Preventive Effect of Meloxicam against Colon Cancer Induced by 1, 2 Dimethyl Hydrazine in Rats

Dr.Abderrhman Ahmed Mohammed Ismeil
Assistant Professor, Department of Physiology, Faculty of Medicine, Sinnar University, Sudan
E-mail: draahmed208@yahoo.com

Abstract
Background:
Colorectal cancer (CRC) is among the most common types of cancer in the world. CRC is common cancer in both males and females representing 4.5% and 3.6% of the total cancers. Hydrazines were manufactured from chemicals such as ammonia, dimethyl amine, hydrogen peroxide, and sodium hypochlorite .A small amount of hydrazine occurs naturally in some plants. 1, 2 Dimethyl hydrazine (DMH) has been used as a research chemical to produce colon cancer in lab animals. A therapeutic area in which NSAIDs use became important was in the treatment and prevention of cancer. Meloxicam was shown to prevent the initiation of chemical-induced tumors, and considered as anticancer agent by virtue of its anti-proliferative effect. Aims: The present study was designed to investigate the protective role of meloxicam which is a non-steroidal anti-inflammatory drug against colon cancer development. Methods: Sixty male albino mice were divided into five groups of 12 mice as follows: Group A: The animals were served as control; received S.C. injections of saline solution. Group B: The animals received S.C. injections of 20 mg 1, 2 DMH/Kg b.w. Group C: The animals received 1, 2 DMH as in group B with ad libitum access to water and high fat diet. Group D: The animals were fed high fat diet and water ad libitum. Group E: The animals received S.C. injections of 1,2 DMH as in group B and oral 15mg/Kg /day meloxicam/0.1 ml saline via gastric tube 1 hour before DMH administration, fed high fat diet and water ad libitum. Results: there was elongation of the crypts with epithelial proliferation. Architectural disturbance, mitotic figures (MF), and inflammatory cells are observed in Colon sections of group C of animals received DMH – high fat diet. Colon sections of rats injected with DMH – Meloxicam -High fat diet in group E showed the majority of colonic glands with regular size and shape. Conclusion: The growth of aberrant crypt foci in colon was delayed or uncompleted after meloxicam use and this was shown to be a good evidence of its effectiveness in colon cancer protection.
Keywords: Colon Cancer, High fat diet, Meloxicam, 1, 2 Dimethyl hydrazine.

Introduction:

Colorectal cancer (CRC) is among the most common types of cancer in the world. In Egypt, CRC is the 6th common cancer in both males and females representing 4.5% and 3.6% of the total cancers. The percentage of young-onset colorectal cancer cases in Egyptians was strikingly high with more than one third of cases occurring

under age 40 years, and the age-adjusted mortality rates in young Egyptians were likewise high [1,2]. Colorectal cancer was divided into sporadic (70-80%) and familial cases. Approximately 5 - 10% of all cancers fall into the familial category [3]. The vast majority of CRC developed from benign precursor lesions through a series of genetic and epigenetic changes [4]. According to the WHO classification, nearly 85% of CRC were usual adenocarcinomas, and10 to 15% were classified as mucinous adenocarcinomas [5].Globally a steadily increasing proportion of elderly people in the world result in approximately 16 million new cases of cancer by the year 2020 (IARC) [6].

Hydrazines were manufactured from chemicals such as ammonia, dimethyl amine, hydrogen peroxide, and sodium hypochlorite .A small amount of hydrazine occurs naturally in some plants[7]. 1, 2 Dimethyl hydrazine (DMH) has been used as a research chemical to produce colon cancer in animals [8]. Also known as symmetrical dimethyl hydrazine N, N' DMH, considered as a highly specific colorectal procarcinogen that undergoes metabolic activation in the liver to DNA-reactive metabolites by a series of reactions through intermediates and to the ultimate carcinogenic metabolite [9]. 1, 2 dimethyl hydrazine was tested for carcinogenicity in mice, rats and hamsters following oral and subcutaneous or intramuscular administration, producing tumors at various sites [10].This carcinogen was metabolized in the liver to azoxymethane (AOM), then to methylazoxy methanol (MAM), which led to methylcarbonium ion , the ultimate carcinogen bound DNA in the colon [11].

A therapeutic area in which NSAIDs use became important was in the treatment and prevention of cancer. Epidemiologic studies in human showed that aspirin use was associated with a significant reduction in the incidence of colon cancer and evidence suggested that the therapeutic effect of NSAIDs on colon cancer mediated by inhibition of COX-2, which was up regulated in many premalignant and malignant neoplasms [12]. Meloxicam was shown to prevent the initiation of chemical-induced tumors, and considered as anticancer agent by virtue of its anti-proliferative effect, capacity for cell cycle arrest, and pro-apoptotic effects, also acted as free radical scavenger, in particular superoxide anion oxidation scavenge [13].

The aim of the present study was to investigate the role of meloxicam which is a no steroidal anti-inflammatory drug (to investigate its protective role) against colon cancer development.

Materials and Methods

The present study was carried out on 60 male albino rats, 5 -6 weeks old at the beginning of the experiment, weighing from 100-120 g and obtained from the animal house of Theodor Bilharz Institute, Cairo. All experiments were performed in line with the ethical considerations recommended by Alexandria University, Egypt.

The animals were kept in the animal house, 4 per cage in a temperature and light controlled room. They were maintained on standard diet and allowed free access

to appropriate diet and water ad libitum throughout the 8 weeks of the experiment.

The animals were divided into 5 groups, 12 animals in each group.

Group A: The animals were served as control; received S.C. injections of saline solution.

Group B: The animals received S.C. injections of 20 mg 1,2 DMH /Kg b.w dissolved in 1ml sterile physiological saline in the inter scapular region using a tuberculin syringe with a 24-gauge needle once weekly for 8 weeks , fed standard diet and water ad libitum .

Group C: The animals received 1, 2 DMH as in group B with ad libitum access to water and high fat diet.

Group D: The animals were fed high fat diet and water ad libitum.

Group E: The animals received S.C. injections of 1,2 DMH as in group B and oral 15mg/Kg /day meloxicam/0.1 ml saline via gastric tube 1 hour before DMH administration[14] , fed high fat diet and water ad libitum.

Standard diet for groups A & B consisted of 5% fat, 53% carbohydrate, 23% protein, with total caloric value 25 kJ/kg and high-fat diet for group C,D & E consisted of 30% fat, 48% carbohydrate, and 20% protein with total caloric value 44.3 kJ/kg were used[15].

At the end of the experiment, colon was dissected out to study:

*Histopathological studies:

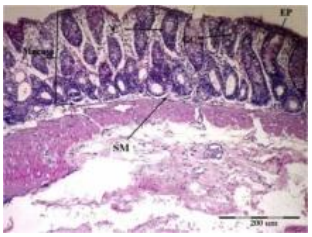
- Hematoxylin and eosin staining procedure
- Alcian blue pH 2.5- acid mucopolysaccharide
- Feulgen nuclear staining of DNA
- Methylene blue – Whole mount staining of colon
- Immunoassay of serum CEA

Results

Histopathological Results:

In control group the normal colon mucosa of albino rat. Typically glandular crypts (C) that invaginate deep in the submucosa (SM). Muscular is externa, serosa layers are also observed (Fig 1).Sections of colon of animals of group B received DMH - Standard diet showed. Crowded tubular glands (*) with irregular shapes and sizes invading the muscularis mucosa layer and abundant chronic inflammatory cells (▼) are also noticed (Fig 2). In addition there was elongation of the crypts (*) with epithelial proliferation. Architectural disturbance, mitotic figures (MF), and inflammatory cells (▼) are observed (Fig 3).Colon sections of group C of animals received DMH – high fat diet. Overcrowding of damaged tubular glands (*) which appeared irregular in shape and size lined by dysplastic epithelium. Some glands are seen lined by normal epithelial cells with round nuclei (Fig 4). As well on colonic glands (*) showed dilated lumen and severe epithelial atypia with slim rod shaped stratified nuclei (Fig 5). Colon sections of rats fed high fat diet and water ad libitum. (Group D) showed aregularly organized glands (*) lined mainly by epithelial and mucus secreting cells (Fig 6).Colon sections of rats injected with

DMH – Meloxicam -High fat diet.Group E showed the majority of colonic glands (*) with regular size and shape (Fig 7).



Fig(1):

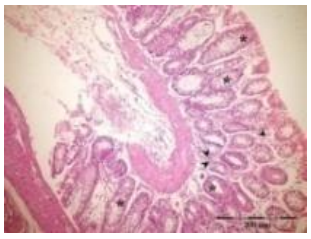
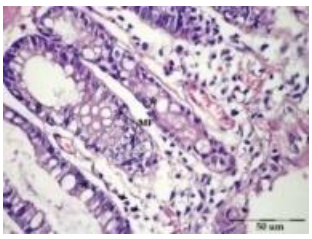
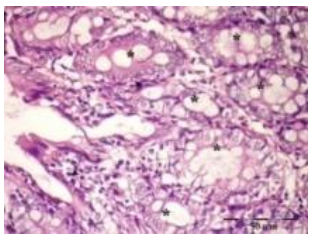


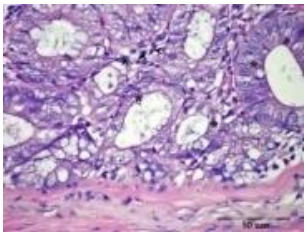
Fig (2):



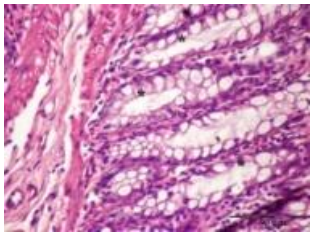
Fig(3):



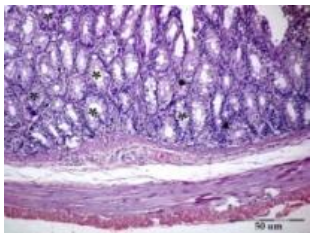
Fig(4):



Fig(5):



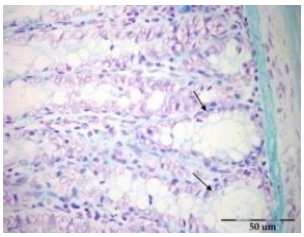
Fig(6):



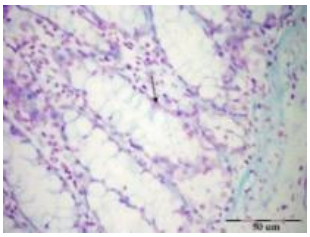
2. Nuclear DNA staining of colon mucosa using Feulgen technique:

Normal rat colon section of group A, showing colon glands lined by columnar epithelium and abundant normal secretory cells. Positively stained nuclei located in the epithelial cells of the crypts (↑) (Fig 8).Colon sections of rats of group B received DMH -Standard diet and ad libitum access to water. Showing crypt multiplicity with parallel moderate increase in DNA (↑) (Fig 9). Colon section of group C of rats received DMH as in group B, fed high fat diet and water ad libitum. Showing aberrant crypts with stratified mucosa, occasional mitotic division and pyknotic nuclei (▼) which expressed marked increase of DNA (Fig 10).Colon section of group D of rats fed high fat diet and water ad libitum. Showing predominance of closely packed glands (↑) with distended goblet cells and normal epithelial cells with small-sized nuclei stained dark purple for DNA (Fig 11).Colon sections of group E of animals received S.C. injections of DMH - Meloxicam - High

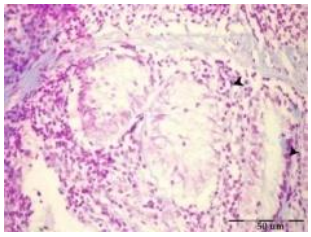
fat diet and water ad libitum .The colon epithelium showed small non dividing nuclei stained for DNA reaction(↑) (Fig 12).



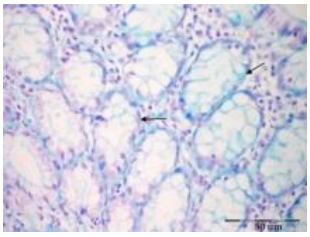
Fig(8):



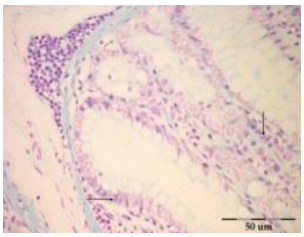
Fig(9):



Fig(10)



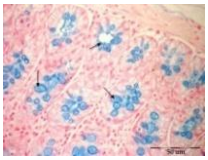
Fig(11)

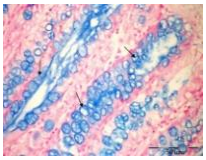


Fig(12)

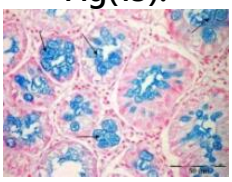
3. Staining of acidic mucin of rat colon secretory cells using alcian blue:

Colon sections of control group of rats received S.C.injections of saline solution. The goblet cells in most of crypts appeared few in number with moderate positive blue staining mucin (←) (Fig 13). Colon sections of group B received S.C.injections of 1, 2 DMH, fed standard diet and water ad libitum. Increased number of goblet cells with increased blue staining mucin (↑) (Fig 14). Colon sections of group C of rats received 1, 2 DMH as in group B -High fat diet. The colon crypts showed numerous proliferated crypts devoid of goblet cells. Mucin is almost completely depleted in most of ACs in rat colon (←) (Fig 15). Colon sections of group D of rats fed high fat diet and water ad libitum. Goblet cells with different sizes condensed in colon crypts lumen stained with different degree of blue coloration and other empty goblet cells after discharging their contents are observed (↑) (Fig 16). Colon sections of group E of rats received S.C.injections of 1, 2 DMH-Meloxicam-High fat diet. Showed colon crypts with few goblet cells which contained less mucin (↑) (Fig 17).

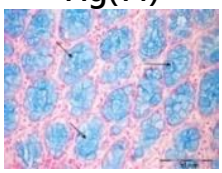




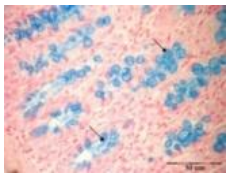
Fig(13):



Fig(14)



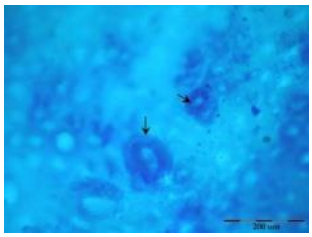
Fig(15)



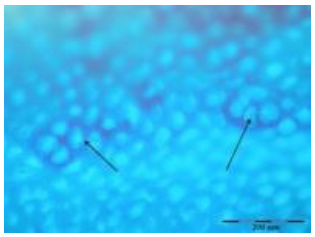
Fig(16)

4. Whole mount methylene blue staining of ACF of rat colon:

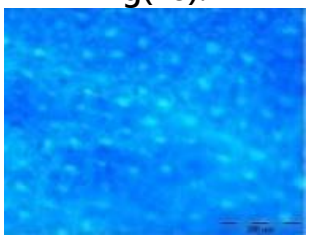
whole colon of Group B of rats received S.C.injections of 1, 2 DMH, fed standard diet showing a small sized focus consisting of thickened crypts(↑), with increased pericryptal area; greater staining intensity due to thickened epithelium (Fig 28). ACF of whole mount colon of group C of animals received 1, 2 DMH as in group B and fed high fat diet. Showing darkly staining proliferated crypt foci with thick epithelial lining (↑) (Fig 29). ACF in methylene blue-stained whole mount colon of group D of rats fed high fat diet and water ad libitum. Showing normal, moderate sized closely packed crypts (Fig 30). Whole colon of group E of animals received DMH- Meloxicam -High fat diet. Showing normal crypts with some thickened lining crypts (↑) (Fig 31).



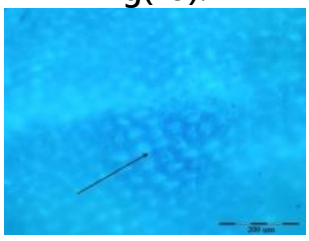
Fig(28):



Fig(29):



Fig(30):



Fig(31):

Discussion

Cancer chemopreventive agents were considered as non-invasive and non-toxic agents that delayed, inhibited, or reversed carcinogenesis. Although many anticancer drugs have been developed, still numbers of mortality and morbidity among cancer patients were high; therefore, it was necessary to implement the golden principle of "prevention is better than cure" [16].

Tumor formation in the large bowel is an intricate, multistep process influenced by an interplay between intrinsic and extrinsic factors, including age, gender, diet

(intake of fat, fiber, alcohol and red meat), co-morbidities (inflammatory bowel diseases, obesity, diabetes mellitus) and lifestyle (physical activity, cigarette smoking) [17].

1, 2 DMH is a common colon carcinogen often used in developing CRC in various rodents [18]. Perše, et al. 2011[19] recorded that 1,2DMH was highly specific for colonic epithelium, inducing colorectal tumors in experimental animals. They stated that it was the most widely used model of chemically induced colon carcinogenesis.

The aberrant crypt foci were considered as an early biomarker lesion for colorectal cancer [21]. In the present study, induced CRC by the administration of 1, 2 DMH to rat model fed balanced diet showed proliferation and crowding of the tubular glands which appeared irregular in shapes and sizes, touching the muscularis mucosa layer. The lining cells showed hyperchromatic nuclei and chronic inflammatory cells in between the glands.

In a related study carried out by Naim, et al. 2009 [22] , ACF were considered as an early neoplastic cell lesion that was characterized by unstable colonic epithelia which encompassed many dysplastic crypts of the ACF that were enlarged and elevated when compared with the adjacent normal crypts. This was in accordance with result of the present study.

In the present study, rats which received 1, 2 DMH and fed high fat diet showed overcrowding of the colonic tubules. The glands showed dysplastic changes evidenced by the absence of goblet cells, stratified absorptive cells with elongated hyperchromatic nuclei, lacking distinct nucleoli and infiltrated by chronic inflammatory cells. In accordance with these findings, previous work by Reddy. 2000 [23] confirmed that diet was one of the major factors accounting for the variability in cancer incidence and mortality at these sites. In addition, studies on different experimental animal models have supported the idea that high fat diet augmented the incidence of colon carcinogenesis. Whereas low fat and high fiber present in fruits and vegetables diet, decreased the risk of colon cancer [24].

In the present work, examination of sections of colon of rats fed high fat diet, the structure of colonic mucosa was nearly similar to the control group. Regularly arranged tubular glands in which the secreting cells outnumbered the absorptive cells. In contrast, Nakagama, et al. 2005 [25] reported that increased quantity of fat presented, could have direct action on the colonocytes, causing significant increase in the colonocytes proliferation index, thus promoting colorectal cancer.

In the current study, colonic gland of the rats group treated with meloxicam (a preferential COX 2 inhibitor) showed slight changes and remained in the form of somewhat irregular size and shape lined by both types of cells. The mucous secreting cells exceeded the tall columnar cells. These results were evaluated in relation to the study done by Brown, ET al.2000 [26] who investigated several NSAIDs agents such as indomethacine, sulindic sulphone, celecoxib, and meloxicam. They found that the chemopreventive efficacy of these anti-inflammatory drugs were independent of cyclooxygenase inhibitor profile.

Chemical carcinogens were found to cause a genetic error by modification of the molecular structure of DNA that led to a mutation during DNA synthesis .DNA adducts formation resulted in either the activation of a proto-oncogene or the inactivation of a tumor suppressor gene which was considered a tumor initiating event [27]. The relevance of DNA damaged and repaired to the generation of cancer became evident when it was recognized that all agents that caused cancer also caused a change in the DNA sequence and thus were mutagens [28]. All the effects of carcinogenic chemicals on tumor production can be accounted for, by the DNA damage and by the errors introduced into DNA during the cells efforts to repair this damage [29].

Sengottuvelan, et al.2009 [30] Indicated that 1, 2 DMH-induced DNA damage and oxidative stress in Wistar male rat colon carcinogenesis were suppressed/ prevented effectively by resveratrol supplementation which ameliorated DNA damage. The role of DNA content as a prognostic factor in colorectal cancer was highly controversial. [31] Buhmeida. 2009 [32] showed that DNA content was not associated with clinical outcome. Others have reported that, some of these discrepant observations might be explained by differences in the technical aspects of recording the DNA contents or by differences of interpretation of the DNA histogram [33].

Cancer cell nuclei has indicated that measurements of nuclear morphometry and investigation of the distribution of histone protein/ DNA complexes within the nucleus can be used to characterize the disease state and predict its progression [34].

In the present work Feulgen staining technique has been used for demonstrating the different degrees of DNA concentration in all the studied groups. The degree of the magenta color of Feulgen reaction revealed moderate DNA concentration in normal group associated with the normal columnar epithelial cells of the colon crypts.

In Rats which were injected with 1,2 DMH received therapeutic dose of meloxicam and fed standard diet,there was occasional nuclear stratification and loss of polarity, irregular foci of increased multinucleated cells, with hyperchromatic nuclei due to increased concentration of DNA. However, in the current study the combinations of high-fat diet and 1, 2 DMH result in a higher incidence of colon aberrant foci with increased multinucleated cells and matched higher level in DNA. Similar observation was reported by Nairooz.et al. 2010 [35] who stated that studies of rats fed high fat diet which differed significantly from the standard diet group showed nuclei typically irregular with positive DNA staining. The same finding was previously shown by Calle, et al. 2004 [36] who confirmed that a high fat diet increased the risk of colon cancer.

Mucins are a family of high molecular weight, heavily glycosylated proteins produced by epithelial tissues [37]. Their key characteristic are their ability to form gels; therefore they are a main component in most gel-like secretions, serving functions from lubrication to cell signalling to forming chemical barriers[38].

Secretory mucins were released from the apical surface of goblet cells by, baseline secretion or simple exocytosis and compound exocytosis. A wide array of bioactive factors, including hormones, neuropeptides, and inflammatory mediators, can induce compound exocytosis [39].

In the present study , the group of rats recieved 1,2 DMH and standard diet showed colorectal mucosa with abundant goblet cells predominant in the luminal epithelium in different phases of secretory activity with acid mucin stained by alcian blue .Corfield, et al.2000 [40] reported both the qualitative and quantitative changes occurred in the mucins in malignant transformation of colon. These changes included reduction in the total mucins output, reduction in sulphation (of neutral mucin), but an increase in sialylated mucin (of acidic mucin). Combination of high-fat diet and 1,2DMH exhibited numerous alcian blue positive mucin stained goblet cells of different phases along the crypts. However, the rats that recieved HFD, showed goblet cells with different sizes condensed in the colon crypts lumens and stained by different degrees of blue color with other empty goblet cells forming mucin depleted foci.

However, the group treated with meloxicam drug, showed predominance of closely packed distended goblet cells, most of them appeared empty in the altered crypts, attained light blue coloration and contained less mucin due to expulsion of mucus. The present results were confirmed by the study of Nairooz.et al. 2010 [35] who reported that goblet cells appeared distended with blue acidic mucin secretion.

Methylene blue can positively stain metaplastic absorptive epithelium, such as intestinal-type metaplasia in the stomach, it does not stain non-absorptive epithelium, such as ectopic gastric metaplasia in a background of positive staining duodenal mucosa. In the gastrointestinal epithelium the dysplastic epithelium areas and cancers absorb methylene blue in a different way than the normal mucosa. Thus, after staining with methylene blue these abnormalities appear as areas of absent or light staining or as a heterogeneous staining pattern against a background of uniformly blue-stained mucosa [41]. Intra-arterial methylene blue injection is recommended as a routine technique in the histopathologic study of colon cancer. Methylene blue–assisted lymph node (LN) dissection as a routine technique in the histopathologic examination of colorectal specimens [42].

Conclusion

The results of the current study showed that meloxicam has a protective effect against CRC moreover, the increased quantity of fat in diet has a deteriorated action on the crypts fission and showed a significant increase in proliferation of cells. Hence, it is highly recommended that high fat diet should be avoided or limited and replaced by a healthy balanced food.

Acknowledgment

Author would like to thank prof. Amany Kazim professor of histopathology for her technical support and also thank for all the staff of animal house and lab in Alexandria Medical Research Institute for their cooperation during this study.

References

1. Jenkinson F, Steele RJ. Colorectal cancer screening-methodology. Surgeon 2010; 8:164-71.
2. Siegel R, Naishadham D, Jemal A. Cancer statistics. CA Cancer J Clin 2013; 63:11-30.
3. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics. CA Cancer J Clin 2010; 60:277.
4. Wilkes G, Hartshorn K. Colon, rectal, and anal cancers. Semin Oncol Nurs 2009; 25:32-47.
5. Veruttipong D, Soliman AS, Gilbert SF, Blachley TS, Hablas A, Ramadan M, et al. Age distribution, polyps and rectal cancer in the Egyptian population-based cancer registry. World J Gastroenterol 2012; 18:3997-4003.
6. Zeeneldin A, Saber M, Seifeldin IA, Frag S. Colorectal carcinoma in gharbiah district, Egypt: comparison between the elderly and non-elderly. Journal of Solid tumors 2012; 2:3.
7. Center MM, Jemal A, Smith RA, Ward E. Worldwide variations in colorectal cancer. CA Cancer J Clin 2009; 59:366-78.
8. Edwards BK, Ward E, Kohler BA, Eheman C, Zauber AG, Anderson RN, et al. Annual report to the nation on the status of cancer, 1975-2006, featuring colorectal cancer trends and impact of interventions (risk factors, screening, and treatment) to reduce future rates. Cancer 2010; 116:544-73.
9. Ferlay J, Parkin DM, Steliarova-Foucher E. Estimates of cancer incidence and mortalityin Europe in 2008. Eur J Cancer 2010; 46: 765-81.
10. Rachet B, Maringe C, Nur U, Quaresma M, Shah A, Woods LM,et al. Population-based cancer survival trends in England and Wales up to 2007: an assessment of the NHS cancer plan for England. Lancet Oncol 2009; 10:351-69
11. Ferguson LR. Meat and cancer. Meat Sci 2010; 84:308-13.
12. Levin TR, Palitz A, Grossman S, Conell C, Finkler L, Ackerson L, et al. Predicting advanced proximal colonic neoplasia with screening sigmoidoscopy. JAMA 1999; 281:161-77.
13. Lang T, Maitra M, Starcevic D, Li SX, Sweasy JB. A DNA polymerase beta mutant from colon cancer cells induced mutations. Proc Natl Acad Sci 2004; 101:6074-9.
14. Triantafillidis JK, Nasioulas G, Kosmidis PA. Colorectal cancer and inflammatory bowel disease: epidemiology, risk factors, mechanisms of carcinogenesis and prevention strategies. Anticancer Res 2009; 29:27-37.
15. Michor F, Iwasa Y, Lengauer C, Nowak MA. Dynamics of colorectal cancer. Semin Cancer Biol 2005; 15:484-93.
16. Gsteiger S, Morgenthaler S. Heterogeneity in multistage carcinogenesis and mixture modeling. Theor Biol Med Model 2008; 5: 13.

17. Hussain SP, Schwank J, Staib F, Wang XW, Harris CC. TP53 mutations and hepatocellular carcinoma: insights into the etiology and pathogenesis of liver cancer. Oncogene 2007; 26:2166-76.
18. Pancione M, Remo A, Colantuoni V. Genetic and epigenetic events generate multiple pathways in colorectal cancer progression. Patholog Res Int 2012; 50:93-8.
19. Perše M, Cerar A et al. Morphological and Molecular Alterations in 1, 2 Dimethylhydrazine and Azoxymethane induced colon carcinogenesis in rats. J Biomed Biotecnol 2011; 10: 473964-78.
20. Klaunig JE, Kamendulis LM, Hocevar BA. Oxidative stress and oxidative damage in carcinogenesis. Toxicol Pathol 2010; 38:96-109.
21. Wood LD, Parsons DW, Jones S, Lin J, Sjoblom T, Leary RJ. et al. The genomic landscapes of human breast and colorectal cancers. Science 2007; 318:1108-13.
22. Naim J et al. Chemoprevention of Induced Colonic Aberrant Crypt Foci in Rats by the Combination of Meloxicam and Grapefruit Juice. J Med J 2009; 43:316- 23.
23. Reddy BS. The Fourth DeWitt S. Goodman lecture. Novel approaches to the prevention of colon cancer by nutritional manipulation and chemoprevention. Cancer Epidemiol Biomarkers Prev 2000; 9:239-47.
24. NaKui H, OKitsu K, Maeda Y, Nishimura. The effect of pH on sonochemical degrafation of hydrazine. Ultrason Sonochem 2007; 14:627-32.
25. Nakagama et al. modeling human colon cancer in rodents using a food-borne carcinogen, PhIP. Cancer Sci 2005; 96: 627-36.
26. Brown et al. Non-steroidal anti-inflammatory drugs with different cyclooxygenase inhibitory profiles that prevent aberrant crypt foci formation but vary in acute gastrotoxicity in a rat model. Journal of Gastroenterology and Hepatology 2000; 15: 1386-92.
27. Sara W. Hydrazine and hydrazine sulfate. National Toxicology Program. Rep Carcinog 2004; 11:145-6.
28. Perše M, Cerar A. The dimethylhydrazine induced colorectal tumours in rat experimental colorectal carcinogenesis. RadiolOncol 2005; 39: 61-70.
29. Sharma A, Sharma KK. Chemoprotective role of triphala against 1, 2-dimethylhydrazine dihydrochloride induced carcinogenic damage to mouse liver. Indian J Clin Biochem 2011; 26:290-5 .
30. Sengottuvelan et al .Resveratrol ameliorates DNA damage, prooxidant and antioxidant imbalance in 1, 2-dimethylhydrazine induced rat colon carcinogenesis. Chem Biol Interact 2009; 181:193-201.
31. Gerner EW. Impact of dietary amino acids and polyamines on intestinal carcinogenesis and chemoprevention in mouse models. Biochem Soc Trans 2007; 35: 322-25.

32. Buhmeida A, Hilska M, Elzagheid A, Laato M, Collan Y, Syrjänen K, et al. DNA image cytometry predicts disease outcome in stage II colorectal carcinoma. Anticancer Res 2009; 29:99-106.
33. Nakagama H, Ochiai M, Ubagai T, Tajima R, Fujiwara K, Sugimura T, et al. A rat colon cancer model induced by 2-amino-1-methyl-6-phenylimidazo [4, 5-b] pyridine, PhIP. Mutat Res 2002; 507:137-44.
34. Bird A. DNA methylation patterns and epigenetic memory. Genes Dev 2002; 16: 6-21.
35. Nairooz S, Ibrahim SH, Omar SMM, Affan M. Structural Changes of the Colonic Mucosa Induced by Orlistat: Experimental Study. Egypt. J. Histol 2010; 33: 635 – 48.
36. Calle EE, Kaaks R. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. Nat Rev Cancer 2004; 4:579-91.
37. Garcia SB, Barros LT, Turatti A, Martinello F, Modiano P, Ribeiro-Silva A,et al. The anti-obesity agent Orlistat is associated to increase in colonic preneoplastic markers in rats treated with a chemical carcinogen. Cancer Lett 2006; 240:221-4.
38. Cowey SL, Quast M, Belalcazar LM, Wei J, Deng X, Given R, et al. Abdominal obesity, insulin resistance, and colon carcinogenesis are increased in mutant mice lacking gastrin gene expression. Cancer 2005; 103: 2643–53.
39. Ikeda K, Mutoh M, Teraoka N, Nakanishi H, Wakabayashi K, Taguchi R. Increase of oxidant-related triglycerides and phosphatidylcholines in serum and small intestinal mucosa during development of intestinal polyp formation in Min mice. Cancer Science2011; 102: 79–87.
40. Corfield Ap, Shukla AK. Mucins: vital components of the mucosal defensive barrier. Genomic/Proteomic Technol 2000; 3: 20–2.
41. Flegal KM, Carroll MD, Ogden CL, Johnson CL. Prevalence and trends in obesity among US adults, 1999–2000. JAMA 2002; 288: 1723–27.
42. Tanaka T. Colorectal carcinogenesis: Review of human and experimental animal studies.J Carcinog 2009; 8:5.