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Thyme Leaves Extract Inhibits Growth Of Colon Cancer In Dietary-Induced Obese Rats Via Obstructing Angiogenesis, Inflammation And Oxidative Pathways

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ABSTRACT

This study investigated the inhibitory effect of thyme leaves extract (200mg/kg) on development of colon cancer in the obese rats. Obesity was achieved by feeding HFD daily for 12 weeks, while colon cancer was induced by subcutaneous injection of dimethylhydrazine (DMH, 40 mg/kg) weekly for the same period. Rats received HFD and those with HFD+DMH showed significantly increased body weight gain, while the reverse was observed in animals with DMH- alone. Significant increase in serum levels of colon tumor marker (CEA), glucose, lipids, leptin, insulin and HOMA-IR, but decreases in values of QUICKI were observed in the studied groups. Serum levels of growth factors (IGF-1, VEGF), inflammatory markers (TNF-α, IL-1, IL-6, COX-2, PGE2) and colon lipid peroxidation exhibited marked increase, while colon antioxidants (SOD,CAT,GSH) and TAC were decreased. Colon histopathological examination revealed signs of pre-neoplastic lesions in HFD-fed rats, with prominent growth of colon neoplastic lesions in the other animals, particularly those with HFD+DMH. Supplementation of thyme leaves extract markedly attenuated changes in the body weight, biochemical and histological abnormalities in colon of the studied groups, thereby reducing growth of colon cancer, even with increased consumption of dietary fats.

Keywords: Obesity, Thyme, Colon cancer, VEGF, COX-2, PGE2.

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INTRODUCTION

Obesity is a metabolic health problem with increasing prevalence worldwide**.** Physiologically, obesity arises from an imbalance between energy intake and energy expenditure, which in turn results in increased fat accumulation in adipose tissue **[1].** Such fat accumulation predisposes individuals to development of certain types of cancers including, colon cancer**.** Evidently, increased body max index (BMI) and central obesity are significantly associated with increased incidence of colon cancer **[2].**

In this area of research, several studies have focused on the role of white adipose tissue as an active endocrine and metabolic organ. Excess adipose tissue as seen with obesity could lead to an increased level of circulating glucose and lipids which are critical determinants for developing insulin resistance and hyperinsulinemia **[3]**. Hyperinsulinemia has shown to increase bioavailability of free insulin like growth factor-1 (IGF-1), which may enhance cell proliferation in colon mucosa, leading to growth of colon cancer **[4].**

Other studies provided evidence that obesity is a chronic inflammatory condition in which hypertrophied adipose tissue serves to increase release of pro-inflammatory cytokines including, TNF-α, IL-2 IL-6 and IL-8, which in turn activate various pathways promoting colon carcinogenesis **[5].** Adipose tissue also plays a key role in enhancing generation of reactive oxygen species (ROS)**.** Increased ROS causes oxidation of lipids, proteins and genetic materials, thereby increasing susceptibility to a variety of diseases including, colon cancer **[6].** It is hence possible that reducing adiposity and associated pathogenic events could additionally reduce initiation and progression of colon cancer.

To date, considerable effort has been done to identify natural plants which may act as preventive and therapeutic agents against various diseases. Among these, thyme (*Thymus vulgaris*) is an aromatic plant of the Mediterranean flora commonly used as a spice in many foods. Thyme has also shown to possess a broad spectrum of medicinal activities through its anti-inflammatory**,** antimicrobial and anticarcinogenic properties**.** Besides, thyme is an effective antioxidant through scavenging ROS and increasing cellular antioxidant capacity **[7]**. In other studies, thyme has defined to lower blood glucose and cholesterol levels in diabetic patients and in animal models of diabetes**.** Thyme is also important for treating ailments that range from bronchitis and sore throat to gastritis and skin disorders **[8].**

In light of the above information, the present study aimed to provide more insight into the relation between obesity and colon cancer and also to investigate the possible modulating effect of thyme leaves extract against such relation.

MATERIALS AND METHODS

Experimental animals

The present study was performed on male Wister albino rats (170-180g), obtained from animal house of the Biological Products and Vaccines (VACSERA, Cairo, Egypt). Rats were housed in stainless steel cages at well controlled environmental conditions (25± 2 ^{0}C and 12 h light/ dark cycle) with standard diet and water *ad libitum*. The study was approved by Mansoura University committee for use and care of Laboratory Animals. All experiments were carried out in accordance with the protocol provided by National Institute of Laboratory Animal Resources, National Research Council **[9].**

Induction of colon cancer

For induction of colon cancer, dimethylhydrazine (DMH) purchased from Sigma Chemical Company (St. Louis, MO, USA) was dissolved in physiological saline and administered subcutaneously (S.C) at a dose of 40 mg/kg b.wt once a week for 12 weeks **[10].**

Preparation of thyme extract

Thyme was obtained as dried leaves from the Egyptian local market (Haraz store, Cairo, Egypt). Dried powdered leaves were macerated in 70% alcohol for 72 hours. The mixture was filtered, then the solvent was evaporated at ambient temperature and the extract was kept at 4ºC until being used **[11].**

Experimental design and animal grouping

After one week of acclimation period, rats were randomly divided into nine groups (six animals each) as follows: The 1st served as normal control group, the 2nd group was injected S.C with isotonic saline as vehicle at dose of 2 ml/kg b.wt once weekly, while the 3rd group was given thyme leaves extract (TLE) by oral gavage at dose of 200 mg/kg b.wt **[12].** Rats of the 4th group were fed high fat diet (HFD) consisted of 68% powdered normal diet, 30% melted animal abdominal fat and 2% extra pure cholesterol **[13]**, while those of the 5th group were injected S.C with DMH at a dose of 40 mg/kg b.wt **[10]** once weekly. The 6th group was fed HFD and received DMH as described in groups 4 and 5. In the other groups (7th, 8th, 9th) rats were given TLE and received HFD, DMH and HFD+DMH, respectively as mentioned in the above groups. All treatments were continued for 12 weeks and the rats were weighed at the start and end the study to obtain the body weight gain. Following the last treatment, all rats were fasted 12 hr and sacrificed under light ether anesthesia. Blood samples were collected, centrifuged at 855g for 10 min and the separated sera were stored at -20 ºC for later biochemical analysis. Animals were then dissected and colon from each rat was removed, rinsed in ice-cold physiological saline and dried with filter paper. Specimens of colon were weighted and homogenized for biochemical analysis, while others were fixed in 10% neutral formalin for histological examination.

Biochemical analysis

Serum glucose, lipids [total lipids (TLs), total cholesterol (TC),triglycerides (TGs)],colon malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and total antioxidant capacity (TAC) were determined using commercial kits from Bio-diagnostic Co. Dokki, Giza, Egypt. Level of carcinoembryonic antigen (CEA) was determined in the serum using enzyme-linked immunosorbent assay (ELISA) kit provided by Cloud - Clone Corp, (Katy, TX, USA**).** Serum leptin ,insulin and tumor necrosis factor-α (TNF-α) were measured using ELISA kits provided by ALPCO (Salem, NH, USA) and KAMIYA BIOMEDICAL (Seattle, WA, USA),while interleukin -1 (IL-1) and interleukin -6 (IL-6) were assessed using ELISA kit provided by BioVision (Minneapolis, MN, USA). Measurement of cyclooxygenase-2 (COX-2) and prostaglandin E2 (PGE2) were performed in the serum using ELISA kits provided by CUSABIO (Baltimore, MD, USA).Insulin-like growth factor-1 (IGF-1) and vascular endothelial growth factor (VEGF) in the serum were measured using ELISA kit provided by Thermo scientific (Waltham, MA USA) and BioVision (Minneapolis, MN, USA), respectively. Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) was calculated as: I_O (µIU/ml) **×** G_O (mg/dL) **/** 405 **[14]**, while Quantitative Insulin Sensitivity Check Index (QUICKI) was calculated as: 1 **/** [(log I_O (µIU/ml) **+** log G_O (mg/dL)] **[15]**, where I_O is the fasting insulin and G_O is the fasting glucose.

Statistical analysis

Data were represented as mean ± SEM. All data were analyzed by one way analysis of variance (ANOVA) followed by Least Significant Difference (LSD) test. The analysis was conducted using SPSS statistical package, version 19.00 software. $P < 0.05$ was considered statistically significant **[16].**

RESULTS

Body weight gain

Results in Table 1 exhibited marked obesity features reflected as significantly increased body weight gain in animals received HFD for 12weeks and those with DMH-induced colon cancer and fed HFD for the same time, while significant body weight loss was observed in animals with DMH- alone, compared to the control group. Administration of TLE to the studied groups (HFD,DMH,HFD+DMH) showed significant reduction in mentioned body weight changes compared to non- administered ones.

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No significant alterations were recorded in the body weight of normal rats that received TLE during the experimental duration compared to the control animals.

Table 1: Effect of thyme extract on body weight gain in different animal groups

	CON	SAL	TLE	HFD	DMH	HFD+ DMH	HFD+ TLE	DMH + TLE	HFD+DMH +TLE
Initial body Weight (g)	174.20 ±0.79	178.12 ±0.70	174.21 ±1.14	175.04 ±1.59	176.21 ±1.01	177.01 ±1.03	177.20 ±1.01	178.11 ±0.87	174.12 ±1.01
Final body weight (g)	297.70 ±2.75	305.20 ±2.27	299.50 ±2.97	359.50[a] ±1.38	232.83[a] ±2.57	314.70[a] ±2.04	308.50[ab] ±2.12	251.83[ac] ±1.85	303.52[d] ±1.97
Body weight gain (g)	123.50 ±1.22	127.08 ±2.18	125.29 ±2.15	184.46[a] ±3.62	56.62[a] ±2.56	137.69[a] ±2.29	131.30[ab] ±2.95	73.72[ac] ±3.09	129.40[d] ±2.36

Values are means± SE of six animals for each group. CON: control, SAL: saline, TLE: thyme leaves extract. HFD: high fat diet, DMH: dimethylhydrazine, a: significant when compared different groups versus control. b:significant when compared HFD+TLE versus HFD. c: significant when compared DMH+TLE versus DMH. d: significant when compared HFD+DMH+TLE versus HFD+DMH.

Serum CEA, glucose and lipid fractions

There was significant elevation in serum levels of CEA, glucose and lipid fractions (TLs, TGs, TC) in all studied groups, particularly those with combination of HFD+DMH. However, a reverse pattern of changes was exhibited following administration of TLE to different animal groups, where significant decreases in the mentioned parameters were recorded compared to non-administered groups. Meanwhile, oral administration of TLE to normal rats did not cause alterations in the levels of CEA, glucose and lipids compared to control group (Tables 2).

Table 2: Effect of thyme extract on serum CEA, glucose and lipid fractions (TLs, TC and TGs) in different animal groups

	CON	SAL	TLE	HFD	DMH	HFD+ DMH	HFD+ TLE	DMH + TLE	HFD+DMH +TLE
CEA(Pgml)	0.88 ±0.05	0.82 ±0.03	0.78 ±0.03	2.34[a] ±0.11	4.41[a] ±0.28	6.08[a] ±0.18	1.24[ab] ±0.10	2.69[ac] ±0.19	3.40[ad] ±0.13
Glucose (mg/dl)	99.60 ±2.49	103.51 ±3.01	99.25 ±1.99	198.42[a] ±3.22	191.31[a] ±3.68	204.80[a] ±4.89	140.51[ab] ±3.11	132.53[ac] ±2.67	147.42[ad] ±2.74
TLs (mg/dl)	494.37 ±26.01	503.89 ±33.24	500.43 ±33.57	886.58[a] ±24.25	715.15[a] ±38.59	1092.77[a] ±20.53	598.29[ab] ±14.30	558.87[c] ±13.11	729.93[ad] ±12.78
TGs (mg/dl)	97.39 ±6.27	100.19 ±6.40	96.05 ±2.59	184.99[a] ±4.78	170.67[a] ±2.35	220.73[a] ±9.87	136.22[ab] ±4.45	128.81[ac] ±5.58	142.32[ad] ±5.52
TC (mg/dl)	91.79 ±4.10	93.82 ±3.94	90.55 ±3.39	181.95[a] ±4.89	164.43[a] ±4.54	243.96[a] ±9.25	133.67[ab] ±2.67	124.89[ac] ±4.07	146.93[ad] ±5.89

Values are means± SE of six animals for each group. CON: control, SAL: saline, TLE: thyme leaves extract. HFD: high fat diet, DMH :dimethylhydrazine, a: significant when compared different groups versus control. b: significant when compared HFD+TLE versus HFD. c: significant when compared DMH+TLE versus DMH. d: significant when compared HFD+DMH+TLE versus HFD+DMH.

Serum leptin, insulin, HOMA-IR and QUICKI

Different experimental groups showed significant elevation in serum levels of leptin, insulin and HOMA-IR, with decreased values of QUICKI which seemed maximal in the animal group with both HFD+DMH. Indeed, marked amelioration was observed in these changes when studied groups where supplemented by TLE, however no significant changes were noticed in normal rats that received TLE (Table 3).

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Table 3: Effect of thyme extract on serum leptin, insulin, HOMA-IR and QUICKI in different animal groups

	CON	SAL	TLE	HFD	DMH	HFD+ DMH	HFD+ TLE	DMH + TLE	HFD+DMH +TLE
Leptin (Pg/ml)	652.91 ±4.26	655.10 ±2.57	661.31 ±3.72	1281.01^{a} ±10.40	787.41^{a} ±7.81	1329.11^{a} ±13.33	879.10^{ab} ±11.51	712.21^{ac} ±3.65	912.10^{ad} ±4.45
Insulin (µIU/ml)	2.79 ±0.40	2.74 ±0.18	2.37 ±0.05	7.49^{a} ±2.78	6.83^{a} ±0.69	9.14^{a} ±1.74	4.90^{ab} ±1.36	3.97^{ac} ±1.38	5.47^{ad} ±1.02
HOMA-IR (µIU×mg/dL)	0.69 ±0.04	0.69 ±0.02	0.58 ±0.03	3.67^{a} ±0.18	3.23^{a} ±0.19	4.61^{a} ±0.16	1.71^{ab} ±0.13	1.31^{ac} ±0.09	1.99^{ad} ±0.07
QUICKI (µIU×mg/dL)	0.41 ±0.005	0.41 ±0.002	0.42 ±0.004	0.31^{a} ±0.002	0.34^{a} ±0.003	0.28^{a} ±0.005	0.36^{ab} ±0.006	0.38^{ac} ±0.004	0.34^{ad} ±0.003

Values are means± SE of six animals for each group. CON: control, SAL: saline, TLE: thyme leaves extract. HFD: high fat diet, DMH: dimethylhydrazine, a: significant when compared different groups versus control. b: significant when compared HFD+TLE versus HFD. c:significant when compared DMH+TLE versus DMH. d:significant when compared HFD+DMH+TLE versus HFD+DMH.

Serum growth factors and inflammatory markers

Significant increases in serum levels of growth factors (IGF-1,VEGF) and inflammatory markers (TNF-α, IL-1, IL-6,COX-2, PGE2) were demonstrated in different studied groups, with the highest changes being recorded in animals with HFD+DMH. Marked improvement was observed in tested parameters following administration of TLE, however no significant changes were recorded when TLE was given orally to the normal untreated rats (Fig.1, 2).

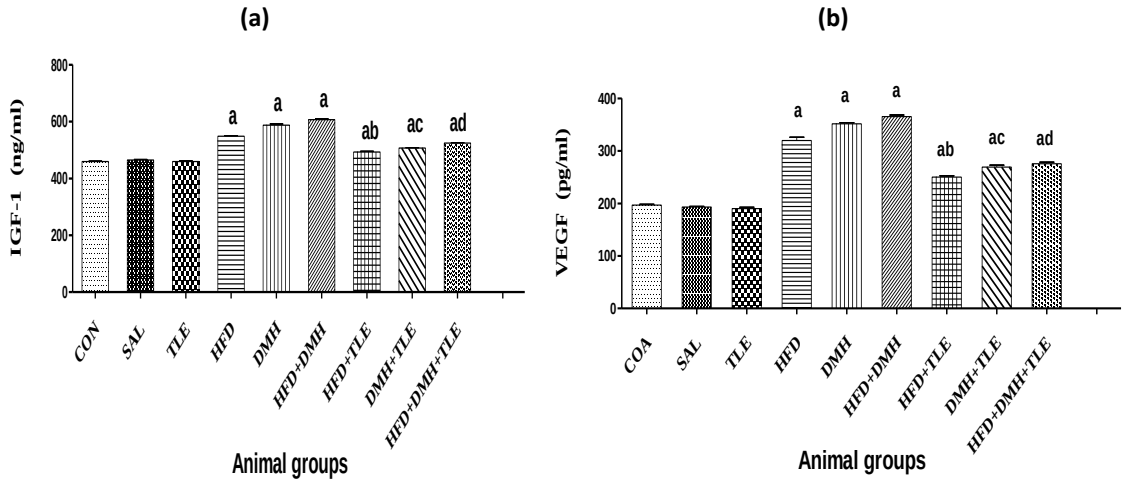

Fig. 1: Effect on thyme extract on serum growth factors (IGF-1) (a) , VEGF (b) in different animal groups. Values are means± SE of six animals for each group. CON: control, SAL: saline, TLE: thyme leaves extract. HFD: high fat diet, DMH: dimethylhydrazine, a: significant when compared different groups versus control. b: significant when compared HFD+TLE with HFD. c: significant when compared DMH+TLE versus DMH. d:significant when compared HFD+DMH+TLE versus HFD+DMH.

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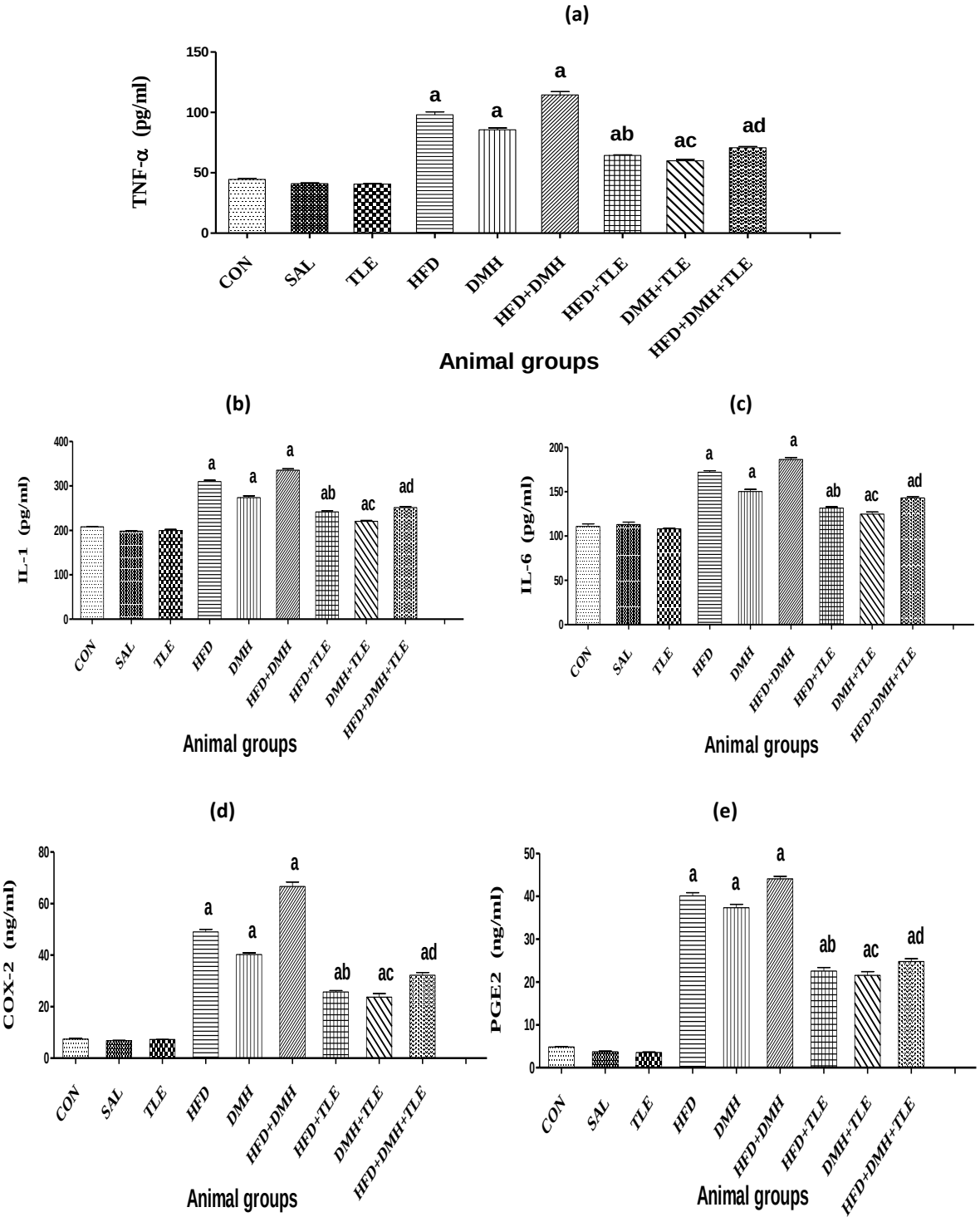

Fig. 2: Effect on thyme extract on serum inflammatory markers (TNF-α (a), IL-1 (b), IL-6 (c), COX-2 (d), PGE2 (e)) in different animal groups. Values are means± SE of six animals for each group. CON: control, SAL: saline, TLE: thyme leaves extract. HFD: high fat diet, DMH: dimethylhydrazine, a: significant when compared different groups versus control. b: significant when compared HFD+TLE with HFD.c: significant when compared DMH+TLE versus DMH. d:significant when compared HFD+DMH+TLE versus HFD+DMH.

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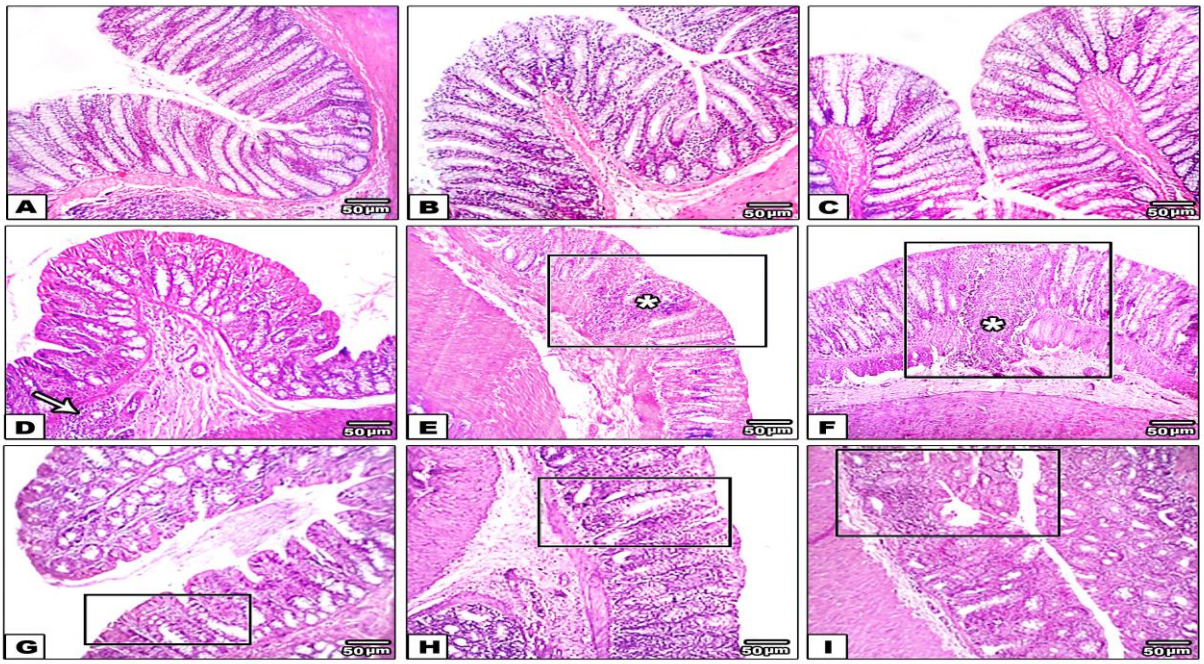

Plate 1: Photomicrograph of H&E stained colon sections in control (A), saline (B) & thyme (C) groups showing normal colon structure. Rats fed on HFD (D) revealed signs of pre-neoplastic lesions (arrow). DMH(E) & HFD+DMH (F) groups showed prominent growth of colon tumor (square) presented by focal neoplastic cells invading the mucosa to reach the sub-mucosal layer (star), with the highest changes being recorded in animals with the combined treatment. Supplementation of TLE markedly attenuated colon histopathological changes in the studied groups, HFD+TLE (G), DMH+TLE (H) & HFD+DMH+TLE (I),suggesting thyme extract as a promising dietary candidate for inhibiting colon cancer, even with increased dietary fats and obesity.

Colon oxidative stress markers

Results showed marked increase in colon MDA, with decreased antioxidants (GSH, SOD, CAT) and TAC in the studied animals, however the most sever changes were noticed in animals with combination of HFD+DMH. These changes tended to be significantly improved when different animals were administered by TLE, however no alterations were observed when the plant extract was given to the normal untreated rats (Table 4).

Table 4: Effect of thyme extract on colon oxidative stress markers in different animal groups

	CON	SAL	TLE	HFD	DMH	HFD+ DMH	HFD+ TEL	DMH + TLE	HFD+DMH +TLE
MDA (nmol/g)	0.35 ±0.01	0.34 ±0.03	0.34 ±0.02	0.54^{a} ±0.03	0.72^{a} ±0.04	0.88^{a} ±0.06	0.41^{b} ±0.02	0.47^{ac} ±0.04	0.54^{ad} ±0.04
GSH (mg/g)	4.33 ±0.30	4.11 ±0.26	4.32 ±0.26	2.23^{a} ±0.13	1.93^{a} ±0.18	1.58^{a} ±0.21	3.00^{ab} ±0.26	2.85^{ac} ±0.12	2.63^{ad} ±0.25
SOD (U/g)	130.99 ±3.22	134.47 ±3.30	131.08 ±3.29	105.31^{a} ±2.36	98.17^{a} ±3.86	91.14^{a} ±3.52	120.07^{ab} ±1.64	117.27^{ac} ±4.66	100.02^{a} ±4.09
CAT (U/g)	48.35 ±2.08	45.40 ±2.22	47.51 ±3.27	32.44^{a} ±0.86	27.78^{a} ±1.08	15.59^{a} ±0.93	45.26^{b} ±2.44	39.85^{ac} ±1.54	31.50^{ad} ±2.81
TAC (Mm/g)	0.77 ±0.03	0.74 ±0.07	0.78 ±0.06	0.47^{a} ±0.05	0.29^{a} ±0.09	0.24^{a} ±0.03	0.67^{b} ±0.05	0.61^{c} ±0.07	0.57^{ad} ±0.05

Values are means± SE of six animals for each group. CON: control, SAL: saline, TLE: thyme leaves extract. HFD: high fat diet, DMH: dimethylhydrazine, a: significant when compared different groups versus control. b: significant when compared HFD+TLE versus HFD. c: significant when compared DMH+TLE versus DMH. d:significant when compared HFD+DMH+TLE versus HFD+DMH.

Histopathological observations

Colon sections of control (A), saline (B) and TLE (C) groups showed normal cellularity of colon mucosa, sub-mucosa and muscular coats. HFD fed rats (D) showed inflamed mucosa with goblet cells metaplasia (arrow) and appearance of pre-neoplastic lesions in the colon tissue. Rats with DMH- alone (E) and those with combination of HFD+DMH (F) showed enhanced proliferative activity with focal neoplastic cells invading the mucosa to reach the sub-mucosal layer (star), reflecting development of colon tumor (square), however the most severe changes were noticed in rats with the combined treatment that showed more proliferation and expanded size of tumor (square), indicating pro-tumorigenic effect of dietary fats. Co-administration of TLE diminished most of the pathogenic changes, where colon sections of HFD+TLE group (G) showed near normal appearance, with reduced proliferation activity and tumor growth (square)in the other groups (H&I), but disorganized mucosal architecture still seen in HFD+DMH+TLE group (I).

DISCUSSION

Obesity is a complex disorder characterized by excessive accumulation of body fat that may impair health. In most cases, changes in dietary pattern, particularly increased consumption of saturated fats is considered as a primary cause of this problem **[13].** Numerous studies suggested that obesity can lead to various types of cancers, including colon cancer **[4].** To clarify involved mechanisms, the present study tended to investigate the effect of HFD -induced obesity on development and progression of colon cancer in DMH treated rats.

Results, showed significantly increased body weight gain in animals with HFD, and those received a combination of HFD+DMH, while a reverse pattern was presented in the animals with DMH- alone compared to control group, most likely due to increased rate of body catabolism and diversion of nutrients to support tumor growth **[17].**

Prior studies revealed a relation between obesity and levels of CEA, which is the most extensively investigated marker for colon cancer and its value is indicative for more advanced disease **[18].** CEA is present in lower limits in normal colorectal mucosa, but is over expressed markedly in cases of colon cancer **[19].** In this respect, the present study showed marked elevation in serum levels of CEA in the studied groups, with the highest level being attained in HFD+DMH group, indicating that incidence of colon cancer continues to increase with increased consumption of dietary fats and development of obesity **[18]**.

Obesity heightens the risk of colon cancer mostly through number of metabolic and hormonal alterations which in all may derive growth and progression of colon cancer **[20].** As such, the present study showed prominent increase in serum glucose, lipids (TLs, TGs, TC), leptin and insulin in the studied animals, particularly those with colon cancer and fed HFD. Similar study revealed marked signs of hyperglycemia and hyperlipidemia with accompanied elevation of both leptin and insulin in rat model of HFD- induced obesity which together promote colon carcinogenesis **[21].**

Hyperglycemia may have a direct role in cancer development as glucose is a key nutrient required for growth of cancer cells. It is needed for proliferating cells, and several types of tumor cells have shown to up-regulate glucose transporters **[22].** Hyperlipidemia may also affect carcinogenesis. Tumor cells often express receptors that attract cholesterol metabolites necessary to support their growth, thus increasing levels of cholesterol in the tissues **[23].**Further evidence for utilizing lipids by cancer cells is the up- regulation of fatty acid synthase, an enzyme that catalyzes synthesis of fatty acids which are required for growth of cancer cells **[24].**

It is also widely accepted that leptin has the potential to act as tumor growth factor. Leptin is a peptide hormone secreted by adipocytes which is primary involved in the regulation of body weight through binding of leptin to its receptors on hypothalamus**.** To add, leptin receptors are identified in colonic epithelium, which have functional importance in regulating specific intracellular pathways that control cell growth, differentiation and angiogenesis involved in development of cancer**.** Dysregulation of these processes, as a result of the obesity-related hyperleptinemia, can lead to colon neoplasia **[25].** Serum leptin is

positively correlated with insulin which is thought to regulate secretion of leptin from adipocytes and is therefore, involved in the association between leptin and colon cancer **[26].**

Further research into insulin-related pathways and their connection to colon cancer has yielded mixed results, which mostly suggested insulin resistance as a major cause **[27].** Insulin resistance is an interrupted state in the biological response to insulin which in turn leads to hyperinsulinemia **[3].**It was reported that chronic hyperinsulinemia in the obese states is associated with various types of cancers such as colon cancer**.** To aid, hyperinsulinemia may lead to decreased IGF-1 binding proteins (IGF-BP), leading to increased level of free IGF-1which can promote cell proliferation, thereby increasing the risk of tumorigenesis **[28].** In an experimental animal study, cancer growth was reduced when IGF-1 receptors were removed or when IGF-1 concentration was reduced, indicating that IGF-1 can directly modulate cancer growth.

Besides, IGF-1 was found to stimulate production of VEGF from adipocytes, which is a necessary step for cancer development**.** VEGF seems to play a crucial role in the proliferation and migration of endothelial cells, providing nourishment to the growing tumor cells and making established continuity with the host vasculature**.** It is hypothesized that over-expression of VEGF protein is a turning point in the development of colon cancer by activating angiogenesis and tumor neo-vascularization **[29].** In this view, the present study confirmed incidence of hyperinsulinemia, insulin resistance (HOMA-IR) and lowered insulin sensitivity (QUICKI), with consequent elevation in serum IGF-1 and VEGF in the studied animals, particularly those with HFD+DMH, suggesting increased susceptibility to develop colon cancer with increased feeding of dietary fats.

For more interpretation, obesity can be viewed as an inflammatory state, which is a key mechanism in the pathophysiology of colon carcinogenesis. Notably, the inflammatory pathways in case of obesity are highly activated, leading to excessive secretion of pro-inflammatory cytokines (TNF-α,IL-1, IL-6, IL-8,). These molecules are secreted by macrophages and are considered as major agents in the transition between acute and chronic inflammation **[30].** A special role in the pathophysiology of obesity-driven colon carcinogenesis is related to the TNF-α **[31].** TNF-α is a pro-inflammatory adipokine produced mainly by activated macrophages, natural killer (NK) cells, and neutrophils, although it can be produced by many other cell types. TNF-α exerts marked impact on the development of colon cancer in the obese states through direct effects on colon cells and indirectly through action on the tumor-promoting microenvironment. TNF-α may also contribute to cancer progression by promoting cell migration and metastasis **[32].**In this respect, a number of in vivo studies have documented that IL-1 is a crucial regulator for production of TNF-α and growth factors such as, VEGF**.** Thereby, proliferation of colon cancer cells is stimulated indirectly by IL-1 **[33].**Besides, IL-6 has documented to be involved in initiation and growth of colon cancer and that IL- 6 encouraged invasiveness of colon cancer cells **[34].**

Importantly, COX-2 is an enzyme that is generally overexpressed at sites of inflammation and in number of cancers including, colon cancer. COX-2 is involved in the regulation of angiogenesis, and tumor cell invasiveness, which appear to contribute to its effects on tumorigenesis **[35].** COX-2 has been found to regulate synthesis of prostaglandins which has shown to stimulate tumorigenic pathways **[36].** PGE2 is a pro-inflammatory mediator and a well established biomarker for determining the risk for colon cancer development. It plays a significant role in colonic crypt cellular expansion and the consequent formation of adenoma. PGE2 is formed from arachidonic acid (AA) via action of cyclooxygenases, namely constitutive COX-1 and inducible COX-2 in the colonic mucosa**.** PGE2 plays a role in regulating the migratory and invasive behavior of cells during development and progression of cancer. Many human cancers exhibit high prostaglandin levels due to up-regulation of COX-2 and prostaglandin E2 synthase-1, the key enzyme in generation of prostaglandins **[37].** As such, current study revealed increased levels of pro-inflammatory cytokines (TNF-α, IL-1, IL-6), COX-2 and PGE2 in serum of the studied groups, where the highest effect was observed in HFD+DMH group. Thus, indicating enhanced inflammatory response with increased dietary fats which can promote development of colon cancer.

Other studies proposed that increased oxidative stress seemed to mediate the ability of HFD to promote colon cancer. Evidently**,** increased lipid substrates in the tissues may increase the metabolic load on such tissues, causing increased oxygen consumption and production of reactive oxygen species (ROS) **[38].** Under normal physiological conditions, ROS can be eliminated or inactivated by different antioxidant systems, however with obesity antioxidants are not provided in adequate amounts to compensate for increased production of free radicals, thereby generating a state of oxidative stress **[39].**Colon cancer is a disease

originating from the epithelial cells lining the bowel mostly due to increased formation of ROS in the intestinal lumen. Continuous exposure of the mucosa to these free radicals, promoting oxidative damage and development of colon carcinogenesis **[40].** Meanwhile, ROS have the ability to oxidize polyunsaturated fatty acids, which take part in cell membrane constitution. This reaction initiates lipid peroxidation, a chain reaction that produces free radicals and other toxic substances, such as malondialdehyde (MDA). Products of lipid peroxidation (particularly MDA) may act as signaling transducers modulating several cell functions, including gene expression and cell proliferation **[41]**.

Specifically, accumulation of MDA with accompanied depletion of cellular antioxidants has emphasized to increase during carcinogenesis **[42].** In relation, the present study showed increased levels of MDA with decreased antioxidants (SOD, CAT, GSH) and TAC in colon tissue of different studied groups, which appeared to be higher in animals with colon cancer and fed HFD. This seemed to be linked with the present histopathological observations characterized by marked mucosal inflammation with signs of pre-neoplastic lesions in colon of HFD fed rats, while rats with DMH- alone and those with combination of HFD+DMH showed prominent endothelial cells proliferation and growth of colon neoplastic lesions, however the most severe changes were noticed in rats with the combination treatment. It is hence logical to confirm the pro-tumorigenic effect of dietary fats and further to underscore the potential importance of controlling associated pathogenic events that mediate this effect.

Nowadays, there is increasing interest in the use of various plant products as a natural therapy for prevention and cure of several diseases. In the present study, administration of TLE was found to reduce incidence of obesity and risk of colon cancer even in the presence of high fat intake, as assessed by diminished levels of serum CEA and improved biochemical and histological abnormalities in the colon tissue of all supplemented groups, compared to un-supplemented ones. Interestingly, thyme extracts have reported to contain abundant amounts of active constituents particularly, terpenoids, flavonoids, glycosides and phenolic acids **[43].** Among these, thymol and carvacrol are monoterpene phenols known to have numerous health benefits, including anticancer properties. Both thymol and carvacrol can be easily converted to thymoquinone, which is now considered as a promising anticancer candidate**.** Thymoquinone has been reported to inhibit growth of various cancer cells through inhibition of proliferation and induction of apoptosis **[44].** Besides, thyme contains several bioactive components such as luteolin, rosmarinic and apigenin which are described to possess anticancer activities **[7].**

Other health attributes may arise from thyme ability to maintain normal metabolic and hormonal status, which may have a direct role in preventing various diseases. Importantly, thymol which is a major constituent of thyme extract has shown to exert anti-obesity effect through inhibiting accumulation of visceral fats, enhancing insulin and leptin sensitivity and improving lipid and glucose profile in HFD-fed animals **[45].** Thymol has also shown to increase the expression of phospho AMP-activated protein kinase, phospho acyl-CoA carboxylase, hormone-sensitive lipase, carnitine palmitoyltransferase and acyl-coenzyme A oxidase, which in all play a key role in glucose and lipid homeostasis **[46].** An effect that seemed to be a direct mean for reducing progression of colon cancer. In relation, the present study demonstrated the ability of TLE to diminish serum glucose, lipids, leptin, insulin and insulin resistance in the different treated groups. Results also showed marked decline in serum IGF-1 by thyme administration. IGF-1 has shown to interfere with various stages of carcinogenesis in different cancer cells. Importantly is that luteolin (as one of the most abundant flavonoids in thyme extract) was found to block colon carcinogenesis via suppressing stimulated tumor growth by IGF-1 **[47].** Flavonoids also inhibit VEGF expression and tumor angiogenesis in vivo **[48].** It seems that more advanced tumor stages actually express higher levels of VEGF protein **[49].**Several flavonoids including apigenin **[50],** genistein and chrysin showed the ability to inhibit VEGF expression in tumor cells. Of importance is that VEGF inhibitors could reduce angiogenesis, cause tumor vessels regression and slow tumor growth. As such, the present study showed lowered serum VEGF in the studied groups by administration of TLE, indicating suppressed angiogenesis and growth of colon cancer.

Direct evidence indicated the anti-inflammatory nature of thyme which might be one of the effective pathways in inhibiting tumor growth **[51].** Many processes involved in inflammation and its suppression are affected by thyme. Thyme when evaluated individually exhibited anti-inflammatory actions against diverse inflammatory stimuli. For example, in mice fed a high-fat diet and supplemented with thyme (0.1% w/w) for 10 weeks, the production of pro-inflammatory cytokines in visceral adipose tissues was suppressed compared to the high-fat controls, probably due to inhibition of toll like receptor 2 (TLR2) and TLR4- mediated signaling**.**

Dietary supplementation of thyme was also able to significantly reverse the high fat diet-induced up-regulation of adipose tissue genes and proteins associated with inflammation. Individual constituents of thyme particularly, thymol and carvacrol have also been evaluated for anti-inflammatory activity. Studies suggested the potential anti-inflammatory effect of thymol and indicated that it could be used in a similar fashion to non-steroidal anti-inflammatory drugs **[52].** Thymol administration showed decreased expression of pro-inflammatory cytokines (TNF-α and IL-6) resulting in suppression of tumorigenesis and cancer cells growth **[53].** Meanwhile, carvacrol has shown to suppress the key mediators of inflammation, such as COX-2 and PGE2 **[54].**Other bioactive constituent of thyme is apigenin which is a flavonoid has been documented to exhibit anti-inflammatory **[55]** and anticancer properties **[56].** In the present study, TLE administration tended to exhibit marked reduction in the pro-inflammatory cytokines, TNF-α, IL-1, IL-6 , accompanied by lowing of COX-2 activity and PGE2 production in colon tissue of different treated groups. Results, thus confirmed the anti-inflammatory influence of thyme which may aid in preventing colon cancer growth.

Thyme has also identified as one among several herbs that contain high total concentration of antioxidants. Antioxidant activity of thyme and its extracts is dependent mainly on its phenolic compounds. It was demonstrated that thymol and carvacrol, the main phenolic constituents of thyme mostly contributed to its antioxidant activity **[57].** One of the most studied effects of thymol includes scavenging of free radicals by increasing the activities of several endogenous antioxidant enzymes; SOD, GPx and GST along with non-enzymatic antioxidants such as vitamin C, vitamin E and GSH **[58].** Similar study revealed that thymol has superoxide and hydroxyl radical scavenging activity, besides protection against oxidative damage to lipids **[59].** Carvacrol as the second major phenolic compound in thyme extracts was found to exhibit antioxidant activity due to its weak acid character that facilitates reaction with the free radicals, thereby donating hydrogen atoms to an unpaired electron producing more stable products **[60].** In the present study, thyme administration showed marked elevation in the antioxidants SOD, CAT and GSH and TAC, along with depletion of MDA accumulation in colon tissue of the studied groups. It is hence possible to suggest reduced oxidative stress by thyme administration which may eventually aid in suppressing tumor growth, as presently reflected by the improved histological features of colon tissue in all studied animals**.**

In conclusion, the present study indicated that thyme could help to suppress the tumorigenic effect of HFD, through modifying various pathogenic events including, hyperglycemia, hyperlipidemia, insulin resistance, elevated growth factors, inflammation and oxidative stress which are potential mediators for tumor development. Result therefore increased thoughts that thyme could be a promising dietary candidate for chemoprevention of colon cancer, even with increased intake of dietary fats.

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