STUDIES ON A NEWLY INTRODUCED DRUG ACTING AS ANTI-CANCER INITIATION ACTIVITY

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Abstract: The aim of this study was to investigate the possible immunomodulatory and chemopreventive effects of a bacterial polysaccharide drug (*Bacillus subtilis* sulphated Levan; BSL) relevant for prevention of tumour development during the initiation stage. Initiation is the first stage of carcinogenesis. The activity of BSL could be tested via a series of *in vivo* and *in vitro* assays; direct scavenging of ROS (Reactive oxygen species), modulation of the carcinogen metabolizing mechanisms, either via inhibiting phase-I drug metabolizing enzymes (particularly CYP 450; Cytochrome P450), which converts of pro-carcinogens to active carcinogens, or via enhancing phase-II conjugating enzymes (*e.g.* GSTs, Glutathione-S-Transferase), for removing carcinogens. In order to examine the anti-initiation activity of BSL, *in vitro* experiments; its effect on the CYP 450, GST and GSH (Glutathione) using Hep-G2 cells and its DPPH (1,1-diphenyl-2-picrylhydrazyl) scavenging capacity was performed. I*n vivo* experiments on adult Swiss albino male mice was performed to investigate its effect on the level of GST, GSH, MDA (Malondialdehyde), GST-P immunochemical and HDAC (Histone Deacetylase) using hepatocarcinogenesis in the mice by i.p. injection of diethyl-nitrosamine (DEN). In addition, BSL activity was also compared with garlic as a natural compound with chemopreventive action. **Results:** Through the *in vitro* studies, statistical inhibition of both CYP1A and GSH activities was noticed in relation to the different doses of BSL. In addition, scavenging capacity of BSL against DPPH radicals was inactive. *In vivo* studies showed that GST activity of saline and control groups were not significantly altered by administration of garlic, while, BSL administration significantly ($P<0.05$) enhanced this activity to 2.4-fold of the control. BSL was proved to have an antioxidant activity proved by enhancing the antioxidant protein GSH content to 1.2-fold in induced group of mice with cancer induction. Also, decreased level of LOP (Lipid peroxidation) values was significantly ($P<0.05$) noticed by BSL administration and was insignificantly altered by garlic administration. GST-P immunohistochemical staining in the mice livers showed absence of initiated cells in mice administered BSL alone and highly decreased initiated cells when administrated BSL before and after DEN injection (the carcinogenic material used). In comparison, treatment with garlic before and after DEN decreased the appearance of the initiated cells to lesser extent than BSL. Results showed that BSL had a highly significant ($P<0.05$) inhibitory effect on HDAC activity (play an essential role in the regulation of cell proliferation and apoptosis). It decreases HDAC activity to 2 fold of control, while, garlic did not possessed any inhibitory effect. C**onclusion:** BSL was represented as a promising cancer chemopreventive agent against initiation of hepatocarcinogenesis.

[Haiam Abd El Raaof[1],Faten S. Bayoumi[1], El Amir A.M. **STUDIES ON A NEWLY INTRODUCED DRUG ACTING AS ANTI-CANCER INITIATION ACTIVITY.** Nature and Science 2012;10(1):1-11]. (ISSN: 1545-0740). http://www.sciencepub.net

Key words: *Bacterial polysaccharide drug, GST-P immunochemical, cytochrome P450, carcinogens, initiation stage.*

1. INTRODUCTION

Cancer is one of the most world-wide spread diseases. Tumour development follows three distinct phases: initiation, promotion, and progression (BerenblumI and Armuth, 1981; Heidelberger *et al*., 1983). The initiation phase is a rapid (within hours or days), and irreversible event. It occurs when a normal cell is exposed to a carcinogen that causes un-repairable DNA damage. DNA damage itself is not mutagenic unless the resulting somatic mutation is recapitulated via mitosis to yield a clone of the mutated cell.

Choice of cancer treatment remains mainly surgical resection, radiotherapy or chemotherapy (Wild and Hall, 2000; Yu *et al*., 2000). Recently, considerable attention has been focused on identifying naturally occurring chemopreventive substances capable of inhibiting, retarding, or reversing the multi-stage carcinogenesis. A wide array of phenolic substances, particularly those present in dietary and

medicinal plants, have been reported to possess substantial anticarcinogenic and antimutagenic activities (Surh, 1999). The majority of these naturally occurring phenolic retain anti-oxidative and anti-inflammatory properties which appear to contribute to their chemopreventive or chemoprotective activity (De Mejí *et al*., 1999). Chemoprevention, by definition, is the use of agents to slow the progression, reverses, or inhibit carcinogenesis, thereby, lowering the risk of developing invasive or clinically significant disease (Incomplete sentence) (Hong and Sporn, 1997; Kelloff *et al*., 2001). Consequently, an effective chemopreventive agent should intervene early in the process of carcinogenesis to eliminate premalignant cells before they become malignant (Smith *et al*., 1995; Wattenberg, 1995; Hong and Sporn, 1997; Kelloff *et al*., 1999a).

Microbial secondary metabolites include antibiotics, pigments, toxins, effectors of ecological competition and symbiosis, pheromones, enzyme inhibitors, immunomodulating agents' receptor antagonists and agonists, pesticides, antitumor agents and growth promoters of animals and plants (Arnold, 1998). *Bacillus subtilis* is a genetically amenable, non-pathogenic Gram-positive bacterium. Levan (natural polymers of the sugar fructose) is produced by *B. subtilis* (natto), which is used to make fermented soybeans (Meng *et al*., 2003). Levan has a number of effects on the immunologic system, including tumour suppression and enhancement of leukocyte antitumor activity (Pileggi *et al*., 1962; Kim *et al*., 1998).

Therefore, the aim of this study was the investigation of the possible immunomodulatory and chemopreventive effects of a bacterial polysaccharide (*Bacillus subtilis* sulphated Levan; BSL) relevant for prevention of tumour development during initiation stage.

2.MATERIALS AND METHODS

2.1. Materials:

2.1.1. Levan Extract:

Levan was isolated from culture filtrate of *Bacillus subtilis* after the stage of fermentation using sedimentation by ethanol. Ethanol (96%) was added to the culture filtrate 2.0:1.0, mixed for 24 hr at room temperature. Sediment was then separated by decantation and used for investigations as levan. Sulphation of levan was carried out with chlorosulfonic acid (Hussein, 1994). The formed product was isolated by precipitation with 3 vols. of MeOH. Purification of the sulphated derivatives was preformed with repeated dissolution in water and reprecipitate with MeOH.

2.1.2. Diethylnitrosoamine (DEN)

DEN is diethyl nitrosamine .It is a potent hepatocarcinogen in experimental animals influencing the initiation stage of carcinogenesis during a period of enhanced cell proliferation induced by hepatocellular necrosis and forming DNA–carcinogen adducts, inducing DNA-strand breaks and in turn HCCs without cirrhosis through the development of putative preneoplastic focal lesions (Tatematsu *et al*.,1988).
DEN was intraperitoneally (i.p.) injected in concentration of 200 mg/kg and promoted by adding 0.05 % sodium phenobarbital to water.

2.1.3. Garlic

Garlic from Tomex, ATOS Pharma, Cairo, Egypt, as tablets (200mg) and orally administered as a suspension in physiological saline to mice in a dose of 250 mg/kg.

2.1.4. Study Samples:

2.1.4.1. *In vitro* Study (Cell Lines)

Hep-G2 cells (ATCC, USA.) were cultured in tissue culture flasks and maintained in RPMI medium (Cambrex Bioscience, Copenhagen, Denmark.) supplemented with 10% fetal bovine serum, 2 µmol/ml L-glutamine, 250 ng/ml fungizone, 100 units/ml penicillin, and 100 units/ml streptomycin at 37°C in a humidified 5% CO_2 atmosphere. The cultures were passed every 4 days by trypsinization using 1.5 ml trypsin/EDTA solution which prepared by dissolving 0.25 mM Trypsin (Cambrex Bioscience, Copenhagen, Denmark) in 1m M EDTA for 5 min at 37°C.

2.1.4.2. *In vivo* Study:

Two hundred adult Swiss albino male mice (25-30g) were divided into 7 groups (16 mice in each.). Hepatocarcinogenesis was performed i.p in mice with DEN. Seven groups were as the following:
**Control gp.*: Mice were normal healthy untreated gp.
**Saline gp.:* Mice were orally received saline twice a week for 6 wk.
**BSL gp.*: Mice were received 80mg/kg body wt. BSL i.p. twice a week for 6 wk.
**Garlic gp.:* Mice were received 250mg/kg body wt. orally twice a week for 6 wk.
**DEN gp.:* Mice were received 200mg/kg body wt. i.p. once, in addition, 0.05% sodium Phenobarbital was added to the drinking water after 2 wk of DEN injection.

**BSL+DEN gp.*: Mice were received 80mg/kg body wt. BSL i.p. 24 hr before and after DEN injection followed by 80mg/kg body wt.BSL i.p. twice a week for six weeks.

**Garlic+DEN gp.*: Mice were received 250mg/kg body wt. garlic 24 hr before and after DEN injection followed by 250mg/kg body wt. garlic orally twice a week for six weeks.

On the 6th week, animals were sacrificed, blood samples were collected, liver tissues were excised and rinsed in cold PBS. One portion was preserved in 10% buffered formalin then embedded in paraffin for immunohistochemistry and pathological investigation. The other portion was used for the preparation of 20% (w/v) liver homogenate by homogenization in ice Ripa buffer, centrifuged using cooling centrifuge at 1000 xg for 10 min at 4°C. The supernatant was stored at -80°C for later assessment of the followed parameters that express the initiation development and the antagonistic effect of BSL

2.2. Methods

2.2.1. *In vivo* and *In vitro* Bioassays Studies

2.2.1.1. Modulation of Glutathione-S-Transferase Activity:

The GST activity was determined spectrophotometrically according to the method described by Habig *et al.* (1974).

2.2.1.2. Estimation of Glutathione Content:

The level of GSH was determined spectrophoto-metrically according to the method of **Griffith (1981).**

2.2.2. *In vitro* Bioassays Studies

2.2.1. Cytochrome P450 1A1:

Cyp1A activity was determined by measuring the rate of dealkylation of 3-cyano-7-ethoxycoumarin (CEC) to the fluorescent 3-cyano-7-hydroxycoumarin (CHC) based on the method of Crespi *et al*. (1997).

2.2.2.2. Determination of Cellular Total Thiols:

The total thiol concentration was measured by an enzymatic cycling procedure based on the oxidation of thiols by 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) to 5-thio-2-nitrobenzoic acid (TNB) and reduction of the oxidized GSH by NADPH in the presence of glutathione reductase (Griffith, 1981), the extent of TNB formation was determined spectrophoto-metrically.

2.2.2.3. Scavenging of DPPH Radicals (Non Cellular Assay):

1,1-diphenyl-2-picrylhydrazyl is a stable deep violet radical due to its unpaired electron. In the presence of an antioxidant radical scavenger, which can donate an electron to DPPH, the deep violet colour decolorize to the pale yellow non-radical form (van Amsterdam *et al.,* 1992)

2.2.3. *In vivo* Bioassays Studies

2.2.3.1. Determination of the LD_{50} values for BSL:

The LD_{50} dose for BSL was evaluated in Swiss albino mice. It was found that all used doses (1g, 1.5g and 2g/kg) were safe for mice. On that basis, 80 mg/kg b. wt. dose was chosen as a safe dose for BSL to be utilized in the *in vivo* animal model.

2.2.3.2. Lipid Peroxidation:

Lipid peroxidation (LPO) was indirectly quantified by measurement of thiobarbituric reactive substances (TBARS) according to Varshney and Kale (1990**).**

2.2.3.3. GST-P Immunohistochemistry:

Initiated cells were detected immuno-histochemically using GST-P as an initiation marker according to the method of Nordstrand and Stenius (1999).

2.2.3.4. Histone Deacetylase Activity Assay (HDAC): The activity of HDAC was measured using a colorimetric assay kit (BioVision, Mountain View, kit no. K331-100).

2.2.4. Statistical Analysis:

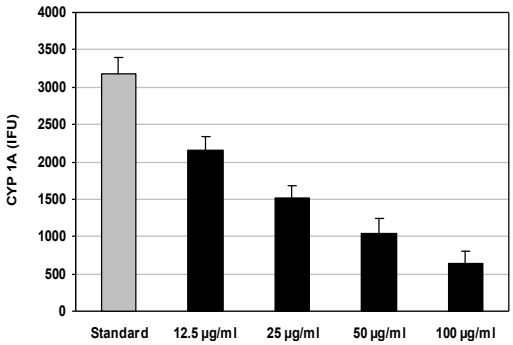
The results were expressed as the mean±SD from n=3. Statistical analysis was preformed with Student's t-test using instate soft ware (Version 3.05). Statistical significance was accepted at the level of $P<0.05$**.**

3.RESULTS

3.1. *In vitro* Studies

3.1.1. Effect of BSL on the Carcinogen Activator Cytochrome P450 Enzyme

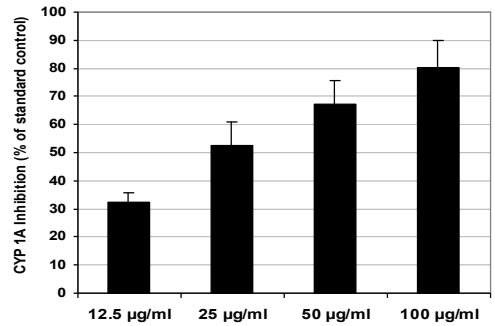
Significant ($P<0.05$) CYP1A activity inhibition to 2180, 1500, 1060 and 680 IUF by BSL doses of 12.5, 25, 50 and 100 µg/ml, respectively where standard control was 3200 IUF (Fig 1).



*Significantly different from control values at ($P<0.05$).

Figure 1: Anti-initiating activity through inactivation of the carcinogen: The effect of BSL on CYP1A. Data are expressed as (IUF) Mean±S.E. and the standard control represents the CYP1A content in β-NF-induced Hep-G2 cells.

Phase 1 enzyme CYP1A activity was inhibited by BSL in a dose dependant manner to a low extent of 19.85 % of the standard control at the dose of 100µg/ml. The IC_{50} of CYP1A by BSL was calculated from the curve equation of the inhibition percentage and valued as 30.47µg/ml (Fig. 2). BSL could be recognized as CYP1A inhibitor ($P<0.05$) starting from the dose 12.5 µg/ml.



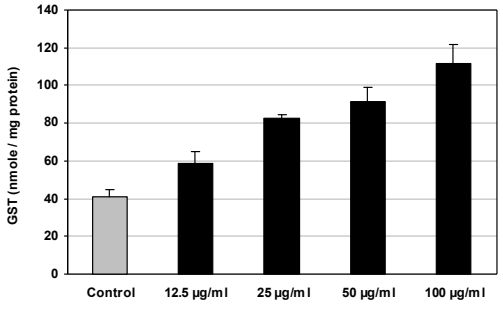
* Significantly different from control values at ($P<0.05$).

Figure 2: The inhibition percentage of CYP1A activity by BSL as compared with untreated standard control CYP1A. In the scale, the control is represented by 0%. Data are represented as Mean± S.D.

3.1.2. The Effect of BSL on the Carcinogen Detoxification Enzyme GST

GST is one of the carcinogen detoxification enzymes (phase II enzymes). GST activity was measured before and after treatment with different doses of BSL (12.5, 25, 50 and 100 µg/ml) and it shows a gradual induction in GST activity to 58, 84, 95 and 109 nmole/mg protein where the control value was 43 nmole/mg protein, considering the GSH content of the cells, which was measured in cells before treatment.

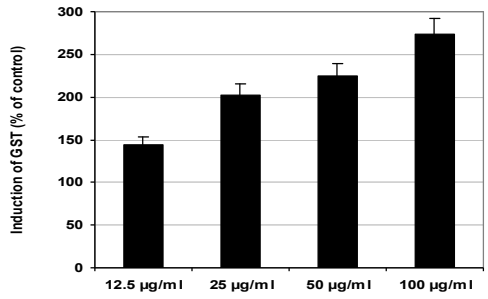
Figure (3), showed that the phase II enzyme GST is significantly enhanced by BSL treatment to 2.21-fold of the control ($P<0.01$) at a peak point at the 50 µg/ml dose.



* Significantly different from control values at ($P<0.05$).

Figure 3: Anti-initiating activity through detoxification of the carcinogen: The effect of BSL on GST in Hep-G2 cells. Results are expressed as (nmole/mg protein) Mean±S.E. and the standard control represents the GST activity of Hep-G2 cells before treatment with BSL.

The increase of dose to 100 µg/ml led to less extent of GST activity induction. BSL could be recognized as significantly ($P<0.05$) GST inducer starting from the dose 25 µg/ml (Fig. 4).



* Significantly different from control values at ($P<0.05$).

Figure 4: The induction percentage of GST activity in BSL treated cells as compared with untreated control. In the scale, the control is represented by 100%

3.1.3. Effect of BSL on the Cellular Antioxidant GSH

The Influence of BSL on the cellular

antioxidant defence mechanisms was estimated through the determination of the non enzymatic antioxidants; total thiols, which were mainly containing GSH. GSH concentration was measured by an enzymatic cycling procedure based on the oxidation of thiols by DTNB to TNB and reduction of the oxidized GSH by NADPH in the presence of GSH reductase. As shown in figure (5), the antioxidant protein GSH is significantly enhanced by BSL treatment to 1.61-fold of the control ($P<0.05$) at the dose 100 µg/ml. The GSH induction with the doses was slow, starting with 32 nmole/mg protein GSH at 12.5 µg/ml BSL dose and increased to 37.3, 41.7 and 43.6 nmole/mg protein GSH with 25, 50 and 100 µg/ml BSL. BSL could be recognized as an inducer of cellular antioxidant capacity ($P<0.05$) at the dose 100 µg/ml (Fig. 6).

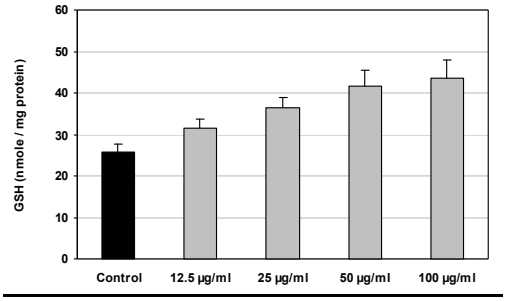


Fig. 5: Antioxidant activity of BSL as investigated by its effect on GSH content Mean±S.E. in Hep-G2 cells. Results are expressed as nmole/mg protein and the standard control represents the GSH content in Hep-G2 cells before treatment with BSL
* Significantly different from control values at ($P<0.05$).

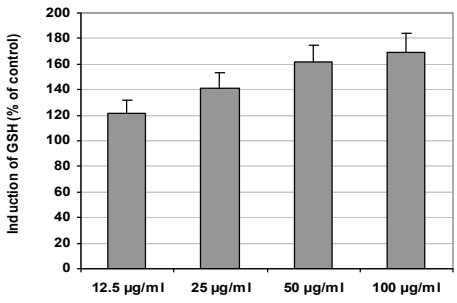


Figure 6: The induction percentage of cellular GSH content in BSL treated cells as compared with untreated control. In the scale, the control is represented by 100%.
* Significantly different from control values at ($P<0.05$).

For the determination of the total protein, a standard curve of BSA was blotted (Fig. 7) to be used in the calculation of the cellular protein after each experiment.

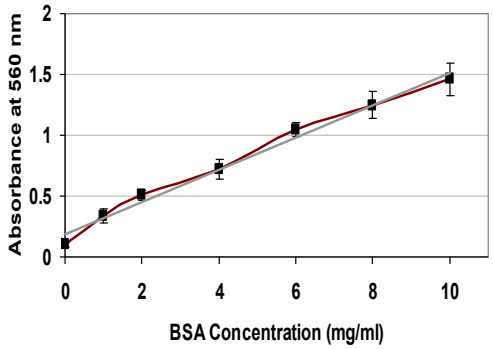


Figure 7: A standard curve for BSA using BCA assay.

1.1.4. Scavenging of DPPH Radicals (Non Cellular Assay)

Scavenging capacity of BSL against DPPH radicals was found to be inactive due to its high SC_{50} value ($SC_{50} > 250$ µg/ml).

3.2. *In vivo* Studies

3.2.1. Determination of Glutathione S Transferase (GST) Activity

As in figure (8), GST activity of the control, saline, garlic, BSL, DEN, BSL+DEN and garlic+DEN groups recorded as 27.8, 27.2, 23, 68.1, 15.8, 45.3 and 25.1 nmole/mg protein, respectively. GST activity of saline and control groups were not significantly altered by administration of garlic, while, BSL administration significantly ($P<0.05$) enhanced this activity to 2.4-fold of the control.

Administration of DEN led to 1.7 times decrease in the total GST activity. Such significant inhibition in GST activity was reversed to significant elevation ($P<0.05$) by receiving BSL and garlic.

3.2.2. Estimation of Glutathione Content

GSH is a major antioxidant that is found in large amounts in all cells. The most important function of GSH is to provide protection against oxidative damage induced by reactive oxygen species.

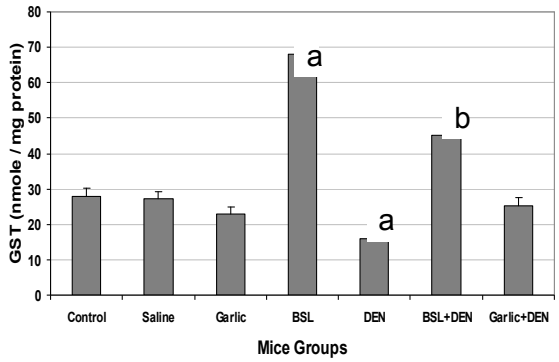


Figure 8: Anti-initiating activity through detoxification of the carcinogen: The effect of BSL on GST in mice liver tissue homogenate. Results were expressed as nmole/mg protein (Mean±S.E.).
a Significantly different from control gp at (*P<0.05*).
b Significantly different from DEN gp at (*P<0.05*).

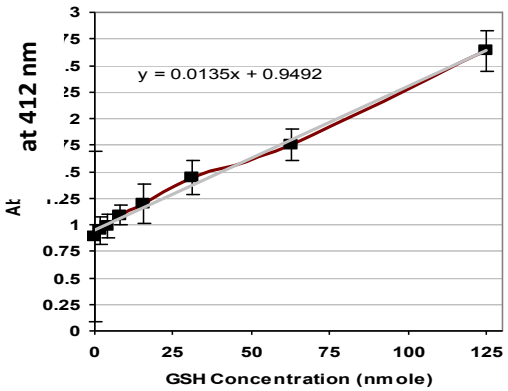


Figure 9: GSH standard curve (nmole)

Control, saline, garlic, BSL, DEN, BSL+DEN and garlic+DEN treated groups recorded 31.3, 29.8, 25.7, 29.2, 17.5, 21.4 and 27.5 nmole/mg protein GSH content, respectively as shown in figure (10). The antioxidant protein GSH content in control and saline groups was not significantly altered by administration of garlic or BSL. Administration of DEN significantly (*P<0.05*) decreased GSH level by 1.8 times. Treatment with BSL after DEN was significantly (*P<0.05*) enhanced GSH level to 1.2-fold of the DEN group. Also, treatment with garlic after DEN was significantly (*P<0.05*) enhanced GSH level compared with only DEN treated group.

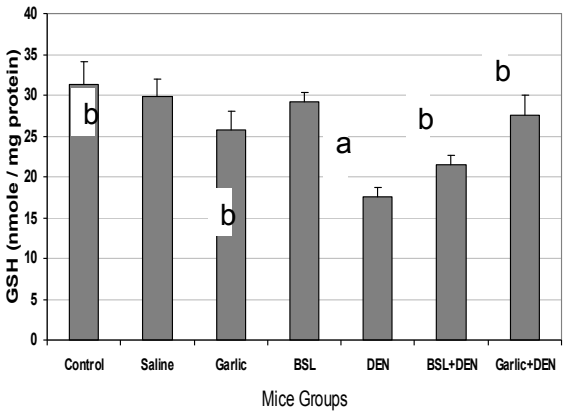


Figure 10: Antioxidant activity of BSL as investigated by its effect on GSH content (Mean±S.E) in mice liver tissue homogenate. Results were expressed as nmole/mg protein
a Significantly different from control gp at (*P<0.05*).
b Significantly different from DEN gp at (*P<0.05*).

3.2.3.. Lipid Peroxidation (LPO)

LPO is a useful marker of oxidative stress because it is linked to increased production of ROS when CYP metabolizes DEN.

LPO was assessed in mice by measuring the thiobarbituric acid reacting substances (TBARS) formations and expressed in terms of malondialdehyde (MDA) formed per mg protein.

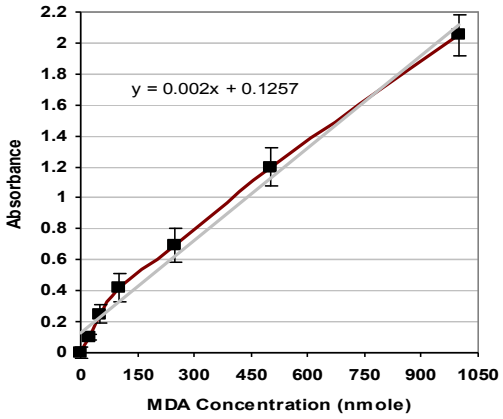


Figure 11: MDA standard curve (nmole)

As shown in figure (12), LPO values were 27.8, 27.2, 23, 68.1, 45.3 and 181.7 nmole of control, saline, garlic, BSL, DEN, BSL+DEN and garlic+DEN groups, respectively. According to these results, BSL could be recognized as significant LPO enhancer (*P<0.05*) to 2.4 fold of the control where DEN

injection elevated significantly ($P<0.05$) LPO to 5.8 fold of control. This elevation significantly ($P<0.05$) decreased by BSL administration and was insignificantly altered by garlic administration.

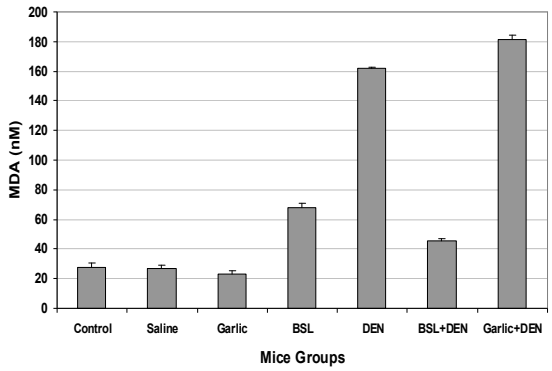


Figure 12: Anti-initiating activity through modulating LPO in the mice: The effect of BSL on MDA in mice liver tissue homogenate. Results were expressed as nmole Mean±S.E.
a Significantly different from control gp at ($P<0.05$).
b Significantly different from DEN gp at ($P<0.05$).

3.2.4. Immunochemical Evaluation of the Initiation Marker Glutathione-S-Transferase-P (GST-P)

The placental form of GST is possibly the best marker for early detection of preneoplastic (initiated) cells in the chemical hepatocarcinogenesis models. GST-P expression appears very early in initiated hepatocytes and persists in foci, nodules, and cancer but not in surrounding normal hepatocytes.

The results of GST-P immunohistochemical staining in the mice livers showed absence of initiated cells (no GST-P expression in the hepatocytes) in control, saline, BSL and garlic groups where, mice received DEN alone showed a high presence of initiated cells which appeared in solitary and colony forms. BSL highly decreased initiated cells when administrated before and after DEN injection in comparison with DEN group where treatment with garlic before and after DEN decreased the appearance of the initiated cells to lesser extent than BSL (Fig. 13).

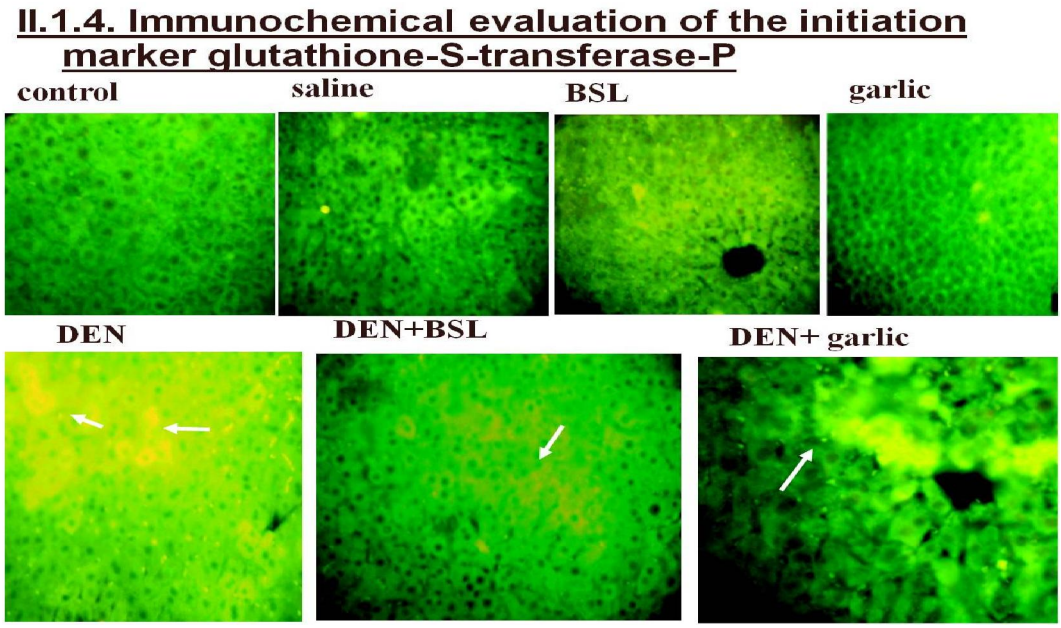


Fig (13): Photograph of a GST-P immunohistochemical staining in the sections of mice livers. (The GST-P positive hepatocytes (initiated cells) are stained yellow while normal hepatocytes are green). Cells were visualized by fluorescence microscope (x 400), black arrows referred to colony initiated cells and white arrows referred to solitary initiated cells.

3.2.5.. Estimation of Histone Deacetylase Activity

HDACs play an essential role in the regulation of cell proliferation and apoptosis. Inhibition of HDACs has been implicated to modulate transcription and to induce apoptosis or differentiation in cancer cells. The activity of HDAC was measured using a colorimetric assay kit. As shown in figure (14), HDAC activity were 109.7, 114, 105.5, 52.6, 155.9 and 67.6 µM deacetylated substrate / mg protein for control, saline, garlic, BSL, DEN, BSL+DEN and garlic+DEN groups, respectively.

The results showed that BSL had a highly significant ($P<0.05$) inhibitory effect on HDAC activity. It decreased HDAC activity to 2 fold of control. Control, saline and garlic groups showed a HDAC activity between 105 and 114 µM deacetylated substrate / mg protein. This value was significantly elevated in DEN group to 1.4 fold and was highly significant ($P<0.05$) inhibited by administration of BSL. Where, garlic was not possessed inhibitory effect on HDAC.

4.DISCUSSION

In recent years, the morbidity and mortality of cancer still reaches a high plateau and is a major public health problem worldwide. Chemoprevention i.e. the use of either synthetic or naturally occurring agents to inhibit pre-cancerous events, has become recognized as a plausible, cost-effective and necessary approach to reduce cancer morbidity and mortality (Hong and Sporn, 1997; Wattenberg, 1997; Sporn and Suh, 2002). So, searching for a new compound for the treatment and prevention of cancer is the aim of numerous studies and the aim of our study. A sulphated levan derived from the bacterial strain "*Bacillus subtilis*" and its chemopreventive and immunomodulatory activities were investigated using series of bioassays involved *in vitro* and *in vivo* assays target the initiation of hepatocarcinogenesis.

DEN, is an electrophilic carcinogen which may interact with the large nucleophilic pool of GSH, thereby reducing the macromolecules and carcinogen interaction (Ketterer and Meyer, 1989). Halting of the carcinogen danger is one of the effective cancer chemoprevention strategies, through targeting the inhibition of the carcinogens metabolic activators, e.g. CYP1A, and the induction of the carcinogen detoxification enzymes, e.g. GSTs (Talalay *et al*., 1995). Phase I enzymes (cytochromes P450) activates xenobiotics by addition of functional groups which render these compounds more water-soluble. A large number of carcinogens are metabolized by the CYP enzymes to chemically reactive species that covalently bind to the DNA and promote carcinogenesis. Another potentially damaging effect of phase I enzymes is the production of oxygen free radicals that occurs as a result of CYP activity (Percival, 1997). In addition, a decrease or inhibition of CYP1a1 mRNA expression is interpreted as the possible mechanism of chemoprevention involving either inhibition of the CYPs required for metabolizing carcinogens, such as the CYP1 family or the induction of phase II enzymes such as the UGT1A1 and UGT1A6 (Kensler, 1997).

In the present study, BSL was demonstrated as an inhibitor of CYP1A activity *in vitro*. The CYP1A inhibition by BSL might be due to the influence of the sulphation. Down-regulation of the CYP1A activity by BSL might likely reduce the initiation of chemically induced carcinogenesis through blocking of the metabolic activation of pro-carcinogens to their ultimate forms. In agreement with this work, several studies demonstrated that natural polysaccharides and modified natural polysaccharides, especially sulphated polysaccharides, that revealed their promising tumour anti-initiating possessed a similar inhibition in CYP1A and induction of GST *(Gamal-Eldeen et al., 2006)*

GSH, the major cytosolic thiol, is a key determinant of the cellular response to oxidative stress, which helps in destruction of hydrogen peroxide, lipid peroxides and free radicals (Aggarwal and Shishodia, 2006). GSH antioxidant system is also a crucial factor in the development of the immune response by the immune cells and is a substrate for eliminating various toxic chemicals during the course of biotransformation in phase II detoxification enzyme systems, e.g. GST (Habig *et al*., 1974; Perquin *et al*., 2000). In the current study, it was found that, BSL significantly enhanced GSH content of HpG2 cells *in vitro*. The elevated level of GSH induced by BSL protected cellular proteins against oxidation (via GSH redox cycle) and also directly detoxified reactive oxygen species and/or neutralized reactive intermediate species generated from exposure to xenobiotics, including chemical carcinogens.

GST is a soluble protein located in cytosol and plays an important role in detoxification and excretion of xenobiotics (Bansal *et al*., 2005). GST catalyzes the conjugation of the thiol functional groups of GSH to electrophilic xenobiotics and results in increasing solubility. The xenobiotic–GSH conjugate is then either eliminated or converted to mercapturic acid (Rao *et al*., 2006). Since GST

increases solubility of hydrophobic substances, it plays an important role in storage and excretion of xenobiotics. Induction of xenobiotic detoxifying enzymes is an additional mechanism by which antioxidant rich extracts may act as anticarcinogens as they compete with steps in xenobiotic activation and metabolize toxic compounds to non-toxic ones (Bergmeyer *et al*., 1974). In the present study, BSL was found inducing GST activity of HpG2 cells *in vitro*. The induction of the detoxification enzyme might be due to activation of the expression of the enzyme. The balance between the abilities of potential chemoprotective agents to inhibit P450s and induce phase II enzymes may be critical for their capacity to function as anticarcinogenic agents (Krajka-Kuzniak and Baer-Dubowska, 2003). In the present study, BSL treatment affected all the components of the antioxidant defence system analysed in the same 'direction', namely towards counteracting oxidative stress. In agreement with our results, Gamal-Eldeen *et al*. (2009) demonstrate that different fractions of water-soluble polysaccharide extract derived from *S. latifolium* were effective as anti-initiation agent via its significant inhibition of CYP1A and induction of GSTs. Estimation of the antioxidant activity of *BSL* indicated that it is inactive scavenger against DPPH.

In the mouse liver carcinogenesis models, a variety of enzyme-altered condition has been studied for their relevance to preneoplastic and neoplastic developments. For example, an immuno-histochemically demonstrable enzyme marker, GST-P has been utilized for the identification of liver preneoplastic focal lesions. Over expression of GST-P has attracted great interest because of its relationship to carcinogenesis and human cancers (Katagiri *et al*., 1993; Hamada *et al*., 1994). Using immunohistochemical examination, we found that GST-P expression decreased in mice treated with BSL before and after DEN injection when compared to DEN induced mice, causing a decrease in the liver initiated cells. Recent studies found that, the molecular mechanisms involved in the regulation of GST-P gene are mediated by an antioxidant-responsive element (ARE) and the activator protein-1-responsive element; both are located on GST-P gene promoter and/or enhancer regions (Okuda *et al*., 1989; Henderson *et al*., 1998). Since, BSL treatment affected all the components of the antioxidant defence system and this effect may be responsible for high decreasing effect of BSL in the GST-P expression. These data are supported by the fact that, butyrate which is colonic fermentation product of non-starch polysaccharides has been shown to induce several isoforms of GST, including GSTP1, in vascular smooth muscle cells (Ranganna *et al*., 2007).

Consequently, it could be concluded that the protective effect of BSL on hepatocellular transformation was more effective than that of garlic, since it inhibited HDAC activity, decreased LPO level where garlic insignificantly altered these components. And garlic increased GST activity and GST-P expression to lesser extent than BSL. Where, garlic was more effective in the enhancement of GSH than BSL. Altogether, the results of the present study indicated that BSL could be represented as promising cancer chemopreventive agent against hepatocarcinogenesis, since it had tumour anti-initiating activity via its protective modulation of carcinogen metabolism.

Acknowledgment:

Thanks for Ass. Prof., Dr. Mona A.Esawy, Ass. Prof. Dr Azza M. Abdel-Fattah and Ass. Prof Dr. Wafaa A. Helmy Department of Natural and Microbial Products Chemistry, National Research Centre, Dokki, Cairo, Egypt For purchasing BSl

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11/12/2011