+++	++
Carbohydrate	++	+	++
Quinones	+	+	+

Here +++- highest value; ++- moderate value; + - lowest value and – indicates absence. All the values expressed are the mean of three replicates.

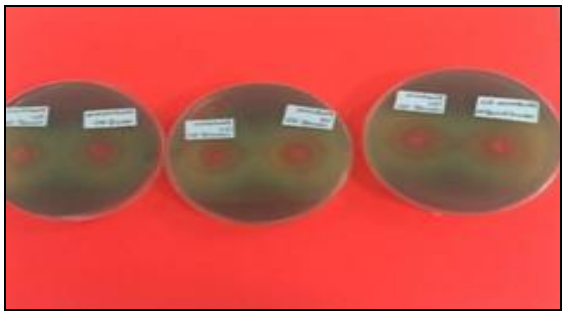
3.2. Antimicrobial efficacy of Different types of honey different synergistic: The antibacterial activities of different Honey with different edible synergistic like turmeric, lemon and zinger against G –ve(*E. coli* and *p. aeruginosa*) and G +ve (*S . aureus*) have shown as effective antibacterial with increased activity. According to the analysis among the different Honey tested alone it was noticed that, NOH against *S. aureus* (36mm), NTH against *P. aeruginosa* (40mm)and CH against *P. aeruginosa* (38mm), *maximum* zone of inhibition was noticed. While the synergistics used in this study to evaluate alone observed that, lemon against *S. aureus* and *P. aeruginosa* showed the highest zone of inhibition 27 mm, while zinger and turmeric shown very less antimicrobial against *E.coli* whereas against *P. aeruginosa* and *S. aureus* too low zone of inhibition was noticed. These results clearly shown in **Table 2** indicate that Honey has high impact of antimicrobial efficacy alone and in combination with various common edible food additives.

Therefore Honey alone comparatively between omani Honey, turkish natural honey and commercial honey available in the market showed that Turkish honey found to be more antimicrobial against *P. aeruginosa* (40mm),while commercial honey against the same microorganism *P. aeruginosa* (38mm) zone of Inhibition was observed. As omani Honey against *S. Aureus* showed maximum zone of inhibition (36mm), while against with *P. aeruginosa* (20mm) less zone of inhibition was observed. These antimicrobial efficacy of Honey is irrespective of synergistics is because of the presence of antimicrobial substances like enzymatic production of hydrogen peroxide. However, another kind of honey, called non-peroxide honey (*viz.*, manuka honey), displays significant antibacterial effects even when the hydrogen peroxide activity is blocked. Its mechanism may be related to the low pH level of honey and its high sugar content (high osmolarity) that is enough to hinder the growth of microbes [15].

The beneficial role of honey is attributed to its antibacterial property with regards to its high osmolarity, acidity (low pH) and content of hydrogen peroxide (H_2O_2) and non-peroxide components, *i.e.*, the presence of phytochemical components like methylglyoxal (MGO) [16, 17]. The antimicrobial agents in honey are predominantly hydrogen peroxide, of which the concentration is determined by relative levels of glucose oxidase, synthesized by the bee and catalase originating from flower pollen [16]. Most types of honey generate H_2O_2 when diluted; because of the activation of the enzyme glucose oxidase that oxidizes glucose to gluconic acid and H_2O_2, which thus attribute the antimicrobial activity [18].



A. AGAINST G+ve AND G BACTERIA **B. AGAINST *S. AUREUS***



C. AGAINST *P. AERUGINOSA*

FIG. 1: ANTIMICROBIAL EFFICACY OF DIFFERENT TYPES OF HONEY AGAINST *E.COLI, P. AERUGINOSA* AND *S. AUREUS.* A. AGAINST *E.COLI.* B. AGAINST *S. AUREUS* AND C. AGAINST *P. AERUGINOSA*

TABLE 2: ANTIMICROBIAL EFFECT OF DIFFERENT HONEY WITH SYNERGISTICS AGAINST THREE DIFFERENT MICROORGANISMS

Type of Honey \ Name of bacteria		*E. coli*	*S. aureus*	*P. aeruginosa*
Natural Omani Honey	NOH	37	36	20
	NOH+Lemon	21	28	36
	NOH+Zinger	40	35	38
	NOH+Turmeric	23	36	40
Natural Turkey Honey	NTH	37	39	40
	NTH+Lemon	17	31	34
	NTH+Zinger	39	31	38
	NOH+Turmeric	37	34	35
Commerica Honey	CH	35	34	38
	CH+Lemon	16	34	38
	CH+Zinger	11	34	35
	CH+Turmeric	11	33	37
Lemon		21	27	27
Zinger		11	-	-
Turmeric		11	-	-
Control		31	-	-

Note: The Zone of Inhibition (mm) expressed in the table are mean average values of three replicates. Here NOH: Natural Omani Honey, NTH: Natural Turkish Honey and commercial Honey.

3.3. Physiological effect of different Honey *in vivo* on Mice: The influence of Honey on mice physiological studies like Body weight, Hemoglobin, blood sugar and WBC count has been carried out to evaluate. Since traditionally Honey Has been used as regular food as well as medicines around globe to treat various disorders , therefore the present study is aimed to investigate the role of Honey and its combination with various synergistic like lemon, turmeric and zinger on blood components and blood sugar level.

The results of the research showed a great avenue to support the use of honey alone and in combination with lemon, zinger and turmeric as traditional medicines to cure various disorders. As the previous findings showed that Honey has many valuable health benefits, according our study we also found that a diverse results as shown in **Table 3**. According to results in the present investigation, it was noticed that the Body weight was not much affected by the tested different honey types but found very less increase in body weight. The increasing of body weight may be due to the presence of androgenic property which causes anabolic metabolism [19]. While hemoglobin levels it was found that the tested natural omani Honey, Natural Turkish Honey and Commercial Honey increased level up to 3 gm/dL. Honey administration trends to increase in Hb level may be due to honey as an anti-anemic and immune-stimulant agent [20].

These results shows that exclusive honey feeding alters hematological parameters like Hb levels and WBC %[21]. In the present study it was observed that increase in WBC % with greatest significance. Among all the tested Honey, Natural Turkish Honey we found more increase of WBC % upto 30% shown in **Table 3**.

TABLE 3: EFFECT OF HONEY ON VARIOUS PHYSIOLOGICAL PARAMETERS

Control	Weight before	Weight after	Hemoglobin before	Haemoglobin after	Blood sugar before	Blood sugar After	WBC % before	WBC % After
Control	27.5	27.5	90	90	6.1	6.1	28	28
NOH	27.5	24.7	90	90	6.1	5.5	28	37
NTH	22.1	26.3	80	83	4.8	5.9	29	36
CH	19.6	21.5	90	90	4.3	5.7	24	44

Note: all the values expressed in the table are the mean of five replicates where NOH; natural Omani honey, NTH: natural turkey honey, CH: commercial honey. The treatment duration is 21 days under controlled conditions. Before: prior to experiment and after: from the day of experiment 21 days.

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CONCLUSION: The present findings of the research concludes that Honey is a potent antimicrobial agent against microorganisms and also with different synergistic it was found that Honey has significant influence on inhibiting the microbial growth. Among The different types of Honey against different microorganisms, the highest zone of inhibition was noticed with natural omani flora Honey against *E.coli* (40mm) while against the same organism the commercial honey showed lowest zone of inhibition (35mm). the addition of synergistics along with the different types honey does not showed any significant increase in the zone of inhibition except that natural omani honey with turmeric shown highest zone of inhibition which is 40 mm against *p. auriginosa*. These findings proved that Honey has highest and effective antimicrobial constituents. While through physiological study the findings confirming that Honey is not effective on blood sugar level and body weight significantly, whereas it has significant influence on hematological parameters like Hb and WBC count. Amongst the tested types, Turkish Honey accelerated more WBC than other types of Honey tested. These findings strongly suggests and correlates with the use of Honey as folklore practices and further the Honey with different synergistic also need to be evaluated more.

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