

## Influence of Honey on Immune Status in Mice-Bearing Ehrlich Carcinoma

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Received date: November 23, 2014, Accepted date: February 12, 2015, Published date: February 19, 2015

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### Abstract

Honey is currently the focus of many research projects due to its varied biological activities including antiinflammatory, antioxidant, antibacterial, antihypertensive and hypoglycemic effects. In the current study antitumor effect of coriander honey was investigated in mature mice bearing Ehrlich ascites carcinoma (EAC) with special reference to immune status. Coriander honey (500 mg/kg) caused decrease in tumor volume, packed cell volume and viable cell count, and caused increase in non-viable cell count and mean survival time thereby increasing life span of EAC bearing mice. The study of the effect of honey on immunological status in mice bearing Ehrlich carcinoma showed that the levels of immunoglobulin M, G and A were increased following the administration of coriander honey. It was also clear that coriander honey increased the phagocytic activity in mice bearing Ehrlich carcinoma. There was reduction in the stimulation indices of lymphocyte transformation of mice bearing Ehrlich carcinoma. Delayed hypersensitivity skin test revealed that the Ehrlich carcinoma reduced the reaction after 72 hours post inoculation with bovine serum albumin. The administration of honey caused the rise in skin thickness as shown in Ehrlich carcinoma and subsequently treated with coriander honey with a rise of 0.61 mm as compared with 0.52 mm in EAC control group. The skin thickness in coriander honey group was the highest among all groups with 0.90 mm thickness. Based on these results, it can be concluded that coriander honey exhibited antitumor effect by modulating cell mediated immune response and immunoglobulin levels, in EAC bearing mice.

**Keywords:** Coriander honey; Immunoglobulins; Anti-tumor; Cell mediated immune response; Delayed hypersensitivity; Phagocytic activity

### Introduction

Cancer continues to represent the largest cause of mortality in the world claiming over 6 million lives every year [1]. An extremely promising strategy for cancer prevention today is chemoprevention. Plants, vegetables and herbs used in the folk and traditional medicine have been currently accepted as one of the main sources of cancer chemoprevention drug discovery and development [2].

There is a growing interest in the pharmacological evaluation of various natural products used in traditional medicine. Flavonoids, terpenoids, and steroids have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and antitumor activity [3,4]. Honey contains numerous phenolic and non-phenolic antioxidants [5,6], the amount and type of which depends largely upon the floral source of the honey. Antioxidants play an important role in inhibiting and scavenging radicals, thus providing protection to humans against infection and degenerative diseases [6,7].

The present study was carried out to evaluate the antitumor activity, immunoglobulin levels, phagocytic activity, lymphocyte transformations and delayed hypersensitivity of coriander honey against Ehrlich ascites carcinoma (EAC) in Swiss albino mice.

### Material and Methods

#### Coriander honey

Coriander honey was purchased from local market in Egypt. Sterile distilled water was used to dilute honey immediately before administration by a stomach tube. Honey was used in a final concentration of 500 mg/kg/mouse.

#### Animals

The experiment was carried out using a total of 210 male Swiss albino mice weighting 22-25 g obtained from Animal House of National Research Center, Giza, Egypt.

#### Ehrlich ascites carcinoma

Ehrlich ascites carcinoma (EAC) cells were obtained from Cancer Biology Section, National Cancer Institute, Cairo, Egypt. The Ehrlich tumor line was maintained, till the time of the experiment in female Swiss albino mice by serial intraperitoneal passage of  $2 \times 10^6$  cells/mouse at 7-10 days intervals [7].

#### Standard anticancer drug

5-Fluorouracil (5-FU) purchased from Calbiochem, USA was used as a standard anticancer drug. 5-FU was injected intraperitoneally to mice at a dose of 20 mg/kg body weight [8,9].

## Experimental design

A total of 210 male Swiss albino mice were divided into 6 groups (n=35) (Table 1) with an average weight 22-25 gm each as follows:

The first group (normal control) received a daily dose of 50 µl/mouse normal saline through oral administration until the end of the experiment. The second group (coriander control) received a daily dose of 500 mg/kg/mouse through oral administration until the end of the experiment. The third group (5-FU control) received a daily dose of 20 mg/kg/mouse of 5-fluorouracil as standard anticancer drug until the end of the experiment. The fourth group (EAC control) was inoculated intraperitoneally with a single dose of EAC cell line ( $2 \times 10^6$  cells/mouse). The fourth group received also the same treatment of normal saline like the normal control group.

Group s	Group Name	Treatment			
		Saline	Coriander honey	EAC	5-FU
1	Normal control	+	-	-	-
2	Coriander control	-	+	-	-
3	5-FU control	-	-	-	+
4	EAC control	+	-	+	-
5	Coriander+EAC	-	+	+	-
6	5-FU+EAC	-	-	+	+

**Table 1:** Experimental groups

The fifth and sixth groups (Coriander+EAC and 5-FU+EAC, respectively) were inoculated intraperitoneally with a single dose of EAC like the EAC control group but, the fifth group further received the same treatment of coriander honey like the coriander control group while the sixth group received 5-FU like the 5-FU control group.

At the second day post inoculation with Ehrlich carcinoma 0.1 ml of bovine serum albumin was inoculated intraperitoneally in each mouse in all groups as priming for the specific antigen used in the skin test.

On 7, 14 and 21 days of the experiment 5 mice from each group were subjected to 18 h fasting before sacrificing. Anti-tumor effect of coriander honey was assessed by observation of changes in body weight, ascites volume, viable and nonviable tumor cell count. The remaining animals in each group were kept to check the Mean Survival Time (MST) of the tumor bearing hosts and increase in Life Span Percentage (ILS%). MST of each group containing five mice were monitored by recording the mortality daily for 7 weeks and ILS % was calculated using the following equation:  $MST = (\text{Day of first death} + \text{Day of last death}) / 2$ .  $ILS\% = [(\text{Mean survival time of treated group} / \text{mean survival time of control group}) - 1] \times 100$  [10,11].

Blood and serum samples were collected from 5 mice from each group at 7, 14 and 21 days intervals post inoculation with Ehrlich effusive carcinoma cells. Blood samples were used to determine blood lymphocytic count and lymphocyte transformation according to

Hegazi et al. [12] as well as the phagocytic activity using the technique adopted [13]. While the sera sample were used for detection of different Immunoglobulin levels by quantitative determination of IgG, IgA and IgM [14]. At 72 hours before sacrificing, 0.1 ml of bovine serum albumin was inoculated in the left footpad of the mice where the right foot pad was inoculated with saline. Skin thickness was measured after 72 hours post inoculation to evaluate the delayed type hypersensitivity [12,15].

**Statistical analysis:** The results obtained in the present study were represented as means  $\pm$  standard error, and were analyzed using analysis of variance (ANOVA). The significance of difference between means at  $P < 0.05$  was calculated using the Duncan Multiple Range Test [16].

## Results

Administration of repeated daily dose of 500 mg/kg/mouse coriander honey during the experiment did not show any abnormal behavioral responses. Coriander honey increased the body weight of the mice in coriander control group (Table 2).

### Antitumor activity

In EAC control group the mean survival time was 22 days with a decrease of 44.3% in expected life span compared to normal control group, while the treatment with coriander honey increased the mean survival time to 34.5 days in coriander+EAC group with a decrease of 12.7% in expected life span compared to normal control group. Meanwhile, the mean survival for 5-FU+EAC group was 36.5 days with a decrease of 7.5% in expected life span compared to normal control group. Treatment with coriander honey reduced the ascites volume ( $1.60 \pm 0.01$  mL) and viable tumor cell count ( $8.84 \pm 0.06 \times 10^{10}$  cells/L) as compared to that of EAC control group ( $3.37 \pm 0.07$  mL and  $12.30 \pm 0.07 \times 10^{10}$  cells/L respectively). On the other hand nonviable tumor cell counts in coriander+EAC were increased when compared with the EAC control (Table 2).

### Hematological status

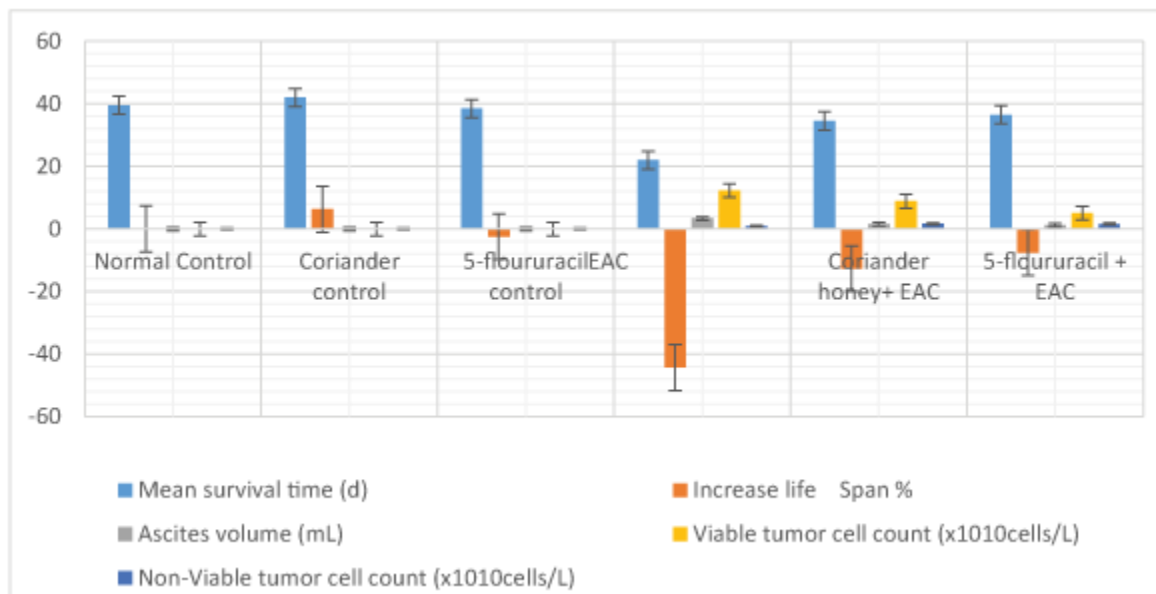
Hematological status in mice bearing Ehrlich carcinoma was evaluated by measuring some hematological parameters including total and differential leukocytic count (Table 2). Administration of coriander honey brought back all hematological parameters near the normal range.

### Immunological status

The results of the effect of coriander honey on Immunological status in the mice bearing Ehrlich carcinoma were illustrated in Table 3. It was observed that the levels of Immunoglobulin (M, G and A) were raised in all groups if compared with the control group all over the experimental period. There was an increase in the level of Immunoglobulins in case of coriander honey treatment, and in carcinoma control group. This increase was in IgM and IgA, but IgG was slightly increased if compared with the normal control group. It was clear that the treatment with coriander honey in case of Ehrlich carcinoma increases the level of different Immunoglobulins but with lesser levels than that in case of coriander honey treatment only.

Parameters	Normal Control	Coriander control	5-FU control	Ehrlich ascites carcinoma		
				EAC control	Coriander+EAC	5-FU+EAC
Body weight (g)	25.70 ± 0.16	28.22 ± 0.16	20.27 ± 0.09	36.70 ± 0.16	34.60 ± 0.19	31.20 ± 0.14
Mean survival time (d)	39.5	42	38.5	22	34.5	36.5
Increase life Span %	0	6.3	-2.5	-44.3	-12.7	-7.5
Ascites volume (mL)	0	0	0	3.37 ± 0.07	1.60 ± 0.01	1.20 ± 0.01
Viable tumor cell count (x10 <sup>10</sup> cells/L)	0	0	0	12.30 ± 0.07	8.84 ± 0.06	5.04 ± 0.04
Non-Viable tumor cell count (x10 <sup>10</sup> cells/L)	0	0	0	0.89 ± 0.06	1.62 ± 0.06	1.57 ± 0.05
WBC/10 <sup>12</sup> /L	4.73 ± 0.09	5.02 ± 0.06	12.51 ± 0.08	17.20 ± 0.03	5.02 ± 0.06	8.97 ± 0.03
Monocyte/%	1.80 ± 0.01	1.80 ± 0.01	1.20 ± 0.03	1.10 ± 0.02	1.80 ± 0.01	1.40 ± 0.01
Neutrophil/%	17.8 ± 0.15	25.10 ± 0.12	53.50 ± 0.19	65.40 ± 0.17	35.10 ± 0.12	39.90 ± 0.12
Lymphocyte/%	80.4 ± 0.23	73.10 ± 0.43	45.30 ± 0.35	33.50 ± 0.42	63.10 ± 0.43	58.70 ± .33

**Table 2:** Effect of coriander honey on body weight, MST, % ILS, ascites volume, viable and non-viable tumor cell count in EAC bearing mice. n=5, Mean ± SE P<0.01 vs. EAC control group.



**Figure 1:** Effect of coriander honey on MST, % ILS, ascites volume, viable and non-viable tumor cell count in EAC bearing mice.

Phagocytic activity of mice bearing Ehrlich carcinoma was tabulated in Table 4. It was clear that the phagocytic activity in mice bearing carcinoma was reduced if compared with the control group. The group treated with coriander honey showed an increase in phagocytic activity. Also the groups treated with coriander honey after inoculation with Ehrlich carcinoma revealed an increase in the phagocytic activity than carcinoma only and control group where the group treated with coriander honey there was an increase in the phagocytic activity. It was observed that the phagocytic activity of

Ehrlich carcinoma and subsequently treated with coriander honey was higher in Ascites state.

Stimulation index evaluated the lymphocyte transformation of mice bearing Ehrlich carcinoma revealed that there was a reduction in the stimulation indices ranged from 0.90+0.03 up to 1.22+0.03 in case of cancer control. It was ranged from 1.11+0.04 up to 1.73+0.02 in coriander honey treated Ehrlich carcinoma (Table 4) if compared with the control group or coriander honey group.

The results of delayed hypersensitivity skin indices were demonstrated in Table 4. Delayed hypersensitivity skin test revealed that the Ehrlich carcinoma reduced the reaction after 72 hours post inoculation with bovine serum albumin. The administration of honey caused the rise in skin thickness as shown in Ehrlich carcinoma and

subsequently treated with coriander honey with a rise of 0.61 mm as compared with 0.52 mm in EAC control group. The skin thickness in coriander honey group was the highest among all groups with 0.90 mm thickness (Figure 1).

Days post inoculation	IgM						IgG						IgA					
	control	honey	fluorouracil	Ehrlich ascites carcinoma			control	honey	fluorouracil	Ehrlich ascites carcinoma			control	honey	fluorouracil	Ehrlich ascites carcinoma		
				EAC	Cor. H +EAC	5-flu +EAC				EAC	Cor. H +EAC	5-flu +EAC				EAC	Cor. H +EAC	5-flu +EAC
7	68	72	58	62	70	64	22	30	23	18	23	23	28	166	70	58	70	70
14	80	88	65	68	81	74	25	36	20	22	22	20	102	80	68	69	82	78
21	82	98	73	78	88	84	28	30	20	23	28	18	78	98	80	50	83	78

**Table 3:** Effect of coriander honey on Immunoglobulin levels (mg/dl) in mice- bearing Ehrlich carcinoma.

Days post inoculation	phagocytic activity						Lymphocyte transformation						Delayed hypersensitivity (mm)					
	control	honey	5-FU	Ehrlich ascites carcinoma			control	honey	5-FU	Ehrlich ascites carcinoma			control	honey	5-FU	Ehrlich ascites carcinoma		
				EAC	Cor. H +EAC	5-FU +EAC				EAC	Cor. H +EAC	5-FU +EAC				EAC	Cor. H +EAC	5-FU +EAC
7	59 ± 0.9	63 ± 0.9	43 ± 0.9	48 ± 0.2	50 ± 0.7	50 ± 0.8	1.18 ± 0.03	1.19 ± 0.02	1.10 ± 0.02	1.41 ± 0.02	1.55 ± 0.04	1.22 ± 0.03	0.21	0.20	0.20	0.21	0.21	0.21
14	60 ± 0.3	66 ± 0.5	38 ± 0.6	35 ± 0.6	55 ± 0.1	52 ± 0.4	2.60 ± 0.05	1.92 ± 0.04	1.00 ± 0.03	1.63 ± 0.05	1.73 ± 0.02	1.01 ± 0.04	0.22	0.30	0.22	0.23	0.24	0.22
21	59 ± 0.8	69 ± 0.2	29 ± 0.8	27 ± 0.3	58 ± 0.6	55 ± 0.5	1.31 ± 0.2	1.22 ± 0.06	0.90 ± 0.03	1.11 ± 0.04	1.23 ± 0.04	0.99 ± 0.05	0.21	0.90	0.30	0.52	0.61	0.41

**Table 4:** Effect of coriander honey on phagocytic activity, lymphocyte transformation and delayed hypersensitivity in mice- bearing Ehrlich carcinoma.

## Discussion

A reliable criterion for judging the value of any anticancer agent is the prolongation of life span of animals [17]. In the current study, coriander honey increased the mean survival time of mice bearing EAC. Coriander honey was found to stimulate mammalian tissue regeneration, as it caused strong activation of mitosis of cells cultured *in vitro* and it enhanced protein biosynthesis [7,18,19]. Honey was found to exhibit an estrogenic activity resembling estradiol in female mice and caused an increase in uterine weight, while in male mice it showed androgenic activity (acetylcholine like action) and stimulated the parasympathetic terminal [20].

Honey was also found to affect the progression of cancer by exhibiting statistically significant antimetastatic effect which can be achieved by oral administration. The findings indicated that honey activates the immune system and its administration may be advantageous for cancer and metastasis prevention. Oral administration of honey before tumor cell inoculation was found to

decrease spreading of tumor [21,22]. The antitumor effect of honey against Ehrlich ascites tumor in mice and the possible mode of antitumor action have been investigated. *In vitro* studies on EAT cells demonstrated inhibitory effect of honey on tumor cell proliferation, viability % of tumor cells as well as the size of solid tumor [23].

Immunosuppression during malignant diseases may cause predisposition of the host to bacterial infection. The immunosuppressive effect of Ehrlich ascites carcinoma was documented to be due to the presence of low molecular weight factors in the ascetic fluid that can cause an impairment of macrophages function [24]. In the current study, it was clear that administration of honey increased the phagocytic activity in mice bearing EAC. The phagocytic function of macrophages, as well as the T- and B-cell function, was found to increase following the administration of honey. Oral administration of honey in mice in concentrations of (10,100 or 1000 mg/100 g BW) every other day for 4 weeks before intraperitoneal inoculation with Ehrlich ascites tumor (EAT, 1 × 10<sup>6</sup> cells) increased

the number of bone marrow cells as well as peritoneal macrophages, but not peripheral blood leukocytes nor splenocytes [23].

Foods with high antioxidant and anti-inflammatory activity are suggested to be cancer preventive agents. Honey contains varying amounts of phenolic compounds which have different *in vitro* anti-inflammatory and antioxidant activities [25]. The fact that antioxidants have several preventative effects against different diseases, such as cancer, coronary diseases, inflammatory disorders, neurological degeneration, and aging, led to search for food rich in antioxidants. Polyphenols found in honey, namely, caffeic acid (CA), caffeic acid phenyl esters (CAPE), Chrysin (CR), Galangin (GA), Quercetin (QU), Kaempferol (KP), Acacetin (AC), Pinocembrin (PC), Pinobanksin (PB), and Apigenin (AP), have evolved as promising pharmacological agents in the treatment of cancer. From a nutritional viewpoint, substrates (amino acids, energy, enzyme co-factors) are needed to support the clonal proliferation of antigen-driven lymphocytes, the recruitment of new monocytes and heterophils from bone marrow, the synthesis of effector molecules (e.g., immunoglobulins, nitric oxide, lysozyme, complement), and communication molecules (e.g., eicosanoids, cytokines) [26,27].

Honey as well as other bee products was found to modulate the immune response against infection. Hegazi et al. [13] studied the effect of some bee products on the immune response of chicken infected with virulent Newcastle Disease Virus (NDV). They found that, the mortality rate was reduced in groups infected with virulent NDV and subsequently treated either with propolis or honey when compared with the infected groups only. The foot pad indices as indicated by skin test as a parameter of cell mediated immune response of chickens was measured. It was obvious that the highest foot pad indices were observed in mice sensitized and inoculated with its specific antigen, while other antigens inoculated in non-sensitized mice showed a slight or moderate reaction. The delayed hypersensitivity skin test showed a reaction in chickens sensitized either by propolis or NDV as specific antigen.

Because of honey's complex and unusual composition, it has several interesting attributes. In addition, honey has some properties, because of its composition, that make it difficult to handle and use. With modern technology, however, methods have been established to cope with many of these problems.

Honeys contain 11 to 21 free amino acids. Proline, glutamic acid, alanine, phenylalanine, tyrosine, leucine, and isoleucine are the most common biological properties, especially its antimicrobial and antioxidant capacities. its polyphenolic composition and other bioactive compounds, such as glyoxal and methylglyoxal. source of natural macro- and micro-nutrients as well as minor constituents, especially phenolic compounds. Several studies have shown that the antioxidant potential of honey is strongly correlated not only with the concentration of total phenolics present, the major flavonoids in manuka honey are: pinobanksin, pinocembrin and chrysin, while luteolin, quercetin, 8-methoxykaempferol, isorhamnetin, kaempferol and galangin have been also identified in minor concentration [4].

While normal inflammation resolves within 1-2 days as the neutrophil number decreases, the accumulation of these cells in the wound site contributes to a disordered network of regulatory cytokines, leaving the wound in a chronic state of inflammation. The effect of honey and its components on the production of inflammatory cytokines has been evaluated in primary human monocytes cells [28]. In these studies, it was shown that manuka honey stimulated the

production of inflammatory cytokines TNF- $\alpha$ , IL-1 $\beta$  or IL-6 via a TLR4-dependent mechanism. For the first time, a 5.8-kDa component responsible for cytokine induction in human monocytes via TLR4 was isolated from manuka honey [29]. chemical composition of also showed that the antibody titers in most of treatment honey which includes huge mixture of flavonoids and groups at each time point were higher than that from phenolic acids which act as antimicrobial agent [6]. Improvements in immunity or functions that support immunity are associated with Zn, Mn, Cu and Se. Dietary Se interacts with vitamin E in antioxidant protection of cells because it is a component of glutathione peroxidase. In addition to antioxidant activity, Selenium (Se) has been shown to impact disease resistance [6].

The antibody titers and phagocytic percentages were increased in chickens infected with virulent Newcastle disease virus following the administration of either propolis or honey [30]. Giurgea et al. [31] found an increase in the formation of antibodies in rats fed with standardized ethanol extract of the propolis compared with rats not fed propolis.

Fauzi et al. [32] found that tualang honey induces apoptosis and disrupts the mitochondrial membrane potential of human breast and cervical cancer cell lines. The increase in Lactate Dehydrogenase (LDH) leakage from the cell membranes indicates that tualang honey is cytotoxic to cancer cells with effective concentrations. This study showed that tualang honey has significant anticancer activity against human breast and cervical cancer cell lines.

The findings of this study suggest that coriander honey can be used as an effective natural product to modulate the immune response against serious diseases including cancer. Further studies are still needed to confirm the results on different types of cancer models and cancer cell lines and to standardize the use of coriander honey as well as other types of honey.

## Acknowledgement

The authors are grateful for the financial support of National Research Centre, Dokki, Egypt.

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