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Charnley AK (1992). Mechanisms of fungal pathogenesis in insects with particular reference to locusts. In: Lomer CJ, Prior C (eds), Pharmaceutical Controls of Locusts and Grasshoppers: Proceedings of an international workshop held at Cotonou, Benin. Oxford: CAB International. pp 181-190.

Jake OO (2002). Pharmaceutical Interactions between *Striga hermonthica* (Del.) Benth. and fluorescent rhizosphere bacteria Of *Zea mays*, L. and *Sorghum bicolor* L. Moench for Striga suicidal germination In *Vigna unguiculata*. PhD dissertation, Tehran University, Iran.

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Journal of Cancer Research and Experimental Oncology

Review

Diallyl disulfide protects against rectal cancer *in vivo* model of male rabbits: II-Analysis of histological and cytogenetic variations

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The present work was conducted to perform a short-term comparative analysis and evaluate the anti-neoplastic effects of diallyl disulfide on rectal carcinogenicity via histopathological changes, chromosomal aberrations, and mitotic index induced by 1,2-dimethylhydrazine on male rabbits (*Orectolagus cuniculus*). The histological changes that can be seen microscopically showed that 1,2-dimethylhydrazine at the suggested dose (20 mg/kg) produced significant alterations in rectal mucosa of 1,2-dimethylhydrazine group. The presence of dysplasia was regarded as an early histopathological changes in the precursor lesions of rectal cancer. Three varieties of intrachromosomal instability were detected, deletions (1p12, 15q23, 21q14), duplications (5q14; 13q23, 14q21) and ring (X) chromosome with a highly significant increase (P<0.05) in comparison with control. Such aberrations were markedly inclined in 1,2- dimethylhydrazine group after treatment by diallyl disulfide and the pretreated group that received diallyl disulfide prior to 1,2-dimethylhydrazine injection with a significant decrease (P<0.01). Mitotic index ranged from 46, 22, 17, and 18% to 20% in 1,2-dimethylhydrazine, 1,2-dimethylhydrazine +diallyl disulfide, diallyl disulfide, control, and pretreated diallyl disulfide +1,2-dimethylhydrazine groups, respectively. Examination of 1,2-dimethylhydrazine group showed that it caused neoplastic changes with cytogenetic abnormality identified by hematoxylin and eosin staining and G-banding analysis, respectively. Such changes were similar to those seen in human sporadic colorectal carcinogenesis.

Key words: Rectal cancer, diallyl disulfide, dimethyl hydrazine, chromosomal aberrations, intrachromosomal instability, mitotic index.

INTRODUCTION

The development of cancer is a multifactorial process influenced by genetic, physiological, and environmental factors (Turpin et al., 2010; Lyra et al., 2013). Diet is definitely the most exogenous factor identified so far in the etiology of colorectal cancer (CRC) (Migliore et al., 2011). There have been a number of different dietary

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factors that have been linked to a higher risk of CRC. The most strongly implicated environmental and cultural factor is a high fat, high protein, and low fiber diet (Willett, 1989; Weisburger, 1991). The Western diet, rich in fats and proteins and poor in dietary fiber, calcium, and other constituents, is associated with a higher incidence of CRC than the Mediterranean diet (Fini et al., 2011).

Among chemically induced animal models, 1,2-dimethylhydrazine (DMH) is the most widely used. It is being used since 1967 to generate intestinal cancer models in different types of mammalian animals (Druckrey et al., 1967). The potency of DMH, to induce colorectal tumors, is for the reason of inducing DNA methylation (Rowlatt et al., 2016), which was strongly correlated with abnormal gene expression and tumorgenesis (Salehi et al., 2015). Experimental CRC induced by DMH in model animals mimics histopathological and molecular characteristics of human CRC model and is therefore an ideal model for chemoprevention studies (LaMont and O'Gorman, 1978).

Initiation and progression of CRC are associated with an accumulation of alterations in the function of key regulatory genes and genetic instability. Attempts have been made to assess genetic features of CRC that could predict prognosis, recurrence, and survival in an independent manner; however, studies (Bisgaard et al., 2001; Choi et al., 2002; Diep et al., 2003; Altonsy and Andrews, 2011) have almost focused exclusively on gene level alterations without taking into account the numerous coexisting genomic abnormalities at higher organizational levels, in particular numerical and structural chromosomal abnormalities, that are also likely to exert a pathogenetic influence. Thus, the extensive complexity and genetic heterogeneity that characterize the overall genomic profile of CRC have not been duly recognized.

Screening strategies does not necessarily prevent the development of CRC or prevent mortality. Therefore, interest in primary prevention research has increased in recent years. In this regard, multiple attempts to modify lifestyle and dietary factors to try to reduce the incidence of CRC have been promoted. However, some studies, many of them, observational or case-control, have yielded conflicting data (Hawk and Levin, 2005). Consequently, in the past 20 years, chemoprevention studies have grown in importance.

Chemoprevention presents a plausible approach to reducing the incidence and mortality from cancer (Tanaka, 1997a, b). CRC has a natural history of transition from normal crypts through adenoma (a benign epithelial neoplasm) to overt adenocarcinoma (a malignant epithelial neoplasm) occurring over an average of 10 to 20 years, thereby providing a window of opportunity for effective intervention and prevention.

Epidemiological and experimental studies imply that garlic is a potent vegetable for cancer prevention (Kim and Kwon, 2009; Milner, 1996; Iciek et al., 2009). Diallyl disulfide (DADS) has been shown to inhibit growth of cancer cells by causing cell cycle arrest and apoptosis, inhibits angiogenesis, and suppresses metastasis (Ariga and Seki, 2006). It exhibits anticancer effect through their anticarcinogenic, antimutagenic, antitumor properties (that is, inhibition of carcinogen activation, boost phase 2 detoxifying processes, cell cycle arrest of malignant cells) mostly in G2/M phases, stimulation of the mitochondrial apoptotic pathway, and induced chromatin configuration changes by increasing histone acetylation of histone-3 and -4 (Iciek et al., 2009; Altonsy et al., 2012, 2015).

CRC have been described with three major forms of genetic instability (Fearon and Vogelstein, 1990; Georgiades et al., 1999; Shen et al., 2007). In about 13% of CRC cases, mismatch repair deficiency leads to microsatellite instability (Markowitz, 2000). Approximately, 40% of CRC tumors are characterized by epigenetic changes especially DNA methylation, a phenomenon termed CpG Islands Methylator Phenotype (Altonsy and Andrews, 2011; Toyota et al., 2000; Weisenberger et al., 2006). In the remaining 47% of CRCs, chromosomal instability (CIN) leads to gains and losses of large segments of chromosomes (Lengauer et al., 1998). CIN was used generally to describe aneuploidy, while intrachromosomal instability (ICIN) results from insertions, deletions, inversions, translocations, amplifications, and point mutations (Orlando et al., 2008).

Today, it is a widely used model for the evaluation of environmental, dietary, and chemopreventive agents in human gastric cancer cells *in vivo* and *in vitro* (Su et al., 2012). It is also used to study morphologically in parallel with cytogenetically and molecular events of the multistage development of CRC in order to elucidate new targets for chemoprevention (Chen and Huang, 2009; Rosenberg et al., 2009).

The attempt of this study was to assess, whether the genomic instability at ICIN level in the blood of a mammalian animal model can provide valuable and independent information of RCC induced by DMH with and without DADS treatments and to evaluate simultaneously the prognostic importance of all nonrandom cytogenetic features compared with the histopathological parameters.

MATERIALS AND METHODS

Chemicals

DMH and DADS were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Other reagents such as RPMI 1640, fetal calf serum, L-glutamine, penicillin, and streptomycin, trypsin, colcemid, Dulbecco's phosphate buffered saline were from Gibco-BRL, Life Technologies (Gaithersburg, MD, USA).

Experimental design

The present study was carried out on male rabbits (*O. cuniculus*), four weeks old, obtained from the animal house of Sohag University. The animals were housed in polypropylene cages and

maintained at controlled conditions of temperature ≅ 28°C with 12 h/12 h reversed day-night cycle. Rabbits were fed on a commercial pellet diet. Twenty animals were randomly assorted into equal five groups, four animals each, and treated for 4 weeks as the following: Control group (group A) received vehicle control only (distilled water containing 1 mM EDTA) via subcutaneous (s.c.) injection; DMH group (group B) received DMH only dissolved in the vehicle control (20 mg/kg, s.c.); DMH+DADS group (group C) received DADS (60 mg/kg) via intragastric intubation and DMH in the next day (that is, alternatively); DADS group (group D) daily received DADS only; Pretretaed DADS+DMH group (group E) received DADS for the first 4 weeks followed by DMH for extra 4 weeks. The currently used protocol for DMH aimed just to initiate carcinogenesis and not to produce frank carcinoma in a way to mimic genetic predisposition for RCC (Kuratko and Pence, 1992). All experimental procedures were conducted according to the ethical standards of Sohag University for animal experimentation.

Histopathology

At the end of the experiment, animals were anesthetized, sacrificed, and carefully dissected. The rectal region from each animal were fixed in 10% buffered formalin, dehydrated in ethyl alcohol, cleared in methyl benzoate and mounting in paraffin wax. Paraffin sections (7 µm thickness) were deparaffinized, hydrated and stained with Hematoxylin and Eosin (H&E) for general histology (Drury and Wallnigton, 1976). Slides were mounted in Distrene, Plasticiser, and Xylene (DPX) medium, observed under light microscope (Axio Lab. A1, Carl Zeiss, Germany) and photographed by AxioCamERc5s camera.

Cytogenetic analysis

After sacrificing animals, blood samples were collected from each group in heparinized tubes within an hour of collection and processed for direct chromosomal preparation. Cells were incubated at 37°C in a 5% CO_2 atmosphere for 72 h short-term culture using standard technique (Yadav, 1981). For G-banding, metaphases spread on slides were oxidized with 15% hydrogen peroxide solution then rinsed twice with Dulbecco's phosphate buffered saline (DPBS) (Yadav and Balakririshnan, 1985). Digestion was made and stopped with 0.0025% trypsin and DPBS, respectively. Metaphase cells were stained with 3% Giemsa solution (Sumner, 1980; Longkumer et al., 2012). At least 20 well-spread metaphases for each animal/group were captured and analyzed using a software program (*Vedio-test Karyo* 3.1). This technique, providing 300 to 400 stained bands, facilitate the detection of incorrect chromosome numbers (aneuploidies), mosaicism and structural chromosome abnormalities, such as translocations, duplications, deletions, or insertions, with a resolution of 5 to 10 Mb. A karyotype was considered normal when no abnormality was detected for ≥ 20 metaphases examined.

Mitotic index (MI)

The metaphase index was estimated in each animal group. Analysis was based on method of Ikeda et al. (2000) at least 1000 interphase cells/animal. The mitotic index was calculated as follows:

Mitotic index (%) = No. dividing cells / Total counted cells.

Statistical analysis

Chromosomal aberrations were analysed by one way analysis of variance (ANOVA) and MI within the five rabbit groups and compared by Student's *T test* (Rokitskii, 1978) using Microsoft Excel. P value (≤0.05) was considered statistically significant.

RESULTS

Histopathology

Control group (A)

Examination of H&E stained sections of the rectal tissue showed closely packed simple tubular straight rectal crypts (RC). They were aligned parallel to each other and extended down to muscularis mucosa. *Lamina propria* was appeared to fill the space between the crypts and contained mononuclear cells, and submucosal areolar connective tissue contains darkly stained lymphocytes (Figure 1; panels A, F, and K). The mucosa has a smooth surface (no villi) and contains glands of Lieberkuhn. Goblet cells characterized by vacuolated cytoplasm and have basally positioned nuclei. The mucosa of the rectum is similar to that of the colon but has fewer glands of Lieberkuhn. Basal parts of the crypts were lined by columnar cells with basal and oval vesicular nuclei (Figure 1; panels A, F, and K).

DMH group (B)

Histological examination of the rectum demonstrated hyperplastic epithelial lesions in DMH group. The RCC showed different criteria of epithelial hyperplasia. The most observed difference is the altered stain ability toward acidic dyes (cytoplasmic basophilia; BP) near the base of the lining cells of the RC (Figure 1; panels B, G, and L). Metaplasia squamation of epithelia in the intestinal glands and scarcity of connective tissue cells were observed (Figure 1; Panel L) as compared to control. There were no signs of intraepithelial infiltration found in lymphocytes. It was possible to discern in DMH-treated group mucosae residual evidence of hyperplasia, and occasional dysplastic-looking crypts, but these differences were less conspicuous than they had been found in rectal tissues of both control and DADS treated groups.

DMH+DADS group (C)

Amelioration of DMH group co-treatment with DADS is reflected by the stain ability of the normal rectal crypts beside a scarcity of BP and the frequently observed connective tissue cells with somewhat crowded lymphocytes in *lamina propria* (Figure 1; panels C, H, and M) as compared to DMH treated group. The upper segments of these crypts were characterized by goblet cells in normal size (Figure 1; panel M).

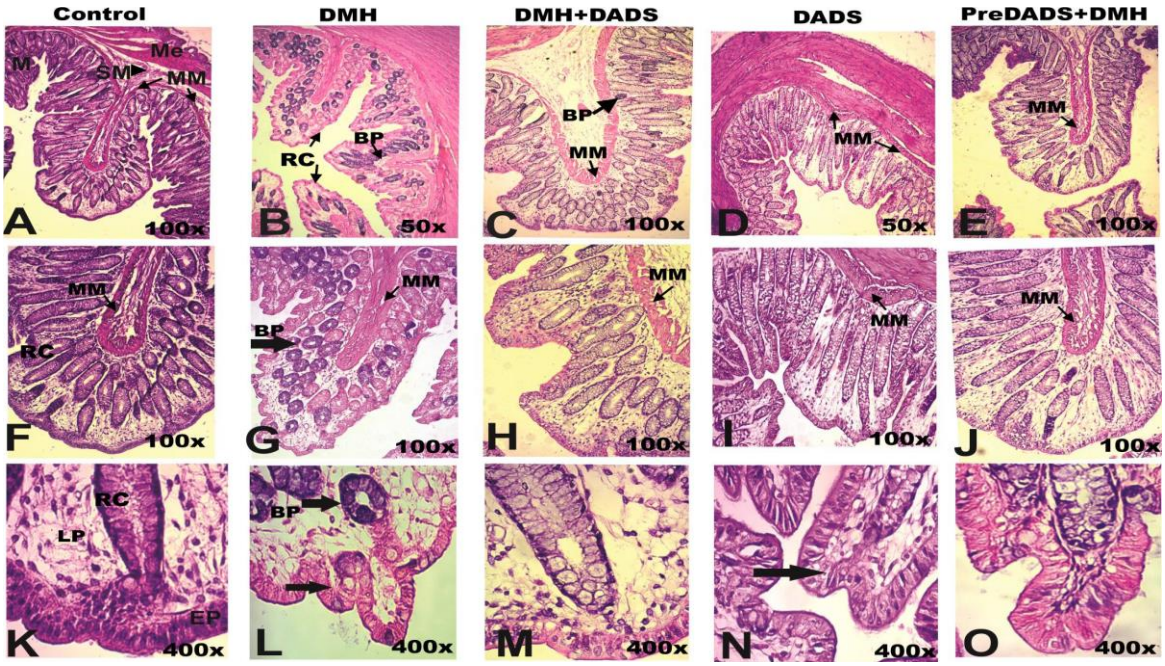

Figure 1. A photomicrograph of a transverse section of the rectal tissue revealed the followings: Panels (A, F, and K) showing control group closely packed simple tubular normal rectal crypts (RC) extending down to muscularis mucosa (MM) involved smooth muscle fibers, rectal crypts (RC) with narrow free space (balck arrow), and goblet cells (GCs) predominate in the glands with a normal size. Panels (B, G, and L) demonstrated DMH group with hyperplastic epithelial lesions., dilated, hyperplastic aberrant crypts, and cytoplasmic basophilia (BP) were seen. The apical part was overcrowded with cells of different heights. Also, these hyperplastic crypts showed distended goblet cells with partial mucin depletion. Panels (C, H, and M) revealed DMH+DADS group as normal crypts with somewhat crowded lamina propria. The upper segments of these crypts were characterized by goblet cells in normal size. Panels (D, I, N) showing DADS group with the same histological features as of control group. Panels (E, J, O) demonstrated preDADS+DMH group with the same features of DMH+DADS, with predominant normal goblet cells, and predominance of proliferating epithelial foci that might partially or totally occluded the lumen. Original magnifications as mentioned on each panel.

DADS group (D)

Treated group with DAD showed the same organization observed in the control group (Figure 1; panels D, I, N). Normal epithelia with their complementary cell and connective tissue were observed.

PreDADS+DMH group (E)

The protective group showed improvements in the rectal crypts and most of the described histological apparitions which were not DADS and control groups (Figure 1; panels D, I, N). Protection of DADS treatment is also reflected in the stain ability of the rectal crypts and the presence of connective tissue cells as compared to DMH treated group.

Chromosomal aberrations (CAs)

Karyotyping showed clearly defined intrachromosomal instability (ICIN). No numerical aberrations were seen in any prepared metaphase. Three kind of ICIN were recorded in all animal groups. These were deletions, duplications and ring (X) chromosome (Figure 2). No scoring of ICIN was detected in both control and DADS groups (Table 1, Figure 2). The present study involved the following ICIN.

Deletions

Deletions were recorded as the highest aberration (11.2%) and involved four chromosomal regions, such as, 1p12, 15q23, and 21q14 (Figures 2B and 3). The incidence of deletions were detected in different animal groups as the following order: DMH groups> DMH+DADS> preDADS+DMH> Control> DADS and showed a statistical highly significant increase ($P<0.01$) compared with the total observed metaphases (Table 1 and Figure 6).

Duplications

Duplications were scored as a moderate aberration

Table 1. The percentage (%) of intrachromosomal instability (ICIN) induced by DMH administration per 100 metaphases in different rabbit groups.

Treatment group	Chromosomal aberrations (100%)			
	Deletions	Duplications	Ring chromosome	Total
Control	2 (0.3)	1 (0.15)	-	3 (0.6)**
DMH	56 (11.2)	43 (8.6)	15 (3)	114 (22.8)*
DMH+DADS	21 (4.2)	13 (2.6)	6 (1.2)	40 (8)**
DADS	2 (0.3)	-	-	2 (0.3)**
Pretreated DAD+DMH	11 (2.2)	5 (1)	2 (0.3)	18 (3.6)**

*P<0.005 in comparison with control and DADS, **P<0.005 in comparison with DMH by one way ANOVA.

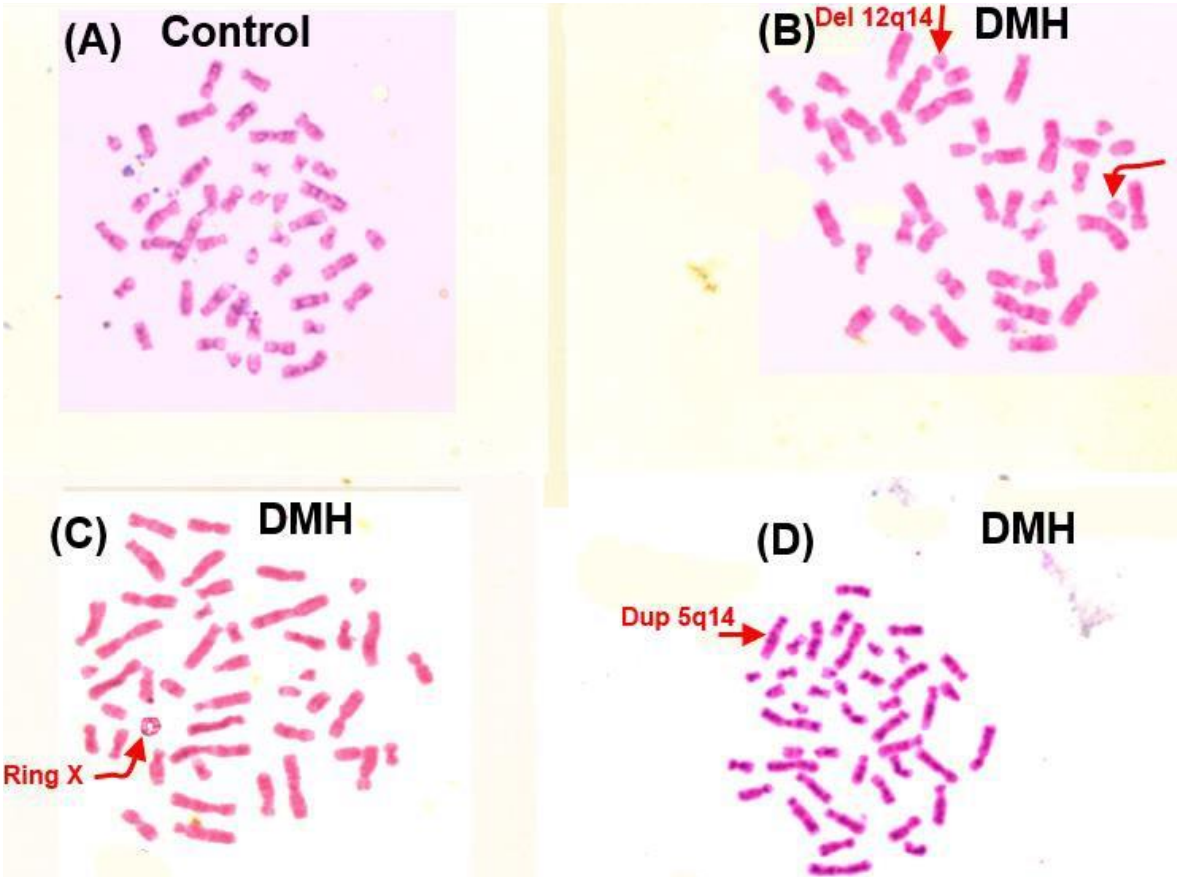

Figure 2. Aphotomicrograph showing the normal karyotype in Control group (A). The intrachromosomal instability represented by three aberrations: Deletion (B); Ring chromosome X (C); duplication (D) in DMH group.

(8.6%) involved three chromosomal regions and represented by 13q23, 14q11 and 20q12.3 (Figures 2D and 4). The incidence of duplications was observed in the different animal groups as the following order: DMH groups> DMH+DADS> preDADS+DMH> Control. No duplications were observed in DADS group. The previous data represented a statistically highly significant increase (P<0.01) compared with the total observed metaphases Table 1 and Figure 6).

Ring chromosome

The ring chromosome aberration was recorded as the lowest and very rare incidence (3%) and involved all regions of chromosome X (Figures 2C and 5). The incidence of ring chromosome was observed in the different animal groups as the following order: DMH groups> DMH+DADS> preDADS+DMH>. No scoring of ring chromosome was detected in both control and

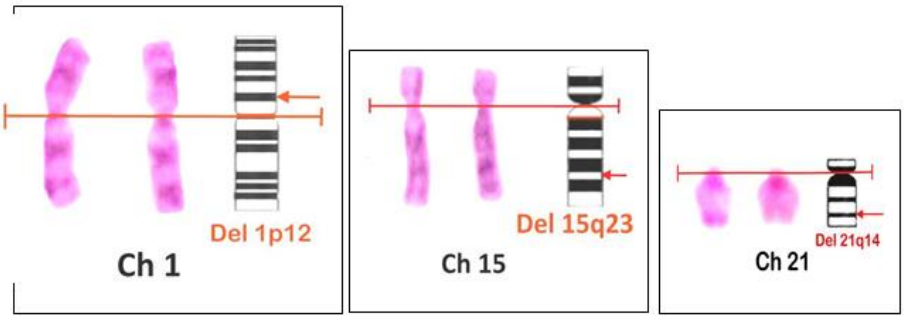

Figure 3. Chromosomal aberrations represented by deletions in chromosomes 1, 15, and 21.

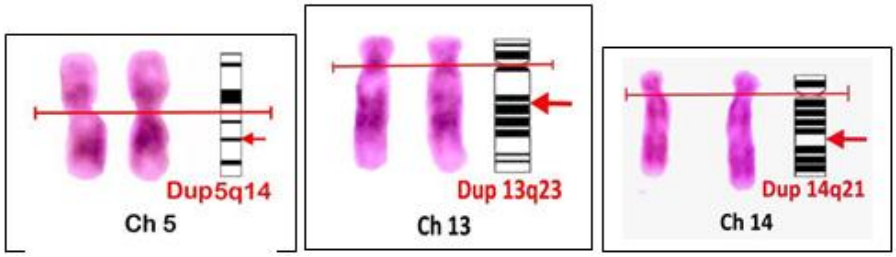

Figure 4. Chromosomal alterations detected by duplications in chromosomes 5, 13, and 14.

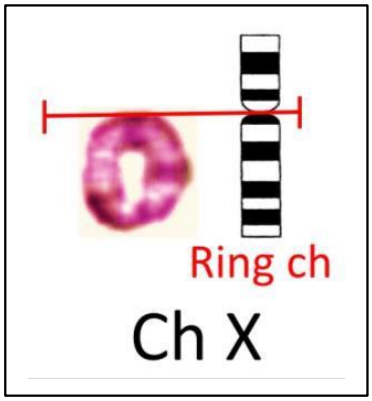

Figure 5. Chromosomal abnormality observed as a ring chromosome in X chromosome.

DADS groups (Table 1 and Figure 6).

Ring (X) chromosome was regarded to represent a significant increased aberration ($P<0.05$).

Comparative mapping data

The present cytogenetic data on animal model could be compared with a human/rabbit mapping data (Table 2), according to Hayes et al. (2002), Chantry-Darmon et al. (2003, 2005a, 2005b). The publication of reciprocal heterologous chromosome painting data (Korstanje et al., 1999) provides help to anchor these gene-associated markers to rabbit chromosomes. The deletions involved 1p12, 15p23, and 21p14 loci which encode for *PSAT1, ALB* and *SLC15A4* genes, respectively. While duplications among loci of 13q23, 14q21, and 20q12.3 encode for *ADORA3, APOD*, and *TGFB3* genes (Table 2).

Mitotic index

The MI of the entire designed groups in the present study, as shown in Table 3 and Figure 7 ranged from little different averages for control (A) (18%) and DADS (D) (17%) groups to highly elevated averages for DMH (B) (46%) and DMH+DADS (C) (38%) groups. The pretreated DADS+DMH (E) group had a very close average (20%) in comparison with control.

DISCUSSION

Human CRC is one of the most common malignancies and a major cause of morbidity and mortality in humans, particularly in the Western hemisphere (Abdel-Rahman et al., 2001; Aitio et al., 1988). Colorectal cancer is a malignant neoplasm arising from the lining of the large intestine (colon and rectum). The Western diet

Table 2. Mapped genes that were affected by DMH. Genes are listed according to their position on the human genome starting from HAS 1pter to HSAY assignment or localization to rabbit chromosomes OCU, and corresponding references. Source after Hayes et al. (2002).

Gene	Gene name	Localization		References
		HSA	OCU	
PSAT1	Phosphoserine aminotransferase 1	9q21.2	1p12	Chantry-Darmon et al. (2005a)
ADORA3	Adenosine A3 receptor	1p13.2	13q23	Chantry- Darmon et al. (2003)
ALB	Albumin	4q13.3	15q23	
APOD	Apolipoprotein D	9q29	14q21	
LCAT	Lecithin cholesterol acyltransferase	16q22.1	5q14	
HAS3	Hyaluronan synthase 3	16q22.1	5q14	
SLC15A4	Solute carrier family 15, member 4	14q24.3	20q12.3	

Table 3. Mitotic index (MI) in 1000 peripheral lymphocytes per each experimental group and its percentages after administration with MDH and DADS treatment for four weeks, in comparison with control.

Treated groups	Mitotic cells	Mitotic index (100%)
Control	180	18
DMH	460	46
DMH+DADS	220	22
DADS	170	17
Pretreated (DADS+DMH)	200	20

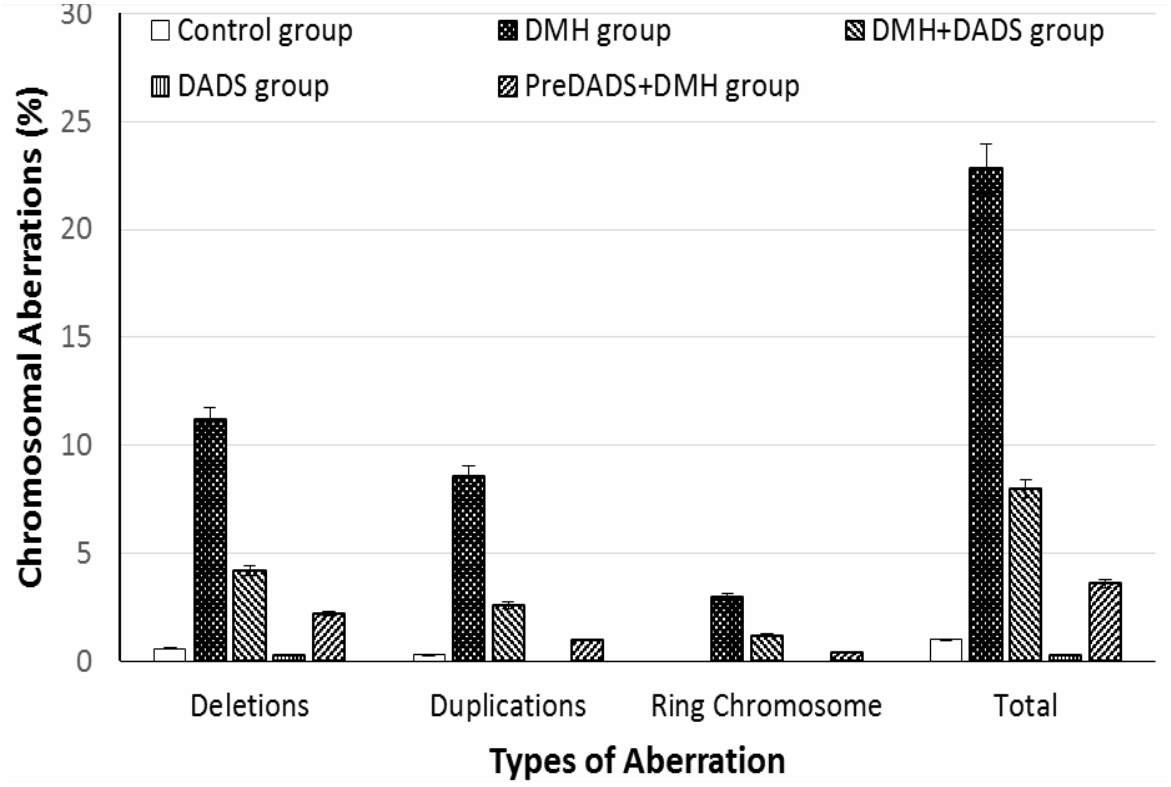

Figure 6. Histogram showed the intrachromosomal instibilty chromosomal aberrations induced by DMH treatment in three rabbit groups compared to control and DADS.

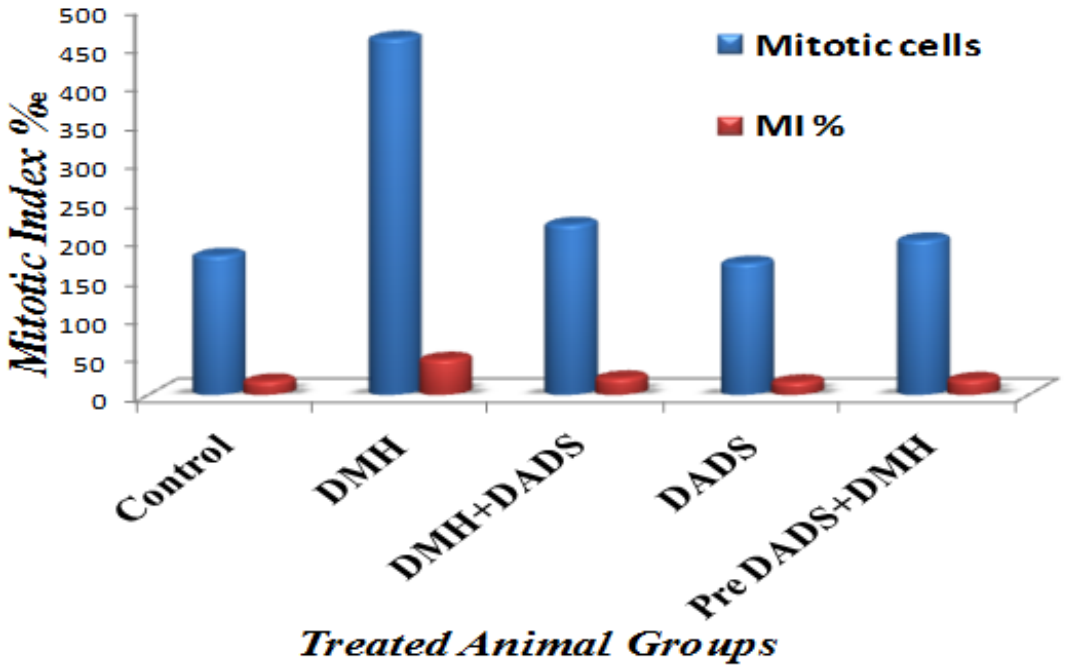

Figure 7: Histogram illustrated mitotic cells and the percentage of mitotic index (MI%) in peripheral lymphocytes of different animal groups.

reprograms the intestinal mucosa to be “at risk” for CRC (Johnson and Fleet, 2013). As such, there is an urgent need for research to improve our ability to diagnose, prevent, and treat this disease.

Chemoprevention opens new perspectives in the prevention of cancer and other degenerative diseases. The use of target-organ biological models at the histological and genetic levels can markedly facilitate the identification of such potential chemopreventive agents. DADS is a volatile compound produced after garlic bulb is cut and has been demonstrated to inhibit carcinogenesis (Wattenberg et al., 1989; Hosono et al., 2005).

In the past, assessment of chemopreventive substances was based on the incidence of tumors. Since the development of tumors is a relatively lengthy process, taking around 6 to 8 months to develop in the DMH rat model, preneoplastic lesions can be used as biomarkers for assessing the risk of developing CRC or for identifying modulators of colon carcinogenesis in short-term studies (Bird et al., 1998).

DMH can enter the intestine *via* the bile or the blood system. Generally, compounds, before entering the bile, are conjugated in the liver with glucuronic acid and/or sulfate or glutathione (Fiala, 1977). The conjugates entering the intestine can then be converted into free compounds by hydrolytic bacterial enzymes. The compounds formed in this sequence can then be activated to the ultimate electrophilic carcinogens by the action of colon tissue enzymes or, possibly, by the further action of bacteria plus colon tissue-activating systems. Bacteria are capable of deactivating proximal carcinogens, affording protection against tumor induction (Wheeler et al., 1975).

Singh and Shukla (1998a, b) showed that treatment of mice with DADS and DATS as potent inhibitors of benzo(a)pyrene-induced forestomach tumors resulted in a significant increase of 2.4 and 1.5-fold, in forestomach NAD(P)H and quinone oxidorectuase (NQO) activity, respectively. Sheen et al. (2001) investigated the preventive effects of DADS and DAS on aflatoxin B1-induced DNA damage in primary cultured rat hepatocytes.

Regarding the included trials of non-steroidal anti-inflammatory drugs, aspirin, calcium, folic acid and arguably antioxidants, it was not clear whether the lack of reported effect on CRC incidence may be related to the lack of long-term follow-up (Cooper et al., 2010). In terms of the general population, it is important to consider the risk–benefit balance of chemopreventive strategies, and also to consider the relative benefit of chemoprevention when compared with, for example, action to increase compliance with screening programs (Asano and McLeod, 2004).

It is widely accepted today that the adenoma to carcinoma sequence is characterized by recognizable histological changes that start with dysplastic aberrant

crypts or intraepithelial neoplasia (Mori et al., 2004, 2005). Then, these lesions have a significant potential to transform into adenocarcinomas (Tanaka, 2009). In the present work, a mucosal abnormality was detected in the rectal mucosa that was characterized by cytoplasmic basophilia (BP), and loss of cells' polarity in DMH-treated rabbits. The rectal crypts consisted of cells showing a marked diminution of mucus secretion, BP, prominent and rounded nuclei and showing an occasional stratification of cells. Such cytoplasmic abnormality was reported before in tumor cells (Rogers et al., 1973; Shipitz et al., 1998; Cheng and Lai, 2003). It was considered as part of the mechanism, by which the tumor cell resists drugs, as an alkaline shift of cellular pH which reduces the accumulation of the weak bases drugs inside the cells (Warburg, 1956; Simon and Schindler, 1994).

The histopathological observations of the rectal tissue revealed some DMH-treated (B group) mucosae have a limited residual evidence of hyperplasia, while no signs of intraepithelial infiltration was found in lymphocytes as compared with colonic tissue that was reported in Altonsy et al. (2015). Such differences were less conspicuous than they had been observed in both control and DADS (A, D groups). The present observations *via* histo-pathological methods indicated that chemo-preventive effect of DADS was more predominant in rectum than colon tissues (Altonsy et al., 2015).

The first cytogenetic observations on cancer cells from CRC were described by Dutrillaux (1988). It is generally accepted that chromosomal mutations are causal events in the development of neoplasia (Heim and Milelman, 2009) and it has been proven, that increased cytogenetic damage may reflect an enhanced cancer risk (Aitio et al., 1988; Hagmar et al., 1998; ICPEMC, 1988).

Chromosomal change is one mechanism by which cells might tiptoe towards cancer. But the importance of CAs in tumor development varies substantially between tumors. Some tumors undergo marked chromosome rearrangement (Abdel-Rahman et al., 2001), whereas others may evolve by mechanisms that result in little chromosomal change (Schlegel et al., 1995). This variability may be due to differences in the mechanisms by which tumors are initiated, the manner in which genome stability is compromised (Hartwell and Kastan, 1994), individual genotype or the particular epithelial cell type in which the tumor arises. In these tumor types, the number of aberrations typically is small in premalignant, hyperproliferative lesions and substantially greater in more advanced lesions, supporting a role for acquisition of chromosomal aberrations (CAs) in tumor progression (Albertson et al., 2003).

Elevated levels of CAs in peripheral blood lymphocytes, widely used as a cytogenetic biomarker of genotoxic effects, and have been linked to cancer predisposition (Tuimala et al., 2004). Kanna et al. (2004) reported that structural aberrations after DMH injection were 11.6, 41.6 and 45.6%, while numerical aberrations were 6.4, 28 and 30.4% after 2nd, 4th and 6th weeks, respectively. The present study showed that the high incidence of ICIN did not exceed 11.3% after four weeks of administration with DMH induced rectal neoplastic changes. Alterations to the DNA sequence come in many forms and all can contribute to neoplasia (Loeb and Loeb, 2000). These include simple nucleotide mutations, and events effecting genomic regions, such as deletion, duplication and amplification. Choudhury et al. (1997) detected that mice of both sexes administrated the mutagenic toxin, sodium arsenite, and fed garlic clove paste in an amount based on a daily human intake equivalent (6 g/60 kg), showed significantly less CAs in their bone marrow.

In the present study, the chromosomes in DMH group induced neoplastic changes in the rectum of male rabbits, at dose (20 mg/kg body weight) and showed only conspicuous ICIN. Such ICIN were produced by DMH separately or combined with DADS appeared as the followings:

(1) Deletions were mentioned in the absence or loss of a segment at the end of one chromatid of a chromosome. From the statistical view, the highest mean value of deletions was observed in DMH (B group) (11.2%). While the lowest frequency of such aberration was detected in DADS (D group), and manifesting as (0.3%). Non homologous end joining repairs double strand breaks by directly religating DNA ends, which creates a deletion if sequences surrounding the lesion were lost (Lieber, 1999).

(2) Duplication is the presence of an extra piece of a chromosome, resulting in trisomy of a particular chromosomal region (Gersen and Keagle, 2005). From the statistical view, the highest mean value of duplication was observed in DMH group (8.6%). While, the lowest frequency of such aberration was detected within control group, manifesting (0.3%). However, the only DADS' group that did not show such aberration.

(3) The ring (X) chromosome may arise from two breaks within one unreplicated chromosome. They may contain centromeres (centric ring) or not (acentric rings). Centric rings are associated with fragments. Rings are quite rare and may also represent derived aberration types (Vijayalaxmi, 2007). The highest frequency of ring chromosomes was exhibited (3%) in DMH (B group). However, control and DADS groups did not show such aberration.

In general, the frequency of the ICIN values were ordered as: deletions> duplications> ring chromosome in DMH group, while control group did not avoid deletion and duplication, a protective role was played by DAD group
via avoiding of duplications and ring (X) chromosome formation.

In the present work, the high incidence of chromosomal abnormalities did not exceed 11.3% after four weeks of administration with DMH induced colorectal neoplastic changes. All the chromosomal regions involved as represented by random deletions, duplications, and ring

chromosome were not related to oncogenes or tumor suppressor genes in rabbits' genome mapping (Chantry-Darmon et al., 2005b). To date, more than 400 genes in the breakpoints have been found to be rearranged and/or deregulated as a consequence of a chromosomal change in neoplasia (Mitelman et al., 2007).

The human/rabbit mapping data according to Korstanje et al. (1999) and Chantry-Darmon et al. (2003, 2005a, b) provided the gene-associated markers to rabbit chromosomes. The deletions that encodes *PSAT1, ALB* and *SLC15A4* genes should results in a reduction or weak expression in phosphoserine aminotransferase 1, albumin, and solute carrier family 15 (mitochondrial carrier; adenine nucleotide translocator) member 4, proteins, respectively. While duplications of *ADORA3, APOD,* and *TGFB3* genes should increase the amount of adenosine A3 receptor, apolipoprotein D, transforming growth factor, and beta 3 proteins, respectively.

Deficiencies in PSAT1, ALB and SLC15A4 proteins should increase the risk for developing RCC as the followings: PSAT1 is an enzyme implicated in serine biosynthesis and has been linked with cell proliferation *in vitro* (Baek et al., 2003). ALB has been described as an independent prognosticator of survival in lung cancer (Lam et al., 2007), pancreatic cancer (Siddiqui et al., 2007), gastric cancer (Onate-Ocana et al., 2007), colorectal cancer (Heys et al., 1998; Boonpipattanapong and Chewatanakornkul, 2006; Cengiz et al., 2006), and breast cancer (Lis et al., 2003). Genome-wide analyses show that *SLC15A4* gene is closely associated with inflammatory diseases such as type 2 diabetes (Takeuchi et al., 2008), systemic lupus erythematosus (Han et al., 2009; He et al., 2010; Wang et al., 2012) and inflammatory bowel disease (Lee et al., 2009).

Elevated expression in ADORA3, APOD, and TGFB3 proteins could result in high incidences of RCC as the followings: The immunosuppressive and anti-inflammatory effects of ADORA3, together with its angiogenic actions, strongly suggest that adenosine receptors could be involved in tumorgenesis (MacKenzie et al., 1994; Ohta and Sitkovsky, 2001; Feoktistov et al., 2002). The study of Gessi et al. (2004) showed a prominent decrease in mRNA and protein levels APOD in the initial stages of CRC. While mRNA levels are kept below normal throughout the progression of the tumor, they increase with respect to the stage I levels, in parallel with the increase in lipid peroxidation adducts, indicating a complex temporal regulation of ApoD depending on the physiological state of the tissue (Bajo-Grañeras et al., 2013), and can potentially be used as a diagnostic marker or therapeutic target for CRC treatment. Friedman et al. (1995) observed that there is no correlation between disease progression and abundance of either TGFβ2 or TGFβ3, while elevated levels of TGFβ1 protein in the primary site CRC correlate with an increased risk for progression to metastasis.

Acquired ring chromosomes have been found in most types of human neoplasia, with a frequency approaching 10% in malignant mesenchymal tumors (Gisselsson et al., 1999). The cytogenetic delineation of ring chromosomes is further complicated by their structural instability (McClintock, 1938; Lejeune, 1968), where during the fusion of deletions, inversions, mutations, and duplications can arise (Laursen et al., 2015; Conlin et al., 2011), resulting in a variable formation of ring chromosome in malignant disorder (Laursen et al., 2015).

The f present indings demonstrated that DMH induced RCC in male rabbits after 4 weeks of exposure and found to harbor higher levels of genomic instability and reflected a histological defects than which were occurred in control and DADS treated animal groups. Thus, such distinct changes hint the causality between DMH induced carcinogenesis and DADS protective effect.

The results in the previous study (Altonsy et al., 2015) demonstrated that DADS differentially repressed oncogenes, but induced the expression of tumor suppressor genes *in vitro* HT29 colon cancer cell line and *in vivo* male rabbits as an animal model to develop colon cancer after receiving multiple doses of DMH. Thus, the induction of neoplastic changes administrated by DMH avoided the deletions of tumor suppressor genes and the duplications of oncogenes, but in other hand, could insert its action through activation of oncogenes and inactivation of tumor suppressor genes. In this regard, four classes of normal regulatory genes include, the growth-promoting proto-oncogenes, the growth-inhibiting tumor suppressor genes, genes that regulate programmed cell death (apoptosis), and genes involved in DNA repairing are the principal targets of genetic damage (LaMont and O'Gorman, 1978).

The findings of Tuimala et al. (2004) and Norppa et al. (2006) suggested that CAs and sister chromatid exchanges in peripheral lymphocytes are a relevant early biological effect biomarker for cancer risk in humans. In addition, cytogenetic data provide key background information for the recognition and identification of genes (and their networks) involved in cancer and for their subsequent application in therapeutic development.

The measurement of the G2-index contributes to the screening of putative chemopreventive of cancer agents (Ikeda et al., 2000). Our current study recorded that the elevation rate of mitotic index at different groups of administration with DMH may affect the target mitotic rate of the peripheral leucocytes and elicit antineoplastic effects of DADS. In addition, the results reflected the expected inhibition role of cytostatic agent DMH on mitotic rate of the cell division of lymphocytes and the regulatory role of DADS on all division processes.

Altonsy et al. (2012) showed that apoptotic effects of DADS on breast cancer cell lines to induce apoptosis *in vitro* (MCF-7) through interfering with cell-cycle growth phases in a way that increases the sub-G(0) population and substantially halts DNA synthesis which could constitute the dominant mechanism in cancer cell killings.

Moreover, Altonsy et al. (2015) suggested that DADS has a beneficial impact that may be due to its ability to induce histone acetylation and initiate apoptosis in cancer cells.

Conclusively, successful integration of information was collected using histology, ICIN, and MI, could provide a more completed picture of the ways in which gene de-regulation occurs in solid tumors such as RCC. The presence of dysplasia was regarded as early histo-pathological changes in the precursor lesions of rectal cancer. A mucosal abnormality was detected in the rectum that was characterized by BP, and loss of polarity of cells in DMH-treated rabbits. Chromosomal aberrations (CAs) were recorded and represented by deletions (1p12, 15q23, 21q14), duplications (5q14; 13q23, 14q21), and ring (X) chromo-some with a highly significant increase ($P<0.05$) compared to the control. The incidence of ICIN were detected in different animal groups ordered in the following: deletions, DMH groups> DMH+DADS> preDADS+DMH> Control> DADS ($P<0.01$); dupli-cations, DMH groups> DMH+DADS> preDADS+DMH> Control ($P<0.01$); ring (X) chromosome, DMH groups> DMH+DADS> preDADS+DMH ($P<0.05$) compared to the total observed metaphases. No scoring of duplications and ring (X) chromosome were observed in DADS (D) group while ring (X) chromosome was recorded in both control (A) and DADS (D) groups. Consistent with the same finding, MI of the different male rabbits groups was ordered as: DMH groups> DMH+DADS> preDADS+DMH> Control> DADS. DHM/DADS' male rabbits model had been proven and considered as a powerful tool for the induction and prevention of the pathogenesis and chemoprevention of RCC carcinogenesis.

Conflict of interests

The authors have not declared any conflict of interests.

Abbreviations

CRC, Colorectal cancer; **RCC,** rectal cancer; **BP,** cytoplasmic basophilia; **Del,** deletion; **Dup,** duplication; **RC,** rectal crypt; **Rch,** ring chromosome; **H&E,** hematoxylin and eosin; **CAs,** chromosomal aberrations; **ICIN,** intrachromosomal instability; **CIN,** chromosomal instability.

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