

## Ameliorative effects of honey and venom of honey bee on induced colon cancer in male albino rats by 1,2 dimethylhydrazine

Nagy S. Tawfek, Diaa B. Al-Azhary, Hanaa F. Hassan and Esraa G. Mostafa

Zoology Department, Faculty of Science, Minia University, Egypt  
[egm\\_1990@yahoo.com](mailto:egm_1990@yahoo.com)

**Abstract: Aim of the work:** The present study was carried out to evaluate the role of bee honey and bee venom (BV) separately or in combination in ameliorating promotion of colon carcinogenesis induced by 1,2 dimethylhydrazine (DMH) in albino rats. **Materials and methods:** Rats were subcutaneously injected by DMH (20 mg/kg b. wt.) once a week for 15 weeks. DMH-treated animals received either oral administration of bee honey (500 mg/kg b. wt.) or intraperitoneal injection of BV (3 mg/kg b. wt.) or both together every other day along the period of DMH- treatment. At the end of 15<sup>th</sup> week treatment, blood samples and colon tissues were taken for biochemical analysis of lipid peroxide, glutathione peroxidase (GPx), alkaline phosphatase (ALP), carcinoembryonic antigen (CEA) and alpha fetoprotein (AFP); and also for histopathological and immunohistochemical investigations. **Results:** The results showed an increase in the levels of lipid peroxide, ALP, CEA and AFP and a decrease of GPx level in DMH-treated rats as compared to control, while honey and BV treatments modulated the DMH-induced changes of these parameters. Moreover, they showed remarkable reduction in dysplasia, inflammatory cells infiltration and loss of acinar patterns of colon glands and abnormalities of P53 expression which were clearly observed in DMH-treated group. **Conclusion:** Findings of the present study indicate significant roles for reactive oxygen species (ROS) in pathogenesis of DMH-induced colon toxicity and initiation of colon cancer. Also, it suggested that honey, BV or the combination of both have a positive beneficial effect against DMH induced colonic cancer in rats. Honey and BV inhibit oxidative stress and enhance antioxidant status suggesting a growing application of these natural compounds as an alternative medicine treatment of colon tumor.

[Nagy S. Tawfek, Diaa B. Al-Azhary, Hanaa F. Hassan and Esraa G. Mostafa. **Ameliorative effects of honey and venom of honey bee on induced colon cancer in male albino rats by 1,2 dimethylhydrazine.** *Cancer Biology* 2018;8(4):9-20]. ISSN: 2150-1041 (print); ISSN: 2150-105X (online). <http://www.cancerbio.net>. 2. doi:[10.7537/marscbj080418.02](https://doi.org/10.7537/marscbj080418.02).

**Key words:** Colon cancer, 1,2dimethylhydrazine, oxidative stress, honey, bee venom.

### 1. Introduction

Colon cancer is a pathological consequence of oxidative stress leading to DNA damage and mutations in cancer-related genes, a cycle of cell death and regeneration, where overproduction of reactive oxygen species (ROS) and reactive nitrogen species is linked [1]. Oxidative stress is a state that happens once the balance between the productions of reactive oxygen species (ROS) overcomes the endogenous antioxidant defense system and inflammation is a complex biological response of tissues to pathogens and damaged cells [2]. The development of colon cancer is a complex pathological process and involves in multiple steps and stages with changes from normal crypt foci to aberrant crypt foci (ACF), adenomas formation, expansion, and eventual development to colon cancer [3].

1,2 dimethylhydrazine (DMH), is a potent colon carcinogen, inducing colorectal tumors in experimental animals and is the most widely used model of chemically induced colon carcinogenesis [4,5]. DMH induced colon cancer is a process involving a series of pathological alterations, such as

oxidative stress, inflammation, formation of ACF and tumor promotion [6].

Recently, a lot of studies reported that many natural products inhibit tumor cell growth and metastasis and induce apoptosis suggesting a growing application of these natural compounds as an alternative medicine treatment of human tumors [7]. Honey has been used since long time in human tradition particularly in medical and nutritional application [8]. It is composed of various sugars, flavonoids, phenolic acids, amino acids, enzymes, proteins and miscellaneous compounds [9]. BV, like many other complementary medicines, has also been used for thousands of years for the treatment of a range of diseases [10]. There are at least 18 active components within the venom which have some pharmaceutical properties. Among these compounds, melittin, a small linear peptide consisting of 26 amino acids, is the major potent toxin of BV, which comprises ~50% of BV [11]. To examine the anti-cancer effects of honey and BV, this study was performed to investigate the modulation of oxidative stress and colon carcinogenesis induced by DMH in albino rats.

## 2. Materials and methods

### **Experimental Animals:**

Forty eight adult male Sprague Dawley white albino rats *Rattus norvegicus* with average weight 120 - 150 g were used. The rats were obtained from the Animal House of the Faculty of Agriculture, Minia University, Minia, Egypt. All animals were housed in well aerated and isolated polypropylene cages under good hygienic laboratory conditions. Rats were provided commercial rodent diet containing all the necessary nutritive elements. Food and water were available throughout the experiment. Animals were housed at  $25 \pm 2$  °C under a 12-h light/dark cycle.

### **Materials:**

1,2-dimethylhydrazine (DMH) used for induction of colon cancer, was purchased from Sigma Chemical Company, Egypt. DMH was dissolved in 1mM EDTA just prior to use and the pH was adjusted to 6.5 with sodium bicarbonate to ensure the stability of the chemical. DMH was injected in rats subcutaneously, in a dose of 20 mg/kg once a week for 15 weeks [12]. Honey (moisture 60%) was purchased from GHADA Company, Borgalarb, Alexandria, Egypt. Sterile distilled water was used to dilute honey instantly before administration by a stomach tube. Honey was orally administered to rats in a final concentration of 500 mg/kg b. wt. [13] every other day for 15 weeks. Lyophilized whole bee venom was purchased from GHADA Company, Borgalarb, Alexandria, Egypt. It was prepared as a solution in sterile distilled water. BV was injected intraperitoneally at dose of 3mg/kg b. wt. [14] every other day for 15 weeks.

### **Experimental Design:**

The rats were divided into eight groups (n=6 in each group) as follows:

**Group 1 (control group):** rats were fed on balanced diet during the experimental period.

**Group 2 (honey treated group):** rats were administered orally with honey (500 mg / kg b. wt.) every other day for 15 weeks.

**Group 3 (BV treated group):** rats were injected intraperitoneally with BV (3 mg / kg b. wt.) every other day for 15 weeks.

**Group 4 (honey and BV treated group):** rats were treated with orally administration of honey (500 mg / kg b. wt.) and intraperitoneally injection of BV (3 mg / kg b. wt.) every other day for 15 weeks.

**Group 5 (DMH treated group):** rats were injected subcutaneously with DMH (20 mg / kg b. wt.) once a week for 15 weeks.

**Group 6 (DMH + honey treated group):** rats were treated with subcutaneously injection of DMH (20 mg / kg b. wt. once a week) and orally administration of honey (500 mg / kg b. wt. every other day) for 15 weeks.

**Group 7 (DMH + BV treated group):** rats were treated with subcutaneously injection of DMH (20 mg / kg b. wt. once a week) and intraperitoneally injection of BV (3 mg / kg b. wt. every other day) for 15 weeks.

**Group 8 (DMH + honey and BV treated group):** rats were treated with subcutaneously injection of DMH (20 mg / kg b. wt. once a week), orally administration of honey (500 mg / kg b. wt. every other day) and intraperitoneally injection of BV (3 mg / kg b. wt. every other day) for 15 weeks.

### **Collection of blood and tissue samples:**

At the end of 15<sup>th</sup> week treatment, rats were deprived of food but not water over night and then sacrificed by cervical decapitation. Blood and tissue samples were collected at once; two blood samples were immediately collected. The first sample is whole blood which was collected into EDTA for preparation of hemolysate for measuring of GPx level. The second one is serum for measuring the levels of lipid peroxide, ALP, CEA and AFP. After animal dissection, the colon was obtained and fixed in 10% formaline solution for histopathological and immunohistochemically studies.

### **Preparation of hemolysate:**

Blood samples were collected in glass tubes provided with EDTA (ethylene diaminetetra-acetic acid) as anticoagulant, then samples were centrifuged at 3000 rpm for 15 min., remaining packed RBCs were washed twice with normal saline to remove the buffy coat. Hemolysate was performed by pipetting out 1 ml of washed red blood suspension in ice cold distilled water. Erythrocyte ghosts were sedimented in a high speed refrigerated centrifuge at 3000 rpm for 40 minutes. The cell content was separated out carefully and used for biochemical studies.

### **Biochemical analysis:**

Lipid peroxide level was determined spectroscopically in serum according to the method described by [15] by using kit purchased from Biodiagnostic, Company, Egypt. GPx level was determined in hemolysate of the blood spectrophotometrically according to the method described by [16]. ALP level was determined spectroscopically in serum according to the method described by [17] using kit purchased from biodiagnostic, Company, Egypt. CEA was quantitatively determined in serum by ELISA according to the method described by [18] using enzyme immunoassay kit, purchased from sigma, Company, Egypt. AFP quantitative measurement was performed in serum by using ELISA according to the method described by [19] using Enzyme immunoassay kit, purchased from USCN life science Inc. Co.

### **Statistical Analysis:**

Collected data were presented as mean  $\pm$  SE and statistically analyzed using one way analysis of

variance (ANOVA). Student "t" test was used for significance according to [20] and statistical significance was set at  $P \leq 0.05$ .

### **Histological and Immunohistochemical studies:**

Formalin fixed tissues were processed for histopathological observations at the light microscopic level. Briefly, following an overnight fixation in buffered formalin, tissues were dehydrated through ascending grades of alcohol, cleared in benzene and embedded in paraffin. Blocks were made and 5  $\mu\text{m}$  thick sections were obtained. The slides were stained with hematoxylin and eosin (H & E) according to the method described by [21]. Formalin-fixed, paraffin-embedded tissue specimens were used for immunohistochemical staining. Procedures for immunohistochemical staining have been described previously by [22].

### **3. Results**

Biochemical analysis of blood demonstrated in (table 1) cleared that there is non-significant increase in groups treated with honey, BV each alone or both together when compared to control group (figure 1(A-E)), while treatment of rats with DMH caused a significant increase in lipid peroxide (figure 1A) and decrease in GPx activity (figure 1B) as compared to control group. Furthermore, treatment with honey and/or BV to DMH-treated rats caused a significant decrease in lipid peroxide and increase in GPx activity when compared to DMH-treated group. Alkaline phosphatase level showed also an observed increase in DMH-treated group when compared to control one, while treatment by honey and/or BV to DMH-treated rats induced a modulatory effect by causing a significant decrease when compared to DMH-treated group (figure 1C). DMH also caused high elevation in the levels of CEA (figure 1D) and AFP (figure 1E) as compared to control group. On the other hand, treatment by honey and/or BV to DMH-treated rats

caused a significant decrease in CEA and AFP as compared to DMH-treated group.

Examination of H & E stained colonic sections of control rats showed normal Lieberkuhn glands with normal mucosal layer and typical colonic architecture with no signs of apparent abnormality (Figure 2A). Colonic sections from DMH treated group exhibiting hyperplastic dysplastic aberrant crypts of colonic mucosa, dysplastic goblet cells with irregular shape and size with inflammatory cells infiltration. Dysplasia and hyperplasia were common features in DMH treated groups (Figure 2(B & C)). Colonic sections of DMH-treated rats supplemented with honey showing clear improvement in mucosal layer with mild infiltration of inflammatory cells and lower degree of hyperplasia (Figure 2D). Colonic sections of DMH-treated rats supplemented with BV rats revealed an evident reduction of dysplasia. The mucosal crypts, lamina propria and muscularis mucosa were well oriented, with few inflammatory cells infiltration (Figure 2E). Co-treatment of DMH-treated rats with honey and BV showed normal mucosal layer with regular crypts and colonic structure more or less similar to the control (Figure 2F).

In the present study, immunohistochemical analyses of p53 were carried out and the brown color indicated specific immunostaining of p53. The colonic section of DMH-treated group (Figure 3(B & C)) showed enhanced immunopositive staining of p53 indicated by brown color as compared to control group (Figure 3A). However, there was reduction in immunopositive staining of p53 in colon sections of rats from DMH + honey treated group (Figure 3D) and DMH + BV treated group (Figure 3E) as compared to DMH treated group. While, co-treatment of DMH-treated rats with honey and BV (Figure 3F) showed no significant difference in the p53 immunostaining as compared to control group.

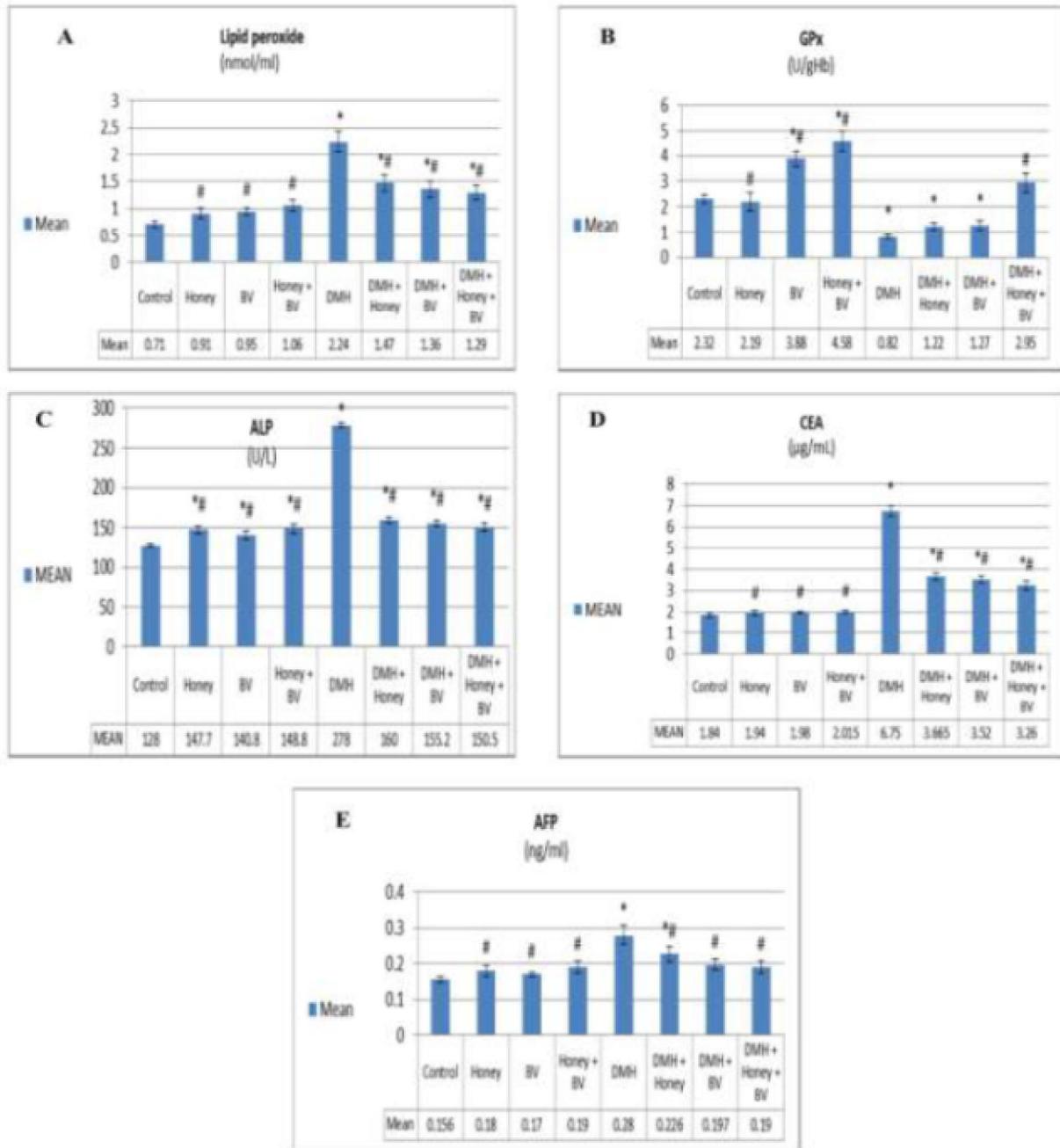
**Table 1: Effects of bee honey and/or venom on some biochemical parameters of DMH-induced carcinogenesis in rats:**

	Control	Honey	BV	Honey + BV	DMH	DMH + Honey	DMH + BV	DMH + Honey + BV
Lipid peroxide (nmol/ml)	0.71 $\pm 0.06$	0.91 $\pm 0.09^{\#}$	0.95 $\pm 0.07^{\#}$	1.06 $\pm 0.11^{\#}$	2.24 $\pm 0.19^*$	1.47 $\pm 0.15^{* \#}$	1.36 $\pm 0.15^{* \#}$	1.29 $\pm 0.13^{* \#}$
Glutathione peroxidase (U/gHb)	2.32 $\pm 0.17$	2.19 $\pm 0.36^{\#}$	3.88 $\pm 0.33^{* \#}$	4.58 $\pm 0.4^{* \#}$	0.82 $\pm 0.09^*$	1.22 $\pm 0.16^*$	1.27 $\pm 0.19^*$	2.95 $\pm 0.375^{\#}$
Alkaline phosphatase (U/L)	128 $\pm 1.9$	147.7 $\pm 4.64^{\#}$	140.8 $\pm 5.63^{* \#}$	148.8 $\pm 6.07^{* \#}$	278 $\pm 2.89^*$	160 $\pm 3.33^{* \#}$	155.2 $\pm 3.92^{* \#}$	150.5 $\pm 5.17^{\#}$
CEA ( $\mu\text{g}/\text{mL}$ )	1.84 $\pm 0.09$	1.94 $\pm 0.11^{\#}$	1.98 $\pm 0.055^{\#}$	2.015 $\pm 0.075^{\#}$	6.75 $\pm 0.24^*$	3.665 $\pm 0.16^{* \#}$	3.52 $\pm 0.16^{* \#}$	3.26 $\pm 0.21^{\#}$
AFP (ng/ml)	0.156 $\pm 0.01$	0.18 $\pm 0.015^{\#}$	0.17 $\pm 0.005^{\#}$	0.19 $\pm 0.02^{\#}$	0.28 $\pm 0.03^*$	0.226 $\pm 0.02^{* \#}$	0.197 $\pm 0.015^{\#}$	0.19 $\pm 0.02^{\#}$

Data are presented as (mean  $\pm$  S.E.). S.E = Standard error

\*  $p < 0.05$ : significant change with respect to the control group.

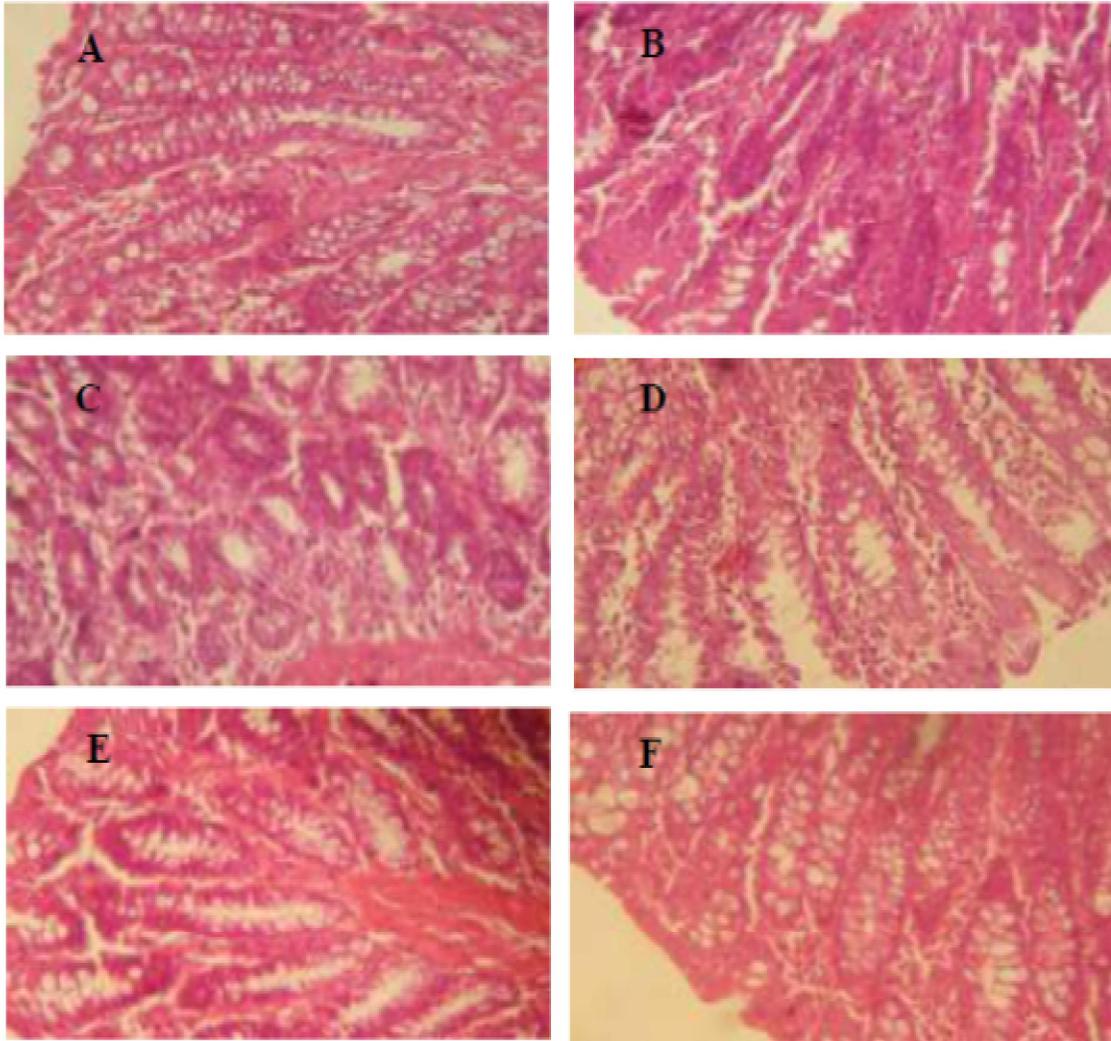
$\#$   $p < 0.05$ : significant change with respect to DMH group.



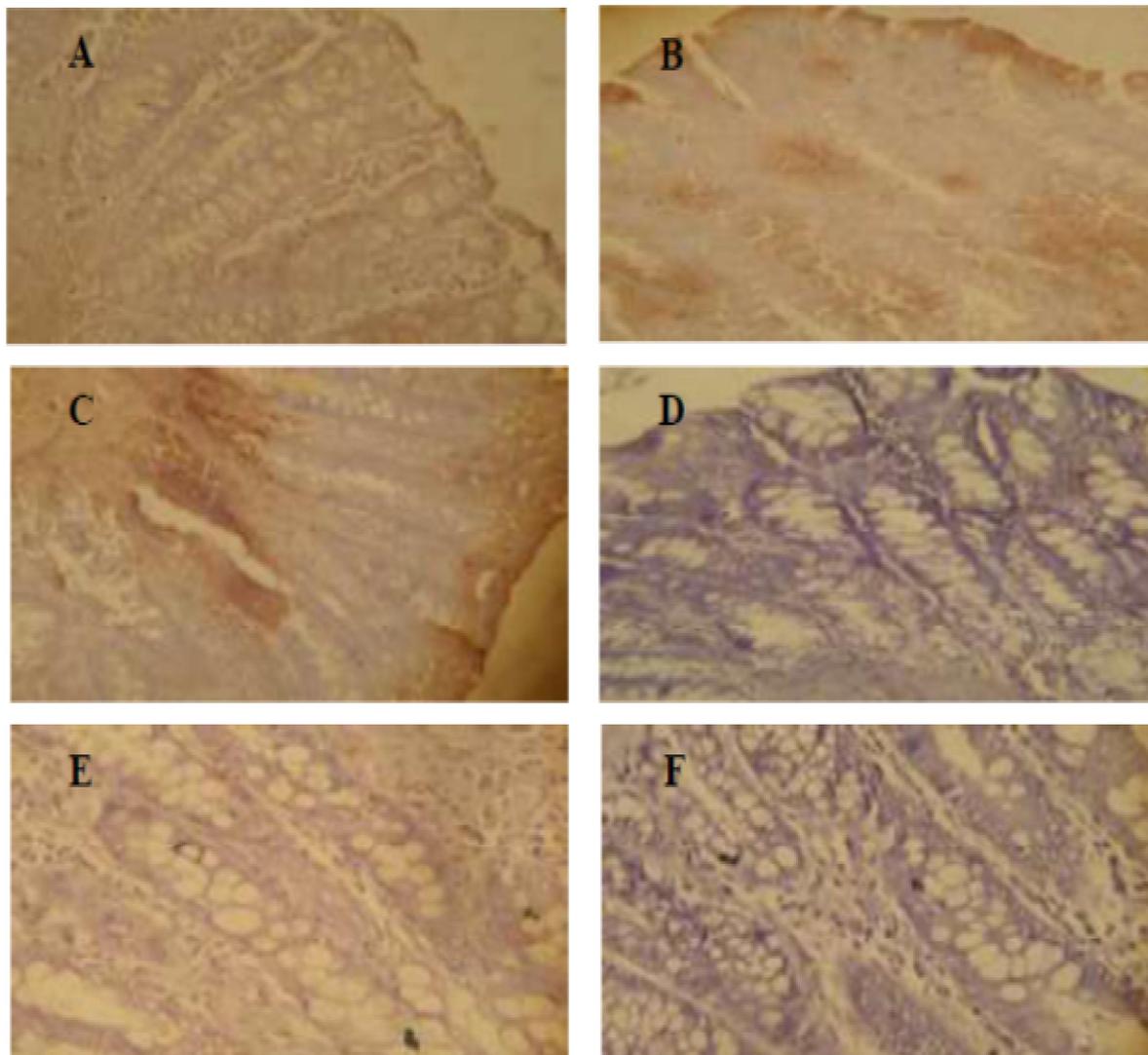
**Figure 1:** Histogram showing serum lipid peroxide content (A) hemolysate erythrocyte glutathione level (B), serum alkaline phosphatase content, serum carcinoembryonic antigen content and serum Alpha-Fetoprotein content in all groups. Data are presented as (mean  $\pm$  S.E.). S.E = Standard error.

\* Significant change at  $p < 0.05$  with respect to the control group.

# Significant change at  $p < 0.05$  with respect to DMH group.



**Figure (2):** Photomicrographs of colon sections of different groups stained by H & E ( $\times 40$ ). (A) Control group showing a normal flat mucosa with its straight crypts of Leiberkuhn. (B & C) DMH-treated group showing hyperplastic dysplastic aberrant crypts of colonic mucosa with reduction of the intercryptic spaces and also showing inflammatory cells infiltration in between the glands of Leiberkuhn. (D) DMH and honey treated group showing mild infiltration of inflammatory cells and lower degree of hyperplasia. (E) DMH and BV treated group showing improvement in the glandular structure and the lining epithelium of mucosa, a few inflammatory cells infiltration was also seen. (F) DMH + honey + BV treated group showing normal mucosa with well-oriented crypts.



**Figure 3:** Photomicrographs of colon sections of different group immunostained with p53 ( $\times 40$ ). (A) Control group showing normal p53 expression. (B & C) DMH-treated group showing strong positive immunostain of p53 indicated by brown colour. (C) DMH and honey treated group showing reduction of p53 immunostain as compared to DMH-treated group. (E) DMH and BV treated group showing less reaction to p53 immunostain as compared to DMH-treated group. (F) DMH + honey + BV treated group showing weakly stained colon tissue indicating less expression of p53

#### 4. Discussion

In the present study, 1,2dimethylhydrazine was used to induce rat colon carcinogenesis. It is utilized to study morphologic and molecular mechanisms of the multistage development of colon cancer in order to elucidate new targets for chemoprevention. Colon carcinogenesis in rat model is similar to histopathological and molecular characteristics of human colon cancer model [23]. The development of both DMH-induced rat colon carcinogenesis and human colon carcinomas include aberrant accumulation of cells with potential for excessive

proliferation, evasion of apoptosis, and genomic instability [24]. So, we used this model to explore the protective effect of honey and BV on experimental colonic damage caused by DMH in rats beside their modulatory effect on different biomolecules altered during colon carcinogenesis.

Reactive oxygen species (ROS) and oxygen free radicals have been discussed as putative mediators in colon carcinogenesis [25]. Colon carcinogenesis is reported to be a pathological consequence of persistent oxidative stress and inflammation [26]. ROS can oxidize polyunsaturated fatty acids (PUFAs), which

take part in cell membrane constitution [27]. The lipid peroxidation and the breakage of lipids with the formation of reactive compounds can lead to changes in the permeability and fluidity of the membrane lipid bilayer and can dramatically alter cell integrity [28] and also leading to DNA damage [29]. DNA damage can result in either cell cycle arrest or induction of transcription, induction of signal transduction pathways, replication errors, and genomic instability, which are all related with colon carcinogenesis [30].

As observed in results of the present study, DMH led to alterations of several parameters of oxidative stress. Earlier study demonstrated that DMH undergoes metabolism in the liver, leading to the generation of methyl diazonium ion, which is known to elicit oxidative stress. This leads to promutagenic events as a result of inflammation and tumor promotion [6]. Lipid peroxidation or MDA formation is one of the critical and relevant markers of oxidative damage and raised level of lipid peroxidation product (MDA) has been found after treatment with DMH [31]. The present results also showed remarkable increase in the level MDA after DMH treatment (table 1, figure 1A).

The observed increase in circulating lipid peroxides of DMH-treated animals, in the present study, correlates with the decline in circulatory antioxidants represented in GPx (table 1, figure 1B). This may be because of their overutilization in detoxification of carcinogenic metabolites of DMH [32]. GPx are related with the direct elimination of reactive oxygen metabolites, which is probably one of the most effective defenses of the living body against diseases. GPx plays a significant role in the peroxyl scavenging mechanism and in maintaining functional integration of the cell membranes [33]. GPx catalyze the reduction of H<sub>2</sub>O<sub>2</sub> or organic hydroperoxides to water or corresponding alcohols using reduced glutathione (GSH) as an electron donor [34].

On the other hand, honey significantly increased the activities of antioxidant enzymes of DMH-treated animals and protected from DMH-induced free-radical toxicity. Many investigators have suggested that honey is an influential antioxidant and strong scavenger of active oxygen species [35, 36]. The antioxidant activity of honey is for the most part attributed to its content of phenolic compounds and flavonoids [37]. Phenolic compounds display antioxidant activity because of their capacity to scavenge free radicals [38]. The antioxidant activities of flavonoids include ROS scavenging, transition metal ion chelation, increase of enzymatic and non-enzymatic antioxidants, and reduction of lipid peroxidation [39]. Honey may lower the risks and impacts of acute and chronic free radical induced pathologies *in vivo* [40]. Other study reported that honey has the ability to decrease

significantly the concentration of lipid hydroperoxides and malondialdehyde (MDA) [41]. Honey supplementation also was reported to restore activities of GPx [42]. In the present study, the oral administration of honey decreased the level of MDA (table 1, figure 1A) and improved the levels of GPx (table 1, figure 1B) of DMH-treated rats.

In the present study, the treatment of DMH-treated rats with BV caused significant decrease in the elevated MDA level (table 1, figure 1A) associated with significant elevation in the declined GPx activities (table 1, figure 1B), indicating that BV has potential antioxidant properties. According to recently published data, BV has strong antioxidant activities such as free radical scavenging activity and inhibition of lipid peroxidation [43]. [44], also stated that BV therapy is a potent antioxidant led to a decline in the levels of reactive oxygen species (ROS), which may be related with the observations of BV affecting glutathione, superoxide dismutase (SOD) and catalase. The antioxidant potential of BV had been confirmed in different studies carried on various pathological conditions [45, 46].

Alkaline phosphatase (ALP) is recognized as a critical marker of differentiation in human malignancies [47]. ALP is representative of colon function and its level serve as prognostic tool which are frequently elevated in colorectal cancer patients [48]. The present study revealed significant increase in the enzyme activity of ALP in the DMH-treated rats compared with normal control group (table 1, figure 1C). The enzyme activity of ALP has been reported to be significantly elevated during carcinogenesis [49]. The exact reason behind ALP elevation is unclear but can be attributed to excessive dephosphorylation of various proteins involved in the regulation of cell division which obviously is high during DMH induced colon carcinogenesis. Also, elevation in the enzyme activity of ALP has been directly connected to increased DNA synthesis [50].

However, treatment with honey considerably reduced the elevated level of ALP in serum of DMH treated animals (table 1, figure 1C). The observed decrease in the level of ALP activities is a clear vindication that the honey is having hepatoprotective and renal protective properties [51]. [52], also stated that, the inhibition in ALT, AST, ALP and GGT enzyme activities in carcinogenic rats administered bee honey may be assigned to the improvement in cellular membrane architecture, thereby suppressing the leakage of enzymes through membranes.

In the present study, upon treatment with BV, a significant moderation was observed in the enzyme activity of ALP in the DMH-treated rats (table 1, figure 1C), which is in corroboration with the observation of [44] who reported that treatment with

BV had significantly lowered the elevation of serum ALT, AST, ALP and LDH levels, demonstrating the hepato-protective effect of BV, which may be explained by the reduction of elevated hepatic nuclear factor kappa B (NF- $\kappa$ B) expression in liver. NF- $\kappa$ B has been considered to play critical roles in managing the genes expression responsible for inflammation, proliferation and apoptosis [53].

CEA is a tumour marker widely measured in colorectal cancer [54]. The obtained results demonstrated that serum CEA level was increased significantly in DMH-treated rats when compared with normal control group (table 1, figure 1D) due to colon cancer induction and this increase was revealed in other previous studies concerned with colon cancer [55, 56]. Furthermore, [57] recorded that; CEA is the best marker in colorectal cancer patients and most thoroughly characterized tumor-associated antigens, in both biochemical and clinical aspects.

AFP is a proven biomarker for hepatocarcinoma with in the serum of adults. Elevated serum levels of AFP can further accompany the growth of malignant lung and bladder tumors and gastrointestinal cancers of the stomach, pancreas, and colon [58]. AFP can also be an indicative of colorectal cancer [23]. In our study, serum level of AFP was significantly elevated in the DMH-treated rats compared with normal control group (table 1, figure 1D). A significantly elevation of AFP in DMH induced rat colon carcinogenesis was also observed in other studies [23].

In this study, administration of honey to DMH-treated rats reduced the level of tumor markers (CEA and AFP) to normal (table 1, figure 1(D & E)). Honey constituents have been reported to exert anti-inflammatory, antioxidant, antiproliferative, antimetastatic, and anticancer effects [8]. Its anticancer activity has been proved against various cancer cell lines and tissues, such as breasts, colorectal, renal, prostate, endometrial, cervical and oral cancer [9]. The cancer preventive property of honey in Wistar breed rats with colon dimethylhydrazinetumorogenesis has also been recorded [59].

The results of the present study also revealed that, treatment with BV to DMH-treated rats reduced the level of tumor markers (CEA and AFP) (table 1, figure 1(D & E)). The presented data support and extend previous findings [60, 61] that BV has direct antitumor effect *in vivo* and *invitro*. BV inhibits the proliferation of carcinoma cells and tumor growth *in vivo* duo to the stimulation of the local cellular immune responses in lymph nodes [62].

In the present study, administration with DMH produced histopathological changes in the form of hyperplastic and dysplastic activity with extensive mucosal layer destruction and intense inflammation.

Other investigation confirmed this result indicating after DMH administration, preneoplastic lesions such as aberrant crypt foci (ACF) were observed [63]. In this study, oral supplementation with honey to DMH-treated rats concealed the inflammatory responses in the colon by decreasing the massive infiltration of the inflammatory cells in the mucosal layer induced by DMH. It also decreased the regional destruction of mucosal layer induced by DMH in the colon. These histological findings exhibited the protective efficacy of honey against damaging effects of DMH in the rat colon. Whereas the colon section of DMH induced rats treated with BV has typical appearance of crypt epithelial cells with goblet formation and colonic tumors multiplicity was greatly reduced in this group. This firmly underlines the efficacy of BV in inhibiting/slowing tumorogenesis in the rat colon. (Figure 2(A-F)).

p53 is a transcription factor that causes cell cycle arrest and apoptosis in response to a variety of cellular damage [64]. It restricts cellular growth by prompting senescence, cell cycle arrest (at G1 and/or G2 phase) or apoptosis. The p53 protein up-regulates the expression of at least two genes, p21 and Bax, whose encoded products can regulate growth arrest and apoptosis, respectively [65]. Mutation within the DNA binding region of p53 accounted for expression of a dysfunctional p53 that has been reported in many tumors including gastric, breast, and colorectal tumors [64]. Mutations in the p53 gene may prevent activation of these downstream effectors [66]. In the present study, a significantly enhanced expression of p53 in the DMH-treated group compared to the normal control group was watched. Various prior studies have reported an over-expression of p53 gene in colorectal carcinomas [65, 66]. A large portion of p53 mutations change single amino acids in the p53 protein, which results in the production of an altered version of the protein that can't control cell growth and division effectively and therefore reduces or eliminates the protein's tumor suppressor function. Because the altered protein is less effective in managing cell growth and division, this results in accumulation of DNA damage. Thus, cells can grow and divide in an unregulated way, which can lead to cancerous tumors [65]. Administration of honey showed a significant decrease in expression of p53 as compared to DMH-treated animals, the result which agrees with [66] who recorded a reduction in p53 expression upon honey administration and attributed it to the anti-mutagenic effect of honey, which minimized the DNA damage. BV also caused decreased expression of P53 as compared to DMH-treated animals and this result corroborated with previous study, indicating the apoptotic and apoptosis-promoting effects of BV [67]. (Figure 3(A-F)).

Honey is characterized by its highly selectivity as it shows up cytotoxicity against cancer cells while it is non-cytotoxic to normal one [68]. Honey prevents the development of cancer by blocking the three principle stages of cancer formation known as initiation, proliferation, and progression [68]. The molecular mechanisms leading to the antiproliferative and anticancer effects of honey include cell cycle arrest, activation of mitochondrial pathway, induction of mitochondrial outer membrane permeabilization, induction of apoptosis, modulation of oxidative stress, reduction of inflammation, modulation of insulin signaling, and inhibition of angiogenesis in cancer cells. Besides, honey indicates potential effects on cancer cell by modulating proteins, genes, and cytokines that promote cancer [36].

A recent study indicating several cytotoxic mechanisms of BV such as apoptosis, necrosis, effects on growth inhibition and proliferation, and cytotoxicity and cell cycle alterations in various malignant cells [69]. [70] observed that melittin, the main compound of BV, inhibitorily affects calmodulin as an antiproliferation agent of BV and contributes to the increased PLA2 activity, calcium influx and necrosis. It additionally exerts a cytotoxic effect on malignant cells by inhibiting tumor growth and inducing the activation of matrix metalloproteinases (MMP) and caspase, which are responsible for apoptosis and necrosis [69]. Finally, data presented in this study confirmed that, the effect of both honey and BV was higher than each of them alone due to their synergistic preventive and modulatory effects.

### Conclusions:

The data of the present study indicated that honey and bee venom have potential antioxidant activity and reduce the risks of free radical induced pathogenesis. They also induced preventive role against colon carcinogenesis, strongly suggesting their use as natural compounds in protection and treatment of colon tumors.

### References

- Inokuma T, Haraguchi M, Fujita F, Tajima Y, Kanematsu T. Oxidative stress and tumor progression in colorectal cancer. *Hepato-gastroenterology*. 2009; 56(90):343-347.
- Eboh AS, Ere D, Chuku LC, Uwakwe AA. Kolaviron an Active Biflavonoid of *Garcinia kola* Extract Prevent 1, 2-dimethylhydrazine Induced Oxidative Stress and Lipid Peroxidation in the Initiation Phase of Colon Carcinogenesis in Wistar Rats. *Journal of Cancer and Tumor International South Korea*. Reviewers. 2015; 2(212):41-49.
- Chen Q, Liang Y, Zhu C, Yan Y, Pang G. Effects of casein glycomacropeptide on the early development of primary colorectal cancer in rats. *Food Science and Human Wellness*. 2013;2(3-4):113-118.
- Newell LE, Heddle JA. The potent colon carcinogen, 1, 2-dimethylhydrazine induces mutations primarily in the colon. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*. 2004; 564(1):1-7.
- Saini MK, Sharma P, Kaur J, Sanyal SN. The cyclooxygenase-2 inhibitor etoricoxib is a potent chemopreventive agent of colon carcinogenesis in the rat model. *Journal of Environmental Pathology, Toxicology and Oncology*. 2009; 28(1):39-46.
- Hamiza OO, Rehman MU, Tahir M, Khan R, Khan AQ, Lateef A, Ali F, Sultana S. Amelioration of 1, 2 Dimethylhydrazine (DMH) induced colon oxidative stress, inflammation and tumor promotion response by tannic acid in Wistar rats. *Asian Pacific Journal of Cancer Prevention*. 2012; 13(9):4393-4402.
- Oršolić N. Bee venom in cancer therapy. *Cancer and Metastasis Reviews*. 2012; 31(1-2):173-194.
- Badria F, Fathy H, Fatehe A, Elimam D, Ghazy M. Evaluate the cytotoxic activity of honey, propolis, and bee venom from different localities in Egypt against liver, breast, and colorectal cancer. *J Apither*. 2017; 2(1):1-4.
- Ahmed S, Othman NH. Honey as a potential natural anticancer agent: a review of its mechanisms. *Evidence-Based Complementary and Alternative Medicine*. 2013; 2013:829070.
- Nabiuni M, Safaeinejad Z, Parivar K, Divsalar A, Nazari Z. Antineoplastic effects of honey bee venom. *Zahedan J Res Med Sci*. 2013; 15(8):1-5.
- Kim YW, Chaturvedi PK, Chun SN, Lee YG, Ahn WS. Honeybee venom possesses anticancer and antiviral effects by differential inhibition of HPV E6 and E7 expression on cervical cancer cell line. *Oncology reports*. 2015; 33(4):1675-1682.
- Manju V, Nalini N. Protective role of luteolin in 1, 2 - dimethylhydrazine induced experimental colon carcinogenesis. *Cell Biochemistry and Function: Cellular biochemistry and its modulation by active agents or disease*. 2007; 25(2):189-194.
- Hegazi A, Al Tahtawy R, Abd Allah F, Abdou A. Antitumor and antioxidant activity of honey in mice bearing Ehrlich ascites carcinoma. *Academic Journal of Cancer Research*. 2014; 7:208-214.
- Liu X, Chen D, Xie L, Zhang R. Effect of honey bee venom on proliferation of K1735M2 mouse

- melanoma cells in - vitro and growth of murine B16 melanomas in - vivo. *Journal of pharmacy and pharmacology*. 2002, 54(8):1083-1089.
15. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical biochemistry*. 1979; 95(2):351-358.
  16. Weinhold LC, Ahmad S, Pardini RS. Insect glutathione Stransferase: A predictor of allelochemical and oxidative stress. *Comp. Biochem. Physiol.* 1990; 95(2):355-363.
  17. Tietz NW. *Fundamentals of clinical chemistry*, W. B. Saunders Co., Philadelphia, 1976.
  18. Bormer OP. Thesis Immunoassays for Carcinoembryonic antigen, Specificity and Interferences. *Scandinavian Journal of Clinical and Laboratory Investigation* 1992; 53(1), 1–9.
  19. Balistreri W, Shaw L. Liver function. In: *Fundamentals of Clinical Chemistry*. Tietz NW ed. 3<sup>rd</sup> ed. W. B. Saunders Company, Philadelphia, 1987. P: 729-760.
  20. Artimage GY, Berry WG. *Statistical Methods*. 7<sup>th</sup> Ed. Ames, Iowa State University Press. 1987; 39-63.
  21. Harris NS. *Anatomy and physiology*. 5<sup>th</sup> ed. St. Louis: 65, 1992.
  22. Ando K, Oki E, Zhao Y, Ikawa-Yoshida A, Kitao H, Saeki H. Mortalin is a prognostic factor of gastric cancer with normal p53 function. *Gastric Cancer*. 2013; 17(2):255–262.
  23. Umesalma S, Sudhandiran G. Differential inhibitory effects of the polyphenol ellagic acid on inflammatory mediators NF -  $\kappa$ B, iNOS, COX - 2, TNF -  $\alpha$ , and IL - 6 in 1, 2 - dimethylhydrazine - induced rat colon carcinogenesis. *Basic & clinical pharmacology & toxicology*. 2010; 107(2):650-655.
  24. Jackson PE, O'Connor PJ, Cooper DP, Margison GP, Povey AC. Associations between tissue-specific DNA alkylation, DNA repair and cell proliferation in the colon and colon tumour yield in mice treated with 1, 2-dimethylhydrazine. *Carcinogenesis*. 2003; 24(3):527-533.
  25. Lauschke H, Tolba R, Burger B, Minor T, Hirner A. Lipid peroxidation as additional marker in patients with colorectal cancer. *European surgical research*. 2002; 34(5):346-350.
  26. Khan R, Sultana S. Farnesol attenuates 1, 2-dimethylhydrazine induced oxidative stress, inflammation and apoptotic responses in the colon of Wistar rats. *Chemico-Biological Interactions*. 2011; 192(3):193-200..
  27. Perše M. Oxidative stress in the pathogenesis of colorectal cancer: cause or consequence?. *BioMed research international*, 2013.
  28. Barrera G. Oxidative stress and lipid peroxidation products in cancer progression and therapy. *ISRN oncology*, 2012.
  29. Branković B, Stanojević G, Nestorović M, Veljković A, Stojanović I, Petrović D, Pavlović D, Kocić G, Đinđić B, Krivokapić Z. Trosative stress parameters in colon cancer tumor, adjacent and healthy tissue. *Actamedica Medianae*. 2016; 55(1):44-50.
  30. Valko M, Rhodes C, Moncol J, Izakovic MM, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-biological interactions*. 2006; 160(1):1-40.
  31. Nabil HM, Hassan BN, Tohamy AA, Waaer HF, Abdel Moneim AE. Radioprotection of 1, 2-dimethylhydrazine-initiated colon cancer in rats using low-dose  $\gamma$  rays by modulating multidrug resistance-1, cytokeratin 20, and  $\beta$ -catenin expression. *Human & experimental toxicology*. 2016;35(3):282-292.
  32. Devasena T, Menon VP, Rajasekharan KN. Prevention of 1, 2-dimethylhydrazine-induced circulatory oxidative stress by bis-1, 7-(2-hydroxyphenyl)-hepta-1, 6-diene-3, 5-dione during colon carcinogenesis. *Pharmacological Reports*. 2006;58(2):229.
  33. Hussein SA, Abdel-Aal SA, Mady HA. Chemopreventive effect of curcumin on oxidative stress, antioxidant status, DNA fragmentation and caspase -9 gene expression in 1, 2-dimethylhydrazine-induced colon cancer in rats. *Benha veterinary medical journal*. 2013; 25(2):125 - 138.
  34. Margis R, Dunand C, Teixeira FK, Margis - Pinheiro M. Glutathione peroxidase family—an evolutionary overview. *The FEBS journal*. 2008; 275(15):3959-3970.
  35. Nagai T, Inoue R, Kanamori N, Suzuki N, Nagashima T. Characterization of honey from different floral sources. Its functional properties and effects of honey species on storage of meat. *Food chemistry*. 2006; 97(2):256-262.
  36. Pasupuleti VR, Sammugam L, Ramesh N, Gan SH. Honey, propolis, and royal jelly: a comprehensive review of their biological actions and health benefits. *Oxidative medicine and cellular longevity*, 2017.
  37. Erejuwa OO, Sulaiman SA, Ab Wahab MS. Honey: a novel antioxidant. *Molecules*. 2012; 17(4):4400-4423..
  38. Seyoum A, Asres K, El-Fiky FK. Structure–radical scavenging activity relationships of flavonoids. *Phytochemistry*. 2006; 67(18):2058-2070.
  39. Almasaudi SB, El-Shitany NA, Abbas AT, Abdel-dayem UA, Ali SS, Al Jaouni SK,

- Harakeh S. Antioxidant, anti-inflammatory, and antiulcer potential of manuka honey against gastric ulcer in rats. *Oxidative medicine and cellular longevity*, 2016.
40. Ahn MR, Kunimasa K, Kumazawa S, Nakayama T, Kaji K, Uto Y, Hori H, Nagasawa H, Ohta T. Correlation between antiangiogenic activity and antioxidant activity of various components from propolis. *Molecular nutrition & food research*. 2009;53(5):643-651.
41. Pérez E, Rodríguez-Malaver AJ, Vit P. Antioxidant capacity of Venezuelan honey in wistar rat homogenates. *Journal of Medicinal Food*. 2006; 9(4):510-516.
42. Yao LK, Razak SLA, Ismail N, Fai NC, Asgar MHAM, Sharif NM, Aan GJ, Jubri Z. Malaysian gelam honey reduces oxidative damage and modulates antioxidant enzyme activities in young and middle aged rats. *Journal of Medicinal Plants Research*. 2011; 5(23):5618-5625.
43. Sobral F, Sampaio A, Falcão S, Queiroz MJR, Calhelha RC, Vilas-Boas M, Ferreira IC. Chemical characterization, antioxidant, anti-inflammatory and cytotoxic properties of bee venom collected in Northeast Portugal. *Food and Chemical Toxicology*. 2016; 94:172-177.
44. Muhammad MMA, Mouchira M, Naglaa RA. Physiological effects of Bee Venom and Propolis on irradiated Albino rats. *Danish Journal of Agriculture and Animal Sciences*. 2015; 11-21.
45. Jeong CH, Cheng WN, Bae H, Lee KW, Han SM, Petriello MC, Lee HG, Seo HG, Han SG. Bee Venom Decreases LPS-Induced Inflammatory Responses in Bovine Mammary Epithelial Cells. *Journal of microbiology and biotechnology*. 2017;27(10):1827-1836.
46. Hanafi MY, Zaher EL, El-Adely SE, Sakr A, Ghobashi AH, Hemly MH, Kazem AH, Kamel MA. The therapeutic effects of bee venom on some metabolic and antioxidant parameters associated with HFD-induced non-alcoholic fatty liver in rats. *Experimental and Therapeutic Medicine*. 2018; 15(6):5091-5099.
47. Xiao B, Guo J, Lou Y, Meng D, Zhao W, Zhang L, Yan C, Wang D. Inhibition of growth and increase of alkaline phosphatase activity in cultured human oral cancer cells by all-trans retinoic acid. *International journal of oral and maxillofacial surgery*. 2006; 35(7):643-648.
48. Hirasaka K, Tokuoka K, Nakao R, Yamada C, Oarada M, Imagawa T, Ishidoh K, Okumura Y, Kishi K, Nikawa T. Cathepsin C propeptide interacts with intestinal alkaline phosphatase and heat shock cognate protein 70 in human Caco-2 cells. *The Journal of Physiological Sciences*. 2008; 58(2):105-111.
49. Saif MW, Alexander D, Wicox CM. Serum alkaline phosphatase level as a prognostic tool in colorectal cancer: a study of 105 patients. *The journal of applied research*. 2005;5(1):88.
50. Ghadi FE, Malhotra A, Ghara AR, Dhawan DK. Chemopreventive effects of selenium on cancer marker indices and ultrastructural changes during 1, 2 dimethylhydrazine-induced colon carcinogenesis in rats. *Journal of gastrointestinal cancer*. 2013; 44(1):54-59.
51. Aliyu M, Odunola OA, Owumi SE, Gbadegesin MA, Choudhary MI, Farooq AD, Rasheed H, Erukainure, o L, Ahmed S. Daily consumption of honey: effects on male wister albino rats. *International Journal of Food Nutrition and Safety*. 2012; 1(2):66-74.
52. Mohamed NZ, Aly HF, El-Salamony HE. Bee Honey Modulates the Oxidant-Antioxidant Imbalance in Diethyl nitrosamine-Initiated Rat Hepatocellular Carcinoma. *Journal of Applied Pharmaceutical Science*. 2016; 6(07):156-163.
53. Shen HM, Tergaonkar V. NFκB signaling in carcinogenesis and as a potential molecular target for cancer therapy. *Apoptosis*. 2009; 14(4):348-363.
54. Duffy MJ, Van Dalen A, Haglund C, Hansson L, Holinski-Feder E, Klapdor R, Lamerz R, Peltomaki P, Sturgeon C, Topolcan O. Tumour markers in colorectal cancer: European Group on Tumour Markers (EGTM) guidelines for clinical use. *European journal of cancer*. 2007; 43(9):1348-1360.
55. Amano K, Kumamoto K, Kuwabara K, Ishiguro T, Ohsawa T, Okada N, Kumagai Y, Baba H, Ishibashi K, Tsuji Y, Haga N, Ishida H. The clinical significance of serum anti-p53 antibody as a monitoring marker in colorectal cancer. *Gan to kagakuryoho. Cancer & chemotherapy*. 2012; 39(12): 2170-2172.
56. Nakatani H, Kumon T, Kumon M, Hamada S, Okanou T, Kawamura A, Nakatani K, Hiroi M, Hanazaki K. High serum levels of both carcinoembryonic antigen and carbohydrate antigen 19-9 in a patient with sigmoid colon cancer without metastasis. *The Journal of Medical Investigation*. 2012; 59(3.4): 280-283.
57. Ogata Y, Murakami H, Sasatomi T, Ishibashi N, Mori S, Ushijima M, Akagi Y, Shirouzu K. Elevated preoperative serum carcinoembryonic antigen level may be an effective indicator for needing adjuvant chemotherapy after potentially curative resection of stage II colon cancer. *Journal of surgical oncology*. 2009, 99(1):65-70.
58. Mizejewski GJ. Alpha-Fetoprotein (AFP) and Gastric Cancer: Why is Lethality More Prevalent in AFP-Secreting than Non-Secreting Tumors?.

- Cancer Therapy & Oncology International Journal. 2018; 9(1):555753.
59. Duleva V, Bajkova D. Preventive action of honey on experimental colon tumorigenesis. Arch. Balkan Med. Union. 2005; 40(1):49–51.
  60. Oršolić N, Šver L, Verstovšek S, Terzić S, Bašić I. Inhibition of mammary carcinoma cell proliferation in vitro and tumor growth in vivo by bee venom. Toxicon. 2003; 41(7):861-870.
  61. Oršolić N, Josipović P, Bašić I. Direct antitumor activity of honey bee venom in vivo and in vitro. Egyptian Journal of Natural Toxins. 2009; 6(1):1-5.
  62. Perše M, Cerar A. Morphological and molecular alterations in 1, 2 dimethylhydrazine and azoxymethane induced colon carcinogenesis in rats. BioMed Research International. 2011; 473964–473978.
  63. Chang FH, Tzeng DS, Lee TM, Chen TC, Hsu LS. Mutations in the p53 tumor suppressor gene in colorectal cancer in Taiwan. The Kaohsiung journal of medical sciences. 2003; 19(4):151-157.
  64. Walia S, Kamal R, Dhawan DK, Kanwar SS. Chemoprevention by Probiotics During 1, 2-Dimethylhydrazine-Induced Colon Carcinogenesis in Rats. Digestive diseases and sciences. 2018; 63(4):900-909.
  65. Hilska M, Collan YU, Laine VJO, Kössi J, Hirsimäki P, Laato M, Roberts PJ. The significance of tumor markers for proliferation and apoptosis in predicting survival in colorectal cancer. Diseases of the colon & rectum. 2005; 48(12):2197-2208.
  66. El-kott AF, Kandeel AA, El-Aziz SFA, Ribea HM. Anti-tumor effects of bee honey on PCNA and P53 expression in the rat hepatocarcinogenesis. Int J Cancer Res. 2012; 8(4):130-139.
  67. Gülmez Y, Aydın A, Can İ, Tekin Ş, Cacan E. Cellular toxicity and biological activities of honey bee (*Apis mellifera* L.) venom. Marmara Pharmaceutical Journal. 2017; 21(2):251 – 260.
  68. Erejuwa OO, Sulaiman SA, Wahab MSA. Effects of honey and its mechanisms of action on the development and progression of cancer. Molecules. 2014; 19(2):2497-2522.
  69. Moga MA, Dimienescu OG, Arvătescu CA, Ifteni P, Pleş L. Anticancer Activity of Toxins from Bee and Snake Venom—An Overview on Ovarian Cancer. Molecules. 2018; 23(3):692.
  70. Gajski G, Garaj-Vrhovac V. Melittin: a lytic peptide with anticancer properties. Environmental toxicology and pharmacology. 2013; 36(2):697-705.

11/6/2018