The Egyptian Journal of Hospital Medicine (January 2018) Vol. 70 (6), Page 994-1006

Combinational Effect of 5-Flourouracil and Resveratrol against N-Nitroso-N-methyl urea Induced Colorectal Cancer

Belal Ahmed Soliman[1], Abdel Razik Hussein Farrag[2], Howayda Al Sayed Khaled[1], Alyaa A.Mostafa Mohamed [1]

[1]Zoology Department, Faculty of Science, Suez University, [2]Pathology Department, National Research Center

Corresponding author: Aliaa Ghaddar, email:aliaa_ghaddar@yahoo.com

ABSTRACT

Background: colorectal cancer is considered to be the third most common cause of deaths in both men and women. The incidence of colorectal cancer cases has been rising at an alarming rate. In most cases, colon cancer treatment involves chemotherapy. However, toxicity and tumor cell drug resistance are outstanding obstacles to this treatment. Scientific studies suggested that combining certain chemotherapy treatments with specific antioxidants at defined dosages can improve drug efficacy or may reduce side effects severity.5-Fluorouracil, which is used in the treatment of breast, stomach and pancreatic cancer, remains the cornerstone of CRC treatment, although widely used in combination with several other drugs. Many effective drugs, including those actually used for cancer treatment, have been developed from botanical sources. Resveratrol is a pleiotropic phytochemical which is belong to the stilbene family. Although, it is only significantly present in grape products. Many preclinical studies investigated its anticancer properties in a plethora of cellular and animal models. **Aim of the work**: in the present study, the anticancer effects of Resveratrol alone or combined with 5-Fu were assessed on experimentally induced colorectal cancer in rats. **Results:** the results of this study indicated that RES had a better therapeutic effect against N-methylnitrosourea induced colorectal cancer than 5-Fu alone and when in combination with each other they diminished the cytotoxic effect of 5-Fu and enhanced normal histological appearance of colon tissue, which could be a promising alternative for resistant colorectal cancer. However, the exact mechanisms involved needs to be further explored. **Conclusion**: our results suggested that both natural compounds could be in the future a possible alternative to enhance the efficiency of 5-Fu in resistant colon cancer cells. This study supports the potential of plant extracts as source of bioactive compounds with biomedical applications.

Keywords: colorectal cancer, resveratrol , 5-flourouracil.

INTRODUCTION

Cancer is an expression used for diseases in which abnormal cells divide without control and are able to invade other tissues[(1)]. Colorectal cancer (CRC) is the second leading cause of cancer-related mortality, with 655,000 deaths worldwide annually [(2,3)]. Large steps have been taken toward the development of an animal model for studying colonic cancer. Intestinal tumours, both adenomas and carcinomas, can be induced in some animals by a variety of methods. Among the most effective were 1,2–dimethylhydrazine and azoxymethane [(4)].

Numerous studies have shown that rats, which rarely develop cancer spontaneously, are good animals to use for the induction of intestinal tumours by these chemicals [(5)]. For the last 70 years, the fluoropyrimidine 5-fluorouracil (5-FU) has been positioned as a first line chemotherapy in the treatment of various cancers including colorectal, head, neck and breast cancers[(6,7)] . 5-FU is a poor tumor selective and therefore its therapeutic use results in high incidence of bone marrow, gastrointestinal tract and central nervous system toxicity. To tackle these problems, a series of 5-Fu prodrugs in which 5-Fu is attached to amino acids, peptides, phospholipids, and polymers have been reported [(8)]. Studies have confirmed that res combined with other chemotherapeutic drugs can be more effective at treating drug-refractory cancer cells [(9)]. In one study , resveratrol potentiated the therapeutic efficacy of temozolomide, an alkylating agent used in cancer therapeutics, in a mouse xenograft model of malignant glioma, through inhibiting ROS/ERK mediated autophagy and enhancing apoptosis [(10)].

The procedure can be used to defeat many problems including poor solubility or absorption, patient acceptability, drug instability and toxicity and especially drug resistance [(11)]. After the entry of a mutual prodrug into a cancer cell, the two active components can reach the target at the same time and are liberated parallel, whereas they might be transported to the same site with different efficacy when administered alone. This type of prodrugs can demonstrate a broader antitumor spectrum, less drug resistance and less toxicity [(12)]. Resveratrol is a naturally occurring phytoalexin, a material synthesized *de novo* by plants, to offset pathogen infections. In preclinical study, resveratrol has been shown to improve vascular health by reducing hypertension and counteract against heart failure and ischemic heart disease in experimental animal

Received: 21/10/2017 DOI: 10.12816/0044352
Accepted: 01/11/2017

models**.** it protects against high fat diet-induced obesity, improve insulin sensitivity, lower serum glucose levels in numerous animal models and improves diabetic kidney disease in rodents [(13)]**.** Similarly, resveratrol has been shown to have neuroprotective property in the experimental model of cerebral stroke [(14)]**.** Even though resveratrol seems to have potential against a variety of diseases/conditions, one of its most obvious health benefits is its capability to obtain chemopreventive as well as therapeutic effect against some cancers**.** Resveratrol affects all three stages of carcinogenesis: initiation, promotion, and progression. Furthermore, resveratrol has been shown to directly induce the apoptotic pathway through several mechanisms [(15,16)]**.**

MATERIALS AND METHODS

Animals

Male western rats, weighing 100-120 gm were used in the current study, they were housed in polystyrene cages in which the ground was covered with sawdust to diminish the risk of painful contact with a hard surface. Rats were kept in a 12 h light-dark cycle. The animals were kept at the room temperature of 25±5ºC.

The animals were obtained from the National Research Centre, Cairo, Egypt and acclimated for one week in a specific pathogen free (SPF) barrier area. Rats were housed with *ad libitum* access typical laboratory diet consisting of protein 21%, lipid 4.59% and cellulose 4.2%.

Chemicals of Tested compounds

N-Methyl-N-nitrosurea:

N-Methyl-N-nitrosurea with chemical formula $C_2H_5N_3O_2$ and molecular weight 103.08 g/mol was purchased from SIGMA and ALDRICH and prepared in distilled water.

Resveratrol

Resveratrol with chemical formula $C_{14}H_{12}O_3$ and molecular weight 228.2 g/mol . resveratrol purity 99% was and purchased from SIGMA and ALDRICH. A 20-µM stock solution of resveratrol was prepared in DMSO.

5-Fluorouracil

5-Fluorouracil (5-FU) with chemical formula $C_4H_3FN_2O_2$ and molecular weight 130.078 g/mol was supplied as one bottle of a colorless, water soluble solution at a concentration of 10 mg/ml.

Formalin solution

The solution was obtained from El-Nasr Pharmaceutical Chemicals Co. a 10 % solution was prepared and used for fixation of tissue specimens for the histopathological examination.

Hematoxyline and Eosin

The stains were obtained from El-Gomhoria Pharmaceutical Chemicals Co, and used for staining histopathological sections.

Experimental design

This study was conducted on 45 rats. They were categorized as follows (5 rats of each):

Group 1: normal healthy rats used as negative control.

Group 2: normal healthy rats used as DMSO treated

Group 3: colorectal cancer group in which rats were intrarectally administered with N-methylnitrosourea in a dose of 2 mg dissolved in 0.5 ml water/rat three times weekly for five weeks [(17)]**.**

Group 4: 5-fluorouracil-treated (5-Fu) group in which rats were intraperitoneally treated with a dose of 12.5 mg/kg on days 1, 3 and 5 with the cycle being repeated every four weeks for 2 month [(18)]**.**

Group 5: resveratrol-treated group (RES) in which rats were orally treated with resveratrol dissolved in DMSO; 200 µg/kg/b.w daily for 2 months [(19)]**.**

Group 6: rats treated with 5-fluorouracil and resveratrol; they were intraperitoneally treated of 5-fluorouracil 6.25 mg/kg on days 1, 3 and 5 with the cycle being repeated every four weeks for 2 months and orally treated with resveratrol dissolved in DMSO; 100 µg/kg BW daily for 2 months [(19)]**.**

Group 7: 5-fluorouracil-treated group in which rats were intrarectally administered with N-methylnitrosourea, then intraperitoneally treated with a dose of 12.5 mg/kg on days 1, 3 and 5 with the cycle being repeated every four weeks for 2 months [(18)]**.**

Group 8: resveratrol-treated group in which rats were administered intrarectally with N-methylnitrosourea, then treated orally with resveratrol which was dissolved in DMSO 200 µg/kg BW daily for 2 months[(19)]**.**

Group 9: rats administered with 5-fluorouracil and resveratrol in which rats were intrarectally administered with N-methylnitrosourea, then intraperitoneally treated with 5-Fluorouracil 6.25 mg/kg on days 1, 3 and 5 with the cycle being repeated every four weeks for 2 months and orally treated with resveratrol which is dissolved in DMSO; 100µ/kg BW daily for 2 months.

Tissue preparation

`Control and treated groups were anaesthetized and dissected immediately. Tissue samples of rat's colon were excised. The colon was removed and washed in ice-cold phosphate-buffered saline. Tissues were fixed in 10% neutral buffered formalin for 24 hrs and then washed for 24 hrs under tap water. The colon samples were

dehydrated using increasing ethanol concentrations then embedded in paraffin wax. Serial sections (5 µm) from five rats per group were prepared for histopathological, histochemical and immunohistochemical using the rotatory microtome (Microtome, Walldorf, Germany).

Hematoxylin and eosin staining

Serial transverse sections of colon (5 µm thick) were cut and mounted on clean glass slides and stained with hematoxylin and eosin [20,21]. Sections were carefully examined and photographed. Microscopic analysis was based on the scoring system [22].Quantifying inflammation was examined as described previously by [23,24] . Briefly, inflammation was graded by extent (focal, multi-focal, diffuse, or extensive areas) and depth/penetration of inflammation into lamina propria, submucosa, and mucscularis propria. The scoring given a numerical value of 0 to 4, where 0 is none observed and 4 is severe inflammation. Definition of categories and general criteria in histomorphological scores for colon histopathology according to the method of [25].

Mucin histochemistry

The number of goblet cells and the composition of mucin were evaluated in crypts of the distal colon. To evaluate the mucin composition in the goblet cells, serial sections were stained 1- by the Periodic acid-Schiff reaction for not substituted glycerol-rich neutral mucin (magenta), and 2- by 1% alcian blue, pH 2.5, for detection of acidic sialo- and sulfomucins (blue). Periodic acid-Schiff/alcian blue staining was performed sequentially to differentiate between neutral and acidic mucins. Dual staining allowed for determination of mucin-type predominance. The AB/PAS-positive areas were determined using Image J processing software to threshold grayscale images, expressing the integrated density of the area of AB/PAS+ mucosubstances per unit length [26,27]. Three randomly selected fields of AB/PAS-stained tissues in each specimen were analyzed. Under ×200 magnification, fields were captured from each section and the images were analyzed. The ratio between the area of AB/PAS-stained glands and total area of tissue was recorded. Data were presented as the mean of 3 fields for each specimen.

Immunohistochemical Examination

After fixation, colon samples were embedded in paraffin and sectioned (4–6 µm thick). After deparaffinization, sections were immersed in 0.3% H_2O_2–PBS for 30 min to block endogenous peroxidase activity and incubated overnight. To reduce nonspecific background staining due to endogenous peroxidase, incubate slide in Hydrogen Peroxide Block for 10 minutes, apply UltraVision protein block and incubate for 5 minutes to block nonspecific background staining. Sections were incubated with primary antibodies (anti-COX-1 and anti-COX-2). then the samples were washed in buffer. HRP Polymer Quanto was applied and incubated for 10 minutes, then colon samples were washed in buffer. 30 µl (one drop) of DAB Quanto chromogen was added to 1 ml of DAB Quanto substrate, Mix by swirling and apply to tissue incubate for 5 minutes. Samples were washed by DI water.

Morphometry analysis

Morphometry were done with the aid of a light microscope (Axiostar plus microscope, Carl Zeiss, Göttingen, Germany) using Canon DC290W camera (Germany) and the digital analysis software Image J (IJ) software (NIH, version v1.45e, USA).

Statistical analysis

Statistics were calculated with SPSS for windows version 17.0, where the mean values obtained in the different groups were compared by one way ANOVA followed by Duncan's. All results were expressed as mean values ± SE and significance was defined as $p< 0.05$ [28].

The study was done after approval of ethical board of Suez university.

RESULTS

Histopathological Results

Control colon tissue showed normal structure. Mucosa is lined by simple columnar epithelium rich in goblet cells. Lamina propria contains tubular intestinal glands or crypts of liberkunn. Crypts of Liberkunn are consisting of stem cells which are active, undifferentiated cells found at the base of lamina propria and Goblet cells (**Fig 1A**). Normal rats did not show evidence of colonic inflammation, injury. The colonic tissues of rats received DMSO (**Fig 1B),** 5-Fluorouracil (**Fig 1C)** and Resveratrol (**Fig 1D**) also showed normal architecture of colonic tissue. In rat received methylnitrosourea showed moderate to severe inflammation, epithelial hyperplasia characterized by crypt damage and inflammatory cell infiltration (**Fig 1F and 1G**). However, tissue sections from methylnitrosourea induced colon cancer rat treated with resveratrol (**Fig 1I)** and 5-Fluorouracil (**Fig 1H**) and their combination (**Fig 1J)** had more intact surface epithelium, normal colon cells and less inflammatory than those in the methylnitrosourea induced colon cancer group.

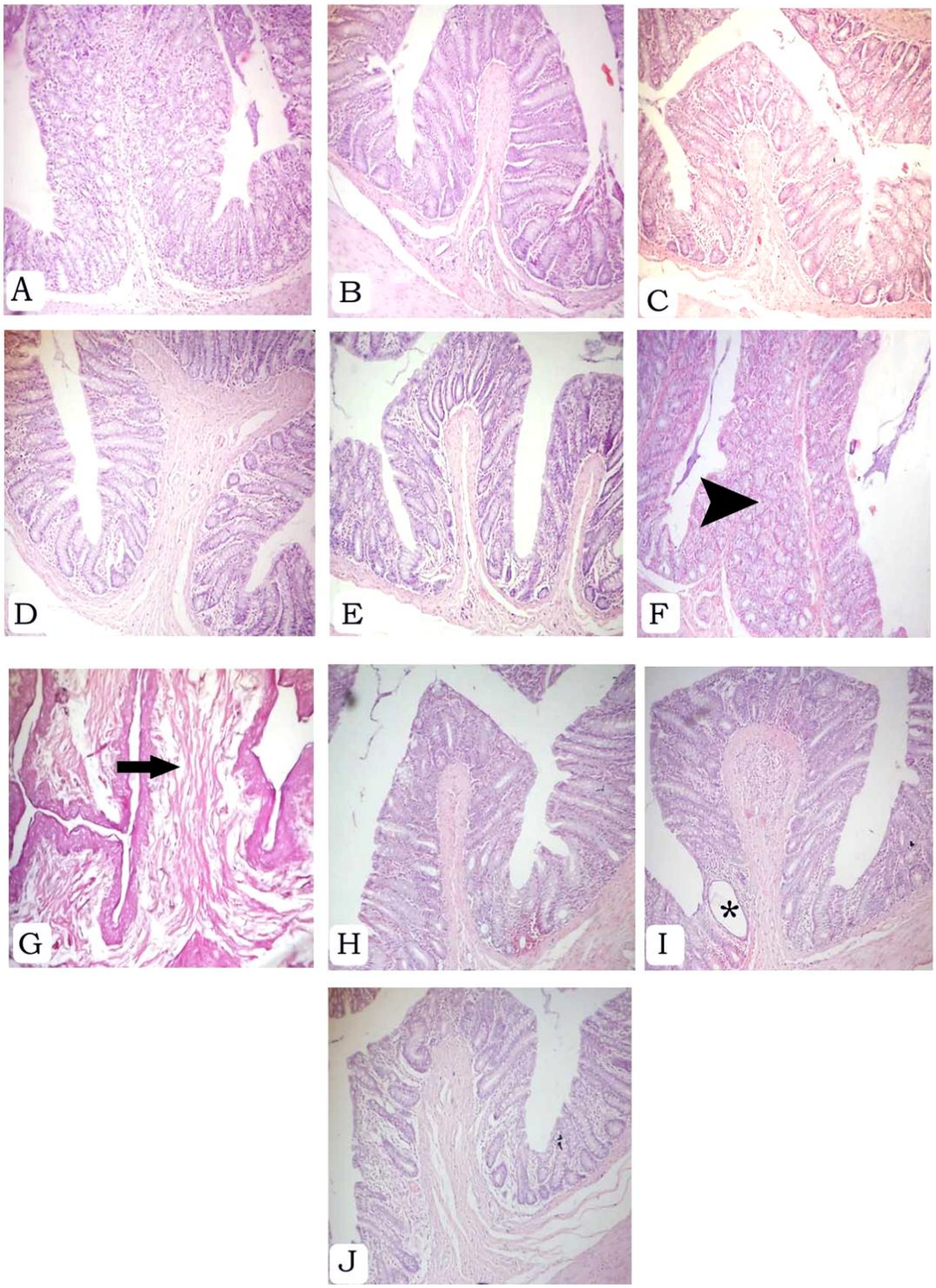

Fig. 1: micrograph of colon sections of : A-control group, B)- DMSO group, C) -5-fluorouracil treated group, D)- resveratrol treated group, E- 5-fluorouracil+ resveratrol treated group (F and G) methylnitrosourea-induced colon cancer, group H)-methylnitrosourea induced colon cancer rat treated with 5-fluorouracil, I)- methylnitrosourea induced colon cancer rat treated with resveratrol, J) -methylnitrosourea induced colon cancer rat treated with 5-fluorouracil in combination with resveratrol (arrow) epithelial hyperplasia, (arrow head) degenerative colon crypts (H & E X 200).

Histopathological score

Microscopically, colon from control group showed the normal histology (**Fig**. **1A).** In cancer group, sections showed typical inflammatory changes in colonic architecture such as crypt and surface epithelial loss. In addition, a complete destruction of the epithelial architecture was observed in some rats (**Figs. 1F and 1G**). The colon cancer group treated with resveratrol and 5-Fluorouracil and their combination showed attenuation in the severity of colon injury, a higher integrity of mucosal architecture and the epithelial

loss. Total histological scores were significantly reduced compared to the colon cancer group (**Table 2**). The inflammatory cells infiltration was lowered in the colon cancer group treated with RES compared to the FUC group, the differences were statistically significant. Histopathological score for normal and methylnitrosourea (MEN) induced colon cancer rats treated with resveratrol and 5-Fluorouracil and their combination were represented in **table 2**. The data showed that inflammatory cell infiltrate, epithelial hyperplasia, irregular crypts were significantly increased in cancer group as compared to the control group.

5-Fluorouracil, resveratrol and their combination treatment revealed significant decrease in inflammatory cell infiltrate, epithelial hyperplasia and irregular crypts as compared t colon cancer rats.

Table 2: Effect of Resveratrol, 5-fluorouracil and their combination on histopathological score of distal colon in N-methylnitrosourea induced colorectal cancer in rats

Parameters	Inflammatory cell infiltrate	Epithelial Hyperplasia	Irregular crypts
Control	0.00±0.00	0.00±0.00	0.00±0.00
DMSO	0.00±0.00	0.00±0.00	0.00±0.00
Colorectal cancer	3.67±0.33[a]	3.33±0.31[a]	2.06±0.58[a]
FUC-treated	0.00±0.00	0.00±0.00	0.00±0.00
RES-treated	0.00±0.00	0.00±0.00	0.00±0.00
FUC+RES-treated	0.00±0.00	0.00±0.00	0.00±0.00
Cancer +FUC	2.23±0.57[b]	2.31±0.37[b]	0.70±0.25[b]
Cancer +RES	0.51±0.28[b]	0.49±0.15[b]	0.33±0.14[b]
Cancer+(FUC+RES)	0.33±0.08[b]	0.33±0.01	0.33±0.09[b]

Values are means ± SE. n=5, One Way ANOVA followed by Duncan multiple comparison tests. a $p<0.05$ compared with normal control group, b $p<0.05$ compared with N-methylnitrosourea induced colorectal cancer group.

Alcian blue and periodic acid-Schiff's stain

Sections of the control, DMSO-treated group Resveratrol-treated and 5-Fluorouracil- treated groups and their combination stained with alcian blue and periodic acid-Schiff's stain showed a moderate diffused cytoplasmic alcian blue staining affinity in the goblet cells lining the intestinal crypts (**Figures 3A- 3E**). There was strong reaction in the methylnitrosourea (MEN) -induced colon cancer in rats colon indicating glandular hypertrophy and proliferation of goblet cells (**Figure 3F).** Sections of methylnitrosourea (MEN) -induced colon cancer group treated with resveratrol-treated and 5-Fluorouracil showed lower reaction and decrease in the number of goblet cells stained with alcian blue, indicating a decrease goblet cell proliferation in the colonic mucosa.

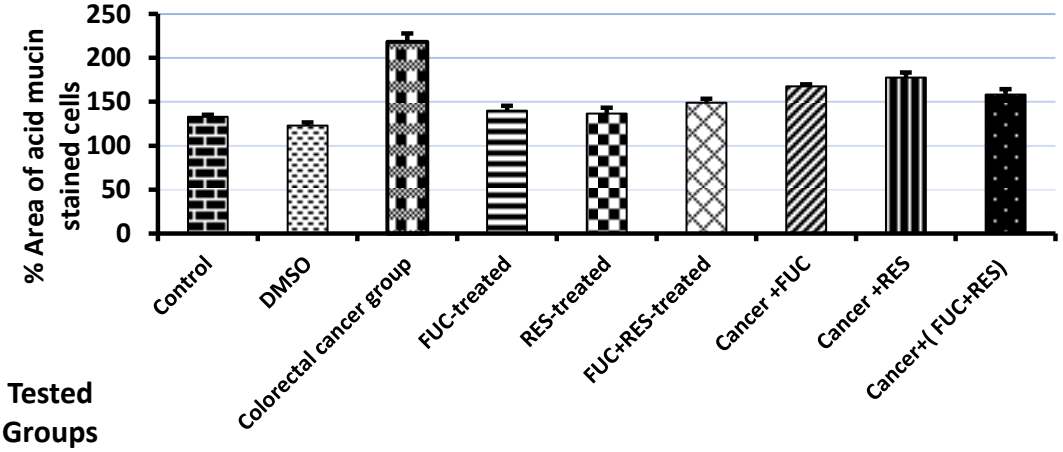

Fig 2: effect of resveratrol, 5-fluorouracil and their combination on acid mucin using alcian blue and periodic acid-Schiff's stain in experimentally induced colorectal cancer rats. Values are means ± SE. n=5, One Way ANOVA followed by Duncan multiple comparison tests. a $p<0.05$ compared to normal control group, b $p<0.05$ compared to N-methylnitrosourea induced colorectal cancer group.

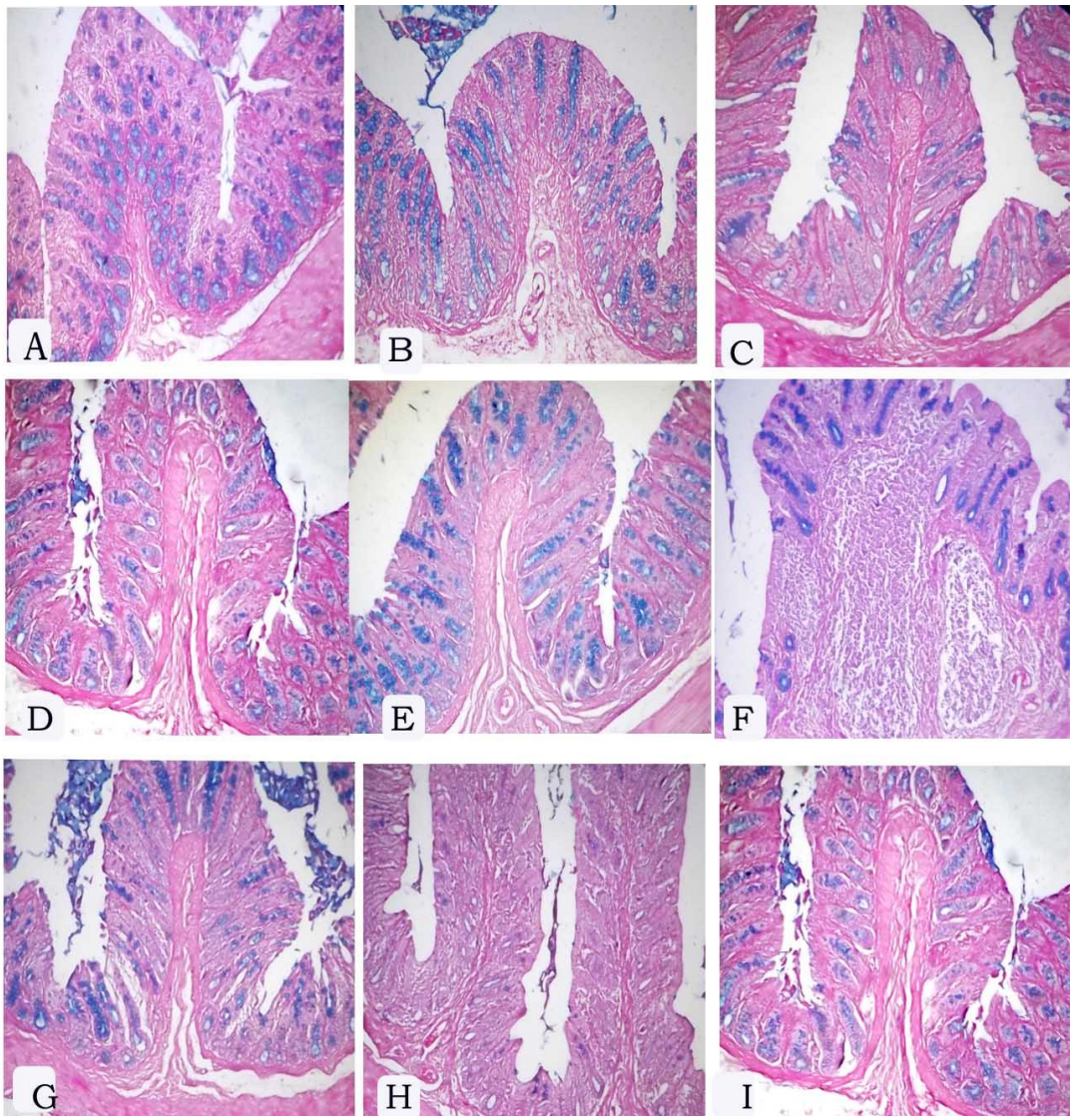

Fig 3: representive photo of alcian blue and periodic acid-Schiff's stain colon sections (A) control group. (B) Dmso treate d group, (C) 5fluorouracil treated group (D) Resveratrol treated group, (E) 5fluorouracil+ Resveratrol treated group (F) methylnitrosourea-induced colon cancer group (G) methylnitrosourea induced colon cancer rat treated with 5-Fluorouracil (H) methylnitrosourea induced colon cancer rat treated with resveratrol (I) methylnitrosourea induced colon cancer rat treated with 5-Fluorouracil in combination with resveratrol (Magnification X200) .

Effects of resveratrol, 5-fluorouracil and their combination on inflammatory-Related Gene Expression of COX1 and COX2 using Immunohistochemical staining

Immunohistochemical examination of COX1 and COX2 in the colon sections of the control group showed a very weak diffused cytoplasmic immunostaining. In colon cancer rats, examination showed strong positive cytoplasmic COX1 and COX2 immunostaining in the epithelial cells lining the crypts. As shown in **figure 3,** expression of COX1 and COX-2 were reduced in colon tissues treated with Resveratrol, 5-fluorouracil or their combination. They significantly modulated the expression of COX1 and COX-2 genes. Our findings indicated that combination Resveratrol and 5-fluorouracil may help prevent cancer in the early stages by increasing anti-inflammatory activities.

COX-1 expression in colorectal tissue

COX-1-positive cells showed brownish yellow granules in the cytoplasm. The positive rate of COX-2 expression was 1.34% in normal control, 1.49%, normal animals treated 5- Fu and 1.70% in RES treated group. In N-methylnitrosourea induced colorectal cancer group. The positive rate of COX-2 expression was 32.23%. In colon tissues treated with Resveratrol, 5-fluorouracil and their combination, the positive expression rate was 7.56%, 18.99% and 5.18% respectively.

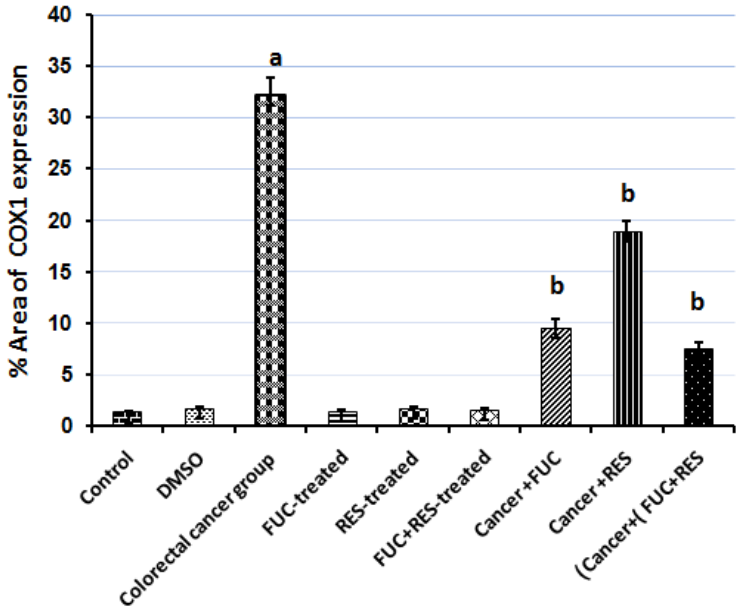

Fig 4: effect of resveratrol, 5-fluorouracil and their combination on COX1 expression in experimentally induced colorectal cancer rats. Values are means ± SE. n=5, One Way ANOVA followed by Duncan multiple comparison tests. a p<0.05 compared with normal control group, b p<0.05 compared with N-methylnitrosourea induced colorectal cancer group.

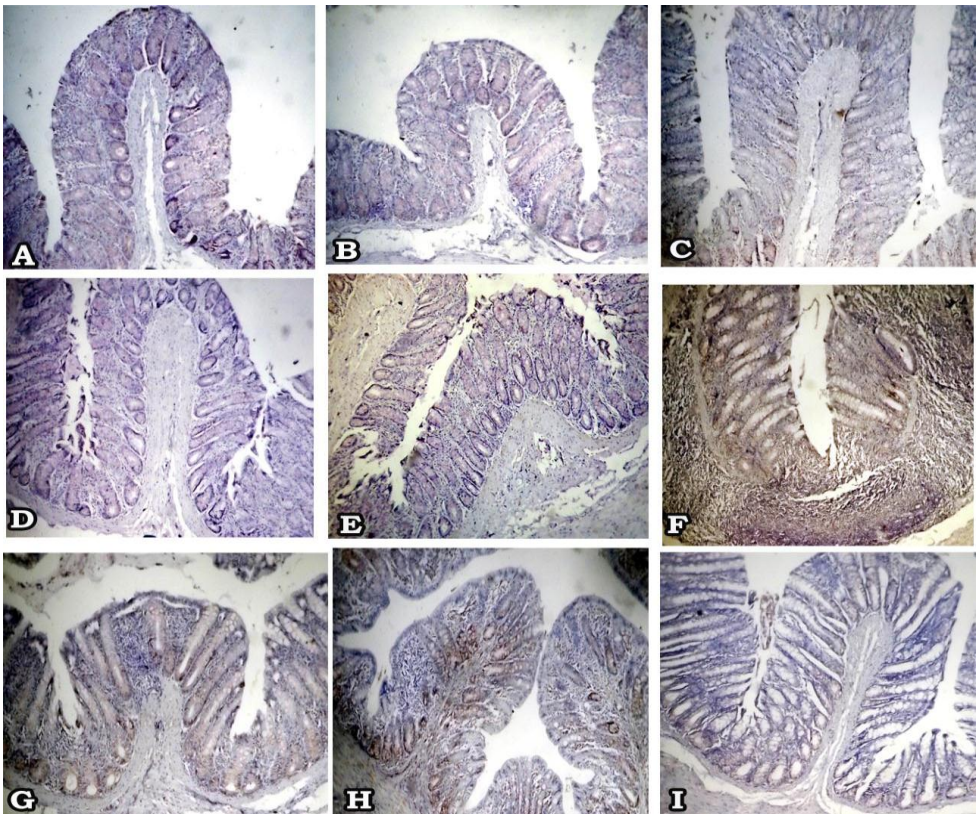

Fig 5: representive photo of COX1 expression using immunohistochemical staining in colon sections (A) control group. (B) Dmso treated group, (C) 5fluorouracil treated group (D) Resveratrol treated group, (E) 5fluorouracil+ Resveratrol treated group (F) methylnitrosourea-induced colon cancer group (G) methylnitrosourea induced colon cancer rat treated with 5-Fluorouracil (H) methylnitrosourea induced colon cancer rat treated with resveratrol (I) methylnitrosourea induced colon cancer rat treated with 5-Fluorouracil in combination with resveratrol (X 200) .

COX-2-positive cells showed brownish yellow granules in the cytoplasm. The positive rate of COX-2 expression was 0.72% in normal control and normal animals treated with either 5- Fu or RES or both of them revealed 2.42%, 1.40 and 1.77%, respectively. In N-methylnitrosourea induced colorectal cancer group. The positive rate of COX-2 expression was 27.16%. In colon tissues treated with Resveratrol, 5-fluorouracil and their combination, the positive expression rate was 6.36%, 9.08% and 0.72%, respectively.

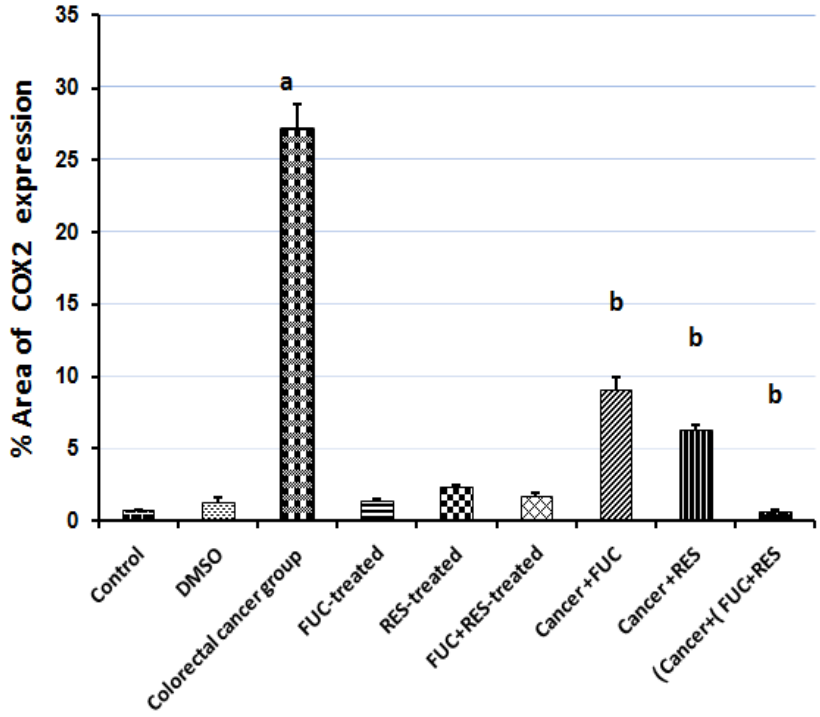

Fig 6: effect of resveratrol, 5-fluorouracil and their combination on acid COX2 expression in experimentally induced colorectal cancer rats. Values are means ± SE. n=5, One Way ANOVA followed by Duncan multiple comparison tests. a p<0.05 compared with normal control group, b p<0.05 compared with N-methylnitrosourea induced colorectal cancer group.

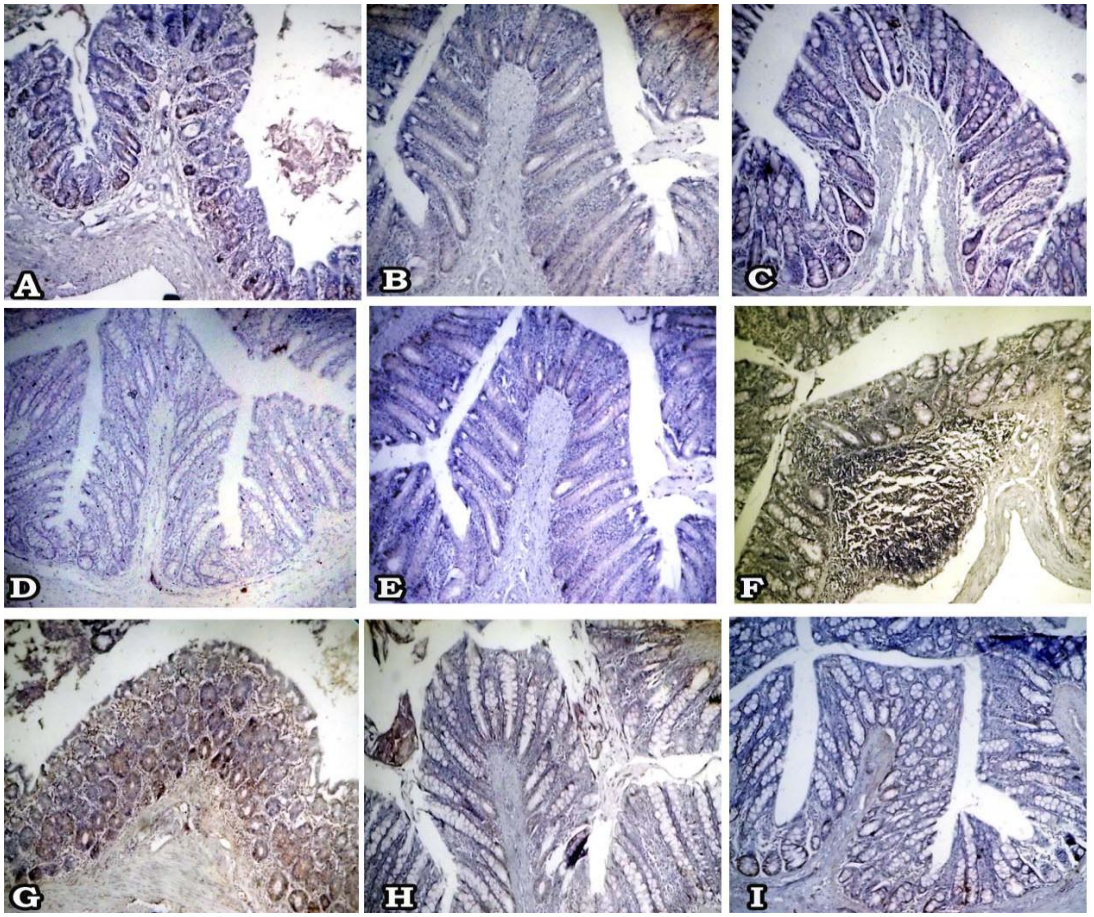

Fig 7: reprehensive photo of COX2 expression using immunohistochemical staining in colon sections (A) control group. (B) Dmso treated group, (C) 5-fluorouracil treated group (D) Resveratrol treated group, (E) 5fluorouracil+ Resveratrol treated group (F) methylnitrosourea-induced colon cancer group (G) methylnitrosourea induced colon cancer rat treated with 5-Fluorouracil (H) methylnitrosourea induced colon cancer rat treated with resveratrol (I) methylnitrosourea induced colon cancer rat treated with 5-Fluorouracil in combination with resveratrol (X 200).

DISCUSSION

Colorectal cancer is considered to be the third most widespread cause of deaths both in men and women [(29)] .Currently, treatment of colorectal cancer involves the combination of surgery with chemotherapy, by administration of cytotoxic drugs and radiation. 5-fluorouracil (5-Fu), an antimetabolite, is one of the most usually used cytotoxic drugs in colorectal treatment. Unluckily, resistance to 5- Fu may emerge during treatment due to several biological mechanisms, namely over expression of thymidylate synthase and alterations in the apoptotic pathway. One of the strategies to overcome drug resistance is the combination with other drugs and/or natural compounds [(30)].

Plants are used as an essential component of conventional medicine [(31)]**.** Still today medicinal plants remain significantly as natural alternatives to synthetic drugs with about 80% of the world population depending upon plants for their primary health care [(32,33)]**.** The use of traditional medicine and medicinal plants in most developing countries for the maintenance of good health, has been widely observed [(34)]**.** Resveratrol or 3, 5, 4' tri-hydroxystilbene is a secondary metabolite produced in limited plant species. *Veratrum grandiflorum* has been reported to synthesize resveratrol and analogues. Several plant species are known to produce resveratrol, in significant to high amounts. Some of them are used as food, i.e. vine plant, peanuts, berries in the vine plant, *Vitis vinifera* [(35)]**.** In the present study, the anticancer effects of resveratrol alone or combined with FUC were assessed on induced colorectal cancer in rat resorting to anti-proliferative, apoptotic, and genotoxic assays. Regarding to histopathological changes in colon tissue, in the present study the N-methylnitrosourea induced colorectal cancer animals exhibited extravasated blood and hemorrhage was observed between the crypts. The mucosa showed inflammatory cellular infiltrations consisting of mononuclear cells and leukocytes,

mainly lymphocyte. The cells lining the crypts showed darkly stained pyknotic nuclei. This could be due to immunological processes and ROS, as had been previously reported by **Osama *et al.*** who stated that immunological processes and ROS, such as peroxide anion, hydrogen peroxide, and hypochloric acid, contribute considerably to the development of tissue injury[36]**.** Oxidative stress and its consequent lipid peroxidation is able to aggravate free radical chain reactions, disrupt the integrity of the intestinal mucosal barrier and activate inflammatory mediators, resulting in tissue damage, as shown in both human and experimental animal studies.

In the present work treatment N-methylnitrosourea induced colorectal cancer animals with 5-Fu and or RES resulting in diminished histopathological changes in colon tissue. The effect of RES combined with 5-Fu displayed more significant reduction for histological tissue alteration. These results are in agreement with results of **Carneiro-Filho *et al.*** who stated that 5-Fu is a kind of chemotherapeutic agent, which is used for colon cancer, esophageal cancer, stomach cancer and pancreatic cancer[37]**.**

In an attempt to explain the effective therapeutic effect of RES **Brooks and Gu** declared that plant-derived resveratrol targets many intracellular molecules in mammalian cells and activates signaling molecules and enzymes in the cell[38]**.** Resveratrol has been shown to slow down and stop various cancer cell lines from dividing indefinitely in vitro [39]**.** Resveratrol binds to various cell-signaling molecules and modulates cell-cycle regulatory genes. It activates transcription factors, inhibits protein kinases and the expression of antiapoptotic genes, as well as angiogenic and metastatic gene products and inflammatory biomarkers, induces antioxidant enzymes, and alters the expression of enzymes such as cytochrome P450s that are involved in drug metabolism [40]**.** Resveratrol increases the activity of endothelial nitric oxide synthase enzyme which synthesizes vasodilator molecule nitric oxide [41]**.**

Mucin is a high molecular weight glycoprotein that is synthesized, stored and secreted by the epithelial mucosal cells, especially the goblet cells [42]**.**Mucin's key characteristic is its ability to form gels; therefore they are a key component in most gel-like secretions, serving functions such as lubrication, cell signaling and forming chemical barriers [43]**.** Their general structure and biochemical composition provides protection for the cell surface and specific molecular structures regulate the local microenvironment near the cell surface. In addition, mucins also communicate the information of the external environment to the epithelial cells via cellular signalling through membrane-anchored mucins [44]**.** It seems that mucins play a role in the processes of tumour progression, invasion and metastasis and also in tumour cell survival and protection against the host immune response [45]**.**Increased mucin production occurs in many adenocarcinoma, including cancers of pancreas, lung, breast, ovary, colon and other tissues [46]**.** According to the WHO definition, at least 50 per cent of the microscopically evaluated area in these tumours must be filled with mucus [47]**.**

Oral administration of RES for a period of five weeks significantly reduced the inflammatory cell proliferation in the methylnitrosourea induced colon cancer rats. This gave an indication of possible effects on inhibition of COX1and COX2 pathway. Most authors who have reported on inflammatory control potential of resveratrol have linked it with its ability to recruit Prostaglandin. Prostaglandin H synthase (PHS) is the primary enzyme responsible for the biosynthesis of prostaglandins and thromboxanes. Resveratrol is a competitive inhibitor of cyclooxygenase and peroxidase activity of PHS in human erythroleukemia cells [48,49]**.**As far as PHS is concerned, both cyclooxygenase and peroxidase activities depend on ferriprotoporphyrin IX [50]**.**The cyclooxygenase inhibition by resveratrol prevents the release of cyclooxygenase products such as prostaglandins and thromboxanes [51]**.**

There are two isoforms of cyclooxygenase (COX) that catalyze the formation of prostaglandins (PGs) from arachidonic acid. COX-1 is a housekeeping gene that is expressed constitutively [52]**.** COX-2 is an immediate, early response gene that is highly inducible by mitogenic and inflammatory stimuli [53]**.**Considerable evidence has accumulated to suggest that COX-2 is important for tumorigenesis. For example, COX-2 is up-regulated in transformed cells [54] and various forms of cancer [55]**.**In the current study N-methylnitrosourea induced colorectal cancer animals exhibited up regulation of COX1 and COX2 gene expression in colon tissue. There are several possible mechanisms that could account for the link between COX-2 and cancer. Enhanced synthesis of PGs, which occurs in a variety of tumors [56]**,** can favor the growth of malignant cells by increasing cell proliferation [57]**,** promoting angiogenesis and inhibiting immune surveillance[58]**.** Overexpression of COX-2 inhibits apoptosis and increases the invasiveness of malignant cells [58]**.**

The results of the present study clearly showed that resveratrol inhibits COX-2 enzyme activity. COX-2 deficiency also protected against the formation of extraintestinal tumors. Thus, COX-2 knockout mice developed approximately 75% fewer chemically induced skin papillomas than the control mice [(59)]. A selective inhibitor of COX-2 caused nearly complete suppression of azoxymethane-induced colon cancer [(60)]. Also **Goel *et al.*** stated that anti-inflammatory compounds modulate the inflammatory response by down-regulating the activity of cyclooxygenase-2 (COX-2) [(61)]. Resveratrol is a phytoalexin found in grapes and other foods that has anti-cancer and anti-inflammatory effects [(62)]. It inhibits the development of preneoplastic lesions in carcinogen-treated mouse mammary glands, for example, and it blocks tumorigenesis in a two-stage model of skin cancer that was promoted by treatment with phorbol ester [(63)]. The anti-inflammatory properties of resveratrol were demonstrated by suppression of carrageenan-induced pedal edema [(64)], an effect attributed to suppression of PG synthesis via direct, selective inhibition of COX-1. In combination, these studies suggest that targeted inhibition of COX-2 is a promising approach to prevent cancer. Therefore, chemopreventive strategies have focused on inhibitors of COX enzyme activity. An equally important strategy may be to identify compounds that suppress the expression of COX-2 [(63)].

According to the present results, there was a significant decrease of the COX2 expression of colon cancer induced animals after the administration of RES and 5- Fu in combination in comparison with cancer animals. This may be an indication of enhancing action of RES of 5-FU and eliminating its toxicity as compared to experimentally induced cancer animals treated with 5-FU alone.

In conclusion, the results of this study indicated that RES had a better therapeutic effect against N-methylnitrosourea induced colorectal cancer than 5-Fu alone and when in combination with each other diminish the cytotoxic effect of 5-Fu and enhance normal histological appearance of colon tissue, which could be a promising alternative for resistant colorectal cancer. However, the exact mechanisms involved needs to be further explored. Our results suggested also that natural compounds could be in the future a possible alternative to enhance the efficiency of 5-Fu in resistant colon cancer cells. This study supports the potential of plant extracts as source of bioactive compounds with biomedical applications.

REFERENCES

1-Hanahan D D and Weinberg R A (2000): The hallmarks of cancer. Cell, 100(1), 57-70.

2- John H , Ash P, Kieran M , Sarah F (2011): Clinical guideline: colorectal cancer: the diagnosis and management of colorectal cancer .
https://www.nice.org.uk/guidance/cg131/documents/colorectal-cancer-full-guideline2

3. Subudhi M B , Jain A, Jain *et al*. (2015): Eudragit S100 coated citrus pectin nanoparticles for colon targeting of 5-fluorouracil . Materials , 8: 832-849.

4-**Corpet D E and Pierre F (2005) :**How good are rodent models of carcinogenesis in predicting efficacy in humans? A systematic review and meta-analysis of colon chemoprevention in rats, mice and men. European Journal of Cancer, 41(13):1911–1922.

5-Papalois E and Paidas C N (2003): Experimental colon cancer .Annals Of Gastroenterology, 16(3):261-264.

6-Grem JL (2000): 5-fluorouracil: forty-plus and still ticking. A review of its preclinical and clinical development. Invest. New Drugs,18:299–313**.**

7- Maria T, Panagiotis A and Ioannis P (2015): Efficacy of 5-FU or oxaliplatin monotherapy over combination therapy in colorectal cancer. J. Cancer Ther., 6: 345-355.

8- Lee J S, Jung Y J and Kim Y M (2001): Synthesis and evaluation of N-Acyl-2-(5-fluorouracil-1-yl)- D,L-glycine as a colon-specific prodrug of 5-fluorouracil. J. Pharm. Sci., 90:1787-1794.

9- Seve M, Chimienti F, Devergnas S, Aouffen M, Douki T, Chantegrel J, Cadet J and Favier A (2005): Resveratrol enhances UVA-induced DNA damage in HaCa T human keratinocytes. Med.Chem. 1:629–633.

10-Lin CJ et al. (2012): Resveratrol enhances the therapeutic effect of temozolomide against malignant glioma in vitro and in vivo by inhibiting autophagy. Free Radic. Biol.Med.,52:377–391.

11- Bill-Cai T, Tang X, Nagorski J, Brauschweiger P G and Wang P G (**2003):** Synthesis and cytotoxicity of 5-fluorouracil/diazeniumdiolate conjugates. Bioorg. Med. Chem., 11:4971–4975.

12-Marx J L (**1986) :** Drug resistance of cancer cells probed. Science, 234: 818–820.

13-Vang O, Ahmad N, Baile CA, Baur J A, Brown K, Csiszar A *et al.* (2011): What is new for an old molecule? Systematic review and recommendations on the use of resveratrol . PLoS One, 6: 19881-19889.

14- Singh N, Agrawal M and Dore S (2013): Neuroprotective properties and mechanisms of resveratrol in in vitro and in vivo experimental cerebral stroke models, ACS. Chem. Neurosci.,4:1151–1162.

15- Aggarwal B B *et al.* (**2004**): Role of resveratrol in prevention and therapy of cancer: preclinical and clinical studies. Anticancer Res., 24: 2783–2840 .

16-Li H *et al.* (2010): 3,3',4,5,5'-pentahydroxy-trans-stilbene, a resveratrol derivative, induces apoptosis in colorectal carcinoma cells via oxidative stress. Eur. J.

Pharmacol.,637:55–61.

17- Narisawa T, Wong CQ, Maronpot RR, Weisburger JH (1976): Large bowel carcinogenesis in mice and rats by several intrarectal doses of methylnitrosourea and negative effect of nitrite plus methylurea. Cancer Research, 36:505–510.

18- Asao T, Takayuki A, Shibata HR, Batist G, Brodt P (1992): Eradic of hepatic metastases of carcinoma H-59 combination chemoimmunotherapy with liposomal muramyl tripeptide, 5- fluorouracil, and leucovorin.Cancer Research, 52: 6254–6257.

19- Tessitore L, Davit A, Sarotto I & Caderni G (2000) : Resveratrol depresses the growth of colorectal aberrant crypt foci by affecting bax and p21(CIP) expression. Carcinogenesis. **,**21: 1619–1622.

20- Drury R A B and Wallington E A (1980): Carleton's Histological Technique. 4th Edn., Oxdford University Press, Oxford, New York.

21-Banchroft J D, Stevens A and Turner D R (1996): Theory and Practice of Histological Techniques. 4th Edn., Churchill, Livingston, New York, London, San Francisco, Tokyo.

22-Neurath MF, Fuss I, Kelsall BL, Stüber E, Strober W(1995): Antibodies to interleukin 12 abrogate established experimental colitis in mice. J Exp Med., 182:1281–1290.

23- Jin Y, Kotakadi VS, Ying L *et al.* (2008): American ginseng suppresses inflammation and DNA damage associated with mouse colitis. Carcinogenesis, 29:2351–9.

24- Kotakadi VS, Jin Y, Hofseth AB *et al.* (2008): Ginkgo biloba extract EGb 761 has anti-inflammatory properties and ameliorates colitis in mice by driving effector T cell apoptosis. Carcinogenesis, 29:1799–806.

25- Erben U, Loddenkemper C, Katja Doerfel, Simone Pieckermann, Dirk Haller, Markus M Heimesaat, Martin Zeitz, Britta Siegmund, Anja A Kühl (2014): A guide to histomorphological evaluation of intestinal inflammation in mouse models. Int J Clin Exp Pathol.,7(8):4557-4576.

26- Harkema JR, Plopper CG, Hyde DM, St George JA (1987). Regional differences in quantities of histochemically detectable mucosubstances in nasal, paranasal, and nasopharyngeal epithelium of the bonnet monkey. J Histochem Cytochem .,**35: 279–286.**

27- Livraghi A, Grubb B R, Hudson E J, Wilkinson K J, Sheehan J K, Mall M A, and Randell S H (2009): Airway and lung pathology due to mucosal surface dehydration in β-epithelial Na+ channel-overexpressing mice: role of TNF-α and IL-4Rα signaling, influence of neonatal development, and limited efficacy of glucocorticoid treatment. The Journal of Immunology, **182(7): 4357-4367.**

28- Field A P (2000): Discovering statistics using SPSS for windows: advanced techniques for the beginner. *Sage publications*, London.

29- Haggar F A, Boushey R P(2009): Colorectal Cancer Epidemiology: Incidence, Mortality, Survival, and Risk Factors. Clinics in Colon and Rectal Surgery, 6(212): 191–197.

30- Duci S B (2015): Justification of the topical use of pharmacological agents on reduce of tendon adhesion after surgical repair. SM Journal of Orthopedics, 1(2): 2–4

31- Fang X, Shao L, Zhang H, Wang S (2005): A comprehensive herbal medicine information system for cancer. Journal of Medicinal Chemistry, 48 (5): 1481–1488.

32- Akerele O (1993): Summary of WHO guidelines for the assessment of herbal medicine. Herbalgram, 28: 13–19.

33- Xutian S, Zhang J, Louise W (2009) : New exploration and understanding of traditional Chinese medicine. Am J Chin Med., 37:411–26.

34- Tiwari A K and Madhusudanarao J (2002): Diabetes mellitus and multiple therapeutic approaches of phytochemicals: present status and future prospects, Curr. Sci., 83: 30–38.

35- Gambini J, Inglés M, Olaso G, Lopez-Grueso R, Bonet-Costa V, Gimeno-Mallench L, Borras C (2015): Properties of Resveratrol: In Vitro and In Vivo Studies about Metabolism, Bioavailability, and Biological Effects in Animal Models and Humans. Oxidative Medicine and Cellular Longevity, **doi:** 10.1155/2015/837042

36- Osama MA, Alaa A, Tarek MA, Shimaa AR, Ayman MM (2012): Ameliorative effects of sildenafil in acetic acid-induced chronic colitis in rats. Life Sci J., 9:354.

37- Carneiro-Filho BA, Oria RB, Wood Rea K, Brito GA, Fujii J, Obrig T, Lima AA and Guerrant RL (2004): Alanyl-glutamine hastens morphologic recovery from 5-fluorouracil-induced mucositis in mice. Nutrition,20: 934-941.

38- Brooks CL, Gu W (2009): How does SIRT1 affect metabolism, senescence and cancer? *Nat Rev Cancer*, 9:123-128.

39-Anand P, Kunnumakara A, Sundaram C, Harikumar K, Tharakan S T, Lai O, Sung B and Aggarwal B B (2008): Cancer is a preventable disease that requires major lifestyle changes. Pharmaceutical Research, 25(9): 2097–2116.

40-Baur J A, Sinclair D A (2006): Therapeutic potential of resveratrol: the in vivo evidence. *Nat Rev Drug Discov*., 5:493-506.

41- Nicholson S K, Tucker G A, Brameld J M (2008): Effects of dietary polyphenols on gene expression in human vascular endothelial cells. *Proc Nutr Soc.*, 67:42-47.

42- Kim YS, Gum JR Jr, Byrd JC, Toribara NW (1991): The structure of human intestinal apomucins. Am Rev Respir Dis., 144: S10-14.

43-Marin F, Luquet G, Marie B, Medakovic D (2008): Molluscan shell proteins: primary structure, origin, and evolution. Curr Top Dev Biol., 80: 209-276.

44- Hollingsworth MA, Swanson BJ (2004): Mucins in cancer: protection and control of the cell surface. Nat Rev Cancer, 4: 45-60.

45- Komatsu M, Yee L, Carraway KL (1999) : Overexpression of sialomucin complex, a rat homologue of MUC4, inhibits tumor killing by lymphokine-activated killer cells. Cancer Res., 59: 2229-2236.

46- Singh AP, Moniaux N, Chauhan SC, Meza JL, Batra SK (2004): Inhibition of MUC4 expression suppresses pancreatic tumor cell growth and metastasis. Cancer Res .,64: 622-630.

47- Jass JR, Sobin LH (1990): Histological Typing of Intestinal Tumours, (2nd edn). Berlin: Springer-Verlag.

48-MacCarrone M, Lorenzon T, Guerrieri P and Agro A F (1999): Resveratrol prevents apoptosis in K562 cells by inhibiting lipoxygenase and cyclooxygenase activity. Eur. J. Biochem., 265(1), 27-34.

49- Knight J , Taylor G W, Wright P, Clare A S and Rowley A F. (1999): Eicosanoid biosynthesis in an advanced deuterostomate invertebrate , the sea squirt (Ciona intestinalis) .Biochim. Biophys. Acta., 1436(3), 467-478.

50- Eling T E , Thompson D C , Foureman G L , Curtis J F and Hughes M F (1990): Prostaglandin H synthase and xenobiotic oxidation. Annu. Rev. Pharmacol. Toxicol., 30:1-45.

51- Schneider C and Pozzi A (2011): Cyclooxygenases and lipoxygenases in cancer. Cancer Metastasis Reviews, 30(0), 277–294.

52- Smith W L, Garavito R M, and DeWitt D L (1996): Prostaglandin endoperoxide H synthases (cyclooxygenases)-1 and -2. J. Biol. Chem., 271: 33157–331602.

53-Kujubu D A, Fletcher B S , Varnum B C, Lim R W, and Herschman H R (1991): a phorbol ester tumor promoter-inducible mRNA from Swiss 3T3 cells, encodes a novel prostaglandin synthase/cyclooxygenase homologue. J. Biol. Chem., 266: 12866–12872.

54- Subbaramaiah K, Telang N, Ramonetti J T, Araki R, DeVito B , Weksler B B, and Dannenberg A J (1996): Transcription of cyclooxygenase-2 is enhanced in transformed mammary epithelial cells. Cancer Res., 56: 4424–442.

55-Kargman S L, O'Neil G P, Vickers P J, Evans J F, Mancini J A, and Jothy S (1995): Expression of prostaglandin G/H synthase-1 and -2 protein in human colon cancer, . 55: 2556–2559.

56- Lupulescu A. (1996): Prostaglandins Leukotrienes Essent. Fatty Acids, 54:83–94.

57- Sheng H, Shao J, Morrow J D, Beauchamp R D, and DuBois R N (1998): Modulation of apoptosis and Bcl-2 expression by prostaglandin E2 in human colon cancer cells. Cancer Res., 58: 362–366.

58- Gately S and Li WW (2004): Multiple roles of COX-2 in tumor angiogenesis: a target for antiangiogenic therapy. Semin Oncol., 2 S (7):2-11.

59- Tiano H, Chulada P, Spalding J, Lee C, Loftin C, Mahler J, Morham S, and Langenbach R (1997): Effects of cyclooxygenase deficiency on inflammation and papilloma development in mouse skin. Proc. Am. Assn. Cancer Res., 38: 1727.

60- Kawamori T, Rao C V, Seibert K, and Reddy B S (1998) : Chemopreventive activity of celecoxib, a specific cyclooxygenase-2 inhibitor, against colon carcinogenesis. Cancer Res., 58: 409–412.

61- Goel A, Kunnumakkara AB, Aggarwal BB (2008): Curcumin as "curcumin": from kitchen to clinic. Biochem Pharmacol., 75:787-809.

62-Chang X, Heene E, Qiao F, Nick P (2011): The Phytoalexin Resveratrol Regulates the Initiation of Hypersensitive Cell Death in Vitis Cell. Plos one, 6(10):e26405.

63-Hasan MM, Cha M, Bajpai V K , Hyun K (2013): Baek Production of a major stilbene phytoalexin, resveratrol in peanut (Arachis hypogaea) and peanut products: a mini review. Reviews in Environmental Science and Bio/Technology.,12 (3): 209–222.

64-Jang M, Cai L, Udeani G O *et al.*(1997) : Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. Science, 275: 218–220.