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Biochemical and Molecular Assessments of Possible Roles of Soybean in 1, 2-dimethylhydrazine-induced Colon Cancer Chemotherapeutic

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Abstract: *Background:* Colon cancer is one of the most common cancers worldwide. 1, 2-dimethyl hydrazine (DMH) is considered as one of the carcinogens that induce colon cancer in animal model. The aim of this work to assess the role of soybean as a preventative and/or therapeutic agent for colon cancer induced by DMH. Methods :sixty male rats were used in this study; the animals were divided into six major groups: (1) control group, (2) carcinogen group, (3) prophylactic (Pro) group, (4) treatment (Tr) group, (5) self limited group and (6) standard group (10 rats in each group). Results: there were over expression of mRNA and the protein of both galectin-3 and C4.4A in both the carcinogenic and self limited groups while low expression in the prophylactic and treated soybean also in. There was also decrease in tissue caspase-3 activity, antioxidant markers of carcinogenic group. While, there was an increase in these markers in 5FU treated group as well as both soybeans prophylactic and treated groups. Conclusion: the biochemical, molecular and histopathological analysis associated with the administration of soybeans exhibit its ability to minimize the frequency of DMH-induced colon carcinomas through increasing the apoptotic tumors cell loss and regulating the level of detrimental oxidative stress markers.

Key words: Colon Cancer · Soybeans · Galectin-3 and C4.4A

INTRODUCTION

Colon cancer is one of the major causes of cancer mortality worldwide, which results from interactions of different factors such as aging, family history and dietary style, colon cancer represents the fourth most common cause of cancer related mortality. The development of colon cancer is associated with (a) perturbations of the delicate balance between cell proliferation and apoptotic cell loss (b) alterations in the oxidative stress state and (c) alterations in proteins related to colon derangement; mainly C4.4A and galectin-3 (Gal-3) [1].

The C4.4A is glycosyl-phosphatidyl-inositol anchored membrane glycoproteins with 30% homology to the urokinase-type plasminogen activator receptor which is implicated in cancer invasion and metastasis [2]. The C4.4A protein was first identified in a highly metastasizing rat pancreatic adenocarcinoma line. In the rat C4.4A is strongly up regulated during implantation of the blastocyst and is highly expressed in the placenta. In the adult, expression is restricted mostly to stratified epithelia of the skin and squamous epithelia of the upper gastrointestinal tract [3].

The advantage of C4.4A in colon cancer diagnosis relies not only on the high frequency and stability of expression, but particularly on the de novo expression of this molecule in colorectal cancer tissues or the strongly up regulated expression as for example in transitional cell cancer [4].

Dramatic changes in cellular glycosylation are a hallmark of cancer cells and significant correlations are now established between some glycosylation patterns and clinical prognosis [5]. Gal-3 is a member of galectins

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that are a family of carbohydrate-binding proteins that share a carbohydrate recognition domain for β-galactosides and are involved in cell adhesion, migration, differentiation, angiogenesis, proliferation and apoptosis [6]. During development, Gal-3 is detected in most types of cells including the hepatocytes in human and mouse embryos and colorectal cells [7].

Alterations in Gal-3 expression have been related to neoplastic transformation and metastatic progression of human colon cancers. Several studies reported a significant increase of galectin-3 content in the tumors that progressed to the metastatic stage relative to the non metastatic tumors or normal colon tissue. However the increased content of Gal-3 in colon cancer and whether it has a role in apoptosis is poorly understood, so we try to find a relation between Gal-3 in colon cancer and apoptosis modulation by some natural products; mainly soybeans [8].

Paret *et al.* [9] explained that Gal-3 associates with C4.4A and that C4.4A ligand binding confers a migratory phenotype that was in line with the supposed metastasis association, in the current study we will try to find their association in colon cancer as candidate markers and their influence by natural product treatment.

In colon carcinogenesis, apoptosis is mediated by several molecules such as caspases. Caspases (cysteinyl-aspartate-specific proteinases), a family of intracellular cysteine proteases causing apoptotic cell death [10]. The activation of caspases then targets their downstream molecules Poly-ADP-Ribose-Polymerase, (PARP) which specifically binds at DNA strand breaks [11].

Oxidative stress induces a cellular redox imbalance which has been found to be present in various cancer cells compared with normal cells. The redox imbalance thus may be related to oncogenic stimulation. DNA mutation is a critical step in carcinogenesis and elevated levels of oxidative DNA lesions (8-OH-G) have been noted in various tumours, strongly implicating such damage in the etiology of cancer [12].

Over the last decades, natural compounds have attracted considerable attention as cancer chemopreventive agents and also as cancer therapeutics. Among their various biological activities, natural products can modulate apoptosis signaling pathways [13]. Soybeans have long been recognized as an excellent source of high-quality protein. The soybean also contains a wide variety of chemical compounds that have potent bioactivity; among these compounds are isoflavones and saponins [14].

Soybean is being considered as a potential chemopreventive agent. In vitro it inhibits transcription and the activity of lipoxygenase or cyclooxygenase enzymes, which facilitate tumor progression. In vivo it is protective in rodent models of chemical carcinogenesis. Soybean contains an á,β-unsaturated ketone, a reactive chemical substituent that is responsible for its repression of transcription [15]. Crude soybean saponin mixtures are growth inhibitory against multiple human colon adenocarcinoma cell lines in vitro. Treatment of human colon cancer cell lines with soyasaponins suppressed growth, induced morphological alterations, increased multiple markers of differentiation and inhibited protein kinase C (PKC) activity [16].

To date, the understanding of the possible protective effects of soybeans in the colon cancer is still incomplete. Therefore, the present study aims to assess the hypothesized alterations by evaluation of (a) mRNA and protein of both C4.4A and Gal-3 (Markers of tumorigenicity), (b) tissue levels of caspase-3 activities (Marker of apoptosis); and (d) lipid peroxidation, NOs, reduced glutathione and superoxide dismutase (Markers of oxidative stress).

MATERIAL AND METHODS

Preparations of Soybean Extract: Soybean extract was purchased from Sigma Aldrich company code no.: (ERMBF-410AK). Soybean extract was prepared by dissolving 100mg in 10 ml distilled water. The rats were given 0.5 ml of Soybean extract twice weekly for one month orally by a dose of (120 mg/kg) according to Raju *et al.* [17].

Treatment and Sampling: Male Albino rats (n= 60) (150±25 g body weight) were purchased from animal house of Assiut University. The dose of 1,2 DMH was purchased from Sigma Aldrich company (20 mg/kg) twice weekly for one month, the dose of 1,2 DMH in our study was determined according to Hiromichi and Michael [18]. Rats were divided into six groups ten rats in each; first control healthy group. The second is carcinogenic group (DMH), were treated subcutaneously with 1,2- DMH. Third Group is prophylactic group (Pro) group, were treated subcutaneously with 1,2- DMH and orally with soybean extract (120 mg/kg) for one month. The fourth Group is soybean treated group (Tr) group, were injected subcutaneously with 1,2- DMH, then treated orally with soybean extract for another month.The Fifth Group is self limited group was treated subcutaneously with 1,2- DMH,

then left to another month without any treatment. The sixth group is 5FU treated group, were treated subcutaneously with 1,2- DMH, then treated with 5FU (100 mg/kg) for another month, the dose of 5FU in our study was determined according to the modified method made by Ragunath *et al.* [19]. At the end of the experiment and after the exact period for each group, rats were anaesethized with light ether then the rats were scarified according to National Institute of Health Guidelines for animal care. Blood samples were collected by cardiac puncturing method, centrifuged and sera were isolated for serological studies. Colon were excised rapidly and used for RNA preparation by homogenization in 20 mM Tris, 100 mM NaCl, 1 mM EDTA and 0.5% Triton X100 buffer. Protein content of colon homogenate was determined using Biuret reagent and bovine serum albumin (BSA) as standard. Samples were aliquotted and stored at -80°C till use.

SDS-PAGE and Western Blotting: Gal-3 and C4.4A antibody was obtained from Santa Cruz Technology. Secondary antibody (Peroxidase labelled antirabbit antibody) was obtained from Amersham™ company (1, 2-DMH and Soybean extract, 5FU were purchased from Sigma Aldrich company. All the other chemicals and solvents used in this study were of analytical grade and obtained from Sigma Aldrich Company. 50 µg from each protein homogenate were denatured by boiling for 5min in 2% SDS and 5% 2-mercaptoethanol and loaded in each lane. SDS–PAGE was done at 100 volts for 2 hrs using 12% gel. The electro-transfer was done using T-77 ECL semidry transfer unit (Amersham Biosciences) for 2 hrs. The membrane was blocked in TBS buffer that contains 0.05 Tween and 5% non-fat milk for one hour. The primary antibodies that used were rabbit polyclonal anti-galectin-3, anti-C4.4A and β-actin (SANTA CRUZ Biotechnology, INC). Polyclonal goat anti-rabbit immunoglobulin, conjugated to alkaline phosphatase (Sigma–Aldrich, Schelldorf, Germany), diluted 1:5000 served as a secondary antibody.

Oligonucleotides Used for Amplifications: NCBI reference sequence: NM_031832.1 and NM_031144.2 were used for design primers for rat Gal-3, C4.4A and β-actin respectively. The coding sequences were used to design the primer pairs and the distance between the two primers was 407, 410 and 500 bases for the Gal-3, C4.4A and β-actin respectively.

The Primer Sets as Following:

- Gal-3 upper: 5'-GGC AGA CGG CTT CTC ACT T-3'
- Gal-3 lower: 5'-GGG CAT ATC GTA GGG CAC T-3'
- C4.4A upper: 5'-TGC TAC AGC TGC GTG CAA-3'
- C4.4A lower: 5'-TTG GAA CTG CGG ATG CTG-3'
- β-actin upper: 5'-CAT GGA TGA CGA TAT CGC TG-3'
- β-actin lower: 5'-CAT AGA TGG GCA CAG TGT GG-3'

RNA Preparation and RT-PCR: Total RNA Kit (Omega Bio-tek) that provides a rapid method for the isolation of total RNA and (Bioron RT/PCR preMix kit (Cat No: 122020-96). PCR was performed using Tag master/high yield (Jena Bioscience). DNA ladder was performed using low range DNA ladder 50-1kbp linear scale (Jena Bioscience). Total RNA fractions were prepared using total RNA extraction kit. The first strand cDNA was synthesized according to the instruction manual of RevertAid™ First strand cDNA synthesis kit (Fermentas) from rat colon total RNA. The PCR was performed using Tag master/high yield (Jena Bioscience) as the following condition: pre-denaturing for 5 min at 94°C then denaturing at 94°C for 30sec, annealing at 55°C and extension at 72°C for one minute. The amplification was carried out in 28 cycles using Biometra cycler (Germany).

Assessment of Serum Oxidative Stress, Caspas-3 and Anti-Oxidants: Blood samples were collected by cardiac puncturing method, centrifuged and sera were isolated for serological studies. Oxidative stress markers (nitrite and MDA), nitrite was determined according the method described by Van Bezooijen *et al.* [20]. and MDA was determined according the method described by Buege *et al.* [21], apoptosis marker (caspase-3), Caspase-3 proteolytic activity was determined using a modified procedure described by Kim *et al.* [22] and some anti-oxidants (GSH and SOD activity), GSH content in colon homogenate was determined using Ellman's reagent according to the method earlier described by Ellman [23], SOD was determined using the method described by Marklund [24], were estimated by using commercially available kits according to the manufacturer's instructions (Biodiagnostic, Egypt).

Histopathological Study: Colon was dissected from each rat and washed several times with 0.9% sterile saline solution to removal any feces. Colon was cut to small

parts and was kept in 10% formalin solution. Formalin-fixed colon specimens were transferred to 70% ethanol and embedded in paraffin. Tissue sections were stained with haematoxylin and eosin (HE) and were examined under Optical microscope for detection of pathological changes.

RESULTS

Detection of C4.4 A by Western Blot Technique: Figure (1) shows C4.4A protein expression by western blot in all experimental stages. C4.4A reached its maximum in Carcinogenic group and Self limited group. On other hand, the expression markedly reduced in group treated with 5-FU and the expression reduced but with less degree in both Prophylactic group and treated with soybean group.

Detection of Gal-3 by Western Blot Technique: The results exhibited over expression of Gal-3 protein in both carcinogenic and self- limited groups. Figure (2) shows the expression levels of Gal-3 protein in total colon homogenate for all experimental groups. Gal-3 expression was reduced in prophylactic and treated group with soybean and 5-FU.

Detection of C4.4A by Rt-pcr Technique: Figure (3) shows the mRNA expression of C4.4A using RT-PCR technique in the all experimental groups (Control, Carcinogenic, Prophylactic, treated with soybean, self limited and treated with 5-FU groups). The expression of C4.4A reached its maximum in Carcinogenic group and Self limited group. But the expression markedly reduced in group treated with 5-FU and the expression reduced but with less degree in both Prophylactic group and treated with soybean group.

Detection of Gal-3 by RT-PCR Technique: The mRNA expression of Gal-3 was detected by RT-PCR technique using upper and lower primers in all experimental stages (Control, Carcinogenic, Prophylactic, treated with soybean, Self limited and treated with 5-FU groups). Figure (13) shows that expression of Gal-3 in colon tissue homogenate of rat after DMH injection. The expression of Gal-3 reached its maximum in Carcinogenic group and Self limited group. In other hand, the expression markedly reduced in group treated with 5-FU and the expression reduced but with less degree in both Prophylactic group and treated with soybean group.

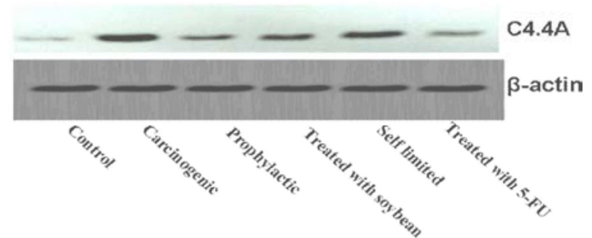

Fig. 1: C4.4A expressions of 50 µg of total colon homogenate were used from (control, Carcinogenic, Prophylactic, treated with soybean, Self limited and treated with 5-FU groups). Rabbit polyclonal antibodies for C4.4A were used in 1:200 dilution. Anti-rabbit secondary antibody conjugated to alkaline phospatase was used in dilution 1:3000. ß-actin re-probed on the same immunoblot to sure the identity of loading.

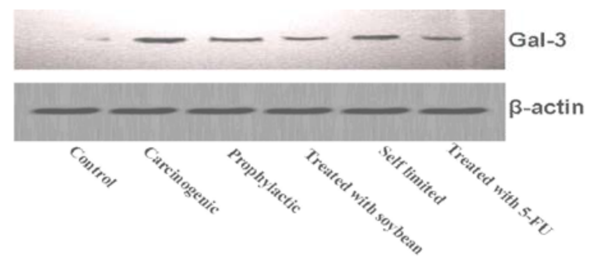

Fig. 2: Gal-3 expressions of 50 µg of total colon homogenate were used from. Rabbit polyclonal antibodies for Gal-3 were used in 1:200 dilutions. Anti-rabbit secondary antibody conjugated to alkaline phospatase was used in dilution 1:3000. ß-actin re-probed on the same immunoblot to sure the identity of loading.

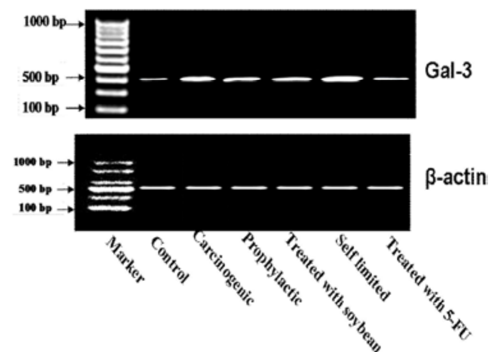

Fig. 3: C4.4A determined by RT-PCR in all experimental stages (Control, Carcinogenic, Prophylactic, treated with soybean, self limited and treated with 5-FU groups).

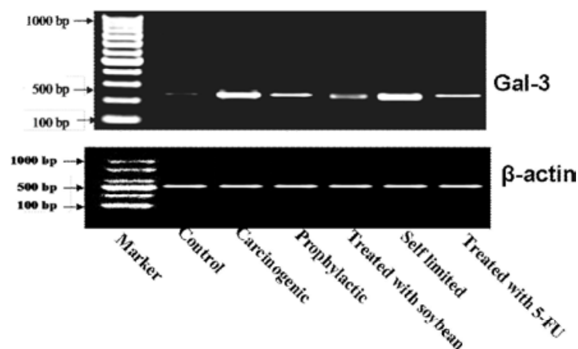

Fig. 4: The expression of Gal-3 by RT-PCR in all experimental stages (control, Carcinogenic, Prophylactic, treated with soybean, self limited and treated with 5-FU groups). ß-actin was used to sure the identity of loading.

Biochemical Alterations: The biochemical alterations included changes in the levels of apoptotic activity, oxidative stress and antioxidants.

Tissue Caspase-3 Activity: As Compared to the control group, there were significant decreased levels of caspase-3, in the DMH, Pro-soybean, treated-soybean and self limited groups (21.89±3.08 vs 9.52±3.80, p<0.001 for DMH group), (21.89±3.08 vs 16.74±2.68, p<0.01 for pro-soybean treated group), (21.89±3.08 vs 14.04 ± 1.94, p<0.001 for soybean treated group) and (21.89±3.08 vs 10.81± 1.51, p<0.01 for self limited group) while no significant difference with 5FU group. These results are summarized in Table (1).

Oxidative Stress Levels: As compared to the control group, there was a statistically significant increase in the tissue levels of NO and MDA respectively in DMH, pro-soybean, tr-soybean, self limited and 5FU-treated group respectively (25.99± 0.574vs 70.24±5.928 and 1.414±0.684 vs. 6.009±0.383, P < 0.001 for DMH group), (25.99± 0.574vs 49.27±6.041 and 1.414 ± 0.684 vs 3.37±0.819, P < 0.001 for pro-soybean group), (25.99± 0.574vs 56.88 ± 5.23 and 1.414±0.684 vs. 6.29±0.77, P < 0.001 for tr-soybean group), (25.99± 0.574vs 72.65±6.40 and 1.414±0.684 vs. 6.009±0.383, P < 0.001 for self limited group) and (25.99± 0.574vs 39.68 ± 5.70 and 1.414±0.684 vs. 2.88±0.62, P < 0.001 for 5FU group) these results are summarized in Table 2.

Anti-oxidant Levels: As compared to the control group, there was a statistically significant decrease in the tissue levels of SOD and GSH in DMH, pro-soybean,tr-soybean and self limited groups respectively (22.44±3.574 vs 10.10±2.63 and 14.55±2.22 vs.5.56±1.94, P < 0.001 for DMH group), (22.44±3.574 vs 15.26±2.86 and 14.55±2.22 vs 10.65±1.94, P < 0.01 for pro-soybean group), (22.44±3.574 vs 13.96±3.36 and 14.55±2.22 vs.7.69±1.132, P < 0.001 for tr-soybean), (22.44±3.574 vs 9.76±2.65 and 14.55±2.22 vs.3.55±1. 46, P < 0.001 for self limited group) and there is no significant difference between control group and 5FU treated group.

Histopathological Results: To assess the changes in colon cells after DMH, soybean and 5-FU administration, HE stained slides were examined and recorded in (Fig.5).

Table 1: Mean tissue levels of caspase-3 (U/mg protein) activity in all studied groups.

Biochemical Indices	Controls	DMH	Pro-soybean	Tr-soybean	Self limited	Tr-5FU
Caspas-3 (U/mg protein)	21.89±3.809	9.528±3.01 p<0.001	16.74±2.683 P<0.01	14.04±1.914 p<0.001	10.81±1.512 p<0.001	18.33±2.894 NS

DMH, 1,2- di-methyl hydrazine; Pro, prophylactic; Tr, treated; NS, non significant. Values represent mean ±10 standard deviation of mean.

Table 2: Mean tissue levels of nitrite (μM/g wet tissue) and MDA (μM/g wet tissue) in all studied groups.

Biochemical Indices	controls	DMH	Pro-soybean	Tr-soybean	Self limited	Tr-5FU
NO (μM/g wet tissue)	25.99±3.5	70.24±5.928 p<0.001	49.27±6.041 p<0.001	56.88±5.235 p<0.001	72.65±6.408 p<0.001	39.68±5.706 p<0.001
MDA (μM/g wet tissue)	1.414±0.7	6.009±0.383 p<0.001	3.376±0.819 p<0.001	4.115±0.842 p<0.001	6.290±0.779 <0.001	2.889±0.620 p<0.001

DMH,1,2- di-methyl hydrazine; Pro, prophylactic; Tr, treated; NS, non significant. Values represent mean ±10 standard deviation of mean.

Table 3: Mean tissue levels of SOD (U/mg protein), GSH (uM/mg protein) and CAT (U/mg protein) in all studied groups.

Biochemical Indices	controls	DMH	Pro-soybean	Tr-soybean	Self limited	Tr-5FU
SOD (U/mg protein)	22.44±3.574	10.10±2.63 p<0.001	15.26±2.86 p<0.01	13.96±3.360 p<0.001	9.764±2.658 p<0.001	18.64±2.953 NS
GSH (uM/mg protein)	14.55±2.228	5.565±1.94 p<0.001	10.65±1.90 p<0.01	7.69±1.132 p<0.001	3.554±1.467 p<0.001	13.63±1.959 NS

DMH,1,2- di-methyl hydrazine; Pro, prophylactic; Tr, treated; NS, non significant. Values represent mean ±10 standard deviation of mean.

Table 4: Correlation between biochemical parameters (ALT, AST, Urea, Albumin, Total tissue proteins, Total tissue lipid, NO, MDA,SOD and GSH).

Biochemical indices	ALT	AST	Urea	albumin	Total tissue proteins	Total tissue lipid	NO	MDA	Caspas-3	SOD	GSH
ALT	-----	0.83283	-0.8334	-0.7751	-0.89076	0.81995	0.87297	0.87322	-0.78002	-0.78101	-0.79565
AST	0.83283	-----	-0.7419	-0.6608	-0.87081	0.86560	0.82984	0.85006	-0.76844	-0.76092	-0.83199
Urea	-0.8334	-0.7419	-----	0.74501	0.74501	-0.74769	-0.72362	-0.73512	0.74569	0.63229	0.69108
albumin	-0.7751	-0.6608	0.74501	-----	0.76438	-0.70051	-0.75414	-0.77758	0.62423	0.65931	0.69108
Total tissue proteins	-0.8907	-0.8708	0.74501	0.76438	-----	-0.828103	-0.86323	-0.85554	0.78852	0.75717	0.84929
Total tissue lipid	0.81995	0.86560	-0.7476	-0.7005	-0.828103	-----	0.83992	0.80352	-0.74792	-0.78617	-0.83691
NO	0.87297	0.82984	-0.7236	-0.7541	-0.86323	0.83992	-----	0.89800	-0.84152	-0.78379	-0.86815
MDA	0.87322	0.85006	-0.7351	-0.7775	-0.85554	0.80352	0.89800	-----	-0.80679	-0.77438	-0.81881
Caspas-3	-0.7800	-0.7684	0.63229	0.62423	0.78852	-0.74792	-0.84152	-0.80679	-----	0.76002	0.72438
SOD	-0.7810	-0.7609	0.63229	0.65931	0.75717	-0.78617	-0.78379	-0.77438	0.76002	-----	0.76878
GSH	-0.7957	-0.8319	0.69108	0.69108	0.84929	-0.83691	-0.86815	-0.81881	0.72438	0.76878	-----

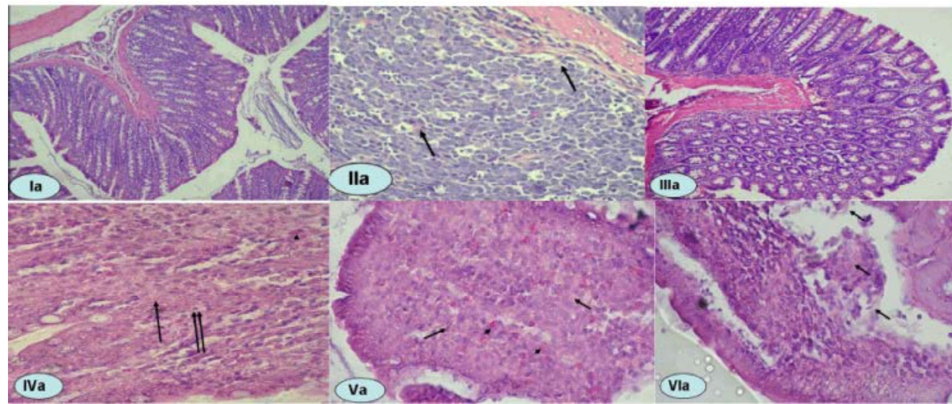

Fig . 5: Histopathological changes in colon tissues. (Ia) Colon section from rats in the control group show normal histological structure (H&E. X 40). IIa) Colon section from rats in the carcinogenic group showing more progressive colon adinocarcinoma, in which the tumor cell (arrows) infiltrating all layers the colon and replacing the normal structure (H&E. X 40). IIIa) Colon section from rats in the prophylactic group showing more or loss normal histological appearance (H&E. X 40). IVa) Colon section from rats in the treated group showing infiltrating tumor cells undergoing either necrosis (arrow) or apoptosis (double arrows) with infiltration of mast cells (arrow head) (H&E. X 40). Va) Colon section from rats in self limited group showing colon adinocarcinoma with tumor cell necrosis and heavy infiltration with mast cells (arrow heads) (H&E. X 40). VIa) Colon section from rats in the 5 FU group showing sever necrosis associated with sloughing of the necrosed tumor cells (arrows) (H&E. X 40).

Examination of colon section from rats in the control group showed normal histological structure (Fig.5,Ia). Examination of colon section from rats in the carcinogenic group revealed colon adinocarcinoma with proliferation of tumor cell from the epithelial layer infiltrating and replacing the colon histological structure. The tumor cells were large polyhedral cell with hyperchromatic nuclei (Fig.5, IIa). Examination of colon section from rats in the prophylactic group show more or less normal histological appearance (Fig.5, IIIa). Examination of colon section from rats in the treated group showed neoplastic cells undergoing either necrosis or apoptosis with infiltration of mast cells (Fig.5, IVa). Examination of colon section from rats in the self limited group showed colon adinocarcinoma with tumor cell necrosis and heavy infiltration with mast cells (Fig.5, Va). Examination of colon section from rats in the 5 FU group showed sever necrosis associated with necrosis and sloughing of the tumor cells (Fig.5, VIa).

Statistical Analysis: Statistical analysis was achieved using Graph Pad In Stat. software Inc, Program, version 4.0 Philadelphia, San Diego CA, (2003). Data were presented as mean ± SD and the levels of significance were accepted with $p < 0.05$. Multiple comparisons were done using one way ANOVA followed by Tukey-Kramer test as multiple comparison post ANOVA test.

DISCUSSION

In this investigation, we hypothesized that in colon carcinogenesis, the administration of soybeans is

associated with molecular, biochemical and histological alterations; these mechanisms include modulation of novel protein markers related to colon derangement, enhancement of apoptosis and inhibition of oxidative stress in colon tissues. To characterize these alterations and to test our hypothesis, we have carried out this investigation.

Our study clearly demonstrated several observations; first,administration of DMH (Carcinogen and self limited) was associated with development of colon carcinoma, over expression of mRNA and proteins of both C4.4A and Gal-3 which are markers of colon tumorgenicity, elevated oxidant markers, decreased antioxidant markers and decreased apoptotic activity. Second, the administration of soybeans was associated with decrease in the frequency of colon carcinoma in rats. Third, administration of soybeans was associated with decreased expression of both mRNA and proteins of C4.4A and Gal-3 markers of colon tumorgenicity, decreased levels of oxidant markers, increased markers of antioxidants and enhanced apoptotic activity.

Administration of the carcinogen DMH in both carcinogenic and self limited groups was associated with the development of colon carcinomas that demonstrated by the over expression of mRNA and proteins of both C4.4A and Gal-3 both are markers of colon tumorgenicity, decrease in apoptotic activity and increase in oxidative stress Concerning C4.4A; The over expression of both mRNA and proteins of C4.4A after the injection of DMH is not only in agreement with previous reports [9], but also suggests their possible diagnostic values in colon cancer.

The mechanism of how C4.4A is induced at colon cancer was explained by Paret *et al.* [25] as they showed that C4.4A bound laminin 5 (LN5) and supported cell migration. As LN5 binds to collagen VII, one possibility is that C4.4A may also be involved in forming an anchor between the cell surface and the collagen matrix. It was also reported by Udayakumar *et al.* [26] that cleavage of LN5 by membrane-type matrix metalloproteinase-1 (MT1-MMP) facilitates cell migration. In this regard, it is suggested that these molecules help cancer cells migrate and invade the cancer-associated stroma in a coordinated manner.

Our finding of the over expression of Gal-3 in DMH groups was in line by the findings of Hill *et al.* [27]. In this regard, the role played by Gal-3 in colon cancer biology was explained by Matarrese *et al.* [28] as they showed that Gal-3 may favor tumor cell spreading and metastasis. Not only did Gal-3 over expression render the tumor cells more adhesive to the activated endothelial cells and to the extra cellular matrix, but the cells themselves exhibited a higher survival ability and resistance to apoptosis and Gal-3 over expression prolongs the survival of the cells and confers resistance to cell death via a mechanism involving an improvement of cell adhesion properties.

The decrease in the tissue levels of caspase-3 activity in the DMH groups as compared to the control group may be explained by the over expression of caspase-3 inhibitors and survivin in tumour cells [29]. Also the relation between Gal-3 and caspase-3 in cancer as Gal-3 also inhibited mitochondrial depolarization and damage after translocation from the nuclei to the cytoplasm, resulting in inhibition of cytochrome c release and caspase-3 activation [30].

On the other hand the antioxidants were assessed by measuring some enzymes as GSH and SOD. Data from this study revealed that rats after treatment with DMH in carcinogenic and self limited groups showed significant decrease in levels of GSH and SOD, in agreement with Ansil *et al.* [32].

The administration of Soybeans was associates with disappearance of the proliferative changes in the colon, decreased levels of markers of tumorgenicity and oxidative stress also markers of antioxidants and apoptotic activity were increased. The ability of Soybeans to decrease the frequency of colon carcinomas in rats was in agreement with previous reports of Davies *et al.* [33-34].

Concerning the role of soybeans on apoptosis in preventing of colon tumor and reduction colon tumor growth. In the current study, our results showed significant increase in tissue caspase-3 activity in both treated and prophylactic groups and that of 5FU treated group when compared with the carcinogenic group these results are in agree with previous study by Dia and Mejia [35], soybean stimulate apoptosis in HT-29 cancer cells through increased caspase-3 activity

In the current study we also observed low expression in both mRNA and protein of Gal-3 in both treated and prophylactic groups with soybeans and 5FU treated group when compared with the carcinogenic group, depending on the explanation of Fukumori *et al.* [30] that Gal-3 leads to inhibition of caspase-3 activation. Taking these results together including the relation between Gal-3 and caspase-3, we can explain both the chemopreventive and the treated effect of soybeans by modulation of apoptosis through activation of caspase-3 and lowering Gal-3.

In another study made by Park *et al.* [36] using treatment hepatic cancer cells with daidzein; one component of soy-extract increased the release of mitochondrial cytochrome- c and activation of caspase-3, indicating that soybeans is a potent inducer of apoptosis in hepatic cancer cells via mitochondrial pathway and these results support our explanation for the involvement of apoptosis mechanism in the chemoprevention of colon cancer using soybeans.

In the current study tissue levels of MDA which is a product of lipid peroxidation and NO which acts as free radical reduced in rats treated with soybean, prophylactic group and rats treated with 5FU. This reduction of NO and MDA levels indicates that soybean reduces the toxic effect of DMH and reduce the colon cancer incidence.

In contrast, levels of GSH and SOD were increased significantly in rats treated with soybean in (Treated group, prophylactic group). This increasing in levels of antioxidants was detected also in rats treated with 5FU. These results interpret an important mechanism of cancer treatment. Decrease levels of oxidative stress and increase antioxidants levels may explain the prophylactic and therapeutic effects of soybean.

Mates *et al.* [37] explored the relation between oxidative stress and carcinogenesis; ROS touch every biological and medical discipline, especially those involving proliferative status, supporting the idea that active oxygen may be increased in tumor cells. In fact, metabolism of oxygen and the resulting toxic byproducts can cause cancer and death. Efforts to counteract the damage caused by ROS are gaining acceptance as a basis for novel therapeutic approaches and the field of prevention of cancer is experiencing an upsurge of interest in medically useful antioxidants.

CONCLUSION

The biochemical, molecular and histological changes associated with the administration of soybeans demonstrated its ability to minimize the frequency of DMH-induced colon carcinomas through increasing the apoptotic tumor cell loss and regulating the level of detrimental oxidative stress markers. These findings suggest possible Prophylactic and therapeutic implications in colon carcinogenesis.

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