Evaluating the Protective Efficacy of Basil seeds, Carob, and Licorice Syrup Against Colonic Cancer in Rats.

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ABSTRACT

The present study was conducted to determine the effect of bioactive compound and antioxidant activity found in basil seeds, carob and licorice against colonic cancer. The biological study was done on 36 of male rats were divided into 6 group (n=6), first group as control (-) fed basal diet only, group 2 as control (+) fed on basal diet and after 3 weeks of experiment injected with 35mg/Kg B.W of DMH twice/ week for 6 weeks to induced colonic cancer. Groups from (3-6) fed basal diet and orally administrated with 10 ml/day of basil, carob, licorice syrup and their mixture respectively, and injected with DMH, at the end of experiment blood sample and tissue were collected. The results found that basil seeds, carob and licorice contain high significant amount of total phenolic, flavonoids, triterpenoids, alkaloids, tannins, saponins and antioxidant activity. The presence of these compounds had a clear effect on the biological study results, which found that decrease lipid profile, improvement the level of liver and kidney function and glucose level, also showed significant decrease the level of (CEA), (CA 19-9) and CA125 compared to control (+) group. Therefore this study recommended to consuming basil seeds, carob and licorice syrup to obtain the health benefits of these plant.

Keywords: Antioxidant, Phytochemicals, Cancer antigen 125, Cancer antigen 19-9, Phytochemicals, Colonic cancer.

تقييم فاعلية التأثير الوقائي لشراب بذور الريحان، الخروب وعرق السوس ضد سرطان القولون في فئران التجارب

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المستخلص:

أجريت هذه الدراسة لتحديد تأثير المركبات الفعالة والنشاط المضاد للأكسدة الموجودة في بذور الريحان، الخروب وعرق السوس ضد سرطان القولون. تمت الدراسة البيولوجية علي ٣٦ فأر من ذكور الفئران تم تقسيمهم إلي ٦ مجموعات (ن=٦)، المجموعة الأولي – المجموعة الضابطة السالبة (–)، تغذت علي الغذاء القياسي فقط، المجموعة الثانية – المجموعة الضابطة الموجبة تغذت علي الغذاء القياسي وبعد ٣ أسابيع تم حقناها مرتين أسبوعيا بجرعه ٣٥ ملجم/كجم بثنائي مثيل هيدرازين لإستحداث سرطان القولون لمدة ٦ أسابيع، المجموعات من ٣–٦ تم تغذيتهم علي الغذاء الأساسي مع تجريعها يوميا ب ١٠ مل من شراب بذور الريحان، الخروب، عرق السوس وخليطهما، علي التوالي، وبعد ٣ أسابيع تم حقنها بنفس الجرعة السابقة من ثنائي مثيل الهيدرازين. في نهاية التجربة تم تجميع عينات الدم والأنسجة لإجراء التحاليل اللازمة. أظهرت النتائج المتحصل عليها أن كلا من بذور الريحان، الخروب وعرق السوس تحتوي علي كميات مرتفعة من المركبات الفعالة (الفينولات، الفلافونيدات، التربينويدات، القلويدات، التانينات والصابونينات، كذلك تميزت بإرتفاع النشاط المضاد للأكسدة. ظهر تأثير هذه المركبات الفعالة علي نتائج الدراسة البيولوجية والتي وجدت أن المعاملة بجرعة يومية من عصير هذه النباتات أدي إلي إنخفاض ملحوظ في مستوي دهون الجسم، تحسن وظائف الكبد، الكلي ومستوي سكر الدم، كما أظهرت النتائج إنخفاضا معنويا في مستويات (CEA)، (CA19–9) و (CA125) مقارنة بالمجموعة الضابطة الموجبة، أكد ذلك النتائج الهستولوجية، خاصة في المجموعة التي جرعت بخليط من الشراب. لهذا نوصي من خلال نتائج الدراسة بضرورة أضافة بذور الريحان الخروب وعرق السوس إلي الغذاء اليومي للحصول علي الفوائد الصحية لهذه النباتات.

الكلمات المفتاحية: مضادات الاكسدة، المركبات الفينولية، مستضد السرطان ١٢٥، مستضد السرطان ١٩-٩، سرطان القولون.

INTRODUCTION:

Cancer of the large intestine (colon) is one of the main causes of death due to an uncontrolled growth of the body's cells can lead to cancer. The numbers for colon cancer are somewhat equal in women and men. Multiple factors are involved in the development of colorectal cancer, such as lack of physical activity, excessive alcohol consumption, old age, family history, high-fat diets with no fiber and red meat, diabetes, and inflammatory bowel diseases, including ulcerative colitis and Crohn's disease **(Lewandowska et al., 2022).** Colon cancer is among the most common malignancy but the third prime cause of cancer associated mortalities especially in developed countries. colon cancer has several stages: 0, I, II, III, and IV, treatment for stages 0 to III typically involves surgery, while for stage IV and the recurrent colon cancer both surgery and chemotherapy are the options. Depending on the cancer stage and the patient characteristics, several chemotherapeutic drugs and diets have been recommended for the management of colorectal cancer **(Puccini et al., 2017).**

Chemotherapy works on active cells, such as cancerous ones, which grow and divide more rapidly than other cells. But some healthy cells are active too, including hair follicle ones, blood and gastrointestinal tract. Most side effects of chemotherapy occur when healthy cells are damaged, as well as headache, fatigue, stomach pain, muscle pain, vomiting and diarrhea, sore throat, blood abnormalities, constipation, damage to the nervous system, memory problems, loss of appetite and hair loss **(Mitchison, 2012).** Surgery and chemotherapeutic interventions are the most used forms of treatment for colon cancer due to the lack of scientifically explored alternatives. However, the development and identification of molecular compounds capable of killing or inhibiting transformed cells, promoting carcinogenesis without inducing being toxic or toxic effects to the normal cells are of utmost significance **(Macharia et al., 2022).**

In light of this, management with dietary supplements derived from plants is beginning to receive due recognition as the most potent approach to lessen the burden of colorectal cancer associated mortality **(Macharia et al., 2022)**. Plants have significant active compounds essential for development and growth in almost all living organisms. They are widely consumed as food and for their medicinal values in virtually all cultures. Most phytochemicals with determined bioactive potential have been associated with plants. Nearly all phytochemicals established on plants are classified into four biochemical classes: glycosides, polyphenols, terpenes and alkaloids **(Banwo et al., 2021).**

For cancer patients, consuming drinks low in sucrose is important due to the metabolic demands and vulnerabilities associated with cancer and its treatments, **Epner et al. (2022)** reported that the mechanistic preclinical studies in multiple cancers show that high sucrose or high-fructose diets activate several mechanistic pathways, including inflammation, glucose, and lipid metabolic pathways, and illustrated that evidence from epidemiologic and preclinical studies demonstrates that excess sugar consumption can lead to development of cancer and progression of disease for those with cancer independent of the association between sugar and obesity. While, **Lashinger et al. (2013)** mentioned that consuming sugar can also increase the production of inflammatory cytokines and insulin-like growth factors (IGFs), which promotes the development and spread of tumors.

Basil is an English name for *(Ocimum basilicum)* while it is called rehan in Arabic language is a medicinal herb of the family *Lamiaceae* that contains a variety of potential bioactive compounds, such as polyphenols, flavonoids, phenolics, and essential oils **(Romano et al., 2022).** Basil can boost phagocytic action of neutrophils and immunostimulant effect, antimicrobial activity due to linalool by having inhibitory action toward all tested microorganism, and additionally, rosmarinic acid shows inhibition in DNA synthesis, as well as protein synthesis when experimented on hepatoma derived cell line, this resulted by lower DNA fragments plus suppression on caspase-3 activation, which blocks apoptosis. Basil, is a culinary plant extensively utilized as a flavoring agent and has been applied for the treatment of gastrointestinal disorders including diarrhea, vomiting, gastrodynia, dyspepsia, flatulence, and gastritis. Relevant studies have identified anti-inflammatory, antioxidant, antiulcerogenic, immunomodulatory, antitumor, antibacterial, antiviral, and antifungal activities of the Ocimum genus **(Makri et al. 2008; Taie and Radwan., 2010; Al-Ali, 2013 and Khojasteh et al., 2020).**

Carob *(Ceratonia silique)* also known as the Mediterranean carob tree is an ever green tree that belongs to the *Leguminosae* subfamily *(Caesalpinioideae*). The fruit is derived from hermaphrodite trees and hard in shape It has been widely exploited since antiquity due to its edible fruits. It is still currently used in agro-food industries and soil restoration purposes **(Tous and Antoni, 2013).** Carob contains high sugar contents in pulp, fat in seed and minerals like potassium, calcium, and phosphorus are present in pods. Polyphenols and antioxidants are abundant in leaves and pods. It can be used for enhancing human health due to its high a profile nutrition, the clinical carob can add as an anticancer, antidiabetic, antidiarrheal, antihyperlipidemia, antibacterial, antimicrobial, antifungal **(Ikram et al., 2023)**.

licorice *(Glycyrrhiza glabra)* is the family *Leguminosae*. The Common names have licorice and glycyrrhiza, and in Egypt licorice known as erq'soos **(Wassef, 2004).** There are more than 30 species of *Glycyrrhiza genus* extensively spread worldwide, it was the most prescribed herb in Ancient Egyptian. There are various beneficial effects of licorice root extracts, such as treating throat infections, tuberculosis, respiratory, liver diseases, antibacterial, antiinflammatory, and immunodeficiency **(Wahab et al., 2021)**. On the other hand, traditional medicines are getting the attraction to treat many diseases. Therefore, it is vital to screen the medicinal plants to find the potential of new compounds to treat chronic diseases such as respiratory, cardiovascular, anticancer, hepatoprotective, etc. **wang et al. (2015)** mentioned that more than 20 triterpenoids and nearly 300 flavonoids have been isolated from licorice. Recent studies have shown that these metabolites possess many pharmacological activities, such as antiviral, antimicrobial, anti-inflammatory, antitumor and other activities. The major active component of licorice root is glycyrrhizin also known as glycyrrhizic acid or glycyrrhizinic acid a triterpene glycoside which is usually found in concentrations ranging from 6% to 10% **(Mittal et al., 2024).**

Choosing drink and syrups low in sucrose may help stabilize blood glucose levels, support immune health, and potentially improve overall outcomes during cancer treatment, also, there are few reports on the effect of antioxidant compounds of basil seeds on human health. **Therefore**, the importance of this research lies in highlighting the effectiveness of adding basil seeds to licorice and carob syrup in preventing colon cancer.

MATERIALS AND METHODS
Materials

Basal diet constituents

Casein, Cellulose, Choline chloride, DL- Methionine, all vitamins, minerals, L. cystine, and choline bitartrate were obtained from Morgan Company, Cairo, Egypt. Starch, corn oil, and sucrose will be obtained from the local market, Egypt.

Plant Materials

The dried basil seed, Carobs and licorice were purchased from herbalist shops in Egypt and were are identified in a Medicinal and Aromatic Plants Research Laboratory - Horticultural Research Institute - Agricultural Research Center., Giza, Egypt.

Chemicals and Reagents

A carcinogen 1, 2-Dimethylhydrazine (DMH), all chemical and reagents for biochemical analysis were purchased from the Gamma Trade Company for Pharmaceutical and Chemical, Dokki, Egypt.

Animals

A total number of thirty-Six adult male Albino rats (Sprague –Dawley strain) weighing (203 ± 5 gm) were purchased from the Animal House of Helwan Farm, Helwan, Egypt.

Ethical Approval:

Ethical Approval of the current study was obtained from Sohag University ethical guidelines for animal care and use in scientific research according to registration number 18-3-12/2024-01.

Methods

Preparation of Basal Diet

The basal diet was consist of protein (14%), corn oil (10%), minerals mixture (3.5%), vitamins mixture (1%), fiber (5%), sucrose (5%), choline chloride (0.25%) and the remainder will be corn starch up to 100%. These constituents will be thoroughly mixed and formulated according to **Reeves et al. (1993)** with some modifications**.**

Preparation of syrup from basil seeds

The seeds were rubbed until the husks were removed, then the seeds (100g) were added to 1liter of water and were absorbed into the gel form according to **Naji-Tabasi and Razavi (2017)** and **Dyab and Abo-Taleb (2024)** with some modified.

Preparation of Carob syrup

Carob syrup was prepared following the traditional method described by **Dhaouadi et sl. (2014),** with some modification. The carob fruits were immediately washed with water and left at room temperature to dry. To prepare the syrup: A cup of crushed fruits will add to a liter of water and left at room temperature for two hours. After two hours, was brought to a boil and simmered for 10 minutes. It was then removed from the heat, left to cool completely before being blended thoroughly using an electric blender. Next, 100 grams of basil seeds were added per liter of carob syrup. The syrup was then bottled in glass containers and stored in the refrigerator until use.

Preparation of licorice syrup

licorice syrup was prepared by add 100g of grinded roots +2.5g sodium bicarbonate +100 ml water mixed well using hand, and kept for 7 hour at room temperature (spontaneous fermentation), the fermented grinded roots mixed with 2.5L of water, filltred and kept in refrigerated until use according to the method of **Olukoge and Donaldson, (1998)** with some modified**.**

Chemical analysis

Total phenolics were determined according to (**Singleton and Rossi, 1965).** Total flavonoids contents in samples powder were estimated according to **Zhisen *et al.* (1999*).*** Total triterpenoids were extracted and measured according to the method of **Schneider et al. (2009).** Tannins were determined by the method of **Van-Burden and Robinson, (1981).** Alkaloids and Saponins were detected according to the method of **Harbome (1973).** Antioxidant activity (AA) of samples powder extracts and standards (α-tocopherol and BHT was determined according to the β-carotene bleaching (BCB) method following the procedure described by **Marco (1968).** Antioxidant activity using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Method DPPH was determined using the method proposed by **Katalinic et al. (2006).** Antioxidants' capacity to convert ferric ions into ferrous ions is assessed using method of **Munteanu and Apetrei (2021).** The total antioxidant activity was estimated using the (ABTS/H_2O_2/HRP) system for $ABTS^{\cdot+}$ radical formation as reported by **Chen et al. (2004)**

Induction of Colon Cancer

A carcinogen 1, 2-Dimethylhydrazine (DMH) were are freshly dissolved in 1Mm EDTA/0.9% NaCl solution to ensure the stability of the chemical just prior to use. Colon cancer was induced by subcutaneous injection of DMH at a dose of 35 mg/kg b.wt for 5 weeks (twice / week) **(Moharib , 2016** and **Hussein et al., 2013).**

Experimental Design and Grouping of Rats

All rats were housed in wire cages at the animal house of the Faculty of Home Economics, Helwan University under controlled environmental conditions of the light/dark cycle (12/12 h) and temperature (22±4°C). The supply of food and water will be uninterrupted during the experimental period. Rats were randomized into six groups (6 of each) as follows:

Group 1: Negative control group, rats were kept as healthy rats and subcutaneously injected with 0.1 ml of the saline solution twice weekly after 3 week of the experimental period.

Group 2: Positive control group, rats feed on the basal diet during the experimental, and after 3 weeks of the experiment rats were subcutaneously injected twice a week with 35 mg/kg b.w.t of DMH to induced colon cancer for 6 weeks.

Group 3, 4, 5 and 6: Rats were orally received 15mL of basil seeds, carob, licorice syrup and their combination; respectively and feed on the basal diet during the experimental period, and after 3weeks rats were injected twice weekly with 35 mg/kg b. wt. of DMH, and continued with orally administration with syrup until the end of the experiment. At the end of the feeding period, rats were fasted over night before scarifying. The serum samples were collected and the samples were centrifuged for biochemical analysis. This experiment was carried out at the animal house of Home Economics Faculty, Helwan University, Cairo - Egypt.

Biochemical Analysis

The biochemical determination were conducted at Graduate Research Labs, Nutrition and Food Science Dept., Faculty of Home Economics, Helwan University. Collected serum blood were used for determination of serum total cholesterol and triglycerides according to **Allain (1974), Fossati et al, (1980)** respectively, high density lipoprotein cholesterol (HDL-c) and low density lipoprotein cholesterol (LDL-c) were assayed according to **Abcam *(2019),*** very low density lipoprotein cholesterol VLDL-c was calculated mg/dl according to **Lee and Nieman (1996)** using the following equation: VLDL-c(mg/dl) = Triglycerides / 5. Liver functions were examined as serum Alanine aminotranferase (ALT) and Aspartate aminotransferase (AST) levels according to **Murray (1984).** The determination of kidney functions: serum urea and creatinine level were assayed according to **Tietz (2006)** and **Young (1990)** respectively. Serum glucose was assayed according to **Carroll (1970).** Serum levels of Carcinoembryonic antigen (CEA), Cancer antigen (CA 19-9) and Cancer antigen 125 (CA125) were determined according to **Bates (1991), Koprowski et al. (1979)** and **Bast et al. (1983)** respectively.

Histological Examinations

Colon specimens of all rats will be removed and prepared for histopathological examination according to the technique described by **Bancroft and Stevens (1996)**.

Statistical Analysis

The obtained results were analyzed statistically using SPSS program, Version (22). Differences between means were determined using ANOVA test. followed by the Duncan's multiple test. Statistical significance was considered at $P < 0.05$ and $P \leq 0.01$. The results are presented as expressed as means ± Standard Error (SE).

RESULTS

Bioactive compounds of Basil seeds, Carob, and Licorice

The data in **Table (1)** illustrated the bioactive compounds of Basil seeds, Carob, and Licorice on dry weight. Meanwhile, the result found that high significant increase ($P<0.01$) in total phenolic and flavonoids in basil seeds recorded 59.15 and 12.59 mg/100g respectively, compared to carob and licorice, while results recorded high significant increase in triterpenoids and alkaloids in carob recorded 971.88 and 801.45 mg/100g respectively, compared to that found in basil seeds and licorice, also results showed high significant increase in tannins and saponins in licorice recorded 4797.89 and 1754.52 mg/100g respectively, compared with basil seeds and carob.

Table (1): Bioactive compounds of Basil, Carob, and Licorice on dry weight

Parameters / Samples	basil seeds	Carob	Licorice	P≤
Total phenolics (mg GAE/100g,	59.13±0.79^{a}	15.44±0.86^{c}	20.03±0.77^{b}	0.01
Flavonoids (mg CE/100g,	12.59 ±0.33^{a}	4.92±0.10^{b}	1.60±0.05^{c}	0.01
Triterpenoids (mg UE.100 g	680.23±10.69^{b}	971.88±12.04^{a}	403.83±7.09^{c}	0.01
Tannins (mg VE/100g	395.14±3.08^{c}	2064.11±33.09^{b}	4797.89±108.80^{a}	0.01
Saponins (mg/100g,	212.32±3.01^{c}	1457.13±6.21^{b}	1754.52±32.73^{a}	0.01
Alkaloids (mg/100g,	63.29±1.95^{c}	801.45±7.50^{a}	80.13±0.77^{b}	0.01

Values are mean ± ES, $^{(a-c)}$: different letters in the same row indicate statistically significant differ ($p \leq 0.01$).

Antioxidant activities of Basil seeds, Carob, and Licorice on dry weight:

The antioxidant activity of the basil, carob and licorice were showed in **Table (2)**, from the data, it could be noticed that basil seeds highest amount of antioxidant activity recorded 92.71% followed by Licorice recorded 85.04% and carob recorded 81.97%.

Table (2): Antioxidant activities (AA) of Basil, Carob, and Licorice on dry weight

Samples / Parameters	basil seeds	Carob	Licorice	P≤
AA (%)	92.71±0.77[a]	81.97±0.77[c]	85.04±0.36[b]	0.01
AA [% of BHT (50 mg/ml)]	106.11±0.88[a]	93.82±0.88[c]	97.33±0.41[b]	0.01
AA [% of BHT (100 mg/ml)]	96.54±0.80[a]	85.36±0.81[c]	88.55±0.37[b]	0.01
α-tocopherol (50 mg/ml)]	94.07±0.78a	83.17±0.78[c]	86.28±0.37[b]	0.01
BHT (50 mg/ml)	87.37±0.006[a]	87.37±0.006 [a]	87.37±0.006 [a]	1.0
BHT (100 mg/ml)	96.02±0.006 [a]	96.02±0.006 [a]	96.02±0.006 [a]	1.0
α -tocopherol (50 mg/ml)	98.56±0.01 [a]	98.56±0.01 [a]	98.56±0.01 [a]	0.98

Values are mean ± ES, [(a-c)]: different letters in the same row indicate statistically significant differences ($p \leq 0.01$).

Antioxidant capacity by DPPH, FRAP and ABST of basil seeds, carob and licorice: -

Free radical scavenging activities were measured by different method, the data in **Table (3)** showed that the highest antioxidant capacity was showed by basil seed in all type of assay DPPH, FRAP and ABST recorded 3.12, 61.43 and 9.05 µmol TE/g sample; respectively followed by licorice recorded 2.99, 38.73 and 9.49 µmol TE/g sample; respectively while the result in carob recorded 2.97, 26.79 and 8.71 µmol TE/g sample; respectively. Also, results observed that in all samples, the antioxidant activity measured by FRAP was higher than that measured by the DPPH and ABST assay

Table (3): Antioxidant capacity by DPPH, FRAP and ABST assay/TEAC

Samples / Parameters	Basil seeds	Carob	Licorice	P≤
Antioxidant activity by 2,2-di-phenyl-1-picrylhydrazyl (DPPH) –TEAC Radical scavenging µmol TE/g sample	3.12±0.01[a]	2.97±0.084 [a]	2.99±0.35 [a]	0.19
Antioxidant activity by monitoring of the reduction power of Fe3+ - (FRAP assay/TEAC) Reduction power µmol TE/g sample d.w	61.43±0.35 [a]	26.79±0.37[c]	38.73±1.21[b]	0.01
Antioxidant activity by - (ABST assay/TEAC) Radical scavenging µmol TE/g sample	9.05±0.04[b]	8.71±0.1[c]	9.49±0.11 [a]	0.01

Values are mean ± ES, (a-c): different letters in the same row indicate statistically significant differ ($p \leq 0.01$)

Effect of administering basil, carob, and licorice syrups on body weight of colonic cancer rats:

The results in **Table (4)** showed the effect of administering basil, carob, and licorice syrup either individually or in combination on the final body weight and body weight gain of colonic cancer rats. The results showed high significant (**P**< 0.01) increase final body weight in control (-) group compared to other experiment groups recorded 127.20 g of body weight gain, also, results showed that injected rats with DMH to induced colon cancer led to significant decrease of body weight gain, this is clearly in control (+) group recorded 40.40g of body weight gain. While showed administering with basil, carob, and licorice syrup either individually or in combination showed moderate improvement of body weight, the observed increase of body weight gain recorded 51, 50.60, 55.67 and 58.50 respectively.

Table (4): body weight of rats administrated with Basil, Carob, and Licorice syrup

Parameters / **Samples**	**Initial body**	**Final body weight /g**	**dy weight gain weight/g**
Control (-)	202.60±1.63[a]	329.80±1.01[a]	127.20±1.71[a]
Control (+)	202.60±1.20 [a]	243.00±1.04[e]	40.40±0.37[e]
Basil syrup	203.75±2.10 [a]	254.75±2.17[d]	51.00±4.26 [d]
Carob	204.80±1.58 [a]	255.40±1.57[cd]	50.60±1.40[d]
Licorice	203.67±1.33 [a]	259.33±0.67[bc]	55.67±1.76[bc]
Mixture of syrup	204.25±1.65 [a]	262.75±1.11[b]	58.50±1.50[b]
P<	0.93	0.01	0.01

Values are mean ± ES, (a-d): different letters in the same column indicate statistically significant differences ($p \leq 0.01$)

Effect of administering basil, carob, and licorice syrups on the lipid profile of colonic cancer rats:

Table (5) presents the effect of administering basil, carob, and licorice syrup either individually or in combination on the lipid profile of colonic cancer rats. The positive control group showed a significant increase ($p < 0.01$) in serum total cholesterol, triglycerides, low-density lipoprotein, and very-low-density lipoprotein compared to the negative control group. The diet supplemented with basil syrup resulted in a moderate improvement in lipid profile parameters, lowering total cholesterol and triglycerides, while raising HDL compared to positive control group. The diet supplemented with carob syrup also showed hypolipidemic activity, with cholesterol at 181.7±0.9 mg/dl, triglycerides at 83.3±0.9 mg/dl, and HDL at 37.15±1.15 mg/dl. The diet supplemented with Licorice syrup had a similar effect, with total cholesterol at 184.0±2.6 mg/dl, triglycerides at 77.6±1.76 mg/dl, and HDL at 38.7±0.9 mg/dl. Among the treated groups, the mixture of syrups showed the most favorable impact, with a significant reduction in total cholesterol (169.3±3.5 mg/dl), LDL (115.27±4.49 mg/dl), VLDL (14.40±0.23 mg/dl), and triglycerides (72.0±1.54 mg/dl), along with a notable increase in HDL (39.7±1.45 mg/dl), approaching values similar to the negative control group.

Table (5): Lipid profile of rats administrated with Basil, Carob, and Licorice syrup

Parameters / Samples	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Control (-)	**184.67**±2.91 ^{b}	**52.33**±1.45^{e}	**44.33**±1.20 ^{a}	**129.87**±4.3 ^{b}	**10.47**±0.29^{e}
Control (+)	**200.0**±2.9^{a}	**97.3**±4.3^{a}	**32.67**±0.9^{d}	**147.9**±2.5^{a}	**19.47**±0.87^{a}
Basil syrup	**185.7**±1.20 ^{b}	**86.00**±1.15 ^{b}	**34.3**±1.2^{cd}	**134.13**±0.71 ^{b}	**17.20**±0.23 ^{b}
Carob	**181.7**±0.9 ^{b}	**83.3**±0.9^{bc}	**37.15**±1.15 $^{b\,c}$	**128.0**±1.05 ^{b}	**16.7**±0.18^{bc}
Licorice	**184.0**±2.6^{b}	**77.67**±1.76^{cd}	**38.7**±0.9 ^{b}	**129.8**±2.00 ^{b}	**15.5**±0.35b ^{cd}
Mixture of syrup	**169.3**±3.5^{C}	**72.00**±1.5^{d}	**39.7**±1.45 ^{b}	**115.27**±4.49 ^{c}	**14.40**±0.23^{d}
P<	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**

Values are mean ± ES, $^{(a-d)}$: different letters in the same column indicate statistically significant differences (p ≤ 0.01)

Effect of administering basil, carob, and licorice syrup on liver functions of colonic cancer rats:

Regarding to liver functions in **Table (6)**, there were significant (P<0.01) increase in serum ALT and AST levels of the positive control group (rats with colon cancer) compared with the negative control group. Feeding rats with basil syrup, Carob syrup, Licorice syrup and mixture of syrup caused a significant (P<0.05) decrease in serum ALT, compared to the positive control group. It was clear that, there was no significant difference in serum ALT level among all the test groups. Moreover, there was no significant difference in serum AST level between the groups fed on basil syrup, Carob syrup, Licorice syrup and mixture of syrups. There was a significant change in serum AST among the groups fed on basil syrup, Carob syrup, Licorice syrup and mixture of syrup and the negative control group. The highest improvements for liver functions were observed at the group that fed on mixture of syrup. Thus our study showed that ingestion of mixture of syrup from basil, Carob and Licorice improved serum liver functions concentration of colon cancer rats.

Table (6): Liver function of rats administrated with Basil, Carob, and Licorice syrup

Parameters / Samples	ALT (u/l)	ASTn (u/l)
Control (-)	**20.67**±1.76[e]	**17.33**±1.76[d]
Control (+)	**46.67**±1.76[a]	**54.00**±3.05[a]
Basil syrup	**41.00**±0.58 [ab]	**43.33**±1.20[b c]
Carob	**38.67**±3.46[b c]	**48.0**±2.31 [b]
Licorice	**32**.67±1.76[cd]	**44.67**±1.76 [b c]
Mixture of syrup	**28.00**±2.31[d]	**40.00**±0.58[c]
P≤	**0.01**	**0.01**

Values are mean ± ES, [(a-d)]: different letters in the same column indicate statistically significant differences ($p \leq 0.01$).

Effect of administering basil, carob, and licorice syrup on kidney functions and blood glucose of colonic cancer rats

Table (7) demonstrates the impact of diet supplemented with basil, carob, and licorice syrup administered individually or in combination on kidney function markers (urea and creatinine) and blood glucose levels in colonic cancer rats. The positive control group had no significant increase in serum of urea while there was significant increase in serum of creatinine and serum glucose compared to the negative control group. The diet supplemented with basil syrup also demonstrated a mild protective effect, as reflected by reductions in urea, creatinine, and glucose. The group fed on carob syrup resulted in moderate improvements, with urea, creatinine and glucose levels. The diet supplemented with Licorice syrup also showed a notable reduction in urea and creatinine, as well as a decrease in glucose, indicating an ameliorating effect on renal and metabolic parameters. The diet supplemented with mixture of syrup group exhibited the most pronounced improvement, showing the lowest levels of urea, creatinine, and glucose, with statistically significant differences ($p < 0.01$) compared to the positive control groups. Statistical analysis revealed significant differences among the experimental groups regarding all three measured parameters (urea, creatinine, and glucose), indicating the potential nephron-protective and hypoglycemic effects of the tested syrup, particularly when used in combination.

Table (7): Kidney function of rats administrated with Basil, Carob, and Licorice syrup

Parameters / Samples	Urea (mmol/L)	Creatinine (mg/dl)	Glucose (mg/dl)
Control (-)	25.67±1.20 [a b]	0.95±0.01[b]	196.33±1.20[b]
Control (+)	28.33±0.67[a]	1.08±0.08[a]	216.67±4.41[a]
Basil syrup	25.33±0.88[b]	0.93±0.01 [b]	190.00±1.15 [b c]
Carob	24.67±0.9 [b c]	0.94±0.03 [b]	186.33±1.85[c d]
Licorice	22.33±0.9[c]	0.91±0.02 [b]	185.67±2.18 [c d]
Mixture of syrup	22.00±0.6 [c]	0.91±0.01 [b]	178.33±4.41[d]
P≤	0.01	0.05	0.01

Values are mean ± ES, [(a-d)]:different letters in the same column indicate statistically significant differences ($p \leq 0.01$)

Effect of administering basil, carob, and licorice syrup on tumor markers of colonic cancer rats:

Table (8) presents the effect of dietary supplementation with basil, carob, and licorice syrup administered individually or as a mixture on serum levels of tumor markers including carcinoembryonic antigen (CEA), cancer antigen 125 (CA 125), and cancer antigen 19.9 (CA 19.9) in rats with induced colonic cancer.

The positive control group exhibited a significant elevation ($P < 0.01$) in all measured tumor markers compared to the negative control group. Serum CEA level in the positive control group was 6.37±0.09 ng/ml, CA 125 was 69.00±0.58 U/ml, and CA 19.9 reached 65.33±1.20 U/ml, indicating a marked tumor progression. On the other hand, the negative control group showed significantly lower values of tumor markers: CEA (0.25±0.20 ng/ml), CA 125 (13.33±2.40 U/ml), and CA 19.9 (20.00±2.31 U/ml). Rats fed on basil syrup demonstrated a moderate reduction in tumor markers, with CEA (5.47±0.44 ng/ml), CA 125 (53.33±0.88 U/ml), and CA 19.9 (52.33±0.87 U/ml) compared to positive control group, suggesting partial anti-tumor activity. While the rats fed on carob syrup group displayed values close to the positive control in terms of CEA (6.30±0.06 ng/ml), but showed improvement in CA 125 (55.33±1.86 U/ml) and CA 19.9 (52.33±1.45 U/ml), implying a limited effect on CEA but some modulation of other markers. The rats fed on licorice syrup supplementation led to further reductions: CEA (5.17±0.20 ng/ml), CA 125 (47.67±1.86 U/ml), and CA 19.9 (51.00±1.73 U/ml), indicating a more noticeable anti-tumor effect compared to the positive control group The group fed on mixture of syrup group demonstrated the most favorable results among the treated groups, with significant reductions in all tumor markers: CEA (3.67±0.18 ng/ml), CA 125 (43.67±1.86 U/ml), and CA 19.9 (45.67±0.88 U/ml). These values were significantly lower ($P < 0.01$)

than those of the positive control and showed a clear trend toward normalization.

Table (8) tumor markers of rats administering with basil, carob, and licorice syrup

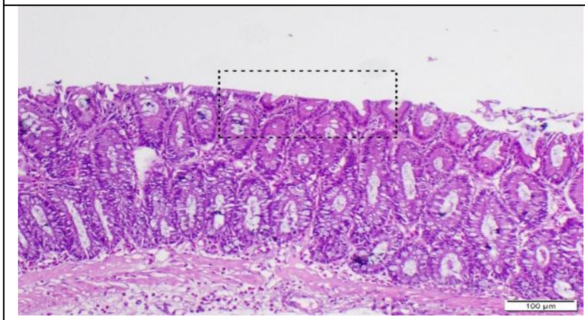
Parameters / **Samples**	CEA ng/ml	CA 125 U/ml	CA19.9 U/ml
Control (-)	٠,٢٥±٠,٢٠ [d]	١٣,٣٣±٢,٤٠ [d]	٢٠,٠٠±٢,٣١ [d]
Control (+)	٦,٣٧±٠,٠٩ [a]	٦٩,٠٠±٠,٥٨ [a]	٦٥,٣٣±١,٢٠ [a]
Basil syrup	٥,٤٧±٠,٤٤ [b]	٥٣,٣٣±٠,٨٨ [b]	٥٢,٣٣±٠,٨٧ [b]
Carob	٦,٣±٠,٠٦ [a]	٥٥,٣٣±١,٨٦ [b]	٥٢,٣٣±١,٤٥ [b]
Licorice	٥,١٧±٠,٢٠ [b]	٤٧,٦٧±١,٨٦ [c]	٥١,٠±١,٧٣ [b]
Mixture of syrup	٣,٦٧±٠,١٨ [c]	٤٣,٦٧±١,٨٦ [c]	٤٥,٦٧±٠,٨٨ [c]
P≤	٠,٠١	٠,٠١	٠,٠١

Values are mean ± ES, [(a-d)]: different letters in the same column indicate statistically significant differences ($p \leq 0.01$)

Effect of administrated with Basil, Carob, and Licorice syrup on colon cancer rats:

The histopathological examination of colon sections of the control (-) group revealed intact mucosa with normal glands lined by mucous secreting cells, intact sub-mucosa and tunica musculosa **(Fig.1 a and b).** In contrast, we showed colon cancer in control (+) group characterized by ulcerated mucosa, glands lined by slightly basophilic cells with elongated nuclei, extensive pleomorphic cells, severe interstitial inflammatory infiltrate in submucosa layer **(Fig.2a and b).** Moreover, we showed in basil seed group sever histopathological changes of colon mucosa characterized by sloughing of tunica mucosa with cell debris, degeneration of intestinal gland, mononuclear inflammatory cells infiltrations and thick layer of mucous exudate **(Fig.3 a and b).** On the other hand, we showed in carob group mild improvement of histopathological alterations of colon layers including desquamation and sloughing of tunica mucosa, and mucous exudate mixed with inflammatory cells if compared with control (-) group **(Fig. 4 a and b).** While the licorice group revealed moderate amelioration of colon mucosa with degeneration of some cells and moderate degeneration of intestinal gland **(Fig. 5 a and b).** Furthermore, the colon layer observed marked improvement of pathological lesions in mixture of syrup group including superficial regeneration of tunica mucosa and mild degeneration of intestinal gland as well as mild inflammatory cells infiltrations **(Fig. 6 a and b).**

control (-)



Fig,1 (a): Showing control group intact mucosa (black square) (x100).

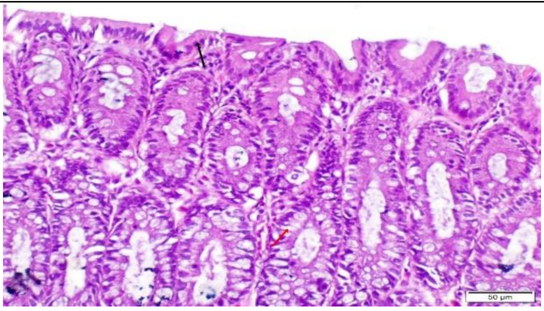
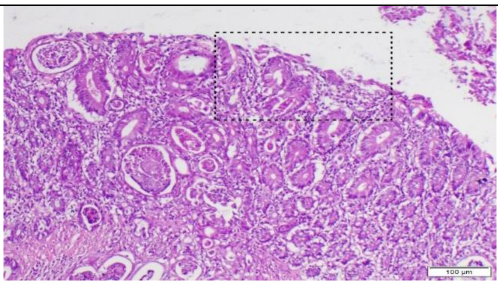
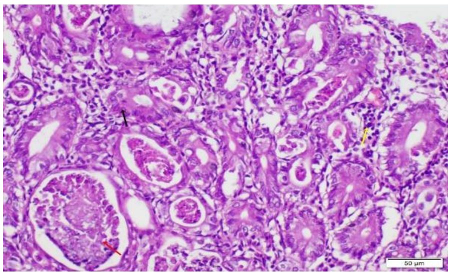


Fig.1 (b): normal mucosa (black arrow) with normal glands lined by mucous secreting cells (red arrow). (H&E, x200).

control (+)



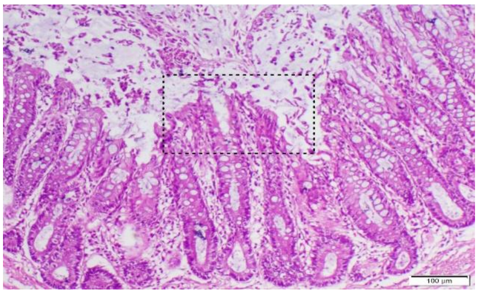
(a) (x100).



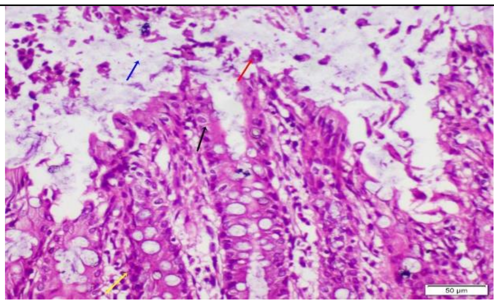
(b) (x200)

Fig. 2: Intestinal glands lined by slightly basophilic cells with elongated nuclei (black arrow), extensive pleomorphic cells, sever interstitial inflammatory infiltrate in submucosa layer (yellow arrow) complete degeneration of intestinal gland (red arrow) (H&E, x200).

Basil group



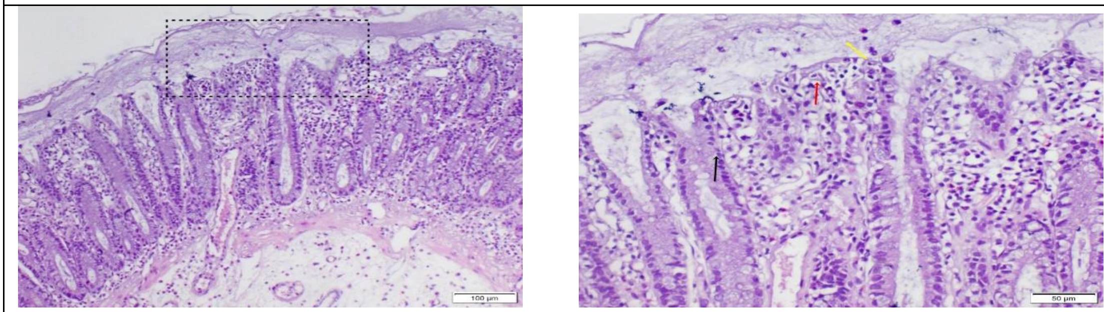
(a) (x100).



(b) (x200)

Fig. 3: Sever degeneration of tunica mucosa (black arrow), cell debris (red arrow), mononuclear inflammatory cells infiltrations (yellow arrow). (H&E, x200).

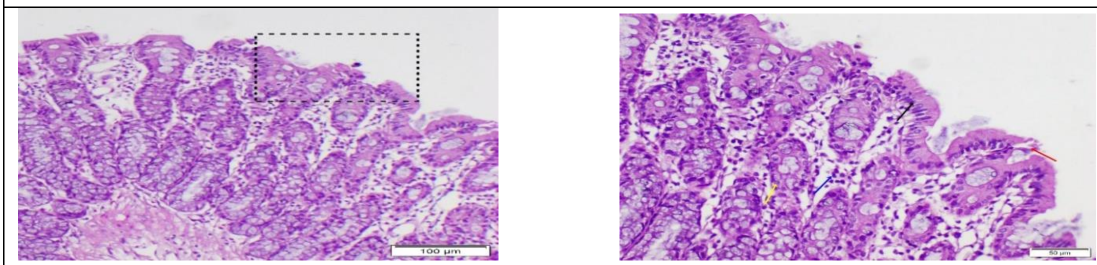
Carob group



(a) (x100). **(b) (x200)**

Fig. 4: Intact mucosa of some parts (black arrow) Superficial desquamation of tunica mucosa (red arrow), mucous exudate mixed with inflammatory cells (yellow arrow) and degeneration of intestinal gland (yellow arrow). (H&E, x200).

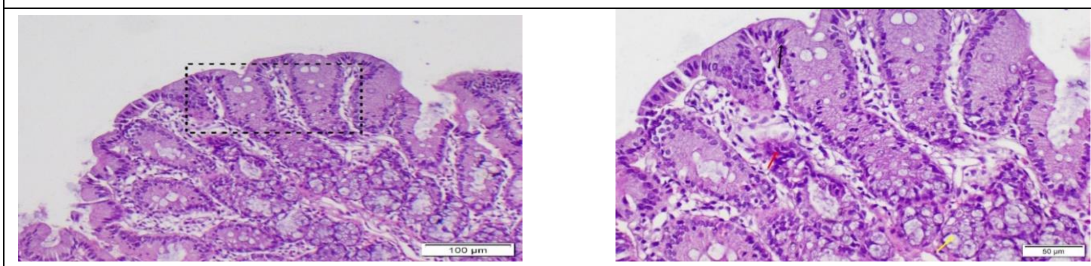
Licorice group



(a) (x100). **(b) (x200)**

Fig. 5: Apparently health of tunica mucosa (black arrow) cell debris (red arrow) and moderate degeneration of intestinal gland (yellow arrow). (x200).

Mixture group



(a) (x100). **(b) (x200)**

Fig. 6: Regeneration of tunica mucosa (black arrow), degeneration of intestinal gland (yellow arrow) and mild inflammation (red arrow). (H&E, x200).

Discussion

The present study demonstrated that the administration of basil, carob, and licorice syrup, whether individually or in combination, the present data found that high significant increase in total phenolic and flavonoids basil seeds, compared to carob and licorice, while recorded high significant increase in triterpenoids and alkaloids in carob compared to that found in basil seeds and licorice, also showed high significant increase in tannins and saponins in licorice compared with basil seeds and carob. This results agree with the results obtained by **Güez et al. (2017)** found that basil seeds extract contain high amount of polyphenol compounds and total flavonoids recorded 23780.00 and 15982.00 µg/mL respectively of extract. Also, **Petrosyan et al. (2023)** how found that flavonoid content in licorice roots collected from 5 countries in Armenia ranged from 4.90 ± 0.03 to 9.80 ± 0.6 mg%, with an average tannin quantity of approximately 4 mg%. also revealed that the composition of these substances is significantly influenced by the soil characteristics of the region. **Ahmed et al. (2019)** found that basil extracts contained appreciable levels of total phenolic contents and exhibited good DPPH radical scavenging capacity higher than that of essential oils, also showed high correlation between antioxidant activity and total phenolic contents of basil extracts.

The present study showed that, basil seeds contain highest amount of antioxidant activity, followed by Licorice and carob, also, in the present study, free radical scavenging activities were measured by different method, and showed that the highest radical scavenging activity was recorded by basil seed followed by licorice and carob recorded. In these study the increment in radical scavenging activity may be due to increase in total phenolic and flavonoids content in the basil seed and licorice. Meanwhile Ahmed et al. (2019) found that high significant increase in radical scavenging activity of Basil seeds and suggests that the antioxidant capacity of basil extracts results from the contribution of phenolic contents. Also Othmen et al. (2019) reported that antioxidant capacity followed the same change pattern shown by the bioactive compounds content which confirms the high correlation between the amounts of bioactive compounds and antioxidant capacity assessed by DPPH test.

The results in **Table (4)** showed administering with basil, carob, and licorice syrup either individually or in combination showed moderate improvement of body weight when compared to control (-) and control (+), the improvement of body weight in all administrated group may be due to fiber content in basil seed and carob, which promotes feelings of fullness and reduces overall food intake. This helps regulate body weight **Farias et**

al. (2024). Additionally, polyphenolic present in carob pulp, such as gallic acid, falvonoids, tannins help improve lipid metabolism, reducing fat accumulation and promoting healthier weight management **chait et al. (2020)** and **Fidan et al. (2020)**. Also, glabridin, a flavonoid found in licorice, has been shown to inhibit the differentiation of fat cells (adipogenesis) and reduce fat accumulation. Glycyrrhizin helps improve insulin sensitivity, which supports better metabolic regulation and prevents fat accumulation. In rats with obesity-related conditions, licorice has been shown to help reduce body weight by modulating lipid metabolism and promoting fat breakdown **(Ahn et al., 2013).** These results agree with **Ameen et al. (2022)** found that increase of body weight gain of peptic ulcer rats treated with licorice aqueous extract compared to control (-) and control (+) group. While, **Cherian, (2019)** mentioned that, the potential of basil seeds to enhance digestion, aid in weight loss, control blood sugar, calm the body, reduce tension, due to cellulose and hemicellulose content of basil, also have a high fiber content, lower cholesterol.

The present data showed that, exerted a significant modulatory effect on the lipid profile of colonic cancer-induced rats. The positive control group exhibited a marked dyslipidemia, characterized by elevated levels of total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), and very-low-density lipoprotein (VLDL), alongside reduced high-density lipoprotein (HDL), which is consistent with findings reported by **Raun et al. (2021)**, who noted that cancer-induced metabolic alterations often lead to significant disturbances in lipid homeostasis. Dietary supplementation with basil syrup led to moderate improvements in lipid parameters, particularly through reductions in TC and TG levels, and an increase in HDL. These findings align with **Dhama et al. (2023)**, who reported that *Ocimum basilicum* possesses bioactive compounds such as flavonoids and polyphenols, which contribute to its hypolipidemic potential by modulating lipid metabolism and enhancing antioxidant defense mechanisms.

Carob syrup demonstrated a more pronounced hypolipidemic effect, which corroborates the findings of **Zunft et al. (2003)**, who found that carob fiber significantly reduced LDL and total cholesterol in hyperlipidemic individuals, attributed to its high polyphenol and dietary fiber content that impedes cholesterol absorption and enhances bile acid excretion. Similarly, licorice syrup produced notable improvements in the lipid profile, in agreement with **Alrefaei and Elbeeh, (2025)** who observed that *Glycyrrhiza glabra* extract decreased serum lipid levels in rats through inhibition of hepatic lipogenesis and enhancement of lipid clearance.

Notably, the combination of basil, carob, and licorice syrup yielded the most favorable lipid profile, nearly restoring the parameters to those of the negative control group. This synergistic effect suggests a potential cumulative or complementary interaction among the bioactive constituents of the three syrups. This result is supported by **Liu, et al. (2022)**, who emphasized the superior efficacy of multi-ingredient functional formulations in modulating dyslipidemia compared to single-component interventions, likely due to broader mechanistic coverage and enhancement of antioxidant and anti-inflammatory pathways.

Overall, the current findings highlight the promising role of plant-based functional beverages in managing lipid disorders associated with colonic cancer and support their inclusion in complementary dietary strategies.

The current study revealed that colonic cancer induction was associated with a significant elevation in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, indicating hepatic dysfunction. These findings are consistent with those of **Laurindo et al. (2023)** and **Jelic et al. (2021)**, who reported that chemically-induced colorectal carcinogenesis is often accompanied by hepatic injury and altered liver enzyme activity due to systemic inflammation, oxidative stress, and hepatic metastasis-related changes.

The administration of basil, carob, and licorice syrups either individually or in combination resulted in a significant reduction in serum ALT levels in all treated groups, while AST levels showed improvement compared to the positive control group. These results support the hepatoprotective effects of these plant extracts previously reported in the literature. For instance, **Chatterjee and Sarkar, (2024).** found that *Ocimum basilicum* extract exerted a hepatoprotective effect in rats by reducing elevated liver enzymes and improving hepatic histology, attributed to its high antioxidant and anti-inflammatory properties. Carob syrup also demonstrated beneficial effects on liver function in the present study. This is in agreement with **Süzek et al. (2017)**, who showed that carob pod extract reduced liver enzyme activity and protected liver tissues in animal models of hepatic injury, owing to its rich polyphenolic content and radical scavenging activity.

Similarly, licorice syrup exhibited hepatoprotective action, in line with the results of **Rehman et al. (2020)**, who reported that *Glycyrrhiza glabra* extract significantly attenuated hepatic damage and decreased serum ALT and AST in rats with induced hepatotoxicity. Glycyrrhizin, a major bioactive compound in licorice, is believed to exert membrane-stabilizing effects on hepatocytes. Notably, the combination of basil, carob, and licorice syrup produced the most significant improvement in liver function, approaching the values observed in the negative control group. This suggests a possible synergistic or additive effect of their bioactive constituents in mitigating liver damage. Similar synergistic hepatoprotective effects of combined plant extracts were observed by **Costea et al. (2022)**, who demonstrated enhanced liver function recovery when multiple antioxidant-rich botanicals were administered together, compared to single-herb treatments. In summary, the current findings support the hepatoprotective potential of basil, carob, and licorice syrups, especially in combination, and highlight their role in mitigating hepatic dysfunction associated with colorectal cancer.

The present study highlights the beneficial impact of basil, carob, and licorice syrups administered individually or in combination on kidney function markers (urea and creatinine) and blood glucose levels in colonic cancer-induced rats. The positive control group exhibited a significant elevation in serum creatinine and glucose levels compared to the negative control, indicating renal and metabolic disturbances commonly associated with tumor progression. in line with the work of **Gonzalez et al. (2017)** who documented renal impairment and hyperglycemia in experimental models of colon cancer, likely mediated by inflammation-induced nephrotoxicity and cancer-related metabolic dysregulation.

The group fed on basil syrup showed mild improvements in kidney and glucose parameters, These findings are consistent with previous reports by **EL-Hassanin (2023)** and **Mnaizel (2024)** who reported that *Ocimum basilicum* extract exhibited nephroprotective effects by modulating oxidative stress and improving glomerular filtration, as well as enhancing insulin sensitivity and glycemic control through its flavonoid content. Carob syrup demonstrated a moderate ameliorating effect on kidney function and glucose levels. These outcomes support findings by **Qasem et al. (2018)**, who found that *Ceratonia siliqua* supplementation in diabetic rats significantly improved renal biomarkers and lowered glucose levels, likely due to its polyphenols, tannins, and dietary fibers which reduce glucose absorption and exert anti-inflammatory effects on renal tissues. Licorice syrup also produced notable protective effects, as evidenced by reductions in serum urea, creatinine, and glucose. This agrees with **Mohamed (2019)**, who reported that *Glycyrrhiza glabra* extract conferred renal protection through antioxidant mechanisms and modulation of renal enzymatic activity.

Remarkably, the combination of basil, carob, and licorice syrups exhibited the most pronounced effects across all measured parameters. The significant reductions in urea, creatinine, and glucose suggest a synergistic interaction among the bioactive compounds of the three botanicals, enhancing both nephron-protective and hypoglycemic actions. Similar synergistic benefits were previously reported by **Bhuyan et al. (2020)**, who demonstrated that multi-herbal formulations targeting oxidative stress pathways offer superior efficacy in restoring kidney and metabolic function compared to single-plant extracts. In conclusion, the current findings emphasize the therapeutic potential of combining basil, carob, and licorice syrups to attenuate renal and metabolic complications associated with colonic cancer.

The present study illustrated the beneficial impact of basil, carob, and licorice on serum levels of tumor markers including carcinoembryonic antigen (CEA), cancer antigen 125 (CA 125), and cancer antigen 19.9 (CA 19.9) in rats with induced colonic cancer. The positive control group exhibited a significant elevation in all measured tumor markers compared to the negative control group. Also, results indicate that all tested syrups possessed varying degrees of tumor marker suppression, with the combination of basil, carob, and licorice syrups yielding the strongest protective effect against cancer-related biomarker elevations. The potential anticancer properties of basil seeds, carob, and licorice syrups in reducing tumor markers in colon cancer, such as CEA, CA 125, and CA 19-9, may be due to potential role of this plant in reducing inflammation and oxidative stress, which are linked to the progression of cancer. Also, high content of polyphenolic compounds in these plant. have been shown to possess anticancer properties by inducing apoptosis (programmed cell death) in cancer cells and inhibiting tumor growth **Bhosale et al. (2020)**. Also, polyphenols in carob, especially gallic acid and tannins, have been shown to inhibit the proliferation of colon cancer cells and reduce tumor growth **Ghanemi et al. (2017)**. Carob may also contribute to lowering CEA, a protein elevated in many colon cancer cases, by inhibiting cancer cell proliferation and promoting apoptosis. Licorice has shown significant anticancer properties, particularly due to its flavonoids and triterpenoids, which have been shown to inhibit cancer cell proliferation and promote apoptosis. Glycyrrhizin, a key compound in licorice, has anti-inflammatory effects that may also help in reducing tumor growth and lowering cancer markers. In animal models of colon cancer, licorice has been shown to lower CEA, a protein that is typically elevated in colon cancer patients **(Wahab et al., 2021)**. **Scalbert et al. (2005)** and **Klenow et al. (2009)** reported that consumption of foods rich in phenolic compounds is associated with various physiological effects, such as preventing cancer and certain chronic diseases. Also, Previous studies conducted with **Seki et al. (2011)** and **Zhu et al. (2016)** confirmed that licorice contains of bioactive compounds such as polysaccharide, triterpenes, tannins and flavonoids, **Wang et al. 2015** and **Wang et al. (2013)** showed that the previous compound have anti-inflammatory, antiviral, anti-inflammatory, anti-tumor, anti-microbial, antidiabetic, neuro-protective, hepatoprotective, immunoregulatory and antioxidant effects.

These results were correlated with the results suggested by **Güez et al. (2017)** Found that basil seeds extract acts as an antioxidant and effectively reverts or subjugates the effects of high oxidizing agents such as hydrogen peroxide. also attributed these actions to its composition, which is rich in polyphenols and flavonoids. **Saeidi et al. (2018)** determined that, anti-inflammatory effect of basil seeds extract on acetic acid-induced colitis in rats. Found that, orally administered of three doses of basil seed (100, 200, and 400 mg/kg) reduced ulcer score, area and index as well as weight of wet colonic tissue compared to the control group also found basil seeds extract could exert more protection against oxidative stress and inflammatory mediators in colitis tissue suggesting a suitable candidate for colitis prevention or treatment as alternative or complementary therapy. **Perna et al. (2022)** mentioned that antioxidants and bioactive compounds such as phenolics, flavonoids, and essential oils in basil leaves show important potential anti-cancer activity regards to cell death and viability inhibition, cytotoxicity, inducing apoptosis, slowing down tumor growth and especially on cell cycle arrest both in vivo and in vitro. **Habib and Aljammaz (2017)** suggest that licorice can be used for the treatment of peptic ulcer and can protect the stomach against nonsteroidal anti-inflammatory drugs induced ulcers. **Ameen et al. (2022)** investigated the protective effect of Licorice against peptic ulcer. Found that licorice protect rats against peptic ulcer through its active components from isoflavonoids, triterpenoids, sterols, lignins, and volatile oils. Also, **El-Zeftawy** and **Ghareeb (2023)** findings indicate that Ceratonia siliqua demonstrated higher scavenging activities, the extract exhibited a cytotoxic effect against prostate cancer, liver cancer, colon cancer, and lung cancer.

The present study evaluate the histopathological changes in colonic tissues of experimental rat groups subjected to chemically induced colon cancer, and to assess the potential protective or ameliorative effects of selected phytotherapeutic agents namely basil, carob, licorice, and their combination.

Histopathological examination of the colon sections in the control group (-) revealed normal histological architecture, with intact mucosa, well-organized intestinal glands lined by mucous-secreting cells, and preserved submucosal and muscular layers **(Fig.1 a and b).** This typical appearance serves as a baseline for comparison and confirms the structural integrity of colonic tissues in the absence of carcinogenic insult.

In contrast, the group (+), which was exposed to a carcinogenic agent without any phyto-therapeutic intervention, showed significant histopathological alterations indicative of malignancy **(Fig.2 a and b).** The most striking features included ulcerated mucosa, extensive pleomorphic tumor cells, and glandular structures lined by slightly basophilic cells with elongated, hyperchromatic nuclei. These findings reflect moderate dysplasia and the morphological hallmarks of moderately differentiated adenocarcinoma (Grade 2), where partial glandular differentiation is retained, yet significant nuclear atypia is present. This pattern is consistent with the classification described by **Bosman et al. (2010)**, where control (+) tumors display both architectural preservation and marked cytological abnormalities.

The high degree of nuclear pleomorphism observed in this group is indicative of increased cellular proliferation and genetic instability hallmarks of tumor aggressiveness and poor prognosis. These observations align with the findings of **Compton et al. (2000),** who reported a direct correlation between increased mitotic activity, pleomorphism, and tumor grade in colorectal cancers. Additionally, the submucosal layer exhibited dense interstitial inflammatory infiltrates, predominantly composed of lymphocytes and plasma cells. This immune cell infiltration may represent a host immune response to tumor antigens, supporting the hypothesis proposed by **Pagès et al. (2005)**, who emphasized the prognostic role of tumor-infiltrating lymphocytes in colorectal carcinoma progression.

Following the confirmation of carcinogenic damage in control (+), the subsequent experimental groups received syrup supplementation with single or combined Basil, Carob and Licorice syrup to evaluate their protective efficacy against colonic damage.

The group treated with basil syrup alone, demonstrated severe histopathological deterioration, including sloughing of the tunica mucosa, presence of cell debris, degeneration of intestinal glands, and thick mucous exudate infiltrated with mononuclear cells. These outcomes suggest that basil, despite its known anti-inflammatory and antioxidant phytoconstituents such as eugenol and linalool may not have been sufficient on its own to counteract the profound epithelial injury in this model. **Kelm et al. (2000)** previously reported basil's ability to modulate oxidative stress and inflammation, but such benefits may be dose-dependent or require prolonged exposure to exert a protective effect.

Conversely, rats in the group supplemented with carob syrup showed mild histological improvements, including reduced mucosal sloughing and a moderate inflammatory response. Carob's rich content of polyphenols and dietary fibers, known to enhance mucosal defense and act as free radical scavengers, may explain this partial protection. **Basharat et al. (2023)** highlighted carob's ant ulcerative and gut regenerative properties, which are consistent with the observed attenuation of mucosal damage in this group.

The group receiving licorice syrup exhibited moderate amelioration in colonic architecture. There was partial preservation of mucosal integrity and moderate glandular degeneration, indicating a more pronounced effect compared to basil and carob syrup alone. This finding supports previous studies demonstrating the anti-inflammatory and mucosa-protective effects of glycyrrhizin and flavonoids in licorice **(Fiore et al., 2005).** These compounds are known to modulate cytokine production, inhibit cyclooxygenase pathways, and reduce oxidative tissue damage, which collectively contribute to mucosal healing.

Notably, the group treated with a combination of basil, carob, and licorice syrup showed the most significant histological improvement. The colonic mucosa exhibited superficial regeneration, minimal glandular degeneration, and mild inflammatory infiltration, indicating a synergistic effect of the combined phytochemicals **(Fig.5 a and b)**. This supports the hypothesis that polyherbal formulations can target multiple molecular pathways simultaneously, offering superior therapeutic outcomes. **Brahma et al. (2024)** emphasized that such combinations can produce additive or synergistic effects through simultaneous modulation of oxidative stress, inflammation, and epithelial regeneration.

Collectively, the data from this study support the protective and restorative potential of herbal therapies in the context of chemically induced colonic injury. The results further highlight that combined phytotherapy may offer enhanced efficacy compared to single agents, potentially through complementary pharmacological actions.

Conclusion:

In conclusion, the present study found that, the administration of basil seeds, carob and licorice syrups has shown beneficial effects in mitigating colon cancer in rats. These natural syrups exhibit significant antioxidant, and anti-proliferative properties, which contribute to the suppression of tumor growth and the protection of colon tissues. These natural substances are rich in cellular protection, provides polyphenols that may modulate gut health and reduce carcinogenic activity, also contains bioactive compounds like glycyrrhizin in licorice, and contain high amount of total flavonoids, triterpenoids, tannins, saponins and alkaloids that help inhibit cancer cell proliferation and induce apoptosis. Together, these finding suggest that incorporating such plant-based compounds may support colorectal cancer prevention and therapy, warranting further investigation for potential clinical application in humans.

References

Abcam. (2019): HDL and LDL/VLDL Cholesterol Assay Kit (Colorimetric/ Fluorometric), Version 11 Last updated 14 June www.abcam.cn

Ahmed, A.F., Attiac, F.A.K., Liua, Z., Li, C., Wei, J. & Kang, W. (2019): Antioxidant activity and total phenolic content of essential oils and extracts of sweet basil *(Ocimum basilicum L.)* plants. Food Science and Human Wellness 8: 299–305. https://doi.org/10.1016/j.fshw.2019.07.004.

Ahn, J., Lee, H., Jang, J., Kim, S. & Ha, T. (2013): Anti-obesity effects of glabridin – rich supercritical carbon dioxide extract of licorice in high fat fed obese mice. (2020): Food Chem Toxicol. 51: 439-445. Doi: 10.1016/j.fct.2012.08.048.

Al-Ali KH, El-Beshbishy HA, El-Badry AA, Alkhalaf M. (2013): Pak J Biol Sci. 2013;16(23):1744-50.

Allain, C. (1974): Cholesterol Enzymatic Colorimetric Method. Journal of Clinical Chemistry, 2, 470.

Alrefaei, A. F., & Elbeeh, M. E. (2025): Hepatoprotective effects of *Glycyrrhiza glabra* in diabetic male rats: addressing liver function, oxidative stress, and histopathological Changes. Biology, 14(3)307. https://doi.org/10.3390/biology14030307.

Ameen, A. A., El-Masry, H. G., & Bashatly, M. Y. M. H. E. (2022): The effect of licorice *(Glycyrrhiza glabra)* on peptic ulcer in rats. International Journal of Health Sciences, 6(S4), 12173–12183. https://doi.org/10.53730/ijhs.v6nS4.11829

Bancroft, J.D. & Stevens, A. (1996): Theory and Practice of histological techniques, 4th Edn. Churchill Livingstone, London.

Banwo, K., Olojede, A.O., Adesulu-Dahunsi, A.T., Verma, D.K., Thakur, M., Tripathy, S., Singh, S., Patel, A.R., Gupta, A.K., Aguilarj, C.N. & Utama, G.L. (2021): Functional importance of bioactive compounds of foods with Potential Health Benefits: A review on recent trends. Food Bioscience 43 (2021) 101320. https://doi.org/10.1016/j.fbio.2021.101320.

Brahma, S., Goyal, A. K., Dhamodhar, P., Kumari, M. R., Jayashree, S., Usha, T., & Middha, S. K. (2024). Can Polyherbal Medicine be used for the Treatment of Diabetes?-A Review of Historical Classics, Research Evidence and Current Prevention Programs. *Current Diabetes Reviews*, *20*(2), 37-91. https://doi.org/10.2174/1573399819666230314093721

Basharat, Z., Afzaal, M., Saeed, F., Islam, F., Hussain, M., Ikram, A., ... & Awuchi, C. G. (2023). Nutritional and functional profile of carob bean *(Ceratonia siliqua):* A comprehensive review. International Journal of Food Properties, 26(1), 389-413. https://doi.org/10.1080/10942912.2022.2164590.

Bast, R.C.Jr., Klug, T.L., St, J.E., et al. (1983): A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer[J].N Engl J Med,1983,309(15):883.-887.

Bates SE. (1991): Clinical applications of serum tumor markers. Ann Intern Med 1991;115:623-38.

Bhosale, P.B., Ha, S.E., Vetrivel, P., Kim, H.H., Kim, S.M. Kim, G,S, (2020): Functions of polyphenols and its anticancer properties in biomedical research: a narrative review. Transl Cancer Res. 9(12): 7619-7631. Doi: 10.21037/tcr-20-2359.

Bhuyan, D. J., Perera, S., Kaur, K., Alsherbiny, M. A., Low, M., Seto, S. W., ... & Zhou, X. (2020): Synergistic effects of Chinese herbal medicine and biological networks. Approaching Complex Diseases: Network-Based Pharmacology and Systems Approach in Bio-Medicine, 393-436. https://doi.org/10.1007/978-3-030-32857-3_17.

Bosman, F. T., Carneiro, F., Hruban, R. H., & Theise, N. D. (2010): WHO classification of tumours of the digestive system (No. Ed. 4, pp. 417-pp).

Carroll, J.A. (1970): Colorimetric serum glucose determination using hexokinase and glucose – 6-phosphate dehydrogenase. Biochem. Med. 4:171-180.

Chait, Y.A., Gunenc, A., Bendali, F.& Hosseinian, F. (2020): Simulated gastrointestinal digestion and in vitro colonic fermentation of carob polyphenols: Bioaccessibillity and bioactivity. *LWT*. 117, 108623.

Compton, C. C., Fielding, L. P., Burgart, L. J., Conley, B., Cooper, H. S., Hamilton, S. R., & Willett, C. (2000). Prognostic factors in colorectal cancer: College of American Pathologists consensus statement 1999. Archives of pathology & laboratory medicine, 124(7), 979-994. https://doi.org/10.5858/2000-124-0979-PFICC..

Costea, L., Chițescu, C. L., Boscencu, R., Ghica, M., Lupuliasa, D., Mihai, D. P.& Gîrd, C. E. (2022): The polyphenolic profile and antioxidant activity of five vegetal extracts with hepatoprotective potential. Plants, 11(13), 1680. https://doi.org/10.3390/plants11131680.

Chatterjee, A. & Sarkar, B. (2024): Polyphenols and terpenoids derived from Ocimum species as prospective hepatoprotective drug leads: a comprehensive mechanistic review. Phytochemistry Reviews,1-43.

Chen, I., Chang, H., Yang, H. & Chen, G. (2004): Evaluation of Total Antioxidant Activity of Several Popular Vegetables and Chinese Herbs: A Fast Approach with ABTS/H2O2/HRP System in Microplates. J Food Drug Analysis 2004; 12: 29-4.

Cherian, R.P. (2019): Health benefits of basil seeds. International Journal of Scientific Research in Science, Engineering and Technology, 6(2), 511–515.

Dhama, K., Sharun, K., Gugjoo, M. B., Tiwari, R., Alagawany, M., Iqbal Yatoo, M. & Farag, M. R. (2023): A comprehensive review on chemical profile and pharmacological activities of *Ocimum basilicum*. Food Reviews International, 39(1),119-147. https://doi.org/10.1080/87559129.2021.1900230.

Dhaouadi, K., Belkhir, M., Akinocho, I., Raboudi, F., Pamies, D., Barrajon, E., Estevan, C. &Fattouch, S. (2014): Sucrose supplementation during traditional carob syrup processing affected its chemical characteristics and biological activities. Food science and technology. 57:1-8. http://dx.dio.org/10.1016/j.Iwt.2014.01.025.

Dyab, A. & Abo-Taleb, H.M. (2024): Evaluation of some nectars supplemented with basil seeds. Egyptian journal of Agricultural Research. 102 (2):226-237. Doi: 10.21608/EJAR.2024.259547.1491.

EL-Hassanin, M. F. (2023): Potential protective effect of basil leaves extract against zinc oxide nanoparticle-induced nephrotoxicity in rats. Scientific Journal of Specifc Education. (2)9: 501-524https://dx.doi.org/10.21608/jsezu.2023.294391.

El-Zeftawy, M. & Ghareeb, D. (2023): Pharmacological bioactivity of *Ceratonia siliqua* pulp extract: in vitro screening and molecular docking analysis, implication of Keap-1/Nrf2/NF-κB pathway. Scientifc Reports. 13:12209 . https://doi.org/10.1038/s41598-023-39034-4.

Epner, M.; Yang, P.; Wagner, R.W.; Cohen, L. (2022): Understanding the Link between Sugar and Cancer: An Examination of the Preclinical and Clinical Evidence. Cancers, 14, 6042. https://doi.org/10.3390/cancers14246042

Farias, C.; Cisternas, C., Caicedo, A., Mercado, L., Valenzuela, R., Calderon, H., Espinosa, A., Videla, L.A. & Munoz, L.A. (2024): High-fiber basil seed flour reduces insulin resistance and hepatic steatosis in high- fat diet mice. NPJ Sci Food. 8 (1): 90. Doi:10.1038/s41538-024-00329-z.

Fidan, **H., Stankov, S., Petkova, N., Petkova, Z., Lliev, A., Stoyanova, M., Ivanova, T., Zhelyazkov, N., Ibrahim, S., Stoyanova, A. (2020):** Evaluation of chemical composition, Antioxidant potential and functional properties of Carob *(Ceratonia silique L.)* seed . J. Food Sci. Technol. 57: 2404-2413.

Fiore, C., Eisenhut, M., Krausse, R., Ragazzi, E., Pellati, D., Armanini, D., & Bielenberg, J. (2008). Antiviral effects of *Glycyrrhiza* species. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 22(2), 141-148. https://doi.org/10.1002/ptr.2295

Fossati, P., Prencipe, L. and Berti, G. 1980. Clin. Chem. 26/2:227-273.

Ghanemi, F.Z., Belarbi, M., Fluckiger, A., Nani, A., Dumont, A., Rosny, C.D., Aboura, I., Khan, A.S., Murtaza, B., Benammar, C., Lahfa, B.F., Patoil, D., Delmas, D., Rebe, C., Apetoh, L., Khan, N.A., Ghringhelli, F., Rialland, M., Hichami, A. (2017): Carob leaf polyphenols trigger intrinsic apoptotic pathway and induce cell cycle arrest in colon cancer cells. Journal of Functional Foods. 33:112-121.

Gonzalez, N., Prieto, I., del Puerto-Nevado, L., Portal-Nunez, S., Ardura, J. A.& Corton, M. (2017): update on the relationship between diabetes and colorectal cancer: epidemiology, potential molecular mechanisms and therapeutic implications. Diabetes Cancer Connect Consortium. Oncotarget, 8(11), 18456. https://doi.org/10.18632/oncotarget.14472.

Güez, C.M., de Souza, R.O., Fischer, P., de Moura Leão, M.F., Duarte, J.A., Boligon, A.A., Athayde, M.L., Zuravski, L., Oliveira, L.F.S. & Machado, M.M. (2017): Evaluation of basil extract *(Ocimum basilicum L.)* on oxidative, antigenotoxic and anti-inflammatory effects in human leukocytes cell cultures exposed to challenging agents. Braz. J. Pharm. Sci. 53(1). http://dx.doi.org/10.1590/s2175-97902017000115098.

Habib, M, K. & Aljammaz, M.H. (2017): Effect of Aqueous Licorice *(Glycyrrhiza glabra)* Extract on Gastric Ulcer Induced by Aspirin in Rats. Journal of Home Economics 27(3),

Harbome, J. B. (1973): Phytochemical Methods. Springer, India, Private Ltd. ISBN: 9788181283108, 9788181283108.

Hussein, S.A., Abdel-Aal, S.A. & Mady, H.A. (2013): Chemopreventive Effect of Curcumin on Oxidative Stress, Antioxidant Status, DNA

Fragmentation and Caspase-9 Gene Expression in 1,2 dimethylhydrzineinduced colon cancer in Rats . BVMJ; 25(2):125-138.

Ikram, A.; Khalid. W.; WajeehaZafar. K-U.; Ali.; A ; Afzal. FM.; Aziz. A.; Rasool, I F.; Al-Farga. A. and Koraqi F.A. (2023): Nutritional, biochemical, and clinical applications of carob: A review. Food Sci Nutr 2023;11:3641–3654.

Jelic, M. D., Mandic, A. D., Maricic, S. M. & Srdjenovic, B. U. (2021): Oxidative stress and its role in cancer. Journal of cancer research and therapeutics, 17(1), 22-28. https://doi.org/10.4103/JCRT.JCRT_862_16.

Katalinic, V., Milos, M., Kulisic, T., Jukic, M. (2006). Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chem. 94, 550–557.

Kelm, M. A., Nair, M. G., Strasburg, G. M., & DeWitt, D. L. (2000). Antioxidant and cyclooxygenase inhibitory phenolic compounds from *Ocimum sanctum Linn*. *Phytomedicine*, *7*(1), 7-13. https://doi.org/10.1016/S0944-7113(00)80015-X

Khojasteh, A.; Mirjalili, M.H.; Alcalde, M.A.; Cusido, R.M.; Eibl, R.; Palazon, J. (2020): Powerful plant antioxidants: A new biosustainable approach to the production of rosmarinic acid. Antioxidants, 9, 1273.

Klenow, S., Jahns, F., Pool-Zobel, B. L., & Glei, M. (2009). Does an extract of carob *(Ceratonia siliqua L.)* have chemopreventive potential related to oxidative stress and drug metabolism in human colon cells? Journal of Agricultural and Food Chemistry, 57(7), 2999–3004.

Koprowsk, H., Steplewski, Z., Mitchell, K, et al. (1979): Colorectal carcinoma antigens detected by hybridoma antibodies. Somat Cell Genet 1979;5:957-972.

Lashinger, L.M., Ford, N.A.& Hursting, S.D. (2013): Interacting inflammatory and growth factor signals underlie the obesity-cancer link, J. Nutr, https://doi.org/ 10.3945/jn.113.178533

Laurindo, L. F., de Maio, M. C., Minniti, G., de Góes Corrêa, N., Barbalho, S. M., Quesada, K. & de Alvares Goulart, R. (2023): Effects of medicinal plants and phytochemicals in Nrf2 pathways during inflammatory bowel diseases and related colorectal cancer: a comprehensive review. Metabolites, 13(2), 243.

Lee, R. and D. Nieman. 1996. Nutritional Assessment. 2nd Edition, Mosby Missouri, USA..

Lewandowska, A., Rudzki, G., Lewandowski, T., Stryjkowska-Góra, A., & Rudzki, S. (2022): Risk Factors for the Diagnosis of Colorectal Cancer.

Cancer control: journal of the Moffitt Cancer Center, 29, 10732748211056692. https://doi.org/10.1177/10732748211056692

Liu, Y., Liu, C., Kou, X., Wang, Y., Yu, Y., Zhen, N. & Xue, Z. (2022): Synergistic hypolipidemic effects and mechanisms of phytochemicals: a review. Foods, 11(18), 2774. https://doi.org/10.3390/foods11182774.

Macharia, J.M., Mwangi, R.W., Rozmann, N., Zsolt, K., Varjas, T., Uchechukwu, P.O., Wagara, I.N. Raposa, B.L. (2022): Medicinal plants with anti-colorectal cancer bioactive compounds: Potential game-changers in colorectal cancer management. Biomedicine & Pharmacotherapy 153 (2022) 113383. https://doi.org/10.1016/j.biopha.2022.113383.

Makri, O.; Kintzios, S. Ocimum sp. (2008): (basil): Botany, cultivation, pharmaceutical properties, and biotechnology. J. Herbs Spices Med. Plants 2008, 13, 123–150.

Marco, G. J. (1968): A rapid method for evaluation of antioxidants. J. Am. Oil Chem. Soc., 45: 594-598.

Mitchison, T.J. (2012): The proliferation rate paradox in antimitotic chemotherapy, Molecular Biology of the Cell. 23 (1): 1-6. DOI: 10.1091/mbc.E10-04-0335.

Mittal, A., Vashistha, V.K. & Das, D.K. (2024): Recent Developments in the Antiviral Activity of Glycyrrhizic Acid and its Derivatives Against SARS-CoV-2: Computational Aspects. Letters in Applied NanoBioScience. 13 (2): 72. https://doi.org/10.33263/LIANBS132.072.

Mnaizel, S. S. (2024): Antidiabetic and antioxidant activity of *Ocimum basilicum* and Gundelia tournefortii extracts in a mouse model. MS.C thesis, (Doctoral dissertation, AAUP). https://repository.aaup.edu/jspui/handle/123456789/1976

Mohamed, N. E. S. (2019): Effect of aqueous extract of *glycyrrhiza glabra* on the biochemical changes induced by cadmium chloride in rats. Biological trace element research, 190, 87-94. https://doi.org/10.1007/s12011-018-1525-5.

Moharib, S. A. (2016): Anticancer and antioxidant effects of fructooligosaccharide (FOS) on chemically induced colon cancer in rats. Ejpau; 19(1):10.

Munteanu I.G. & Apetrei, C. (2021): Analytical methods used in determining antioxidant activity: A review. International Journal of Molecular Sciences 22(7): 3380.

Murray, R. (1984): Alanine aminotransferase. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1088-1090>

Naji-Tabasi, S. & Razavi, S.M.A. (2017): Functional properties and applications of basil seed gum: an over view. Food Hydrocoll. 73. 313-325.

Olukoges, A. & Donaldson, D., (1998): Historical perspectives on health the history of liquorice: the plant, its extract, cultivation, commercailisation and etymology. J R Soc promot Health.4: 118-300

Othmen, K.B., Elfalleh, W., Lachiheb, B. & Haddad, M. (2019): Evolution of phytochemical and antioxidant activity of Tunisian carob *(Ceratonia siliqua L.)* pods during maturation. The EuroBiotech Journal, 3 (3): 135-142. DOI: 10.2478/ebtj-2019-0016.

Pagès, F., Berger, A., Camus, M., Sanchez-Cabo, F., Costes, A., Molidor, R., & Galon, J. (2005). Effector memory T cells, early metastasis, and survival in colorectal cancer. New England journal of medicine, 353(25), 2654-2666.https://www.nejm.org/doi/full/10.1056/NEJMoa051424#:~:text=DOI%3A%2010.1056/NEJMoa051424.

Perna, S.; Alawadhi, H.; Riva, A.; Allegrini, P.; Petrangolini, G.; Gasparri, C.; Alalwan, T.A.; Rondanelli, M. (2022): *In Vitro* and *In Vivo* Anticancer Activity of Basil *(Ocimum spp.):* Current Insights and Future Prospects. Cancers 2022, 14, 2375. https://doi.org/10.3390/cancers14102375

Petrosyan, H.R.; Nigaryan, A.A.; Hovhannisyan, A.M.; Soloyan, V.V. & Martiryan, A.I. (2023): Evaluation of antioxidant activity and heavy metals content in licorice *(Glycyrrhiza glabra L.)* growing wild in Armenia. Heliyon 9 (2023) e22442. https://doi.org/10.1016/j.heliyon.2023.e22442.

Puccini, A.; Berger, M.D.; Zhang, W.; Lenz, H.J. (2017): What We Know About Stage II and III Colon Cancer: It’s Still Not Enough. Target Oncol. 2017, 12, 265–275.

Qasem, M. A., Noordin, M. I., Arya, A., Alsalahi, A., & Jayash, S. N. (2018): Evaluation of the glycemic effect of *Ceratonia siliqua* pods (Carob) on a streptozotocin-nicotinamide induced diabetic rat model. PeerJ, 6, e4788. https://doi.org/10.7717/peerj.4788

Raun, S. H., Buch-Larsen, K., Schwarz, P. & Sylow, L. (2021): Exercise—a panacea of metabolic dysregulation in cancer: physiological and molecular insights. International Journal of Molecular Sciences, 22(7), 3469. https://doi.org/10.3390/ijms22073469.

Reeves, P.G., Nielsen, F.H. & Fahey, G.C. (1993): AIN-93 purified diets for laboratory rodents: final reports of the American institute of nutrition AD Hoc writing committee on the reformulation of the AIN- 76A rodent diet. Journal of Nutrition. 123 (11). Doi: 10.1093/jn/123.11.1939.

Rehman, M. U., Farooq, A., Ali, R., Bashir, S., Bashir, N., Majeed, S., ... & Ahmad, A. (2020): Preclinical evidence for the pharmacological actions of glycyrrhizic acid: a comprehensive review. Current drug metabolism, 21(6), 436-465. https://doi.org/10.2174/1389200221666200620204914.

Romano, R., Luca, L.D., Aiello, A., Pagano, R., Pierro, P.D., Pizzolongo, F. & Masi, P. (2022): Basil *(Ocimum basilicum L.)* Leaves as a Source of Bioactive Compounds. Foods 2022, 11, 3212. https://doi.org/10.3390/foods11203212.

Saeidi, F., Sajjadi, S.E. & Minaiyan, M., (2018): Anti-Inflammatory Effect of *Ocimum Basilicum Linn.* Seeds Hydroalcoholic Extract and Mucilage on Acetic Acid-Induced Colitis in Rats. Journal of Reports in Pharmaceutical Sciences. 7 (3): 295-305.

Scalbert, A.; Manach, C.; Morand, C.; Rémésy, C.; Jiménez, L. Dietary polyphenols and the prevention of diseases. Crit. Rev. Food Sci. 2005, 45, 287–306.

Schneider, P., Hosseiny, S.S., Szczotka, M., Jordan, V. & Schlitter, K. (2009): Rapid solubility determination of the triterpenes oleanolic acid and ursolic acid by UV-spectroscopy in different solvents. Phytochemistry Letters 2(2):85-87.

Seki, H., Sawai. S., Ohyama, K., Mizutani, M., Ohnishi, T., Sudo, H., Fukushima, E.O., Akashi, T., Aoki, T. & Toshiya, M. (2011): Triterpene functional genomicsin licorice for identification of CYP72A154 involved in the biosynthesis ofglycyrrhizinPlant Cell 23 (2011) 4112–4123. https://doi:10.1105/tpc.110.082685.

Singleton, V. & Rossi, J. (1965): Colorimetry of total phenolics with phosphomolybdicphosphotungstic acid reagents. Am. J. E.

Süzek, H., Celik, I. & Doğan, A. (2017): Nephroprotective hepatoprotective potential and antioxidant role of carob pods *(Cerotonia siliqua L.)* against carbon tetrachloride-induced toxicity in rats. https://doi.org/10.5530/ijper.51.2.37.

Taie, H.A.A.; Radwan, S. (2010): Potential activity of basil plants as a source of antioxidants and anticancer agents as affected by organic and bio-organic fertilization. Not. Bot. Horti. Agrobot. 2010, 38, 119–127.

Tietz, N.W. 2006. Clinical guide to laboratory test, 4 th Ed:316-321

Tous, J., & Antoni, C. S. (2013): The Carob Tree: Botany, Horticulture, and Genetic Resources. Horticultural Reviews, First Edition, 41(500 mm), 385–456.

Van-Burden, T.P. & Robinson, W.C. (1981): Fonnation of complexes between protein and tannic acid. Journal of Agricultural and Food Chemistry 1, 77.

Wahab, S., Annadurai, S., Abullais, S.S., Das, G., Ahmad, W., Ahmad, M.F., Kandasamy, G., Vasudevan, R., Ali, S. & Amir, M. (2021): *Glycyrrhiza glabra* (Licorice): A Comprehensive Review on Its Phytochemistry, Biological Activities, Clinical Evidence and Toxicology. Plants 2021, 10, 2751. https://doi.org/10.3390/plants10122751.

Wang, K.L., Hsia, S.M., Chan, C.J., Chang, F.Y., Huang, C.Y., Bau, D.T. & Wang, P.S. (2013): Inhibitory effects of isoliquiritigenin on the migration and invasion of human breast cancer cells, Expert Opin. Ther. Targets 17 (2013) 337–349. https://doi:10.1517/14728222.2013.756869.

Wang, L., Yang, R., Yuan, B., Liu, Y. & Liu, C. (2015): The antiviral and antimicrobial activities of licorice, a widely-used Chinese herb, Acta Pharm. Sin. B 5 (4) 310–315. https://doi:10.1016/j.apsb.2015.05.005.

Wassef, H.H. (2004): Food habits of the Egyptians: newly emerging trends. J. Eastern Mediterranean Health, 10: 898- 915.

Young D.S. (1990): Effect of drugs on clinical laboratory test, 4^{th} Ed:3-609.

Zhishen, J., Mengcheng, T. & Jianming, W. (1999): The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food chemistry, 64(4), 555-559.

Zhu, Z.H., Tao, W.W., Li, J.P., Guo, S., Qian, D.W., Shang, E.X., Su, S.L. & Duan, J.A. (2016): Rapid determination of flavonoids in licorice and comparison ofthree licorice species, J. Separ. Sci. 39 (2016) 473–482. https://doi:10.1002/jssc.201500685.

Zunft, H. J. F., Lüder, W., Harde, A., Haber, B., Graubaum, H. J. & Koebnick, C. (2003): Carob pulp preparation rich in insoluble fiber lowers total and LDL cholesterol in hypercholesterolemic patients. European Journal of Nutrition, 42(5), 235–242. https://doi.org/10.1007/s00394-003-0427-0