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Original Research Article

Histopathology and Immunohistochemistry Evidence of Protective Effects of *Arachis hypogaea* Seed on 1, 2- Dimethylhydrazine-Induced Carcinogenesis in Male and Female Rat Colon

Abigail O. Isoje [1]*, Frederick O. Obi [2,3], Gerald I. Eze[4]

[1]*Department of Biochemistry and Molecular Biology, Faculty of Science, Dennis Osadebay University, Asaba, Delta State, Nigeria.*
[2]*Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria.*
[3]*Department of Biological and Chemical Sciences, Faculty of Natural and Applied Sciences, Michael and Cecilia Ibru University, Agbarha-Otor, Delta State, Nigeria.*
[4]*Department of Anatomy, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Edo State. Nigeria.*

ABSTRACT

The study investigated the potential of a peanut-supplemented diet to reverse 1, 2- dimethyl hydrazine (DMH)-induced carcinogenesis linked tissue morphology and biomolecular alterations in rats' colon. Fifty-six healthy Wistar rats of both sexes used for this study were divided into seven (7) groups of 4 rats each. Group A (control) rats were maintained on normal rat feeds. Groups B and C were maintained on normal rat feed and administered DMH (25 mg/kg body weight per week subcutaneously) for 12 and 24 weeks, respectively. After 12 weeks of DMH administration, a peanut-supplemented diet was provided for the remaining 12 weeks for Group D rats. Group E rats received DMH and a peanut-supplemented diet concomitantly for 24 weeks. Group F rats were on a peanut-supplemented diet for 12 weeks, followed by DMH administration for the remaining 12 weeks. Group G rats were maintained on a peanut-supplemented diet only for 24 weeks without DMH administration. At the end of the treatment period (24 weeks), the rats were sacrificed under mild anaesthesia, and portions of the colon were collected and fixed for histopathological and immunohistochemical examination. Colon histology revealed that DMH treatment without peanut supplementation caused severe histoarchitectural changes that were ameliorated by a peanut-supplemented diet. Immunohistochemistry of the colons showed that rats administered DMH only were positive for cytokeratin 20, indicating cancerous cells, and those exposed to DMH and peanut-supplemented diet were cytokeratin 20 negative. These results suggest that *Arachis hypogaea* seeds via peanut-supplemented diet protected rats' colons against 1, 2-dimethylhydrazine-induced colon carcinogenesis.

Keywords: *Arachis hypogaea*, 1, 2- dimethylhydrazine (DMH), Colon carcinogenesis, Histopathology, Cytokeratin 20

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Introduction

Colorectal carcinoma was previously considered a relatively uncommon malignancy in Africa. Unfortunately, extant and evolving data show an increased incidence rate in the last 2- 4 decades. [1-5] Colorectal cancer development involves a complex interplay of multiple pathophysiological mechanisms. These include uncontrolled cell growth, disrupted cell differentiation, evasion of programmed cell death, invasion of surrounding tissues, and the potential for distant metastasis.[6] Colon cancer is often attributed to prolonged oxidative stress and inflammation.[7-8] The procarcinogen 1, 2-dimethylhydrazine (DMH) is a well-established toxic agent that is metabolically activated to form methyl-diazonium ion, a potent carcinogen with a high specificity for colon tissue. In experimental models, DMH has been shown to induce colonic tumours reliably.[9-10]

*Corresponding author email: abigail.isoje@dou.edu.ng,

Tel: +234-8032553611

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Studies using animal models show that colorectal tumour lesions induced by DMH exhibited striking similarities to human colorectal carcinomas, sharing comparable characteristics in terms of histological features, morphological structure, and architectural arrangement of the colonic mucosa.

Furthermore, the microscopic pathology and immunobiological profiles of DMH-induced lesions closely mirrored those of human colorectal cancer.[9,11] DMH and azoxymethane (AOM) are well-characterised, indirect carcinogens that specifically target the colorectal tissue, initiating and promoting the carcinogenic process in the colon of rats, mice, and hamsters, and the tumour lesions produced are usually dose-dependent. In rats, 100% of tumour lesions have been reported in treated rats.[10] Histology is vital in clinical medicine and research, providing crucial information on biological tissue function and disease diagnostics in toxicological studies.[12] Colorectal epithelial tumours are histologically categorised as adenomas and carcinomas.[13] Adenomas are characterised by hypercellularity, distended, hyperchromatic nuclei, nuclear stratification, loss of polarity, and reduced mucin excretion. Dysplasia in adenomas is classified into mild, moderate, and severe based on nuclear stratification and abnormal nuclear morphology.[11] Carcinomas infiltrate the muscularis mucosa into the submucosa and are grouped into well, moderately, and poorly differentiated adenocarcinomas.[14]

Light microscopic examination of Hematoxylin & Eosin-stained tissue sections remains fundamental in cancer pathology diagnosis globally.[15] However, immunohistochemistry has emerged as a vital complementary investigative technique that exploits antigen-antibody interactions to identify altered cellular or tissue constituents occasioned

by cancer incidence. This technique employs direct or secondary labelling methods to visualise antibody binding sites.[16] Cytokeratin 20 (CK20) is an intermediate filament protein that helps identify certain colorectal cancers.[17] It plays a crucial role in diagnosing the origin of colorectal cancer (CRC), especially in identifying CRC origin in carcinomas of unknown primary (CUP) sources, where histopathology may be inconclusive. [18]

Diet plays a pivotal role in the aetiology of colorectal cancer.[9,19-21] Diets characterised by high red meat intake, insufficient fruit and vegetable consumption, and inadequate fibre intake have been identified as key risk factors.[22] Chemoprevention strategies leveraging dietary supplements and natural compounds have emerged as promising avenues for early-stage cancer, especially colorectal cancer prevention.[23-27] Reports of studies of foods indicate that Mediterranean foods, which are composed of fruits, vegetables, fish, and olive oils, might reduce cancer occurrence risk by 12%. This gives credence to the belief that nutritional modifications might be a feasible strategy for preventing cancer development.[28]

Arachis hypogaea (peanut) seeds are rich in dietary fibres, good fats, and bioactive compounds such as flavonoids, stilbenoids, and phenolic acids, which have potential anticancer properties.[29,30] Since colorectal cancer is aetiologically closely linked to diet,[1,9] and also histopathology and immunohistochemistry are vital and complementary tools in the diagnosis of cancer,[15,16] this study aimed at evaluating the potential chemopreventive effects of *Arachis hypogaea* seed-supplemented diet on 1,2-dimethylhydrazine (DMH)-induced precancerous ultrastructural changes in the colon of exposed male and female rats.

This study addressed the absence of information on the long-term investigation of the effects of A*rachis hypogaea* seed on DMH-induced morphological and immunohistochemical alterations in male and female rat colon, offering a comprehensive understanding of its efficacy in preventing colon cancer development. Long-term assays typically take about 20-40 weeks to complete, giving a true reflection of the efficacy of any test substance on cancer development in the colon.[11]

Materials and Methods

Chemicals

1,2-Dimethylhydrazine Dihydrochloride, DMH, was obtained from Sigma Aldrich, Germany, and halothane (Piramal Healthcare Limited, India). All of the reagents and chemicals used were of analytical grade.

Plant Material

The *Arachis hypogaea* seeds used in this study were purchased at Uselu market, GPS Location, 9JF7+Q9F, A 232, Uselu, Benin City, Edo State, Nigeria, in June 2017. For proper verification, the seed was planted. Seeds and plants were authenticated by Dr. Henry A. Akinnibosun at the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Edo State, Nigeria. The voucher sample was stored at the university herbarium with the designation UBH_A352.

Plant seeds preparation

The peanut seeds were sorted, cleaned, and air-dried for 48 hours. The raw peanuts were then blended into a fine, coarse powder using a blender and then stored in air-tight containers in the fridge at 4℃.[30] *Arachis hypogaea* seed powder was incorporated into the rat diet at a 20% level (20 g of peanut powder to 80 g of rat feed).

Preparation of 1, 2- dimethylhydrazine dihydrochloride (DMH) solution

Stock DMH was dissolved in 1 mM ethylenediaminetetraacetic acid (EDTA- disodium salt)-saline solution (adjusted to pH 6.5 with 1 M NaOH just before use) to ensure that the carcinogen was stable at the time of use.

Animal Model and Experimental Design

Fifty-six healthy (56) healthy Wistar albino rats of both sexes were used for this study. They were purchased from the Department of Biochemistry, Animal Unit, Faculty of Life Sciences, University of Benin, Benin City, Nigeria. The rats were housed in well-ventilated cages and maintained in a controlled environment with a 12-hour light-dark cycle. The rats were fed a standard diet (Bendel Feeds and Flour Mills Ltd, Ewu, Nigeria) and provided water *ad libitum* for two weeks before the experiment commenced.

Established Animal care and handling procedures and guidelines were strictly adhered to.[31] The rats were divided into seven groups, each consisting of four rats. The male and female rats were housed separately. 1,2-Dimethylhydrazine (DMH) was administered subcutaneously at a dose of 25 mg/kg body weight.[32] The experimental design consisted of seven groups:

Group A (control): Normal feed and weekly subcutaneous injections of the vehicle (EDTA-saline) were received.

Group B: Received normal feed and weekly DMH injections for 12 weeks.

Group C: Received normal feed and weekly DMH injections for 24 weeks.

Group D: Received DMH and normal feed for 12 weeks, followed by a peanut diet for 12 weeks.

Group E: Received DMH and a peanut diet concomitantly for 24 weeks.

Group F: Received a peanut diet for 12 weeks before DMH administration.

Group G: Received a 20% peanut diet throughout the 24-week experiment and weekly injections of the vehicle (EDTA-saline).

Rats were fed their respective diet (peanut-supplemented or normal rat chow) daily and given free access to water throughout the experiment. At the end of the 24-week experimental period, rats in each group were sedated with halothane. While under anaesthesia, the abdominal and thoracic regions were opened, and sections of the colon were obtained, weighed, and fixed for histological examination.

Colon Histopathology

The dissected colon sections were fixed in 10% formal saline. Fixed tissues were completely dehydrated in ascending concentrations of alcohol (70, 90, 96, and 100%). The tissues were placed in xylene to remove the alcohol, impregnated, and embedded with molten paraffin wax. They were allowed to solidify before sectioning into 4 µm using a microtome (Leica RM 2235, UK). The 4-5 µm sections were placed on microscope slides and stained with hematoxylin-eosin dye.[33] Stained tissues on the microscope slides were viewed using an optical photomicroscope (Olympus 230 V 50/60 He, Germany) and camera (Eakins 12Mega pixels, UK) at ×100 magnifications.

Preparation of Sections for Immunohistochemistry

Immunohistochemistry for the detection of CK20 was carried out using the method described by Perry *et al.*[34] Based on the histomorphology of the stained colon sections, selected paraffin blocks prefixed in 10% neutral buffered formalin and containing embedded tissue were sectioned using the thermo-scientific semi-automated rotary microtome. The freshly cut paraffin sections were allowed to dry overnight. Tissues were further dried in a hot air oven at 60 ℃ for 1 hour. The slides were passed through 3 10-minute washes of xylene to give a cumulative wash period of 30 minutes. Slides were subsequently dipped in graded alcohols ranging from 100%, 80%, down to 70%. The slides were then immersed in two changes of deionised water and left standing in deionised water for 5 minutes. Antigen retrieval using the EZ automated retrieval system in 10 mM citrate buffer pH 6.0 was carried out for 20 minutes.

Immunohistochemical staining was conducted on tissue sections with a thickness of 5 µm. To prepare the sections, xylene was used for deparaffinisation, followed by dehydration utilising a series of ethanol solutions with increasing concentrations. Endogenous peroxidase activity was blocked by treating the sections with a 3% hydrogen peroxide solution for 10 minutes. To minimise non-specific staining, the sections were treated with a protein-blocking agent for 15 minutes. Antigen retrieval was performed as necessary, and the sections were then incubated with the primary antibody, cytokeratin 20 (CK20) Type-I keratin IgG2a Monoclonal antibody, at room temperature for 30 minutes. The sections were washed again in PBS thrice and further

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incubated with a secondary antibody amplifier, Quanto, for 10 minutes at room temperature. The signals were labelled with HRP polymer Quanto for 10 minutes and detected using diaminobenzidine as chromogen. Negative controls were performed with the primary antibody replaced by PBS. Following immunohistochemical staining, the slides were counterstained with haematoxylin, then subjected to a series of dehydration steps using ethanol, cleared with xylene, and finally coverslipped. Scoring of reactions and photomicrographs of stained sections were done using the Leica DM 750 and camera (Eakins 12Mega pixels, UK) at ×100 magnifications.

Immunostaining assessment

CK20 immunoreactivity was evaluated by assessing both the staining intensity and the percentage of positively stained cells per field. The staining intensity was categorised as either positive or negative based on the percentage of stained cells. CK20 expression was considered positive when more than 10% of epithelial cells exhibited dark brown cytoplasmic staining and negative when less than 10% of epithelial cells showed cytoplasmic staining.[17]

Statistical analysis

The results obtained from the biochemical assays were expressed as mean ± standard deviation (SD). Analysis of variance (ANOVA) was done using SPSS software (Version 21.0) to compare means from different groups, while the LSD multiple range test was done to evaluate significant differences between means employing the same SPSS computer software. Statistical significance was considered as $p \leq 0.05$.

Results and Discussion

The weight of the organ (colon) was obtained from both the treated groups and the control group after the experimental period of 24 weeks. As shown in Table 1, the males showed no significant difference in the colon-to-body weight ratio compared to the control group in all the treatment groups. The insignificant changes in the organ weight-to-body ratio may be a result of the variation in the size of internal organs or body weight of the animals.[12,35]

In the females, the colon-to-body weight ratio of rats administered DMH for 12 weeks and maintained on normal chow for the experimental period (group B) increased significantly ($p \leq 0.05$) relative to the control. This increase in the colon weight may be due to mucosa thickening and the formation of neoplasia.[36] Intervention with the peanut-supplemented diet after, simultaneously with, and before DMH administration (Groups D, E, and F) respectively reduced the colon to body weight significantly ($p \leq 0.05$) relative to the group that was maintained on normal rat feed after 12 weeks exposure to DMH (group B). This may be attributed to the ability of the phytochemicals present in peanuts to restore the cellular metabolic dysfunctions occasioned by DMH.[37]

Table 1: Relative Organ Weights of Toxicological Evaluation in Male and Female Rats

Colon/Body weight ratio×10^{-3} (n= 4)			
Group Designation	**Treatment**	**Rats (Male)**	**Rats (Female)**
A	Control	5.52±0.63	6.92±0.83
B	DMH_{12}/NC*	6.39±2.11	8.83±1.81^{a**}
C	DMH_{24}/NC	6.20±1.18	7.89±1.38
D	DMH_{12}/PNT_{12}	5.98±0.74	6.53±0.63^{b}
E	$DMH_{24+}PNT_{24}$	6.43±0.65	6.44±1.30^{b}
F	PNT_{12}/DMH_{12}	4.91±1.26	7.12±1.05^{b}
G	PNT_{24}	6.43±1.34	5.74±1.12$^{b, c}$

Values are expressed as mean ± SD

*Control = Normal rat chow (NC) and water with weekly subcutaneous injection of EDTA-saline solution.

DMH_{12}/Normal chow = Maintained on normal chow (NC) while treating with 1, 2- dimethylhydrazine (DMH) for 12 weeks.

DMH_{24}/Normal chow = Maintained on normal chow but treated with 1, 2-dimethylhydrazine (DMH) for 24 weeks. DMH_{12}/PNT_{12} = Treated with DMH for 12 weeks while on normal rat chow and later maintained on peanut diet (PNT) for 12 weeks.

DMH_{24}+PNT_{24} = Treated with DMH and peanut simultaneously for 12 weeks

PNT_{12}/DMH_{12}= Maintained on peanut diet for 12 weeks, followed by DMH treatment for 12 weeks.

PNT_{24} = Maintained on peanut diet for 24 weeks with weekly subcutaneous injection of EDTA- Saline solution.

**Values with superscripts a, b, c, d, e, or f are significantly different from the value of the group with the corresponding upper case letter A, B, C, D, E, or F ($p \leq 0.05$).

Results show that colon sections from male and female control rats that were administered EDTA saline (vehicle) showed no evidence of loss of normal histoarchitecture. That is, there were no signs of apparent abnormality (Figure 1). The administration of DMH to Wistar rats of both sexes induced varying degrees of dysplasia in the colonic mucosal epithelial lining and crypts. The dysplasia ranged from mild to moderate and moderate to severe dysplasia, depending on the duration of exposure. Moderate dysplasia when administered for 12 weeks (Figure 1) and severe dysplasia (carcinoma-in-situ) when administered for 24 weeks (Figure 1). The dysplastic changes appeared to be of a comparable degree in both male and female rat colons. The histological observation in this study is in agreement with earlier reports that DMH and its derivative azoxymethane induce precancerous lesions in the colon of experimental animals.[38, 39] The result obtained from this study also agrees with previous reports by Sengottuvelan and Nalini[40] that administration of DMH to rats caused irregular disposition of the crypts, reduced inter-cryptic spaces as well as lymphoid–glandular complexes characterised by severe inflammatory cell infiltrations of stained-colon sections. Colon histology of rats also showed severe dysplasia in the epithelial lining coupled with loss of polarity; the nuclei were randomly placed, which were also observed in the DMH-alone treated group in this study (Figure 1). Dysplasia was used to describe structural and cytological alterations in the epithelium that predispose an organ to cancer development.[41] Jucá *et al.*[42] also reported that DMH exposure led to varying degrees of dysplasia in the stained colon sections of rats. Dysplastic crypts have been observed and identified as early lesions that ultimately lead to colon cancer.[40]

Interventions using peanuts diet via different modalities (curative, ameliorative, and protective) achieved varying degrees of success (Figure 2), especially when administered curatively for 12 weeks following the administration of DMH (for 12 weeks), colon histoarchitecture was restored to a fairly mild degree (Figure 2). Histological examination of colon tissues of rats administered peanut diet and DMH simultaneously for 24 weeks revealed fairly normal colon histoarchitecture (Figure 2). The observation of normal colon histoarchitecture where peanut and DMH were provided simultaneously to the rat must have been due to the presence of phytochemicals in the peanuts. Their presence must have prevented the commencement and sustained progress of the promotion stage of

carcinogenesis.[30, 40] Ascribing the integrity of colon histoarchitecture to the phytochemicals present in peanut is plausible because the colon of rats exposed to DMH-only-treated groups showed severe dysplastic crypts and carcinoma *in situ* (Figure 1) that were absent in the group treated simultaneously with peanut diet and DMH (Figure 2). Consumption of a supplemented peanuts diet before DMH administration (Figure 2) achieved the best result, with the mucosal lining epithelial and crypt cells assuming almost the same level of differentiation as that of the control group in either sex of rats. Although the precise mechanism by which peanuts inhibited the development of precancerous lesions in the large intestine is not completely known, it could, however, be due to the rich phytochemicals that have been reported to be potent in inhibiting the onset of cancer development.[30, 38] The cellular metabolism of a chemical carcinogen is crucial in the initiation process of carcinogenesis.[43] Veceric and Cerar[32] reported that DMH is activated in the liver through a chain of reactions involving the intermediates azomethane (AOM) and methylazoxymethanol (MAM) to the final carcinogenic metabolite, methyldiazonium ion, which is highly reactive. Polyphenols, which are abundant in peanut seeds, could hinder the formation of procarcinogen molecules *in vivo* and also enhance isozymes of $P450_s$, which in turn modulate phase II biotransformation enzymes, scavenge electrophilic molecules, and enhance repair *in vivo.* [24,43-44] From the histological observation, supplementing the rat diets with peanuts before DMH administration restored the colon to near-normal architecture in males and total restoration to normal colon histoarchitecture in the female rats (Figure 2). This could be due to the polyphenolic contents of peanuts ability to inhibit the carcinogen-activating enzymes, thus resulting in a decrease in the conversion of the procarcinogen (1, 2-dimethylhydrazine) to the ultimate carcinogen (methyl-diazonium ion), thereby inhibiting the initiation process.[4] Hence, polyphenol-mediated enzyme activity is likely to decrease the reactivity and carcinogenicity of the ultimate carcinogen.[45] Hormonal factors may be responsible for the better outcome of the peanut-supplemented diet in female rat colon.[9] Studies have shown the protective role of estrogen, a sex hormone predominantly found in females, against colon cancer.[46-48] Certain plants contain phytoestrogen compounds,[48] which, although they are implicated as risk factors for some neoplasms, notably breast and endometrium cancers, appear to play a protective role in the development of colorectal cancer.[49] The effect of estrogen is mediated by estrogen receptors (ERs), namely ERα and ERβ, of which the colon majorly expresses ERβ.[47] The Erβ is closely associated with the anti-inflammatory effect of estrogen. The estrogen receptor (Erβ) has been reported to suppress COX-2 and inflammatory enzymes, thereby exerting a protective role against colorectal cancer.[49] The rats that received a peanut diet only for 24 weeks showed normal colonic histoarchitecture in both the stained and female colon sections (Figure 2). However, there was a heavy infiltration of lymphocytes in the lamina propria in the male stained-colon section. The presence of these lymphocytes in the absence of inflammation can be linked to their crucial role in monitoring and maintaining healthy regeneration of colon mucosal tissues[50]

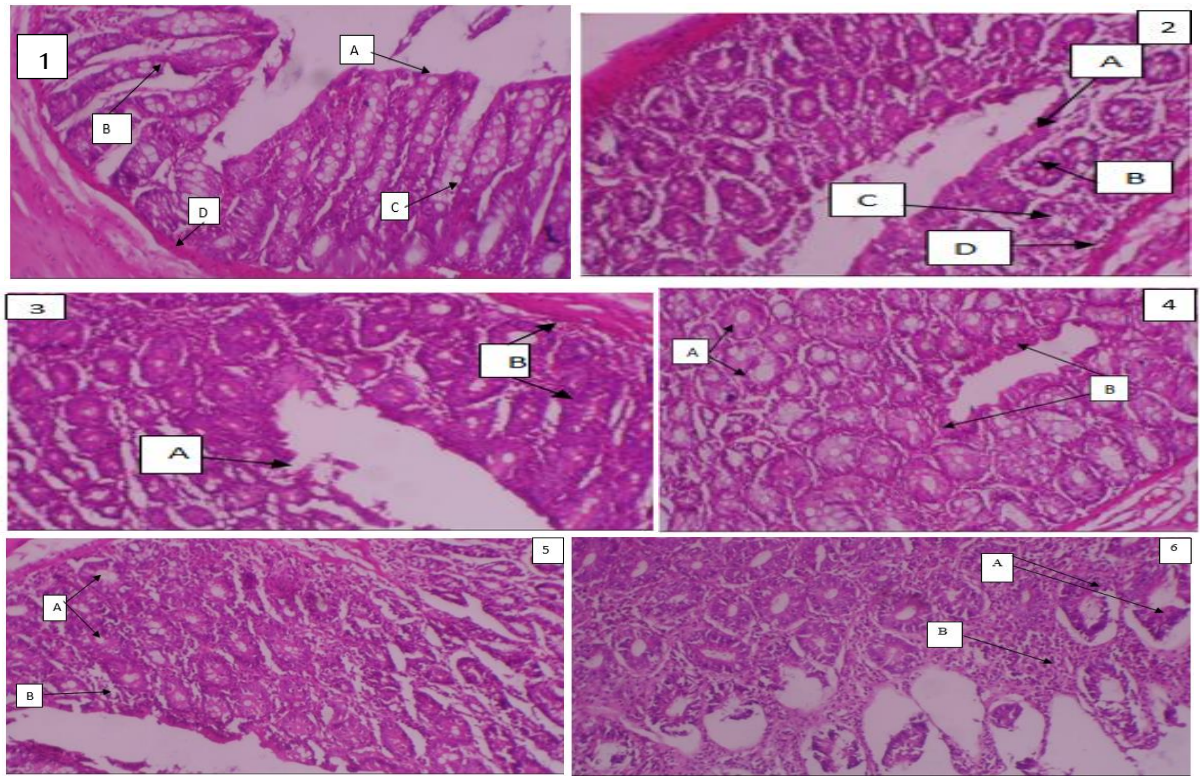

Figure 1: Effect of DMH on male and female rat colon (H&E x100)

Photomicrograph of colon section of control male rat showing Columnar epithelium (A), crypt lined by goblet cells (B), goblet cells (C), and muscularis mucosa (D)
Photomicrograph of colon section of control female rat: Columnar epithelium (A), crypt lined by goblet cells (B), goblet cells (C), and muscularis mucosa (D)
Photomicrograph of colon section from male rat treated with DMH for 12 weeks followed by maintenance on normal diet for 12 weeks: Moderate epithelial lining dysplasia (A), moderate crypt dysplasia (B)
Photomicrograph of colon section from female rat treated with DMH for 12 weeks followed by maintenance on normal diet for12 weeks: Moderate epithelial lining dysplasia (A), moderate crypt dysplasia (B), Moderate crypt dysplasia (A), Moderate epithelial lining dysplasia (B)
Photomicrograph of colon section from male rat treated with DMH for 24 weeks: Moderate epithelial lining dysplasia (A), moderate crypt dysplasia (B), severe crypt cell dysplasia (carcinoma *in-situ*) (A) and heavy lamina propia lymphocyte mobilisation (B)
Photomicrograph of colon section from female rat treated with DMH for 24 weeks: severe crypt cell dysplasia (carcinoma *in situ*) (A) and heavy lamina propia lymphocyte mobilisation (B)

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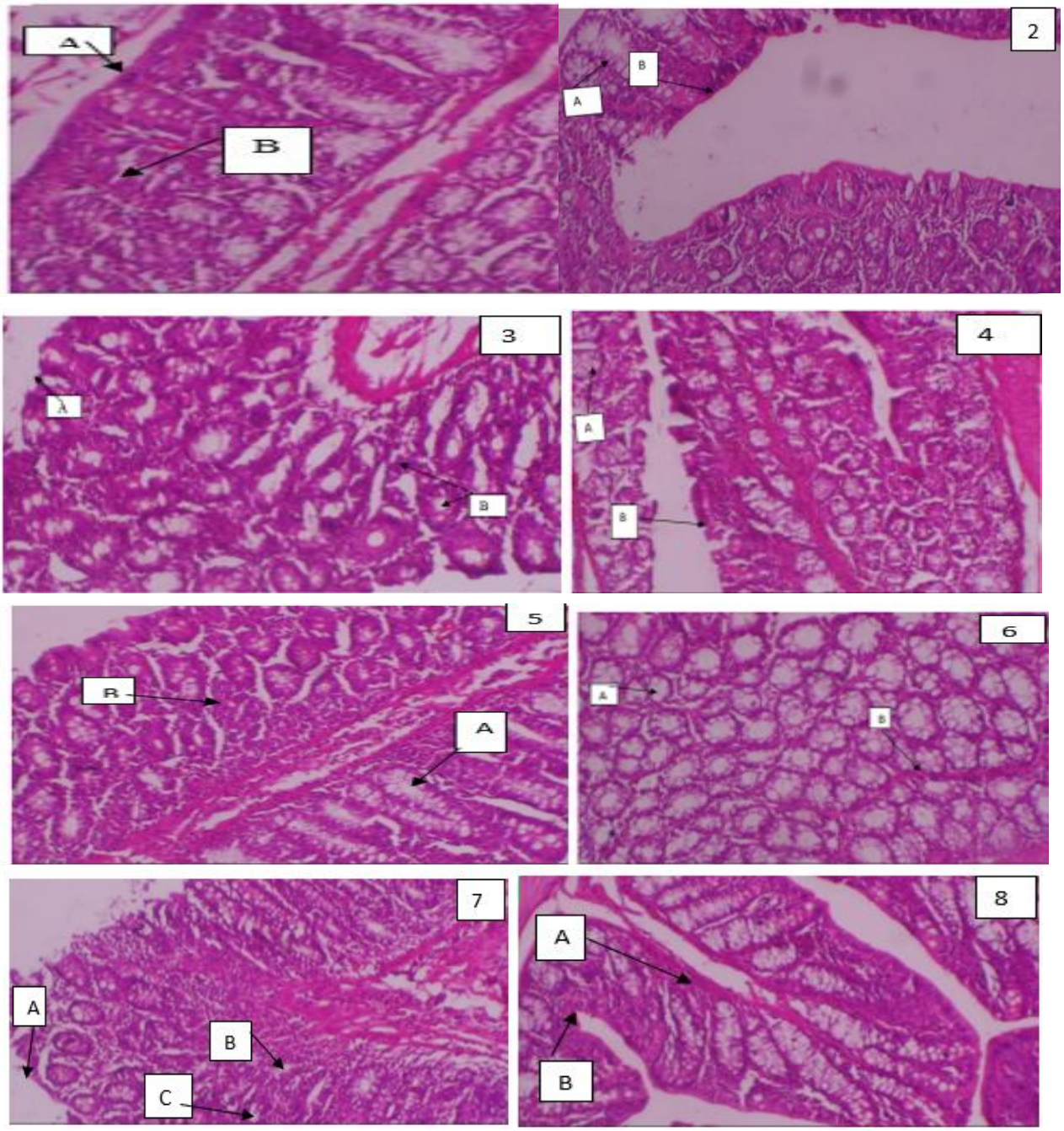

Figure 2: Effect of peanut diet on DMH-induced carcinogenesis in rat colon. (H&E x 100)

Photomicrograph of section of colon from male rat treated with DMH for 12 weeks followed by peanut diet for 12 weeks: mild epithelial dysplasia(A) and mild crypt cell dysplasia (B)
Photomicrograph of section of colon from female rat treated with DMH for 12 weeks followed by peanut diet for12 weeks: mild crypt cell dysplasia(A) and epithelial dysplasia (B)
Photomicrograph of section of colon from male rat treated with DMH and maintained on peanut diet simultaneously for 24 weeks: Mild epithelial lining dysplasia(A) and mild crypt cell dysplasia (B)
Photomicrograph of section of colon from female rat treated with DMH and maintained on peanut diet simultaneously for 24 weeks: Mild crypt cell dysplasia (A) and Mild epithelial lining dysplasia(B)
Photomicrograph of section of colon from male rat maintained on peanut diet for 12 weeks followed by DMH treatment for 12 weeks: Normal crypts (A) and mild lamina propria lymphocyte mobilisation (B)
Photomicrograph of section of the colon from female rat maintained on peanut diet for 12 weeks followed by DMH treatment for 12 weeks: Normal crypts (A) and mild lamina propria lymphocytic mobilisation (B).
Photomicrograph of colon section from male rat maintained on peanut diet for24 weeks: Normal epithelial lining (A), normal crypts (B), and heavy lamina propia lymphocyte mobilisation (C)
Group G (Female)- Peanut diet for 24 weeks: Normal crypts (A) and normal epithelial lining(B)

Figure 3 shows the results of the immunohistochemical staining (CK20), which reveals that the group administered DMH only for 12 weeks (group B) but maintained on normal rat chow was negative for cytokeratin 20 (CK 20) in the male rats, but positive for female rat

stained-colon sections. Stained colon sections of the group that received DMH only and kept on a normal diet for 24 weeks (group C) were positive for CK 20 in both male and female rat-stained colon sections. Simultaneous consumption of the peanut diet with DMH administration for 24 weeks (group E) was negative for CK20 staining in both male and female rat colon sections. The simple epithelial intermediate filament keratin 20 (cytokeratin 20) is characteristically present in the non-neoplastic epithelium of the colon and rectum and is thus expressed in cancers originating from these sites.[18,51] CK20 is a tumour marker that is employed in the diagnosis and monitoring of the treatment of colorectal cancer.[52] Reports from research findings have shown that CK20 is expressed in 95-100% of colon carcinoma.[53-54] CK20 could thus provide a suitable marker for localisation and therapy checks in colorectal carcinoma, and its level reflects the success of surgery, radiotherapy, and chemotherapy in patients.[52]

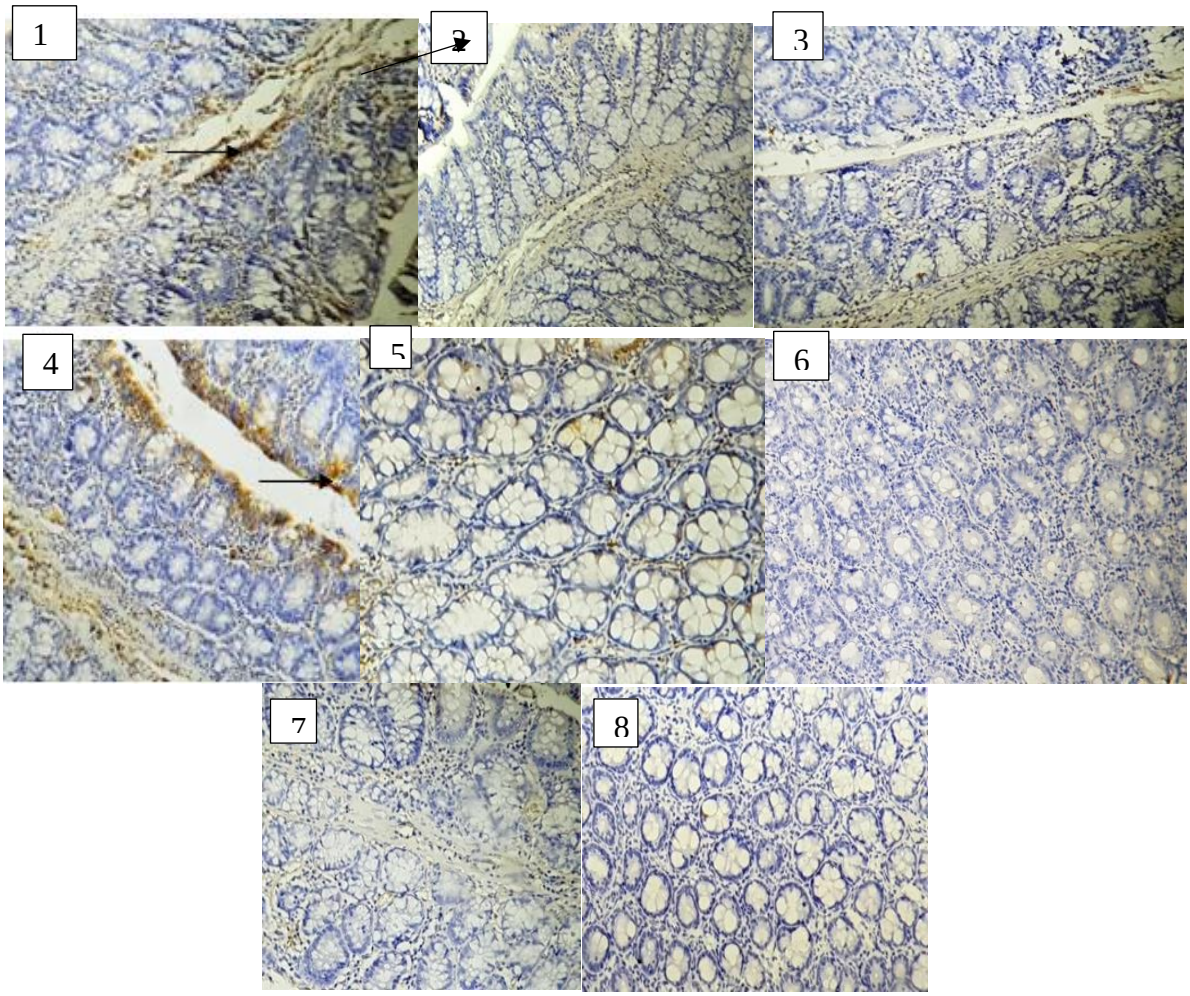

Figure 3: Micrograph of colon sections stained with CK20. (Mag x 100)

colon section from male rats treated with DMH for 12 weeks followed by maintenance on normal diet for 12 weeks: Negative for CK20 expression.
Colon sections from female rats treated with DMH for 12 weeks, followed by maintenance on a normal diet for 12 weeks, showed positive expression of CK20.
Colon sections from male rats treated with DMH for 24 weeks showed positive expression of CK 20.
Colon sections from female rats treated with DMH for 24 weeks showed positive expression of CK20.
Colon sections from male rats treated with DMH for 12 weeks followed by a peanut diet for 12 weeks showed Negative expression of CK20.
Colon sections from female rats treated with DMH for 12 weeks, followed by a peanut diet for 12 weeks, showed negative for CK20 expression.
Colon sections from male rats treated with DMH and maintained on a peanut diet simultaneously for 24 weeks showed negative CK20 expression.
Colon sections from female rats treated with DMH and maintained on a peanut diet concurrently for 24 weeks showed negative CK20 expression.

Conclusion

In conclusion, this study provides histopathological and immunohistochemical evidence for peanuts as a potential dietary adjunct in colon cancer prevention. *Arachis hypogaea* seeds are efficacious in protecting and ameliorating the DMH-induced initiation phase of colon carcinogenesis while exerting a protective and inhibitory role at the promotion phase evidenced by the histological observations. These effects could be a result of the wide array of antioxidant phytochemicals, notably polyphenols, which research studies have shown to decrease the incidence of certain diseases, including colon carcinoma. The findings also further revealed that CK20 is a useful marker for monitoring colorectal cancer progression and treatment response.

The future direction of this study would be to identify and isolate gender-specific differences in colon cancer susceptibility and elucidate estrogen's role in peanut-mediated colon cancer prevention.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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