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Potential effects of asparagus (*Asparagus officinalis* L.) shoots and effective microorganisms in colon carcinogenesis induced by azoxymethane in rats

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Abstract: Shoots of white asparagus (*Asparagus officinalis* L.) are a popular vegetable dish, known to be rich in many bioactive components reported to possess antioxidant, and anti-inflammatory and antitumor activities. The present study aims to investigate the potential effects of asparagus shoots powder (ASP) and effective microorganisms in colon carcinogenesis induced by azoxymethane in rats. Chemical analysis indicated that ASP are rich in antioxidant vitamins (A, C and E), mineral and different classes of bioactive compounds such phenolics, flavonoids, carotenoids, oligosaccharides and inulin which are giving such food high significant as an important functional food. Treatment of animals with azoxymethane caused a significant decreased ($p \leq 0.05$) in body weight gain (BWG, -68.70%), feed intake (FI, -16.97%), feed efficiency ratio (FER, -67.21%), hemoglobin (Hb, -36.99%), packed cell volume (PCV, -23.40%) and intestinal mucosal metabolizing enzyme (cytochrome P_{450} reductase, Cyto P_{450} rd, -74,56%) while significant increased ($p \leq 0.05$) in cytokines [Interleukin-1, IL-1(259.29%) and tumor necrosis factor-alpha, TNF-α (242.68%)] levels and cyclooxygenase ($Cox\text{-}_2$, 163.41%) activity which inducible and plays an important role in inflammation and intestinal tumorigenesis was recorded. Supplementation of the rat diets with ASP or ME and their mixture enhanced all of these parameters by different rates. The highest improvement was recorded for the mixture treatment (EM+ASP). Taken together our data highlight the chemopreventive potential of ASP on colon carcinogenesis through its ability to promote normal cellular homeostasis and antioxidant defense systems. Therefore, we recommended ASP to be included in our daily diets, drinks and food products.

Keywords: Bioactive compounds, body weight gain, feed intake, hemoglobin, IL-1, TNF-α, cytochrome P_{450}.

Introduction

Colon, or colorectal, cancer is cancer that starts in the large intestine (colon) or the rectum (end of the colon). Other types of cancer can affect the colon. These include lymphoma, carcinoid tumors, melanoma, and sarcomas (Cunningham *et al.*, 2010). Cancers of the colon and rectum altogether are the third most common tumour type worldwide. Cancer of the colon is more frequent than rectal cancer: in high-risk populations the ratio is 2: 1, while in low-risk countries rates are generally similar (Labianca *et al.*, 2010). Aetiology and risk factors of colon cancer dietary factors and non-dietary factors which include smoking tobacco, chronic use of non-steroidal anti-inflammatory drugs (NSAIDs) and some conditions and genetic predispositions. Possible Complications could be included: blockage of the colon causing bowel obstruction, cancer returning in the colon, cancer spreading to other organs or tissues (metastasis) and development of a second primary colorectal cancer (NCCN, 2010). Treatment depends on many things, including stage of the cancer. Treatments may include: Surgery (most often a colectomy) to remove cancer cells, chemotherapy to kill cancer cells and radiation therapy to destroy cancerous tissue. Surgical interventions including colon resection and percutaneous ablation are regarded as the most effective approach with curative potential for colon cancer. Unfortunately, due to numerous lesions, and extra colon metastasis, only limited of colon cancer patients are suitable for surgery. On the other hand, chemotherapeutic drugs for colon cancer are limited but their clinical benefits remains modest and drug resistance developed within last years (NCCN, 2010). Furthermore, problems such as colon toxicity, recurrence, drug resistance and other adverse effects exist in current therapeutics, which urge researchers to find alternative treatment.

Many of authorities and academic centers of research pay more attention towards the area of cancer chemoprevention compounds. One of the most impressive findings in the field of chemoprevention is the very large number of compounds that have been demonstrated to prevent the occurrence of cancer. Many of these classes are lies in an enlarged group of compounds called phytochemicals (*phyto* is Greek for plant). Phytochemicals are the bioactive compounds of plants that do not deliver energy and are not yet classified as essential nutrients but possess healthful properties beyond their use as macronutrients or micronutrients. Plants usually produce such low-molecular-weight ingredients for their

protection against pests and diseases, for the regulation of their growth, or as pigments, essence, or odor (Perez-Vizcaino *et al.,* 2006). Scientists have identified thousands of phytochemicals, including flavonoids, glucosinolates (isothiocyanates and indoles), phenolic acids, phytates, and phytoestrogens (isoflavones and lignans), in vegetables, fruits, grains, legumes, and other plant sources. A vast variety of phytochemicals that are present in the daily human diet have been found to possess substantial antimutagenic and anticarcinogenic properties (Surh, 2002). The chemopreventive effects of the majority of edible phytochemicals are often attributed to their antioxidative or anti-inflammatory activities. Besides the edible chemopreventives in vegetables, fruits, herbs, and spices, some phytochemicals in diverse plants also have other beneficial health effects such as anti-obesity, lipid-lowering, and/or antidiabetic properties (Surh et al.,2001).

Asparagus (*Asparagus officinalis* L.) is a popular vegetable often used in soups, salads and vegetable dishes. Several studies revealed numerous pharmacological activities associated with *A. officinalis*, such as anti-inflammation, anti-mutagenicity, and cytotoxicity. Polysaccharides, steroidal saponins and flavonoids extracted from the plant were suggested to be main constituents responsible for its bioactivities (Yue *et al.,* 2016). Asparagus bioactive compounds have been clinically adopted to treat various cancers including breast cancer, leukemia, and lung cancer. For example, Xiang *et al.*, (2014) reported that the asparagus polysaccharide selectively inhibited cell proliferation of HepG2 (IC50, 5.7 mg/mL) and Hep3B (IC50, 9.39 mg/mL) cell lines with less toxicity on normal human hepatocellular 7702 cells (IC50, 20.92 mg/mL). Mechanistic study revealed that the induction of G2/M phase arrest and apoptosis by asparagus polysaccharide via modulation of Bax, Bcl-2 and capase-3 contributed to the effects. Also, asparanin A, a steroidal saponin isolated from A. officinalis, has displayed antiproliferative activities against many cancers, such as esophageal cancer, gastric cancer, lung cancer and leukemia (Huang *et al.,* 2008). Asparanin A also exerted dose- and time-dependent inhibition against HepG2 cells with IC50 at 6.20 _ 0.56 _mol/L. The treatment induced G2/M cell cycle arrest through downregulating Cdk1, Cdk4, and cyclin A and simultaneously upregulating p21WAF1/Cip1. Besides, the promotion of apoptosis via both the intrinsic and extrinsic pathway was observed upon asparanin A treatment to HepG2 cells (Liu *et al.,* 2009).

The microbial community resident in the human colon is a highly complex consortium of many different bacterial species. However, the reduction in carcinogen-induced tumour incidence was my be associated with the modification of gut bacterial activities related to the formation of carcinogens or tumor promoters in the gut. In this direction, Do *et al.*, (2007) feed the rats an effective microorganisms (EM), being a commercial mixture of photosynthesizing bacteria. EM consists of a mixture of lactic acid bacteria, photosynthetic bacteria, yeasts and fungi were accompanied by a reduction in the incidence colon carcinoma. According to our knowledge, the studies regarding the potential effects of asparagus on colon disease/cancer are so limited. Therefore, in this study, we examined the potential preventive effects of asparagus shoots powder (ASP) against colon carcinogenicity in rats induced by azoxymethane. Also, the mixing of some effective microorganisms with ASP on such carcinogenicity rate will be in the scope of this investigation.

Materials and Methods

Materials

Asparagus (*Asparagus officinalis* L.) samples were obtained from Mansoura local markets, Mansoura, Egypt.

Azoxymethane (Methyl-methylimino-oxidoazanium)© : was purchased from Sigma Chemical Co. (St. Louis, MO, Company agent, Cairo, Egypt).

Effective microorganism (EM)®: were obtained from Microbiological Resource Center Cairo Mircen. Faculty of Agriculture, Ain Shams University, Cairo, Egypt. EM consists of *Bifidobacterium longum*, *Saccharomyces boulardii* and *Lactobacillus casei.*

All organic solvents, buffers and other chemicals of analytical grade were purchased from El-Ghomhorya Company for Trading Drugs, Chemicals and Medical Instruments, Cairo, Egypt.

Throughout this study a SP Thermo Separation Products Liquid Chromatograph (Thermo Separation products, San Jose, CA) was used with a Consta Metvic 4100 pump, a Spectra Series AS100, Spectra System UV 1000 UV/Visible Spectrophotometer Detector, Spectra System FL 3000 and a PC 1000 system software. The columns used (Alltech, Deerfield, IL, USA) were a Spherosorb ODC-2 (5 µm, 150 x 4.6 mm I.d.) for glutathione fractions; a reversed phase water Adsorbosil C18 (5 µmol/L, 100 mm x4.6–mm internal diameter) for vitamin C; and

normal Ultrasphere Si (5 µmol/L, 250 mm x4.6–mm internal diameter) for analysis of vitamins A and E, and curcumin.

Methods:

Asparagus shoots powder (ASP) preparation

Asparagus shoots were washed and then dried in a hot air oven (Horizontal Forced Air Drier, Proctor and Schwartz Inc., Philadelphia, PA) at two stages 50 ^{0}C for 6 hrs followed by 40 ^{0}C for 10 hrs. The dried shoots were ground into a fine powder in high mixer speed (Moulinex Egypt, Al-Araby Co., Egypt). The material that passed through an 80 mesh sieve was retained for use.

Chemical analysis of ASP

ASP samples were analyzed for moisture, protein (T.N. × 6.25, micro - kjeldahl method using semiautomatic apparatus, Velp company, Italy) , fat (soxhelt miautomatic apparatus Velp company, Italy , petroleum ether solvent), ash, fiber and essential oil (using rotary evaporator apparatus, Velp company, Italy) contents were determined using the methods described in the A.O.A.C. (1995). Carbohydrates calculated by differences:
Carbohydrates (%) = 100 - (% moisture + % protein + % fat + % Ash + % fiber).

Total phenolics, carotenoids and total dietary fiber in ASP samples were analyzed as follow: TRP was extracted with 80% acetone and centrifuged at 10,000g for 15 min. For biscuits samples, one gram of biscuit powder was extracted with 20 ml of 80% acetone and centrifuged at 8000g at room temperature. The supernatant obtained from both samples were used for the analysis of total phenolics, carotenoids, curcumin and antioxidant activity.

Total phenolics were determined using Folin-Ciocalteu reagent (Singleton and Rossi, 1965). Two hundred milligrams of sample was extracted for 2 h with 2 mL of 80% MeOH containing 1% hydrochloric acid at room temperature on an orbital shaker set at 200 rpm. The mixture was centrifuged at 1000g for 15 min and the supernatant decanted into 4 mL vials. The pellets were combined and used for total phenolics assay. One hundred microliters of extract was mixed with 0.75 mL of Folin-

Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand at 22 0C for 5 min; 0.75 ml of sodium bicarbonate (60g/L) solution was added to the mixture after 90 min at 22 0C, absorbance was measured at 725 nm. Results are expressed as ferulic and equivalents. The total carotenoids in 80% acetone extract were determined by using the method reported by Litchenthaler (1987). Total dietary fiber content in the ASP was estimated according to the method described by Asp *et al.* (1983).

All vitamins (A, C, and E) were extracted according to methods previously detailed (Epler *et al.,* 1993; Moeslinger *et al.,* 1994 and Hung *et al.,* 1980) and were analyzed by HPLC techniques. For vitamins A and E, the chromatographic conditions were as follows: flow rate, 1.5 mL/min; detection, UV absorption at 265 nm, volume of injection, 20 µL; temperature, room temperature; and the mobile phase composition was an isocratic system of isopropanol:hexane (1:99). For vitamin C, the conditions were: flow rate, 1 mL/min; detection, UV absorption at 254 nm, volume of injection, 20 µL; temperature, room temperature, and mobile phase composition was an isocratic system of 100% methanol. Retention times and absorbance ratio against those of standards were used to identify the separated vitamins. Quantitative determination of each vitamin was determined from its respective peak area and corresponding response factor. The percent recoveries of vitamins were also studied by adding each vitamin to plasma after sample preparation and HPLC determination. Under such chromatographic conditions, mean values (±SD) of vitamins A, C and E recoveries were 90.56 ± 1.17, 89.65 ±2.17 and 87.09 ±1.56%, respectively.

Oligosaccharides and inulin were extracted and determined according to the methods mentioned by Henk *et al.,* (2000)

Antioxidant activity

Antioxidant activity of ASP extracts and standards (α-tocopherol, BHA, ans BHT; Sigma Chemical Co., St. Louis, Mo) was determined according to the β-carotene bleaching method following a modification of the procedure described by Marco (1968). For a typical assay, 1mL of β-carotene (Sigma) solution, 0.2 mg/mL in chloroform, was added to round-bottom flasks (50 mL) containing 0.02 mL of linoleic acid (J.T. Baker Chemical Co., Phillipsburg, NJ) and 0.2 mL of Tween 20 (BDH Chemical Co., Toronto, On). Each mixture was then dosed with 0.2 mL of 80%

MeOH (as control) or corresponding plant extract or standard. After evaporation to dryness under vacuum at room temperature, oxygenated distilled water (50 ml) was added and the mixture was shaken to form a liposome solution. The samples were then subjected to thermal autooxidation at 50 ^{0}C for 2 h. The absorbance of the solution at 470 nm was monitored on a spectrophotometer (beckman DU-50) by taking measurements at 10 min intervals, and the rate of bleaching of β-carotene was calculated by fitting linear regression to data over time. All samples were assayed in triplicate. Various concentrations of BHT, BHA, and α-tocopherol in 80% methanol was used as the control. Antioxidant activity (AA) was all calculated as percent inhibition ralative to control using the following equation (Al-Saikhan *et al*., 1995).

$$AA= (R_{control} - R_{sample}) / R_{control} \times 100$$

Where: $R_{control}$ and R_{sample} were the bleaching rates of beta-carotene in reactant mixture without antioxidant and with plant extract, respectively.

Biological Experiments

Animals

Animals used in this study, adult male albino rats (150±8.7 g per each) were obtained from Research Institute of Ophthalmology, Medical Analysis Department, Giza, Egypt.

Basal Diet

The basic diet prepared according to the following formula as mentioned by (AIN, 1993) as follow: protein (10%), corn oil (10%), vitamin mixture (1%), mineral mixture (4%), choline chloride (0.2%), methionine (0.3%), cellulose (5%), and the remained is corn starch (69.5%). The used vitamins mixture component was that recommended by Campbell, (1963) while the salts mixture used was formulated according to Hegsted, (1941).

Experimental design

All biological experiments performed a complied with the rulings of the Institute of Laboratory Animal Resources, Commission on life Sciences, National Research Council (NRC, 1996). Rats (n=30 rats) were housed individually in wire cages in a room maintained at 25 ± 2 ^{0}C,

relative humidity (55±5%), a 12-hr lighting cycle and kept under normal healthy conditions. All rats were fed on basal diet for one-week before starting the experiment for acclimatization. After one week period, the rats were divided into two main groups, the first group (Group 1, 6 rats, as a negative control group) still fed on basal/standard diet and injected with the vehicle alone (5 ml/kg body weight) and the other main group (24 rats) was challenged with an ip injection two times of azoxymethane (AOM, 12.5 mg/kg body weight) dissolved in 0.9% NaCI solution containing 0.1% Tween 20 to induce colon impaired rats according to Rowland *et al.,* (1998), then classified into four sub- groups as follow:

- Group (2): fed on standard diet only as a positive control
- Group (3): fed on standard diet plus 5 ml/kg BW of EM by oral intubations.
- Group (4): fed on standard diet containing 5 g/kg BW of ASP.
- Group (5): fed on standard diet containing 5 g/kg BW of ASP plus 5 ml/kg BW of EM.

At the end of experimental period (8 weeks), the rats were anaesthetized by diethyl ether and sacrificed. Blood samples were collected in clean test tubes and left for coagulation then centrifuged at 3000 rpm for 15 minutes to obtain serum. Body weights of the rats were measured three times a week during four weeks. Daily changes in body weights as percentages were recorded. The percentage of daily changes in body weights and Food efficiency ratio (FER) were calculated.

Hematological analysis

Hemoglobin (Hg) and packed cell volume (PCV) were determined according to Vankampen and Ziglstra (1961) and Mc Inory, (1954), respectively.

Serum levels of total nitric oxide (NO), interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-α) were determined according to Griess *et al.* (1982), Grassi *et al.* (1991) and Beutler *et al.* (1985), respectively.

Colons mucosal of cytochrome P_{450} reductase (Cyto P_{450}) activity, cyclooxygenase (Cox-$_2$) activity, prostaglandin E_2 (PGE_2) concentration and were determined according to Mc-Lean and Day (1974), Hemler and Lands (1976) and Hamberg and Samuelsson (1973), respectively.

Statistical Analysis

The obtained data were statistically analyzed using computerized SPSS (Statistic Program Sigmastat, Statistical Soft-Ware, SAS Institute, Cary, NC). Effects of different treatments were analyzed by one way ANOVA (Analysis of Variance) test using Duncan's multiple range test and $p<0.05$ was used to indicate significance between different groups Snedecor and Cochran (1967).

Results and Discussion

Proximate chemical composition, minerals, vitamins and bioactive compounds contents of ASP

The proximate composition of ASP is shown in Table (1). The results showed that the moisture content was 10.03%, total protein was 16.53%, crude fat was 2.33%, crude fiber was 12.76%, ash content was 6.47% and total carbohydrate content was 51.88%. The proximate composition reported was not in accordance with that observed by Negi *et al.,* 2010 and Olivier *et al.,* 2017). These data reflected the effect of asparagus varieties on the chemical composition of shoots. All of these components in ASP might be important from the nutrition point of view. Therefore, enrichment of different food products with ASP would enhance the nutritional quality of the product better than many food sources. Also, the total phenolics content, total carotenoids, minerals etc in the ASP are giving such food high significant as an important functional food. These data are confirmed with that recorded by Olivier *et al.,* (2017).

On the other side, there are the antioxidant nutrients such as vitamins A, C and E, *β*-carotene and many bioactive compounds (found in ASP) for which there are Dietary Reference Values (DRVs). In this direction, Javeed *et al.,* (2011) reported that of phytochemical of asparagus the presence of saponins, carbohydrates, glycosides and mucilages. However, there are thousands of other bioactive compounds in foods that have antioxidant activity but are not classified as "nutrients." These "non-nutrient antioxidants" include phenolic compounds (found ASP) (Ajila *et al.,* 2008). Also, many studies indicated that there was a positive and significant ($p< 0.01$) relationship between all of the previous bioactive compounds and the antioxidant activity in different plant parts (Jaggi, 2012 and Elhassaneen *et al.,* 2013). Plant-based foods generally

Table 1. Proximate chemical composition, minerals, vitamins and bioactive compounds contents of ASP (as a dry weight basis)

Component	**Content**
Chemical composition (g/100g)	
Water	10.03 ± 2.17
Total protein	16.53 ± 1.89
Crude fat	2.33 ± 0.55
Ash	6.47 ± 1.74
Crude fiber	12.76 ± 1.06
Carbohydrate	51.88 ± 3.76
Minerals (mg/100g)	
Se	0.035 ± 0.012
Ca	200.50 ± 20.76
Mg	117.54 ± 10.65
Na	23.88 ± 5.69
K	1743.56 ± 58.95
P	435.58 ± 37.42
Zn	4.97 ± 0.67
Fe	18.29 ± 1.75
Mn	1.82 ± 0.33
Cu	2.33± 0.28
Vitamins	
Vitamin C (Ascorbic acid, mg/100g)	47.58 ± 9.32
Vitamin A (mg/100g)	57.69 ± 6.93
Vitamin E (mg/100g)	9.96 ± 2.20
Bioactive compounds	
Inulin (g/100g)	23.19 ± 2.89
Oligosaccharides (g/100g)	13.58 ± 1.11
Total carotenoids (mg.100g)	46.39 ± 8.05
Antioxidant activity (AA, %) - Methanolic extract analyzed by β-carotene bleaching method	74.39 ± 5.20
Total flavonoid (mg RE/g)- Methanolic extract	6.18 ± 0.96
Total phenolics content (mg GAE/g) - Methanolic extract	8.76 ± 1.07

Each value represents the mean of three replicates ±SD. GAE, gallic acid equivalent, Re, rutin equivalent

are considered important sources of antioxidants in the diet. Antioxidants help protect cells from the potentially damaging physiological process known as "oxidative stress" (damage to healthy cells or DNA by unpaired electrons known as free radicals). Oxidative stress is thought to be associated with the development of chronic diseases including cancer, heart disease, diabetes, rheumatoid arthritis, obesity, conditions of ageing including neurodegenerative diseases such as Parkinson's and Alzheimer's disease (Halliwell, 1991, Van Gaal *et al.,* 1998, Chaitanya *et al.,* 2010 and Elmaadawy *et al.,* 2016).

Effect of ASP and effective microorganism (EM) on body weight (BWG), feed intake (FI), feed efficiency ratio (FER) of rats

FI, BEG and FRE of the animals subjected to ASP and EM treatments are shown in Table (2) and Figure (1). From such data it could be noticed that treatment of animals with azoxymethane caused a significant decreased ($p \leq 0.05$) in BWG (-68.70%), FI (-16.97%) and FER (-67.21%) compared to normal controls. Supplementation of the rat diets with EM or ASP and mixture of them prevented the decreasing of BWG, FI and FER by different rats. The highest improvement was recorded for the mixture treatment (EM+ASP). These findings conformed with those of Lan *et al.,* (2003) who showed that increases in BWG was efficient utilization of EM provoked assimilation. It is worthy to mention that probiotics have great effect on the main physiological functions of the gastrointestinal tract as well as a reinforcement of the intestinal mucosal barrier against various deleterious agents. Asparagus are content of gross chemical, minerals, vitamins and bioactive compounds may be improvement FI, BWG and FRE of all treated groups. The enhancement of BWG as the result of bioactive compounds feeding such as found in ASP was previously reported by Elmaadawy *et al.,* (2016).

Effect of ASP and EM on blood hemoglobin (HB) and packed cell volume (PCV) of rats

Data in Table (3) and Figure (2) are shown the effect of ASP and EM on blood hemoglobin (HB) and packed cell volume (PCV) of rats.

Table (2): Effect of asparagus shoots powder (ASP) and effective microorganism (EM) on body weight (BWG), feed intake (FI), feed efficiency ratio (FER) of rats

Groups	BWG		FI		FER	
	%	% of change	g/day	% of change	%	% of change
Group 1: Control (-)	25.69±2.18a	-----	18.98±0.64a	-----	0.0244±0.03a	-----
Droup2: Control (+)	8.04±3.31b	-68.70	15.76±1.39b	-16.97	0.0080±0.03b	-67.21
Group 3: EM	22.81±2.69 a	-11.21	18.65±1.24a	-1.74	0.0189±0.03a	-22.54
Group 4: ASP	24.58 ±4.59a	-4.32	19.07±0.45a	0.47	0.0190±0.04a	-22.13
Group 5: EM + ASP	25.61 ±9.65a	-0.31	19.43±0.67a	2.37	0.0205±0.04a	-15.98

Means with different letters in the same column indicate significant difference at P≤0.05

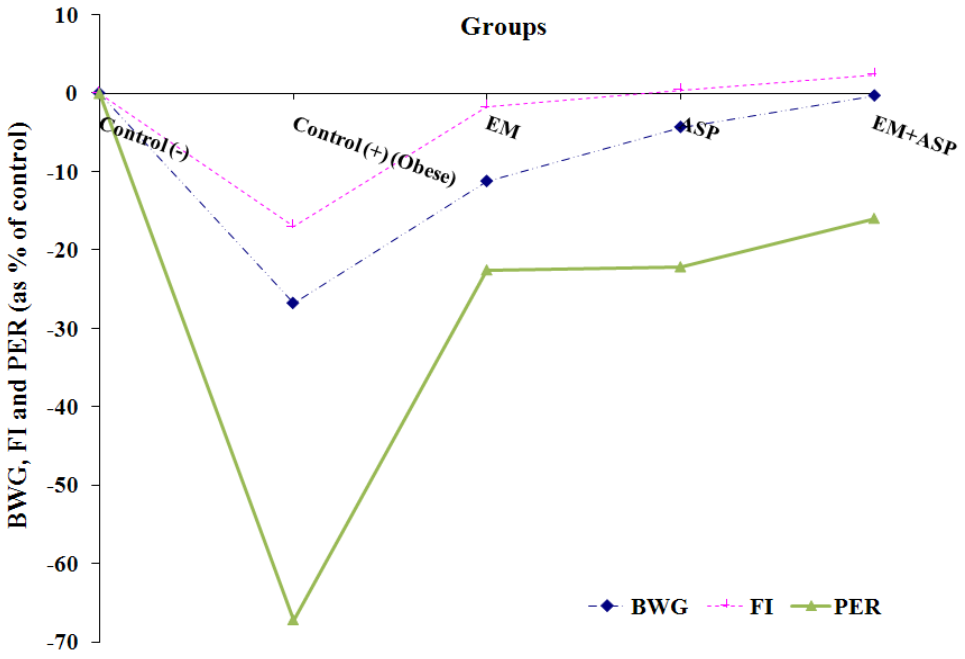

Figure (1): Effect of asparagus shoots powder (ASP) and effective microorganism (EM) on body weight (BWG), feed intake (FI), feed efficiency ratio (FER) of rats

Table (3): Effect of asparagus shoots powder (ASP) and effective microorganism (EM) on blood hemoglobin (HB) and packed cell volume (PCV) of rats

Groups	Hb		PCV	
	g/dl	% of change	%	% of change
Group 1: Control (-)	12.68±2.18 [a]	-----	37.61±3.82 [a]	-----
Droup2: Control (+)	7.99±1.39 [c]	-36.99	28.81±3.55[c]	-23.40
Group 3: EM	10.14±1.4 b	-20.03	33.79±3.47 [b]	-10.16
Group 4: ASP	11.11±2.01 [a]	-12.38	36.51±4.11 [a]	-2.92
Group 5: EM + ASP	12.31±1.82 [a]	-2.92	36.91±3.17 [a]	-1.86

Means with different letters in the same column indicate significant difference at P≤0.05

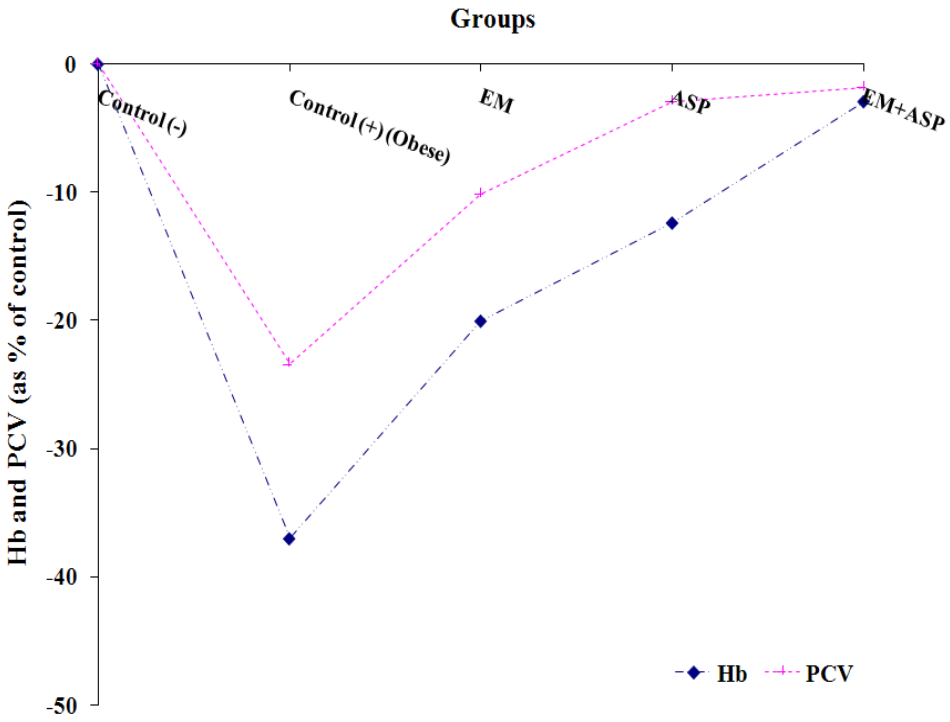

Figure (2): Effect of asparagus shoots powder (ASP) and effective microorganism (EM) on blood hemoglobin (HB) and packed cell volume (PCV) of rats

From such data it could be noticed that treatment of animals with azoxymethane caused a significant decreased ($p \leq 0.05$) in Hb (-6.99%) and PCV (-23.40) compared to normal controls. Supplementation of the rat diets with ME or ASP and mixture of them prevented the decreasing of mean blood Hb and PCV levels. The rate of preventative was recorded for mixture treatment. Such data are in accordance with that observed by Koji *et al.,* (2006) who found that inulin (a bioactive compound found in ASP) effective with improving iron in hemoglobin and PCV.

Effect of ASP and EM on total nitric oxide (NO), Interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-α) of rats

Data in Table (4) and Figure (3) are shown the effect of ASP and EM on total nitric oxide (NO), Interleukin-1 (IL-1)and tumor necrosis factor-alpha (TNF-α) of rats. From such data it could be noticed that treatment of animals with azoxymethane caused a significant increased ($p \leq 0.05$) in NO (67.62%), IL-1 (259.29%) and TNF-α (242.68%) compared to normal controls. Supplementation of the rat diets with ME or ASP and mixture of them prevented the increasing of mean NO, IL-1 and TNF-α levels in serum. The rate of preventative was recorded for mixture treatment. In similar studies, Whittle, (2003) and Hsu and Liu, (2004) found that excessive release of NO from colon epithelial cells induced by methyl-methylimino-oxidoazanium. NO plays an important role in the colon of gastric blood flow as well as in the maintenance of colon mucosal integrity. Although NO is required for normal gastrointestinal functions, there is also some evidence that a large excess of NO may have deleterious effects on the gastrointestinal tract (Lloyd and Taylor, 2010).

It is widely reported that cytokines (IL1β, TNF-α), matrix metalloproteinase (MMP-7, MMP-9) are involved in chronic inflammation, which creates a microenvironment favoring colon carcinogenesis (Lu et al., 2006). The role of these molecules have been linked to all steps involved in tumorigenesis, including initiation, cellular transformation, promotion, survival, invasion, and metastasis. Furthermore, Souad *et al.,* (2013) observed that Asp drastically reduced *IL1β* and TNF-α expression, which are known to induce an upregulation of

Table (4): Effect of asparagus shoots powder (ASP) and effective microorganism (EM) on total nitric oxide (NO), Interleukin-1 (IL-1)and tumor necrosis factor-alpha (TNF-α) in rats

Groups	NO		IL-1		TNF-α	
	pg/mg	% of change	pg/ml	% of change	pg/ml	% of change
Group 1: Control (-)	37.06±2.94 d	0	13.46±1.06 c	0	4.10±0.81 e	0
Droup2: Control (+)	62.12±6.76 a	67.62	48.36±1.34 a	259.29	14.05±1.94 a	242.68
Group 3: EM	42.67±5.29 b	15.14	25.64±2.69 b	90.49	7.46±0.66 d	81.95
Group 4: ASP	40.27±6.67 bc	8.66	22.97±2.72 b	70.65	8.48±0.63 c	106.83
Group 5: EM + ASP	39.35±4.83 c	6.18	20.40±6.65 b	51.56	9.37±0.31 b	128.54

Means with different letters in the same column indicate significant difference at P≤0.05

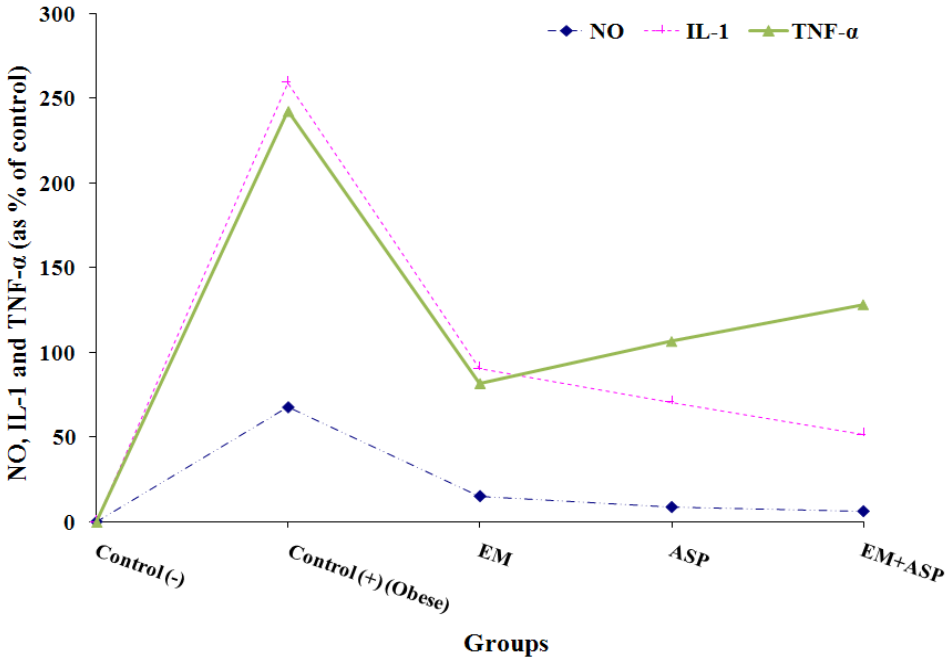

Figure (3): Effect of asparagus shoots powder (ASP) and effective microorganism (EM) on total nitric oxide (NO), interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-α) in rats

MMPs (Friedl and Wolf, 2003). Bioactive asparagus constituents such as saponins or fructo-oligosaccharides are well recognized as potent immune stimulators (Rajput *et al.,* 2007 and Sabater-Molina *et al.,* 2009). These effects might be in part modulated by host-defense components of the innate immune system such as α-defensin-5 (*DEF-5*) and lipocalin 2 (*LCN2*), which are considered as active weapons against several cancer cell types (Papo and Shai, 2005). On the other side, the expression of receptors belonging to the super family of tumor necrosis factor (TNF) receptors such as TNF-related apoptosis such as TNF-related apoptosis inducing ligand (TRAIL) receptors DR4 and DR5, are often altered in patients with colon cancer (Cawston and Wilson, 2006). So, alterations of the processes controlling apoptosis extend the life span of cells and may favor cell neoplastic expansion independently of cell division (Reed, 1999).

Effect of ASP and EM on cytochrome P450 reductase (Cyto P_{450} rd) activity, cyclooxygenase (Cox-$_2$) activity and prostaglandin E2 (PGE_2) concentration of rats

Data in Table (5) and Figure (4) are shown the effect of ASP and EM on cytochrome P450 reductase (Cyto P_{450} rd) activity and prostaglandin E2 (PGE_2) concentration of rats. From such data it could be noticed that treatment of animals with azoxymethane caused a significant decreased ($p\leq0.05$) in Cyto P_{450} rd (-74.56%) and PGE_2 (-41.08%) compared to normal controls. Supplementation of the rat diets with ME or ASP and mixture of them prevented the increasing of mean Cyto P_{450} rd and PGE_2 levels in serum. The opposite direction was recorded for cyclooxygenase (Cox-$_2$) activity. The rate of preventative was recorded for mixture treatment. COX-2, the inducible isoenzyme of COX, is induced in the presence of intestinal tumorigenesis inflammation. Although it has been reported that COX-2 derived prostaglandins are involved in inflammation and intestinal tumorigenesis (Oshima and Taketo, 2002). In the present study, azoxymethane caused increased level of gastric COX-2. This is consistent with many previous studies which reported that non-steroidal anti-inflammatory drugs (NSAIDs) can rapidly up-regulate COX-2 expression in the gastrointestinal (Pereira *et al.,* 2010).

Table (5): Effect of asparagus shoots powder (ASP) and effective microorganism (EM) on cytochrome P450 reductase (Cyto P_{450} rd) activity, cyclooxygenase (Cox-$_2$) activity and prostaglandin E2 (PGE_2) concentration of rats

Groups	Cyto P_{450} rd		Cox-$_2$		PGE_2	
	ng/mg	% of change	pg/mg	% of change	pg/mg	% of change
Group 1: Control (-)	2.87±0.32 a	------	5.74±0.45 d	------	525.07±45.31 a	------
Droup2: Control (+)	0.73±0.07 c	-74.56	15.12±1.23 a	163.41	309.37±15.54 c	-41.08
Group 3: EM	1.86±0.23 b	-35.19	6.81±0.35 c	18.64	473.37±21.92 b	-9.85
Group 4: ASP	2.13±0.48 a	-25.78	7.92±0.90 bc	37.98	489.53±33.60 b	-6.77
Group 5: EM + ASP	2.69±0.58 a	-6.27	8.79±0.50 b	53.14	520.23±58.68 ab	-0.92

Means with different letters in the same column indicate significant difference at P≤0.05

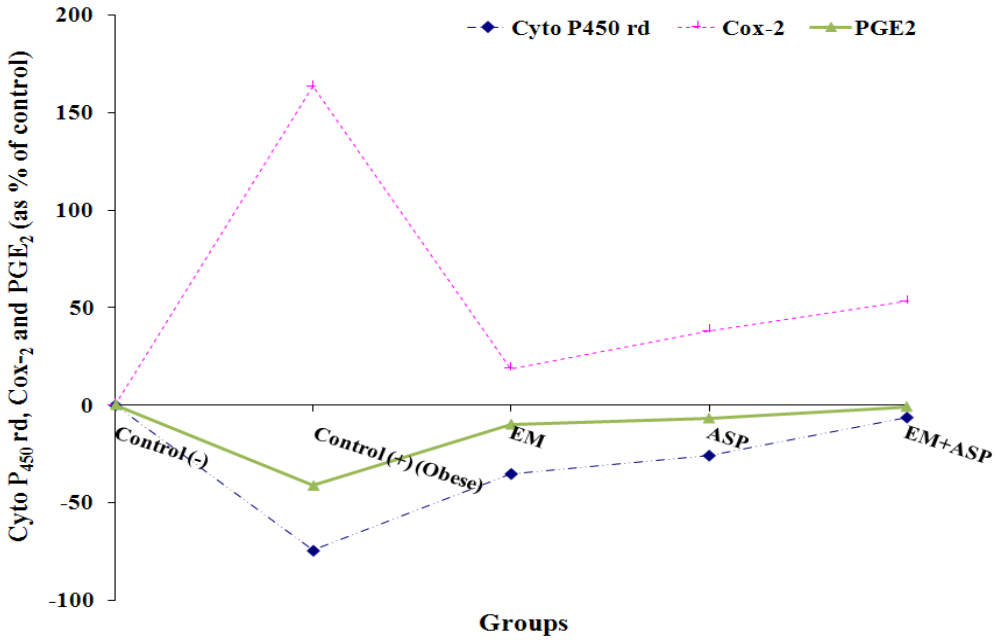

Figure (4): Effect of asparagus shoots powder (ASP) and effective microorganism (EM) on cytochrome P_{450} reductase (Cyto P_{450} rd) activity, cyclooxygenase (Cox-$_2$) activity and prostaglandin E2 (PGE_2) concentration of rats

Previous research studies have demonstrated that NSAIDs causes the intestinal tumorigenesis by decreasing the prostaglandins (PGE2) which inducible and plays an important role in inflammation and intestinal tumorigenesis. Asparagus can help relieve musculoskeletal as well as bowel pain and inflammation (Samad *et al.*, 2014). Another study by Park *et al.*, (2011) who reported that the asparagus has an inflammatory-cascade normalizing action that promotes the healthy metabolism and activity of arachadonic acid, prostaglandins, leukotrienes and platelets. Also, asparagus is a natural COX-2 enzyme modulator, by stopping the cascading effect caused by COX-2 enzyme. It is also an antioxidant that helps to support the body's functions and maintain them in a normal range by neutralizing free radicals.

Conclusion

In conclusion, this study demonstrates that bioactive asparagus shoot constituents such as phenolics, antioxidant vitamins, saponins and oligosaccharides are well recognized as potent immune stimulators. These effects might be in part modulated by host-defense components of the innate immune system, which are considered as active weapons against several cancer cell types. Taken together our data highlight the chemopreventive potential of ASP on colon carcinogenesis and its ability to promote normal cellular homeostasis and antioxidant defense systems. Therefore, we recommended ASP to be included in our daily diets, drinks and food products.

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التأثيرات المحتملة لسيقان الهليون الأبيض والكائنات الحية الدقيقة الفعالة على حدوث سرطان القولون المستحث بالأزوكسى ميثان فى الفئران

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الملخص العربى:

تعد سيقان الهليون الأبيض من أطباق الخضار الشعبية التى تتميز بغناها بالعديد من المركبات النشطة حيويا والتى تبدى أنشطة مضادة للأكسدة، والإلتهابات والأورام .لذلك تهدف الدراسة الحالية إلى إستكشاف التأثيرات المحتملة لسيقان الهليون الأبيض والكائنات الحية الدقيقة الفعالة على حدوث سرطان القولون المستحث بالأزوكسى ميثان فى الفئران. ولقد أشارت نتائج التحليل الكيميائي إلى أن مسحوق سيقان الهليون غنى بالفيتامينات المضادة للأكسدة (ا، جـ، هـ) والمعادن والمجموعات المختلفة من المركبات النشطة بيولوجيا مثل الفينولات، الفلافونويدات، الكاروتينات، سكرات الأوليجو والإينولين التي تعطي مثل هذا الغذاء أهمية كبيرة كغذاء وظيفي مهم. وقد تسببت معاملة الحيوانات بالأزوكسى ميثان الى حدوث انخفاض معنوي ($p\leq0.05$) في وزن الجسم (-٦٨.٧٠٪)، تناول العلف (-١٦.٩٧٪)، نسبة كفاءة العلف (-٦٧.٢١٪)، وهيموجلوبين الدم (-٣٦,٩٩%) ، حجم الخلايا المعبأة (-٢٣.٤٠٪) وانزيم استقلاب الغشاء المخاطى للأمعاء – سيتوكروم P450 ريدوكتاس (-٧٤,٥٦%). فى حين سجلت زيادة معنوية ($p\leq0.05$) فى السيتوكينات [إنترليوكين -١ (٢٥٩.٢٩٪) وعامل النخر الورمى -ألفا، (242.68٪) ومستويات الأكسدة الحلقية (١٦٣.٤١٪) والتى تحفز وتلعب دورا هاما في التهاب وتورم الأمعاء .ولقد أدى تدعيم وجبات الفئران مع مسحوق سيقان الهليون والكائنات الحية الدقيقة الفعالة أو كلاهما معا الى تحسن فى جميع المقايس السابقة وبمعدلات مختلفة .تم سجلت أعلى درجات التحسن فى حالة إستعمال المخلوط. ومن النتائج السابقة يمكن تسليط الضوء على إحتمالية الوقاية الكيميائية لمسحوق سيقان الهليون فى تسرطن القولون وذلك من خلال قدرته على تعزيز التوازن الخلوي الطبيعي وأنظمة الدفاع المضادة للأكسدة .لذلك، توصى الدراسة أن يتم تضمين مسحوق سيقان الهليون في وجباتنا اليومية والمشروبات والمنتجات الغذائية

الكلمات المفتاحية: المركبات النشطة حيويا، الزيادة فى وزن الجسم، تناول العلف، نسبة كفاءة العلف، الهيموجلوبين، إنترليوكين -١، عامل النخر الورمى –ألفا ، سيتوكروم P450 .