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Progression of 1,2-Dimethylhydrazine-Induced Colonic Pathological Lesions In Albino Rats: An Experimental Model

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Abstract

COLORECTAL CANCER (CRC) is a widespread malignant neoplasm kill millions of people worldwide. 1,2-dimethylhydrazine (DMH) is a potent carcinogen used for induction of a CRC model mirroring the pathological features of various stages of colorectal carcinogenesis in human sporadic CRC. However, various steps involving CRC by DMH are poorly defined. Therefore, this study aimed to characterize the multistep progression of colonic lesions and associated immunohistochemical markers during DMH-induced CRC development. Fifty rats were allocated into 2 groups: 25 rats in the control group and 25 rats in the DMH group. Each rat in the DMH group was administered DMH (40 mg/kg body weight I/P) once weekly for 12 weeks, and 5 rats were euthanized from both groups at 8, 12, 16, 20, and 24 weeks from the first DMH treatment. Hyperplasia, low grade dysplasia and high grade dysplasia were detected in DMH exposed group at 8,12 and 16 weeks post exposure respectively. Advanced and invasive carcinoma were also detected at 20 and 24 weeks post exposure. In addition, immunohistochemical analysis revealed a decrease in caspase-3 expression and an increase in β-catenin expression in the colonic sections. In conclusion, the macroscopic mucosal elevations could be detected after 16 weeks of DMH exposure. Additionally, the microscopic preneoplastic dysplastic lesions started at 12 weeks while the typical neoplastic changes could be noticed at 20 weeks post exposure. Subsequently, knowing the time dependent changes and the associated molecular targets could be a key approach to treat CRC.

Keywords: CRC, 1,2 dimethylhydrazine (DMH), caspase 3 and β-catenin.

Introduction

Colorectal cancer (CRC) is categorized as the third most predominant cancer overall and the second biggest reason of global mortality, accounting for around 0.9 million deaths in 2020 [1-3]. The spread and advancement of colorectal cancer are thought to be significantly influenced by several predisposing risk factors including dietary and lifestyle habits. Among these habits are high fats, carbohydrates and meats consumption along with alcoholic drinks, overweight, obesity and low vegetables, fruits, dietary fibers consumption beside lack of physical exercise. Additionally bowel inflammatory lesions as ulcerative colitis, Crohn's disease together with microbial exposure, altered composition of gut microbiota and family history [1, 2, 4, 5].

It has been reported that the pathological features of various stages of colorectal carcinogenesis in human sporadic CRC could be reproduced through administration of 1,2-dimethylhydrazine (DMH) [6, 7]. Metabolic activation of the procarcinogens, DMH and its derivative azoxymethane (AOM), leads to formation of DNA-reactive products. DMH and AOM converts to the final reactive carcinogen methylazoxymethanol, or MAM [8] after xenobiotic-metabolizing enzymes action and oxidation hydroxylation stages. Then, MAM produces methyldiazonium ions that undergo liver macromolecules alkylation then carried into the colon with the bile flow, where they cause oxidative stress and DNA alkylation that results in gene mutation and carcinogenic metabolites [7, 9].

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During the carcinogenic process, cancer cells can escape apoptosis via evading the cytotoxic action resulting from oncogene activation and downregulation or complete loss of the caspase‑3 expression [10, 11]. Caspase‑3 belongs to cysteine proteases division, and is an essential member of apoptosis cascade after caspase‑8 or caspase‑9 initiators activation [12, 13]. Therefore, many anticancer treatments, such as immunotherapy, radiation, and cytotoxic medications, can kill tumor cells by activating caspase-3 [14]. The Wnt/β-catenin pathway is considered a crucial pathway in the development of colorectal cancer [15]. Wnt ligands, also known as Wnts, are a group of 19 synthesized glycoproteins that can activate the canonical or noncanonical Wnt pathway. The β-catenin protein is considered the central molecule of the canonical Wnt pathway and an important component of the cytoskeleton [16, 17]. The β-catenin protein remains at its basal level when Wnt ligands are not present, thanks to ubiquitin-dependent proteasome degradation [18]. The β-catenin destruction complex, including Adenomatous Polyposis Coli (APC), Casein kinase 1 (CK1), Axin ,and Glycogen Synthase Kinase (GSK3β). When the Wnt ligand is not present, β-catenin is phosphorylated by GSK3β, trapped inside the destruction complex, and marked for proteasome breakdown. Meanwhile, Wnt causes β-catenin to break, detach from the complex and move into the nucleus, where it combines with T-cell factor and lymphoid enhancer factor 1 to control the transcription of several oncogenes. It was reported that β-catenin gene mutations are closely linked to the initiation and evolution of CRC [17, 19] as they recorded in up to 77% of colon tumors found in rats administered DMH [20]. Similarly, mutations in β-catenin destruction complex particularly, GSK3β and APC genes were recorded in 33% of colon tumors in rats given DMH [21]. Additionally, cytoplasmic and nuclear expression of β-catenin is observed to be elevated in all dysplastic epithelial lesions but not in hyperplastic lesions [8, 22]. Furthermore, it was demonstrated that Wnt prevents the activation of caspase-9 brought on by chemotherapy medications, which in turn decreases the apoptosis of colon cancer cell lines (CCC) [23]. As a result, a number of chemotherapeutic medications have been prepared to target this pathway in many hematological and solid tumors [24].

The current study aimed to examine the multistep morphological alterations in the colon and to describe the immunohistochemical expression of caspase-3 during this sequence. Additionally, the upstream factor of Wnt signaling β-catenin nuclear translocation as a possible specific molecular target during CRC development in rats induced by DMH was analyzed.

Material and Methods

Animals

Fifty adult male albino rats, between 120 and 130 gram and five to six weeks old, were purchased from the Medical Experimental Research Center (MERC) animal house in Faculty of Medicine, Mansoura University, and were kept one week for acclimatization before the start of the experiment in the Faculty of Veterinary Medicine animal room, Mansoura University, Egypt. Rats were maintained under controlled standard temperature (22 ± 2 ◦C) and relative humidity condition (50± 5%) with 12-h light-dark cycle. clean balanced pellet diet is provided with fresh tap water *ad libitum*.

Chemicals

1,2 *N*,*N*-Dimethylhydrazine (DMH) was obtained from Sigma-Aldrich Co. (Spruce St. Saint Louis, MO, United States, D161802). Anti caspase-3 rabbit pAb (dilution 1:1000, GB11532, Servicebio, Wuhan, China) and anti- β-catenin rabbit pAb (GB11015, dilution 1:2000, Servicebio, Wuhan, China) were used for immunohistochemical analysis.

Colorectal cancer induction

DMH was freshly dissolved in 1 mM/L EDTA before use, the pH was brought to 7.0 by NaOH diluted solution, and given in a dosage of 40 mg/kg body weight, intraperitoneal (I/P) once weekly for 12 weeks [26-28].

Animals grouping and Experimental design

Fifty adult healthy accommodated albino rats were divided into 2 groups: 25 rats in the control group and 25 rats in the DMH group, as follows:

The control group: All rats received a balanced pellet ration and clean tap water without any medication. Five rats were randomly selected and euthanized after 8, 12, 16, 20, and 24 weeks.

The DMH group: Each animal was administered

DMH (40 mg/kg body weight I/P) once every week for 12 consecutive weeks. Five rats were randomly selected and euthanized after 8, 12, 16, 20, and 24 weeks following the first DMH shot.

Histopathological examination

All animals from all experimental groups underwent mild ether anesthesia, and the colons were removed after dissection, cleaned in ice-cold isotonic saline and examined for the presence of any gross abnormalities then fixed immediately for 24 h in 10% neutral buffered formalin for histopathological examination. Paraffin blocks were prepared and 3-5 µm thick sections were cut. Hematoxylin and eosin were used for staining the sections after deparaffinization process then examined by light microscope (Optika,Italy, B-290TB) with adjusted camera [29, 30].

Immunohistochemical (IHC) examination of caspase 3 and β-catenin

Colon sections were deparaffinized using xylene, rehydrated in ethanol descending grades, and incubated in a 3% H_2O_2 solution for ten minutes to inhibit colon endogenous peroxidase. Next, slides were embedded in phosphate-buffered saline (PBS) for washing, heated for five minutes in a citrate buffer solution (pH = 6) inside an 800-watt power microwave, incubated in 5% donkey serum for 20 minutes to avoid nonspecific staining of the background, and dipped once in PBS**.** Subsequently, primary antibodies against caspase-3 and β-catenin were incubated with the slides overnight in a humid chamber, washed 3 times in PBS. After that, the slides flooded with five drops of secondary antibodies and left together for 10 min in room temperature, then dipped thrice in PBS. Next, the sections were dipped in streptavidin peroxidase solution at room temperature for 10 min, followed by a PBS wash. A drop of 3–3′-diamino-benzidine-tetra-hydrochloride (DAB) chromogen was mixed with DAB substrate and used to cover the section for 15 min. Finally, Mayer's hematoxylin was used for sections counterstaining and the slides were dehydrated in ethanol ascending series, then mounted on glass slides using di-n-butyl-phthalate-polystyrene-xylene (DPX) [31, 32].

Pictures of immunostained colonic sections for caspase-3 and *β-catenin* were picked up at magnification X:100 and X:400 then were imported to the ImageJ software (National Institutes of Health, Bethesda, MD, USA; https://imagej.nih.gov/ij/) and analyzed morphometrically. Expression% of caspase-3 and B catenin were statistically analyzed by One way ANOVA followed by tukey's test.

Statistical analysis

The obtained data were quantitatively analyzed by one way ANOVA followed by Tukey's adjustment test to compare all means using the GraphPad Prism software, *p*-value <0.05 was considered statically significant.

Results

Gross findings

Macroscopic pictures of the rat colon from the control group showed normal appearance of the exposed mucosa. Meanwhile rats' colons of DMH-administered group revealed remarkable progression of lesions after 16 weeks (Fig. 1).

Histopathological findings

Microscopic pictures of H&E-stained colon sections from the control and DMH groups were shown in Fig. 2. Colon sections of control group revealed normal histological appearance of all colonic layers without any abnormalities. Also, mucosal acini were normal, abundant and lined by numerous goblet cells. On the other hand, colon sections of DMH group showed progression of the lesions from epithelial crypt hyperplasia accompanied with numerous inflammatory cells in the lamina propria at 8 weeks post exposure. The hyperplastic or non-dysplastic crypts showed hypercellularity, lined by slender, normal and uniform epithelium with enlarged and crowded nuclei without stratification. Additionally, the goblet cells appeared normal with small, basally oriented nuclei and apical mucus localization. At 12 weeks post exposure, low grade colonic dysplasia were observed, it represented by thickened mucosal layer, dilatation, and elongation of the crypts with epithelial proliferation forming adenoma. Meanwhile, high grade dysplasia was noticed at 16 weeks post exposure, represented by intraluminal polypoid growth and atypical tubular hyperplasia. Additionally, severe dysplastic changes, crypt distortions, irregularity in shape and size, and depletion of goblet cells beside marked lymphoid hyperplasia in the submucosa were also detected. After 20 weeks post exposure, advanced carcinoma was identified by invasion of the colonic glands beyond the muscularis mucosa into the submucosa. After that, invasive carcinoma became more aggressive, the malignant acini destructed the muscular coat and became numerous after 24 weeks, along with many free malignant cells can be seen in the stroma.

Immunohistochemical findings

Immunohistochemical data of caspase-3 expression in the colon sections was demonstrated in Fig. 3. The analysis showed mild caspase-3 positive reaction in the mucosal acini of the control group and markedly increased in mucosal acini in the group received DMH after 8 weeks, then gradually decreased with progression of lesions to be moderate after 12 weeks, mild after 16 weeks and 20 weeks, and weak after 24 weeks. Moreover, quantitative analysis of caspase-3 expression % significantly increased after 8 weeks of DMH administration, then gradually decreased to reach control levels 24 weeks after DMH administration, as shown in Fig. 4.

Immunohistochemical data of β-catenin expression in the colon sections revealed a positive β-catenin reaction in mucosal acini in the control group and in the DMH group after 8 weeks, in hyperplastic lesion and low grade dysplasia after 12 weeks, then started to increase in mucosal acini with progression of lesions in high grade dysplasia after 16 weeks, advanced carcinoma after 20 weeks and invasive carcinoma after 24 weeks, as shown in Fig. 5. In addition, quantitative analysis of β-catenin expression % revealed the expression percentages in the control group and in the DMH group after 8 weeks and after 12 weeks were up to 10%. Then started to increase in mucosal acini with progression of lesions in high grade dysplasia after 16 weeks to

be 10-20%, 20-30% in advanced carcinoma after 20 weeks and 40-50% in invasive carcinoma after 24 weeks as shown in Fig. 6.

Discussion

Colon cancer ranked as the third most prevalent cancer worldwide and the second most common cause of cancer-related fatalities [33].The current study aimed to examine the multistep morphological alterations and immunoexpression of caspase-3 as a key effector of apoptosis in the colon. Additionally, studied the progression of β-catenin expression and its nuclear translocation as a possible specific molecular targets during CRC development in rats induced by DMH (40 mg/kg BW) after 8, 12, 16, 20, 24 weeks.

The histological examination of intestinal tissue of DMH group showed progression of lesions from epithelial crypt hyperplasia accompanied with infiltration of inflammatory cells in the lamina propria after 8 weeks distinguished by their slightly dilated lumen and hypercellularity of slender, normal, uniform epithelium with enlarged nuclei, or by being occasionally crowded without stratification beside goblet cells appear normal with small, basally oriented nuclei and apical mucus localization, to low grade dysplasia after 12 weeks represented by thickened mucosal layer, dilatation, elongation of the crypts with epithelial proliferation forming adenoma. These findings are in line with those of [34-36] who noticed inflammatory cells infiltration in lamina propria and numerous lymphatic nodules in the mucosal and submucosal layers in addition to epithelial crypt hyperplasia. Moreover, tubular adenoma and obvious loss of goblet cell along with dilated, distorted colon crypts with moderate dysplasia in Sprague–Dawley rats induced subcutaneously by DMH 30 mg/kg once a week for 9, 11, and 13 weeks and in rats subcutaneously injected with 30 mg/kg of DMH for 12 weeks respectively.

In this study, high grade dysplasia was noticed after 16 weeks represented by polypoid growth into intestinal lumen and atypical tubular hyperplasia, severe dysplastic changes, crypt distortion, irregularity in shape and size, depletion of goblet cells beside marked lymphoid hyperplasia in the submucosa. These findings were similar to those reported by [37-39] Who documented high grade dysplasia, signs of anaplasis and hyperchromasia in the irregularly distorted mucosal acini with marked loss of goblet cells beside mucosal erosions and infiltration of inflammatory cells in rats injected with DMH 20 mg/kg for 15 weeks, 25 mg/kg once weekly for 16 weeks and in rats intraperitoneally injected with DMH 40 mg/kg twice weekly for 16 consecutive weeks.

After 20 weeks in the current study advanced carcinoma was identified by invasion of the colonic glands beyond muscularis mucosa into submucosa. After that invasive carcinoma became more aggressive, the malignant acini destructed the muscular coat and became numerous after 24 weeks along with many free malignant cells can be seen in the stroma. This results in agreement with [40] who noticed medullary carcinoma which invading the submucosal layer as sheet-like cellular growth and the tumor cells exhibited amphiphilic cytoplasm, nuclear pleomorphism with dispersed chromatin in rats subcutaneously injection with DMH 40 mg/kg and euthanized after 19 weeks, and with [9, 41, 42] who noticed mucosal ulceration along with abnormal crypts lined with neoplastic cells that deeply invaded the muscular layer forming invasive adenocarcinoma with some mucus producing malignant acini. Furthermore, endophytic tubular adenocarcinoma with widespread submucosal invasion, the nuclei of malignant cells were enlarged, hyperchromatic with diffused mitotic changes after 22 and 30 weeks of DMH administration.

Apoptosis, often recognized as programmed cell death, is involved in numerous physiological and pathological processes[43]. The removal of damaged cells is one of the many vital roles that apoptosis plays[44]. Genetically damaged cells are able to survive when apoptosis is inhibited, which leads to an imbalance in normal tissue homeostasis and promotes cell growth, tumor development and progression [45].

Findings from the current work showed high expression of caspase-3 in the mucosal acini exhibited hyperplastic lesion in group received DMH after 8 weeks then decreased with progression of the lesions from dysplastic to advanced and invasive carcinoma after 12, 16, 20 and 24 weeks. This data is in agreement with other previous studies [46-49] who reported that carcinogenesis is accompanied by decrease in the spontaneous apoptosis. In this context some *in vitro* and *in vivo* studies concerned with colon carcinogenesis proved that there was gradual resistance of the cells to apoptosis induction. So the tumor resistance to apoptosis may be attributed to abnormal expression or function of "Inhibitor of Apoptosis" (IAP) proteins which are a group of endogenous caspase blockers consists of eight members including XIAP, cIAP2, cIAP1, survivin, livin (ML-IAP), Bruce (apollon), NAIP, and ILP-2 [50, 51].

It is interesting to attributed the elevation in caspase-3 expression in the hyperplastic lesion to the non-autonomous caspase-induced proliferation mechanisms which identified as apoptosis-induced proliferation (AiP) or compensatory proliferation [52]. AiP plays important role in tissue regeneration and wound healing process [53-55], additionally it promotes the abnormal proliferation and growth of neoplastic cells. In fact, researchers documented that AiP has a role in carcinogenesis by promoting tumor repopulation and treatment resistance [56-59].

caspase-3 and caspase -7 activation in apoptotic cells leading to cleavage and activation of calcium-insensitive phospholipase A2 triggering prostaglandin E2 (PGE2) production which is a potent stimulator of AiP results in malignant cells proliferation and progression particularly when exposed to radiation [56, 60, 61] or chemotherapy [62, 63]. Additionally, caspase-3 dependent PGE2 production promotes anti-cancer immunity, epithelial–mesenchymal transition, and chemotherapeutic resistance [59].

β-catenin immune-staining in the colon tissues showed normal positive brown reaction in mucosal acini in control group and in DMH group after 8 weeks in hyperplastic lesion, low grade dysplasia after 12 weeks then the expression increased with progression of the lesion after 16 weeks in high grade dysplasia, advanced and invasive carcinoma after 20 and 24 weeks. This data in agreement with [8, 22, 25, 64] Who mentioned that the cytoplasmic and nuclear β-catenin colon immunoexpression in the DMH rat model was elevated in all dysplastic colorectal epithelial lesions as dysplastic dark and flat aberrant crypt foci, mucin depeleted foci together with adenomas, and adenocarcinomas), but not noticed in hyperplastic lesions. The variation in the expression of β-catenin might be linked to any alteration in Wnt related proteins, earlier regulators directing the Wnt cell signaling pathway in addition to APC or β-catenin genes mutations [65].

It is interesting to mentioned that the regulation of β-catenin interaction with T cell factor by the Wnt pathway is implicated in the regulation of apoptosis [66]. As stimulation of Wnt pathway signals inhibits the onco-protein β-catenin phosphorylation and degradation leading to its nuclear translocation where it binds with T cell factor and lymphoid enhancer factor which in turn altering pro-apoptotic and anti-apoptotic genes expression [67].

Conclusion

In conclusion, we have described the multistep pathological alterations and the time dependant changes induced by DMH in rats. These alterations started with hyperplasia, then progressed to low- and high-grade dysplasia until advanced and invasive colon carcinoma. In addition, we found the expression of caspase-3 to decrease and that of β-catenin to increase with progression of the lesions. Taken together, developing therapies targeting apoptosis and modulating the Wnt/β-catenin pathway, which inhibits tumor cell multiplication and progression, could represent a key approach to treat colorectal tumor.

Acknowledgment

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Conflicts of interest

There is no conflict of interest to state.

Funding statement

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Ethical approval

This experiment was accomplished after agreement of the Institutional Animal Care and Use Ethical Committee of Faculty of Veterinary Medicine, Mansoura University, Egypt.

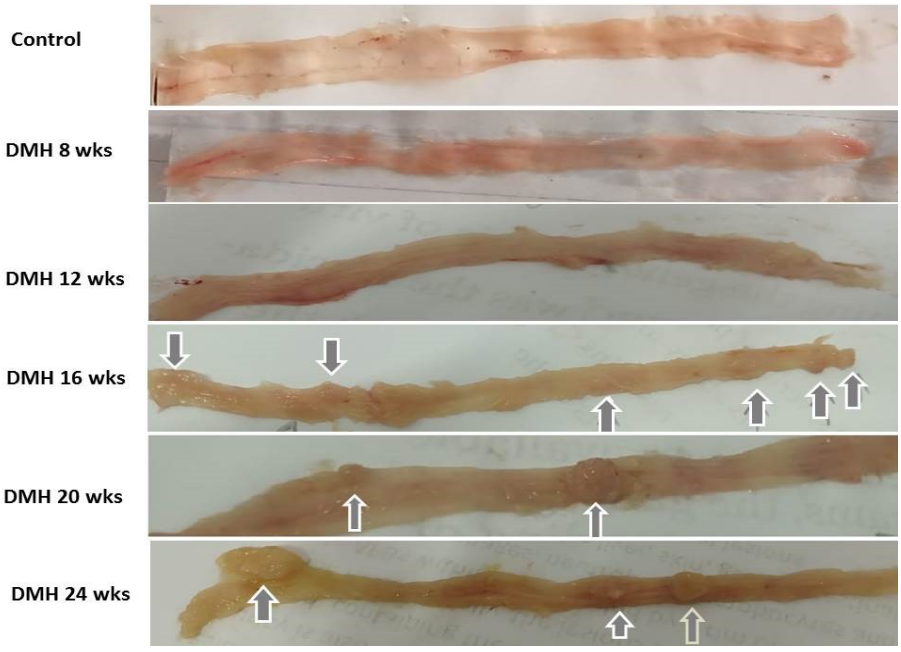

Fig. 1. Macroscopic pictures of rats' colon from the control group and the DMH-received group showing progression of lesions after 16 weeks in the DMH group (thick arrows)

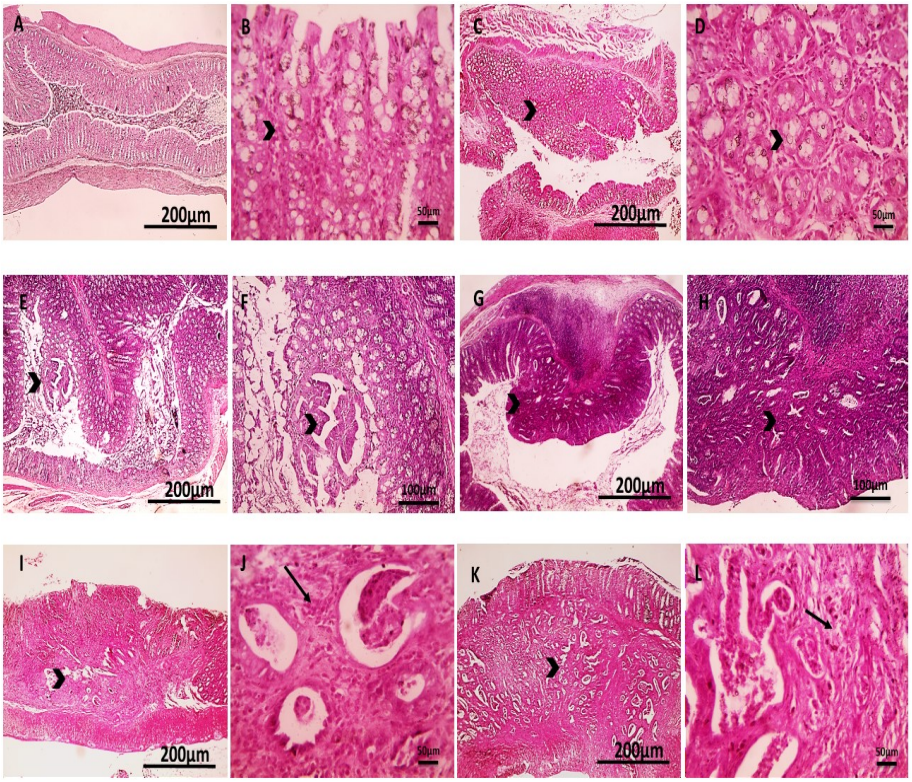

Fig. 2. Microscopic pictures of H&E-stained colon sections from the control group (A&B) and the group received DMH showing progression of lesions: hyperplasia (arrowheads) after 8 weeks (C&D), low grade dysplasia (arrowheads) after 12 weeks (E&F), high grade dysplasia (arrowheads) after 16 weeks (G&H), advanced carcinoma (arrowheads) (acini break down muscularis mucosa and invade submucosa) after 20 weeks (I&J), and invasive carcinoma (arrowhead) after 24 weeks (K&L). Note the tumorous acini breaking down the muscular coat and the several free tumor cells infiltrating the stroma (arrows) after 24 weeks. Magnification: X: 40, bar=200 µm; X: 100, bar=100 µm; and X: 400, bar=50 µm.

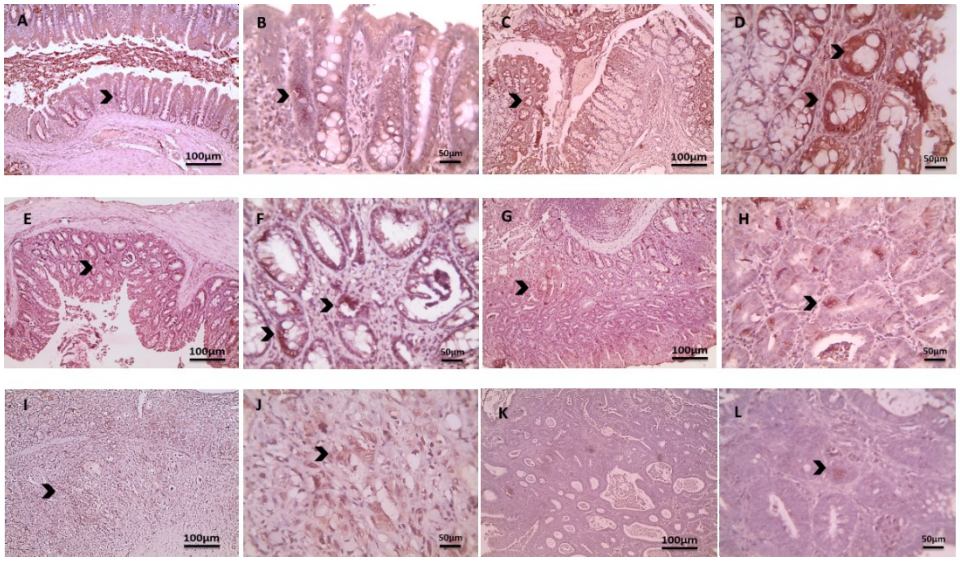

Fig. 3. Microscopic pictures of immunostained colon sections against caspase-3 showing mild caspase-3 positive reaction in mucosal acini (as indicated by arrowheads) in the control group (A&B), markedly increased in mucosal acini in the group received DMH after 8 weeks (arrowheads) (C&D), then gradually decreased with progression of lesions to be moderate after 12 weeks in low grade dysplasia (arrowheads) (E&F), mild after 16 weeks and 20 weeks in high grade dysplasia (G&H) and in advanced carcinoma (I&J), and weak in invasive carcinoma after 24 weeks (K&L). IHC counterstained with Mayer's hematoxylin. Magnification: X: 100, bar=100 µm; and X: 400, bar=50 µm.

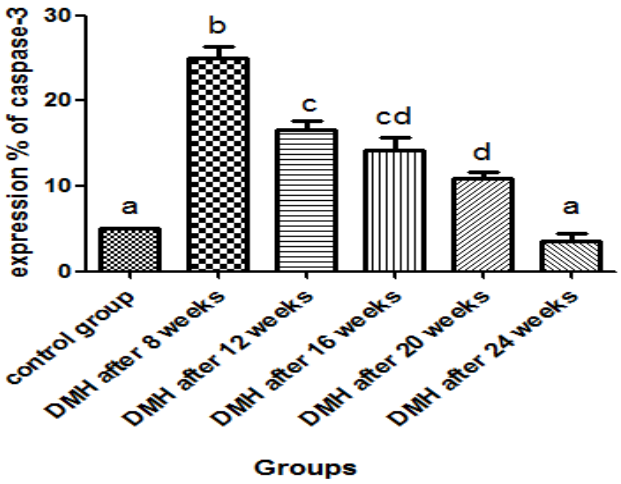

Fig. 4. Quantitative analysis of caspase-3 expression % analyzed by one way ANOVA followed by Tukey's test to compare all means. Different small alphabetical letters mean significant difference, P<0.05. Caspase-3 expression % significantly increased after 8 weeks of DMH administration, then gradually decreased to reach control levels 24 weeks after DMH administration.

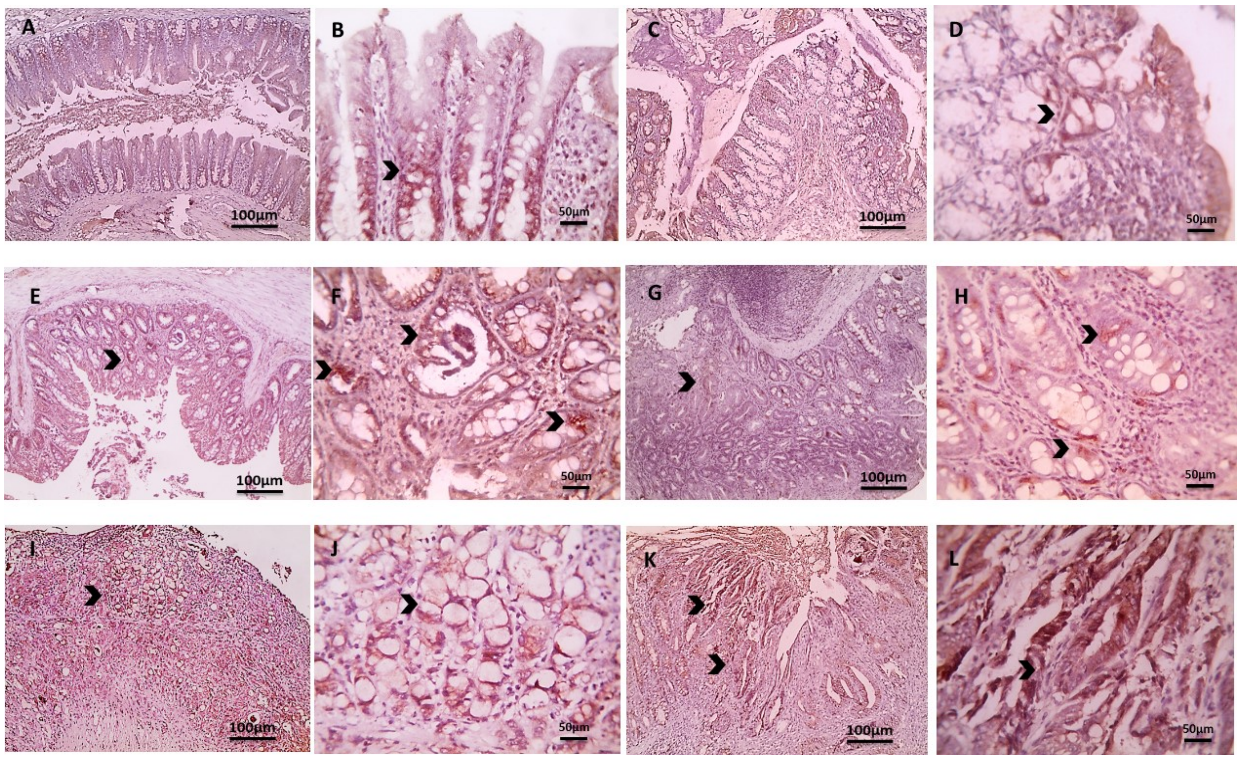

Fig. 5. Microscopic pictures of immunostained colon sections against β-catenin showing positive reaction in mucosal acini (as indicated by arrowheads) in the control group (A&B) and in the DMH group after 8 weeks (arrowheads) (C&D) and low grade dysplasia after 12 weeks (arrowheads) (E&F), then started to increase in mucosal acini with progression of lesions in high grade dysplasia after 16 weeks (G&H), advanced carcinoma after 20 weeks (I&J), and invasive carcinoma after 24 weeks (K&L). IHC counterstained with Mayer's hematoxylin: X: 100 bar 100 and X: 400 bar 50.

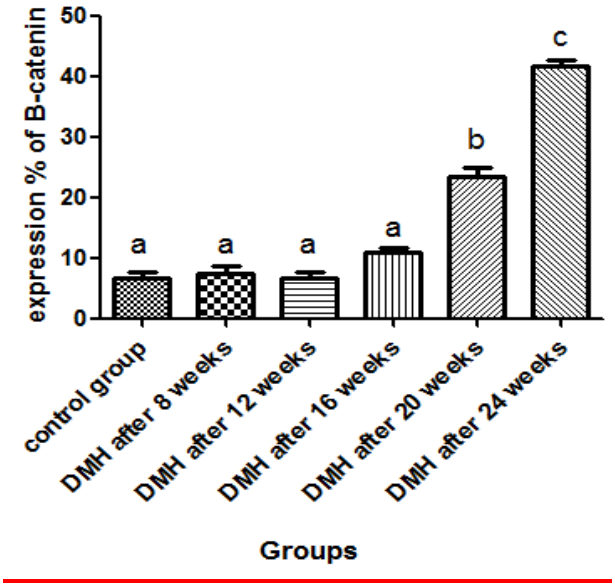

Fig. 6. Quantitative analysis of β-catenin expression % analyzed by one way ANOVA followed by Tukey's test to compare all means. Different small alphabetical letters mean significant difference P<0.05. β-catenin expression % significantly increased only after 20 and 24 weeks of DMH administration.

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تطور السمات المرضية للقولون المستحثة بمادة 1و2 ثنائى ميثيل هيدرازين فى الجرذان البيضاء

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الملخص

يصنف سرطان القولون والمستقيم على أنه ثالث أكثر أنواع السرطان انتشارًا في جميع أنحاء العالم والسبب الثاني الأكثر شيوعًا للوفيات المرتبطة بالسرطان. تشبه السمات المرضية للمراحل المختلفة من سرطان القولون والمستقيم في الانسان تلك التي لوحظت في تسرطن القولون الناجم عن2,1 ثنائي ميثيل هيدرازين. تم تصميم الدراسة الحالية لمعرفة تسلسل الصورة المرضية للقولون والتغيرات المعتمدة على الوقت في التعبير المناعي الكيميائي لكاسبيز 3 وبيتا كاتينين في الغشاء المخاطي للقولون أثناء التسرطن الناجم عن مادة ثنائي ميثيل هيدرازين. تم تخصيص خمسون فأرًا إلى مجموعتين، 25 فأرا في المجموعة الضابطة و25 فأرا في مجموعة ثنائي ميثيل هيدرازين بجرعة 40ملجم / كجم من وزن الجسم /خلال الغشاء البريتونى) مرة واحدة في الأسبوع لمدة12 أسبوعًا متتاليًا وتم القتل الرحيم لخمسة فئران من كلتا المجموعتين بعد 24,20,16,12,8 أسبوعا. ثم أظهرت نتائج الصور المجهرية للقولون المصبوغ بصبغة الهيماتوكسلين والايوسين من المجموعات الفرعية المتلقاه مادة ثنائي ميثيل هيدرازين تطور الصورة المرضية حيث زادت اعداد الخلايا بشكل ملحوظ بعد 8 اسابيع واظهرت الخلايا اختلافا فى الشكل والحجم والترتيب من منخفض الدرجة بعد 12 اسبوع الى عالى الدرجة بعد 16 اسبوع بعد ذلك استطاعت الخلايا الخبيثة غزو الطبقة تحت المخاطية بعد تكسيرالطبقة العضلية المخاطية وكونت سرطان متقدم بعد 20 اسبوع وسرطان غازى يحتوى على عدد اكبر من الحويصلات السرطانية وله قوة غزو عالية حيث استطاع الوصول الى الطبقة العضلية السفلية بعد 24 اسبوع. بالاضافة الى ذلك انخفض التعبير المناعى للكاسبيز 3 فى حين زاد التعبير المناعى للبيتا كاتينين مصاحبا لتطورالصورة المرضية. لذلك فى الختام يعتبر تطوير علاجات تستهدف موت الخلايا المبرمج والمسارالخلوى للبيتا كاتينين التي تمنع تكاثر الخلايا السرطانية وتطورها بمثابة نهج رئيسي لعلاج سرطان القولون.

الكلمات الدالة: سرطان القولون ،1 ،2 ثنائى ميثيل هيدرازين ،بيتا كاتينين ، كاسبيز 3.