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Anticarcinogenic Effect of *Raphanus sativus* on 1, 2 Dimethylhydrazine (DMH) Induced Colon Cancer in Rats

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ABSTRACT

Background: Colon cancer is a major health problem worldwide and rated among as one of the top frequent cancers and is a major cause of morbidity and mortality. Treatment outcome is still not satisfactory and other treatment options are strongly needed. Carbohydrate protein interactions play a major role in numerous biological processes including cancer. However, the seeds of *Raphanus sativus* have never been tested for its anticancer effects on colon cancer. Therefore, this study aimed at elucidating the anti-carcinogenic effect of *Raphanus sativus* in combating chemically (DMH) induced colon cancer.

Materials and methods*:* polysaccharides extract of *Raphanus sativus* (RS) was tested for its ability to reverse the carcinogenic effects of 1, 2 dimethylhydrazine (DMH) which is used for chemical induction of colon cancer in animal models. Rats were injected with DMH and the effects of RS extract either concomitant with or after the administration of DMH were evaluated. . We measured the serum Carcinoembryonic antigen and Carbohydrate antigen as a tumor markers, Glutathione reducatse and Glutathione S transferase as markers of antioxidant activity and liver functions.

Results: RS significantly reduced Serum CEA ($P<0.01$) and CA19-9 ($P<0.01$) as an evidence of its anticarcinogenic effect in colon cancer. Moreover, RS was able to significantly increase activity of Glutathione reductase ($p<0.01$) and Glutathione- S- transferase ($p<0.01$) as a measure of antioxidant compared with DMH untreated group. Also RS was able to significantly increase activities of ALAT, ASAT and ALP ($p<0.01$). Chemical analysis of RS done by NMR and paper chromatography revealed that the polysaccharide in RS was β-galactan and the type of bond that exists between sugar residues is 1, 3 and 1, 6 glucosidic linkages.

Conclusions: This study shows that β-galactan of *Raphanus sativus* has pronounced cytotoxic effects on colon cancer cell line. Moreover, it reduces serum tumor markers and enhancing antioxidant activity in the DMH injected animals. This polysaccharide might be a suitable candidate as a chemopreventive and as an adjuvant therapy for colon cancer.

Key words: Raphanus sativus **,** β-galactan**,** DMH, colon cancer, tumor markers**.**

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer in males and second most common in females worldwide **[1]**. Colon cancer is the third most commonly diagnosed cancer and the second leading cause of cancer death in men and women combined in the US. The American Cancer Society estimates that 142,820 people will be diagnosed in 2013 and that 50,830 will die from colon cancer in the United States **[2].** Hospital-based studies in Egypt showed low incidence of colorectal cancer and high proportion of young-onset disease **[3]**. In comparing rates of Egyptian colorectal cancer to the Surveillance Epidemiology and End Results Program (SEER) of the United States, Egypt had higher rates up to age 30-34 years. The results also shows significantly lower incidence of colorectal cancer in subjects over age 40 years compared to the same age group in the United States SEER **[3].**

Most colon cancers are sporadic with only a few types having a familial basis, such as the familial adenomatous polyposis (FAP) and hereditary non-polyposis colorectal cancer (HNPCC) which accounts for about 5 % of all colorectal cancers, this multistep carcinogeneis process starts with normal colonic cells which acquire genetic alterations in important genes. The mutated cells can grow stepwise and eventually to carcinomas and invade other tissues **[4]**.

1, 2 Dimethyl hydrazine (DMH), is a potent colon carcinogen, inducing colorectal tumors in experimental animals and is the most widely used model of chemically induced colon carcinogenesis **[5, 6]**. DMH induced colon cancer is a multistep process involving a series of pathological alterations, such as formation of aberrant cryptic foci **[7]**. DMH is metabolized in liver to form azoxymethane and methylazoxymethanol which is further transported to colon via bile or blood to generate its ultimate carcinogenic metabolite, diazonium ion which elicits an oxidative stress by methylating biomolecules of colonic epithelial cells and leads to promutagenic events as a result of inflammation **[8]**.

Polysaccharide-rich fungi and plants have been employed around the world for their dietary and medicinal benefits **[9, 10]**. Recent research has indicated that polysaccharides have potent antitumor effects in colon cancer**.** It has been shown that apple oligosaccharide attenuated HT29 cell viability by inducing cell apoptosis and cell cycle arrest **[11]**. Biopolymers mainly polysaccharides from Boletus edulis biologically active biopolymers induce cell cycle arrest in human colon adenocarcinoma cells **[12]**. The antitumor activities of polysaccharides have been reported to result primarily from their immunopotentiation effects **[13] [14]**.

Radish, scientifically classified as Raphanus Sativus, is an annual vegetable found throughout the world and now commonly recognized as a small bulbous red root. The leaves and roots of R. Sativus L. are used as vegetables in different parts of the world. Apart from this, the roots and leaves of the plant have been reported to possess a wide range of pharmacological activities, like gut stimulatory effect **[15]**, hepatoprotective activity **[16]**, cardioprotective effect **[17]**, antioxidant activity **[18]** and antiurolithiatic activity **[19]**.

Furthermore, the freshly squeezed root juice of R. Sativus has been reported to possess antiulcer activity **[20]**. However, polysaccharides from the seeds of *Raphanus sativus* have never been tested for their anti-tumoral effects on colon cancer. This study aims to investigate the Anticarcinogenic and chemopreventive effects of polysaccharides extracted from the seeds of *Raphanus Sativus*.

MATERIALS AND METHODS

Animals

Male Albino rats (National Research Center, Eldoki, Egypt) weighing 80-90 g were used. Animals were housed at 25 ± 2 °C under a 12-h light/dark cycle. Rats were provided with a pellet concentrated diet containing all the necessary nutritive elements. Food and water were available throughout the experiment. Rats were left to acclimatize for 1 week before starting the experiment **[21].**

Preparation of polysaccharides extract

Radish (*Raphanus sativus*) seeds were grounded to fine powder. About 2000 g of radish powder in 3000 ml of mixture of (Methanol/Ethanol) with ratio 2:1 for one hour in order to extract lipids then make centrifugation. Precipitate was washed with 1500 ml of the mixture (Methanol/Ethanol) before centrifugation. Polysaccharides were extracted from the precipitate of the first step by adding 5000 ml of boiling water then put in boiling water bath for eight hours **[22]**. The supernatant was recovered after centrifugation and diluted with equal volume of ethanol (98%) at 4^{o}C for 18 hours to precipitate polysaccharides and centrifuged in order to recover it. Precipitate was resuspended in 2000 ml of 20% TCA (Tri Chloro Acetic acid) for one hour to remove protein **[23]**. Three volume of ethanol 98% was added to the supernatant at 4^{o}C for 18 hours, pellet was recovered after centrifugation, all polysaccharides pellet was dissolved in water, dialyzed against tap water for 72 hours at 4^{o}C **[22, 24]**. The pellet was finally freeze dried.

Analysis of extract

Estimation of total carbohydrates in the extract

Total carbohydrate content was estimated by phenol- Sulphoric acid method **[25]**.

Qualitative examination of the hydrolysed products by paper chromatography

This was examined by chromatography of the resulted hydrolyzate on using Whatman No.1 filter paper, and the solvent system

including n-butanol-acetone-water (4:5:1v/v) **[26]**. For comparison, authentic samples of D-glucuronic acid, D-galactose, D-glucose, D-mannose, L-arabinose, D-xylose, L-rhamnose, were co-chromatographed as reference substances. Detection of spots was achieved by spraying with aniline-phthalate reagent **[27]**.

Proton Nuclear Magnetic Resonance (PNMR) of extract

The NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer. 1H spectra were run at 300 MHz and 13C spectra were run at 75.46 MHz in deuterated chloroform (CDCl3) or dimethyl sulfoxide (DMSO-*d6*). Chemical shifts were quoted in δ and related to that of the solvents **[28]**.

Potential Cytotoxicity by Sulfo-Rhodamine-B stain (SRB) assay.

Radish extract may serve as lead as sources for new pharmaceuticals. Thus, the obtained pure compounds were evaluated in a diverse set of bio-assay. This include specific assays, e.g. for cytotoxicity performed in cooperation with other research groups in Pharmacology unit, National Cancer Institute, Cairo University

Cytotoxicty assay was performed in cooperation with other research groups in the pharmacology unit of the National Research Center. Potential Cytotoxicity of the compound (β galactan) was tested by using Sulfo-Rhodamine-B stain **[29].**

Experimental protocols

The animals were randomly divided into five groups. . *Group I.* Control group, rats were subcutaneously injected with normal saline (.9%Nacl). *Group II,* β- (*Raphanus sativus* extract (RS) Galactan group: rats were orally administrated with 100 mg extract /kg body weight twice a week for 16 consecutive weeks. *Group III,* Rats were subcutaneous injected of DMH (50 mg/kg body weight) once a week for 8 consecutive weeks. *Group IV.* Rats were injected with DMH for 8 weeks followed by orally administration with ß-galactan for 8weeks. *Group V,* Rats were orally administrated with ß-galactan for 8 weeks and comitant with DMH injection until the end of experiment. Five rats from each group were sacrificed at the end of first five weeks, second five weeks and at the end of study (16 weeks). Schematic representation of the experimental design is shown in Figure (1).

Biochemical analysis

Blood samples collected from the orbital plexus using heparinized capillary tubes in clean centrifuge tubes to separate the serum by centrifugation for 10 min. at 5000 rpm, and the supernatant serum was immediately separated and the following markers were evaluated as the following.

a) Tumor markers

CEA and CA 19-9 were assayed by Immunoenzymometric method by ELISA using available commercial kits (Adaltis S.r.l kit, Italy) according to the method described by **Wild [30].**

b) Antioxidant markers

Glutathione reductase was assayed by U.V methods using available commercial kits (Biodiagnostic, Egypt) according to the method described by **Goldberg *et al.* [31].** Glutathione S- Transferase was assayed by U.V methods using available commercial kits (Biodiagnostic, Egypt) according to the method described by **Habig *et al.* [32].**

c) Liver function tests

Alanine transaminase (ALAT) and Asparatate transaminase activity was assayed by the Kinetic method using available commercial kits (Spectrum-diagnostics, Egypt) according to the method described by **IFCC. [33].** Alkaline Phosphatase was assayed by the colorimetric method using available commercial kits (Biodiagnostic, Egypt) according to the method described by **Belfield and Goldberg, [34]**.

Statistical analysis

Statistical differences were calculated with t-test using the SPSS software (version 15.0). Values are expressed as mean and SEM) and a *P*-value < 0.05 was considered significant.

RESULTS

Chemical analysis of extract

Polysaccharides content of radish extract represents about 84.32% of the total crude extract as depicted by figure (2). Paper chromatography was employed for qualitative determination of monosaccharide constitution of RS polysaccharide in which one major component with mobility identical to galactose was identified.

Figure (3) shows the 1D proton NMR spectrum of radish extract. The 1D proton NMR spectrum of extract showed one signal in the anomeric region at 5.20 ppm with *coupling constant (J)* >2 (5.22) indicating that the polymer subunits are galactose in β form **[35, 36].** NMR results also showed that β- galactan is the only sugar present in this extract which is a polymer of galactose monomers linked by 1, 3 and 1, 6 glycosidic linkages.

Biological evaluation of active compounds

The obtained pure compound was evaluated for potential cytotoxicity on four different cancer cell lines. The cells lines used were colon carcinoma cell line (HCT116), liver carcinoma cell line (HEPG2), cervix carcinoma cell line (HELA) and breast carcinoma cell line (MCF7). Each cell line was treated with serial concentrations of 0, 5, 12.5, 25 and 50 µg/mL of β- galactan, and incubated at 37 °C in 5% CO2. Viable cells were counted after 48 h from incubation. β- galactan extract from the *Raphanus Sativum* was tested for any cytotoxic activity on the cell lines from the results depicted from Figure (4). The extract has strong cytotoxic effect on colon, liver, cervix and breast viable infected cells and the concentration that kill nearly 50% of viable cancer cells in colon, hepatic, cervix and breast cancer is 9.2, 13.4, 15.8 and 18.6µg/mL. Indeed, β-galactan extract had the highest cytotoxic activity on a colon cancer cells and therefore its effects were tested on colon cancer induced by DMH throughout the study.

Biochemical analysis

In group II (β- Galactan only) there was no significant change in serum CEA, CA19-9, GR, GST, ALAT, ASAT and ALP throughout the three time experimental intervals when compared with corresponding values of control rats (group I).

A) Tumor markers

CEA and CA19-9 were significantly higher in group III (DMH only), IV (DMH then β-Galactan) and group V (β-Galactan then DMH with β-Galactan compared to control group (group 1). However, when their levels were compared with cancer control (DMH only), there was significant decrease in serum CEA and CA-19-9 ($P<0.001$) (tables 1&2).

B) Antioxidant markers

Serum Gluthione reductase (GR) and Glutathione S Transferase (GST) activity in group III (DMH injected animals) showed significant decrease ($P \leq 0.01$) at three time intervals when compared with corresponding values of control group. Also there was significant decreases ($P \leq 0.01$) in serum GR and GST activities in group IV (DMH then β- Galactan) at the three time intervals when compared with corresponding values of control group (group I). However, when these values were compared with those of, the cancer control (group III), there was significant increases at the 2nd and 3rd time intervals ($P \leq 0.01$) (Tables 3&4).

Serum GR and GST activity in group V (β- Galactan before then DMH with β-Galactan) decreased significantly at the 2nd and 3rd time intervals with ($P \leq 0.01$) and ($P \leq 0.05$) respectively when compared with control group (group I). In contrast, serum GR and GST activities in group V increased significantly when compared with group III ($P \leq 0.01$) at three time intervals.

C) Liver function tests

Serum ALAT, ASAT and ALP activities are represented in tables (5 and 6). Serum ALAT, ASAT and ALP activities in group III (DMH injected animals) and group IV (DMH then β- Galactan) showed significant increase ($P \leq 0.01$) at the three time intervals respectively when compared with corresponding values of control group. Serum ALAT, ASAT and ALP activities in group V (β-Galactan before and during DMH injection) showed a significant increase ($P \leq 0.01$) only at the second time interval (10th week).

On the other hand, serum ALAT, ASAT and ALP activities in group IV (β-Galactan treated animals after DMH injection) decreased significantly ($P \leq 0.01$) at the 2nd and 3rd time intervals, compared with untreated DMH injected animals. Also serum ALAT, ASAT and ALP activities in group V (Chemopreventive β-Galactan before and during DMH injection of animals) showed a significantly decrease at

the three time intervals compared with the untreated DMH injected animals.

DISCUSSION

Colon cancer (CRC) represents a major public health problem, accounting for more than one million new cases diagnosed each year and approximately a half million deaths worldwide **[1]**. Much work remains to be done to improve outcomes for patients with this disease. A skillful and optimal surgical procedure is a basic requirement in the management of patients with CRC. In addition to its role in staging, surgical removal of lymph nodes, more specifically the number of nodes removed, has also been directly correlated with patient survival**[37]**. Despite surgery with curative intent, many patients still have a high risk of tumor recurrence. Search for the most effective strategies, including adjuvant therapy to ameliorate treatment out come.

It is now well established that carbohydrate protein interactions play a major role in numerous biological processes **[38, 39]**. Galactans exhibit a broad range of medicinal effects. Their structures are taxonomically dependent, and their therapeutic actions include benefits in inflammation, coagulation, thrombosis, angiogenesis, cancer, oxidation, and infections **[40]**. Radish (*Raphanus Sativus*) has been used in various parts of the world to treat cancer and as antimicrobial and antiviral agents **[41]**. Previous studies have shown that, radish extract exhibits antioxidant activity **[42]**.

In this study, radish's most bioactive ingredients analyzed by paper chromatography and H^1NMR including polysaccharides mainly (84.3%) and consisted mainly of β- galactan polymer of galactose subunits linked by 1, 3 and 1, 6 glycosidic linkage. Therefore, the present study targeted the evaluation of radish polysaccharides water extract as a natural substance combating colon cancer.

The available body of data regarding subcutaneous injection of Albino rats by dimethyl hydrazine (DMH) which is well defined as potent inducer for colon cancer by DNA alkylation in the colonocytes. It is believed that alkylation of specific sites in DNA leads to promutagenic events which may result in tumour initiation **[43]**.

CEA and CA19-9 are well known as the most common tumor markers of colon cancer, and levels are used not only for preoperative assessment of extent and outcome of cancer, but also postoperative monitoring of recurrence **[44].** Experimental data showed that serum CEA and CA19-9 were increase significantly in DMH-untreated animals due to colon cancer induction and this increased was revealed in other previous studies concerned with colon cancer **[44, 45, 46].**

These tumor markers in the DMH-treated animals revealed significant decrease proven that β galactan has antitumoral effect and the mechanism of this effect may be due to inhibitory effect of β galactan to galectin (Gal 3) mediated cellular interaction. Gal3 is a member of a family of evolutionary-conserved proteins widely found in a range of species from lower invertebrates to mammals. The mammalian lectin Gal3 contains carbohydrate recognition domain (CRD) with an affinity for galactoside residues. The various functions that have been attributed to Gal3 suggest that it plays important roles in several stages of cancer progression and metastasis. For cancer cells to detach from primary tumors, they have to attach to the extracellular matrix and then invade the blood vessels. Basement membrane in which epithelial cells are embedded is rich in such glycoconjugates as laminin and fibronectin. These proteins, through binding to Gal3, allow Gal3 to promote adhesion of the cells. Gal3 is able to play important roles in detachment of cells from primary tumors and attachment of cells in the development of secondary tumors. Furthermore, Gal3 expressed at cell surfaces can mediate self-aggregation of cells when induced by asialofectuin **[47]**.

Another possible mechanism for antitumor effect is Arabinogalactan-mediated enhancement of NK cytotoxicity was not initiated directly but was found to be governed by the cytokine network. Generally, arabinogalactan pretreatment induced an increased release of interferon y (IFNγ), tumor necrosis factor, interleukin-1, interleukin-3 and IL-6 but only IFNγ was involved in enhancement of NK cytotoxicity **[48]**.

It has been reported that GR and GST constitute a mutually supportive team

of defense against reactive oxygen species (ROS) **[49, 50]**. GR is concerned with the maintenance of cellular level of GSH (especially in the reduced state) by effecting fast reduction of oxidized glutathione to reduced form. GST binds to liophilic compounds and acts as an enzyme for GSH conjugation reactions **[51]**.

Data of the study revealed that significant depletion of GR and GST in DMH-untreated rats as compared with control rats throughout the experimental period. This depletion might because the GSH level in rats treated with DMH showed significant decrease **[52, 53]**, as a result levels of antioxidant enzymes decreased **[52, 54]**. Also DMH produces free radicals in blood, liver and the large intestine of experimental models **[55]**.

The data obtained from the present study indicated that radish water extract (β galactan) increase in GR and GST activity in DMH treated with extract as compared with DMH-untreated group. This may achieve by increasing the levels of reduced glutathione in liver and the blood as well. High levels of reduced glutathione protect the cell from oxidative stress and damage **[56]**. Another mechanism suggest that galactose polymer have been proved to possess antioxidative activities in scavenging hydroxyl free radical, scavenging superoxide anion radical and inhibiting lipid peroxidation in various chemical assays **[57, 58]**.

In the present study, severe increase in serum ALAT, ASAT and ALP activities were observed in DMH-untreated rats as compared with control rats, and the maximal ALAT, ASAT and ALP activities were noted in the 15th week. The increase in serum enzymes (ALAT & ASAT) activities might be due to the loss of cellular functional integrity of hepatocytes membrane resulted from highly reactive electrophiles i.e., carbonium ions and alkyl free radicals which severely damage the liver causing necrosis and fatty infiltration, methylate nucleobases and disrupt the polysomal assembly and hence enzymes that are located in liver cells leak out and make their way into the general circulation **[59]**.

The oral administration of radish water extract (β galactan) to DMH injected rats according to the present experimental regimen demonstrated a significant amelioration in liver function indicators (decreased serum ALAT, ASAT and ALP activities a) when compared to their matched values in DMH-untreated group. Such amelioration in the activities of these enzymes is suggesting a significant hepato-protective effect of β galactan **[60, 61]**. This amelioration of liver parameters might be due to β galactan combated oxidative stress resulted from DMH.

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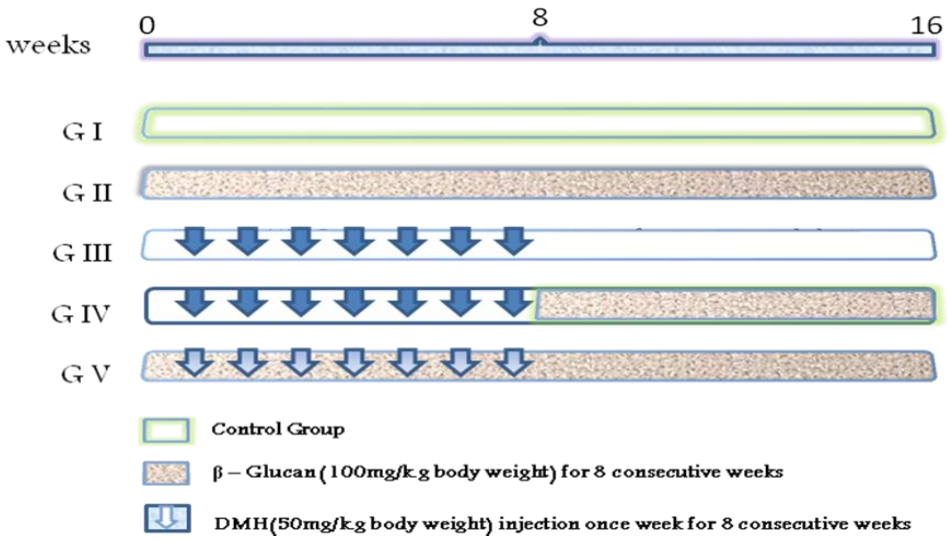

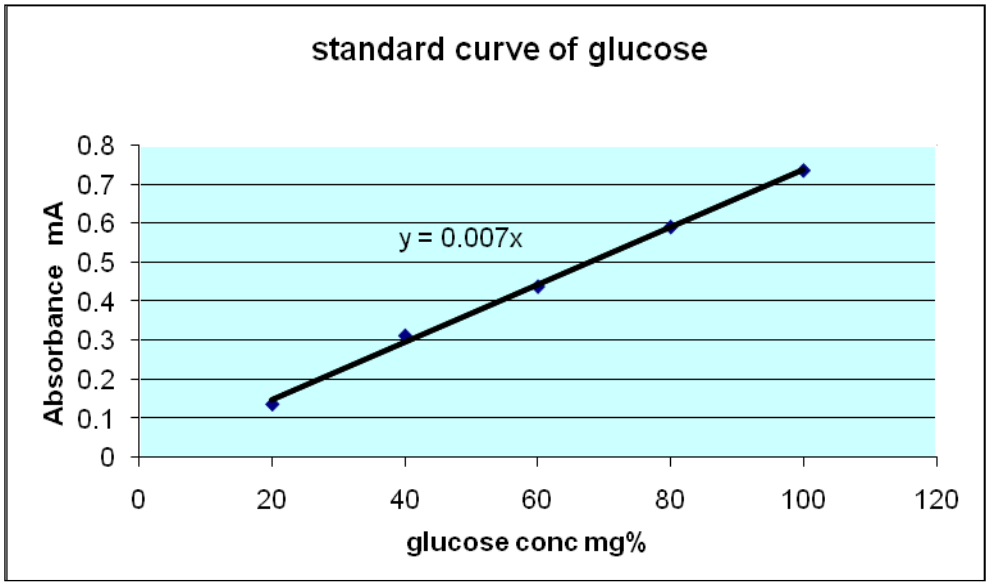

Figure (2): Calibration curve of glucose.

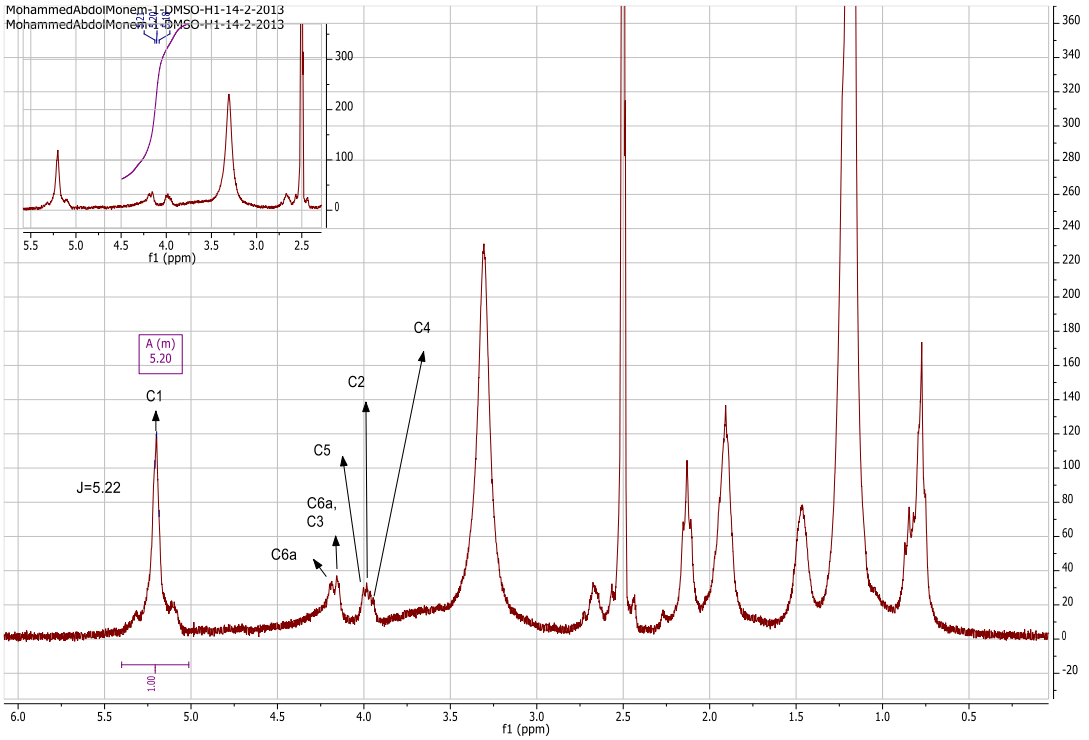

Figure (3): H1 NMR chart of Radish extract (β- galactan).

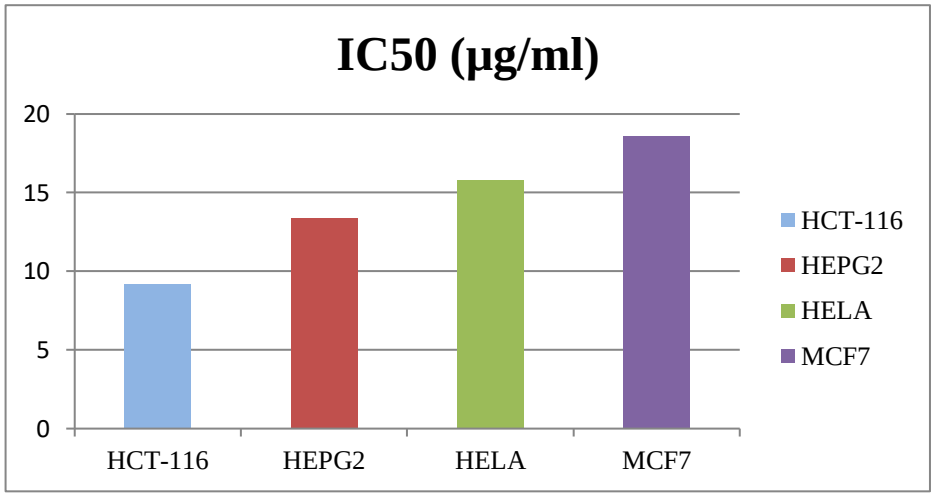

Figure (4): Effect of different concentrations of β- galactan on viability of 4 different types of tumor cell lines.

Table (1): **Serum Carcinoembryonic antigen (CEA) and Carbohydrate antigen 19-9 (CA19-9) in different animal groups.**

Animal Groups		CEA	CA19-9
		16 weeks	16 weeks
Group I (Control)	Mean ±SE	5.68 ± 0.90	20.44 ± 1.40
	%change	---	---
	P-P-value	---	---
Group II (β-Glucan)	Mean ±SE	5.87 ± 0.91	20.91 ± 1.23
	%change	3.3	2.3
	P-P-value	N.S	N.S
Group III (DMH)	Mean ±SE	136.84 ± 1.43	400.64 ± 2.63
	%change	2309.2	1860.1
	P-P-value	P ≤ 0.01	P ≤ 0.01
Group IV (DMH then β-Glucan)	Mean ±SE	59.31 ± 1.01	188.80 ± 2.89
	%change	944.2	823.7
	P-P-value	P ≤ 0.01	P ≤ 0.01
Group V (β-Glucan then DMH with β-Glucan)	Mean ±SE	16.88 ± 0.91	68.89 ± 1.91
	%change	197.2	237.0
	P-P-value	P ≤ 0.01	P ≤ 0.01

Data Expressed as:
Mean ± standard error, % = percentage of change, N.S = non significant, (+) = Increased from control, (-) = Decreased from control, P ≤ 0.01 is considered highly significant and P ≤ 0.05 is considered significant.

Table (2): Serum Carcino embryonic antigen (CEA) and Carbohydrate antigen 19-9 (CA19-9) in β-Glucan treated and untreated DMH injected animals

Animal Groups		CEA	CA19-9
		16 weeks	16 weeks
Group III (DMH)	Mean ±SE	136.84 ± 1.43	400.64 ± 1.68
	%change	---	---
	P-P-value	---	---
Group IV (DMH then β-Glucan)	Mean ±SE	59.31 ± 1.01	188.80 ± 2.25
	%change	- 63.2	- 52.9
	P-P-value	P ≤ 0.01	P ≤ 0.01
Group V (β-Glucan then DMH with β-Glucan)	Mean ±SE	16.88 ± 0.91	68.89 ± 2.00
	%change	- 87.7	- 82.8
	P-P-value	P ≤ 0.01	P ≤ 0.01

Data Expressed as:
Mean ± standard error, % = percentage of change, N.S = non significant, (+) = Increased from control, (-) = Decreased from control, P ≤ 0.01 is considered highly significant and P ≤ 0.05 is considered significant.

Table (3): Serum Glutathione Reductase (GR) and Glutathione S Transferase (GST) in different animal groups.

Animal Groups		GR			GST		
		5 weeks	10 weeks	16 weeks	5 weeks	10 weeks	16 weeks
Group I (Control)	Mean ±SE	170.02 ± 5.62	178.6 ± 3.20	185.58 ± 3.19	126.22 ± 4.14	134.2 ± 4.49	141.46 ± 4.78
	%change	---	---	---	---	---	---
	P-value	---	---	---	---	---	---
Group II (β-Glucan)	Mean ±SE	165.66 ± 4.24	176.4 ± 4.50	188.3 ± 3.30	122.88 ± 5.08	135.4 ± 4.05	139.8 ± 3.68
	%change	- 2.6	- 1.2	- 1.5	- 2.6	0.9	- 1.2
	P-value	N.S	N.S	N.S	N.S	N.S	N.S
Group III (DMH)	Mean ±SE	83.34 ± 3.12	79.2 ± 4.28	73.88 ± 4.08	76.2 ± 4.49	65.4 ± 5.59	58.6 ± 6.32
	%change	- 51	- 55.7	- 60.2	- 39.6	- 51.3	- 58.6
	P-value	P ≤ 0.01	P ≤ 0.01	P ≤ 0.01	P ≤ 0.01	P ≤ 0.01	P ≤ 0.01
Group IV (DMH then β-Glucan)	Mean ±SE	86.28 ± 5.27	106.6 ± 4.30	148 ± 5.61	73.4 ± 5.53	88.96 ± 4.23	107.2 ± 4.83
	%change	- 49.3	- 40.3	- 20.3	- 41.8	- 33.7	- 24.4
	P-value	P ≤ 0.01	P ≤ 0.01	P ≤ 0.01	P ≤ 0.01	P ≤ 0.01	P ≤ 0.01
Group V (β-Glucan then DMH with β-Glucan)	Mean ±SE	164.82 ± 5.91	147.4 ± 3.41	168.42 ± 4.33	121.2 ± 4.40	103.8 ± 4.76	123.24 ± 3.54
	%change	- 3.1	- 17.5	- 9.25	- 4	- 22.7	- 12.9
	P-value	N.S	P ≤ 0.01	P ≤ 0.05	N.S	P ≤ 0.01	P ≤ 0.05

Data Expressed as:

Mean ± standard error, % = percentage of change, N.S = non significant, (+) = Increased from control, (-) = Decreased from control, $P \leq 0.01$ is considered highly significant and $P \leq 0.05$ is considered significant.

Table (4): Serum Glutathione Reductase (GR) and Glutathione S Transferase (GST) in β-Glucan treated and untreated DMH injected animals

Animal Groups		GR			GST		
		5 weeks	10 weeks	16 weeks	5 weeks	10 weeks	16 weeks
Group III (DMH)	Mehan ±SE	83.34 ± 3.12	79.2 ± 4.28	73.88 ± 4.08	76.2 ± 4.49	65.4 ± 5.59	58.6 ± 6.32
	%cange	---	---	---	---	---	---
	P-value	---	---	---	---	---	---
Group IV (DMH then β-Glucan)	Mean ±SE	86.28 ± 5.27	106.6 ± 4.30	148 ± 5.61	73.4 ± 5.53	88.96 ± 4.23	107.2 ± 4.83
	%change	3.5	34.6	100.3	- 3.7	36	82.9
	P-value	N.S	P ≤ 0.01	P ≤ 0.01	N.S	P ≤ 0.01	P ≤ 0.01
Group V (β-Glucan then DMH with β-Glucan)	Mean ±SE	164.82 ± 5.91	147.4 ± 3.41	168.42 ± 4.33	121.2 ± 4.40	103.8 ± 4.76	123.24 ± 3.54
	%change	97.8	86.1	128	59.1	58.7	110.3
	P-value	P ≤ 0.01	P ≤ 0.01	P ≤ 0.01	P ≤ 0.01	P ≤ 0.01	P ≤ 0.01

Data expressed as:

Mean ± standard error, % = percentage of change, N.S = non significant, (+) = Increased from control, (-) = Decreased from control, $P \leq 0.01$ is considered highly significant and $P \leq 0.05$ is considered significant.

Table (5): Serum Alanine Transaminase (ALAT), Aspartate Transaminase (ASAT) and Alkaline Phosphatase (ALP) in different animal groups.

Animal Groups		ALAT			ASAT			ALP		
		5 weeks	10 weeks	5 weeks	10 weeks	16 weeks	16 weeks	5 weeks	10 weeks	16 weeks
Group I (Control)	Mean ±SE	12 ± 1.14	11.4 ± 0.98	13.34 ± 0.78	12.4 ± 1.08	13.12 ± 0.80	14.3 ± 0.94	137.4 ± 2.82	109.6 ± 2.54	121 ± 2.61
	%change	---	---	---	---	---	---	---	---	---
	P-P-value	---	---	---	---	---	---	---	---	---
Group II (β-Glucan)	Mean ±SE	11.6 ± 1.43	11 ± 1.00	14.04 ± 1.07	12.8 ± 1.02	11.8 ± 1.16	13.3 ± 1.21	132 ± 2.41	108.2 ± 2.31	129.2 ± 2.58
	%change	- 3.3	- 3.5	5.2	3.2	- 10.1	- 7	- 3.9	- 1.2	6.8
	P-P-value	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S
Group III (DMH)	Mean ±SE	38.8 ± 2.60	62.8 ± 2.94	73.82 ± 2.51	28.6 ± 2.80	46 ± 2.30	49.8 ± 2.96	258.2 ± 2.71	293.2 ± 2.80	311.4 ± 2.87
	%change	223.3	450.9	453.4	130.6	250.6	248.3	87.9	167.5	157.3
	P-P-value	P ≤ 0.01	P ≤ 0.01	P ≤ 0.01	P ≤ 0.01	P ≤ 0.01	P ≤ 0.01	P ≤ 0.01	P ≤ 0.01	P ≤ 0.01
Group IV (DMH then β-Glucan)	Mean ±SE	38.12 ± 2.84	44.4 ± 2.38	23.4 ± 3.04	25.2 ± 2.40	29.3 ± 2.85	24 ± 2.86	265.8 ± 2.75	217.8 ± 2.24	134.4 ± 2.91
	%change	217.7	289.5	75.4	103.2	123.3	67.8	100.7	98.7	11.1
	P-P-value	P ≤ 0.01	P ≤ 0.01	P ≤ 0.05	P ≤ 0.01	P ≤ 0.01	P ≤ 0.05	P ≤ 0.01	P ≤ 0.01	P ≤ 0.01
Group V (β-Glucan then DMH with β-Glucan)	Mean ±SE	11.8 ± 1.43	26.7 ± 1.59	17.6 ± 1.72	12.2 ± 1.59	25.8 ± 2.92	18.6 ± 1.96	142.6 ± 2.91	188 ± 2.17	126 ± 2.19
	%change	- 1.7	134.2	31.9	- 1.6	96.6	30.1	3.8	71.5	4.1
	P-P-value	N.S	P ≤ 0.01	N.S	N.S	P ≤ 0.01	N.S	N.S	P ≤ 0.01	N.S

Data expressed as:

Mean ± standard error, % = percentage of change, N.S = non significant, (+) = Increased from control, (-) = Decreased from control, P ≤ 0.01 is considered highly significant and P ≤ 0.05 is considered significant.

Table (6): Serum Alanine Transaminase (ALAT), Aspartate Transaminase (ASAT) and Alkaline Phosphatase (ALP) in β-Glucan treated and untreated DMH injected animals.

Animal Groups		ALAT			ASAT			ALP		
		5 weeks	10 weeks	16 weeks	5 weeks	10 weeks	16 weeks	5 weeks	10 weeks	16 weeks
Group III (DMH)	Mean ±SE	38.8 ± 2.60	62.8 ± 2.94	73.82 ± 2.51	28.6 ± 2.80	46 ± 2.30	49.8 ± 2.96	258.2 ± 2.71	293.2 ± 2.80	311.4 ± 2.87
	%change	---	---	---	---	---	---	---	---	---
	P-P-value	---	---	---	---	---	---	---	---	---
Group IV (DMH then β-Glucan)	Mean ±SE	38.12 ± 2.84	44.4 ± 2.38	23.4 ± 3.04	25.2 ± 2.40	29.3 ± 2.85	24 ± 2.86	265.8 ± 2.75	217.8 ± 2.24	134.4 ± 2.91
	%change	- 1.8	- 29.3	- 68.3	- 11.9	- 36.3	- 51.8	2.9	- 25.7	- 56.8
	P-P-value	N.S	P ≤ 0.01	P ≤ 0.01	N.S	P ≤ 0.01	P ≤ 0.01	N.S	P ≤ 0.01	P ≤ 0.01
Group V (β-Glucan then DMH with β-Glucan)	Mean ±SE	11.8 ± 1.43	26.7 ± 1.59	17.6 ± 1.72	12.2 ± 1.59	25.8 ± 2.92	18.6 ± 1.96	142.6 ± 2.91	188 ± 2.17	126 ± 2.19
	%change	- 69.6	- 57.5	- 76.2	- 57.3	- 43.9	- 62.7	- 44.8	- 35.9	- 59.5
	P-P-value	P ≤ 0.01	P ≤ 0.01	P ≤ 0.01	P ≤ 0.01	P ≤ 0.01	P ≤ 0.01	P ≤ 0.01	P ≤ 0.01	P ≤ 0.01

Data expressed as:

Mean ± standard error, % = percentage of change, N.S = non significant, (+) = Increased from control, (-) = Decreased from control, P ≤ 0.01 is considered highly significant and P ≤ 0.05 is considered significant.