

Egypt. Acad. J. Biolog. Sci., 12(2): 59-75(2020)



Egyptian Academic Journal of Biological Sciences D. Histology & Histochemistry ISSN 2090 – 0775 <u>http://eajbsd.journals.ekb.eg</u>



DMBA and *Lagenaria Siceraria* Biological Effects: 1: Is it Possible for *Lagenaria Siceraria* to be a Treatment for The Carcinogenesis in The Ileum of DMBA-Induced Male Swiss Albino Mice

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ARTICLE INFO

Article History Received:8/10/2020 Accepted:18/12/2020

Keywords:

DMBA; *Lagenaria siceraria*; Cell proliferation; Tumor, Cell Biology; Histochemistry; Immunohistochemistry; Histopathology.

ABSTRACT

Nowadays, tumors are recorded a high percentage among human beings due to exposure to different carcinogens. DMBA and mouse will be established models for study tumorigeneses. A trial was done to suppress cell proliferation using Lagenaria siceraria (LS). Mice were divided into C: control animals received a single injection of corn oil and a daily dose of distilled water, D: animals received a single dose of DMBA and 91 days ago, mice received morning and night daily oral dose of distilled water for 30 days, and DL: Animals received a single dose of DMBA (10 mg/ 100 g b.w.) and 91 days ago, mice received morning and night daily oral dose of LS (100 mg/ Mouse) for 30 days. Our results showed a single dose of DMBA (10 mg/ 100 g b.w.) has a stimulatory effect on the cellular activity, DNA synthesis, and cell proliferation of epithelial cells in the ileum, however, LS mostly treated this effect. BAX and BCL2 immunohistochemistry revealed DMBA induced excessive cell proliferation and hyperplasia (dysplasia), while LS almost treated this effect of DMBA. These results suggest LS modulates the toxic effect induced by DMBA in the ileum. A promising data for cancer herbal therapy in human beings.

INTRODUCTION

Cancer is defined as a genetic disease caused by cellular genomic alterations including insertions, deletions, and chromosomal aberrations. These cellular alterations generated uncontrolled cell growth that is evident in tumors (Stransky and Galante, 2010).

Moreover, poor prognosis, diagnosis, and therapies of cancer are associated with severities, location, and resistance against drugs amongst others (Duojiao *et al.*, 2012). Tumor growth is correlated with the invasion and spread of cells from the original sites to other sites in the body through a process termed metastasis (Alberts *et al.*, 2014).

Polycyclic aromatic hydrocarbons such as DMBA are considered carcinogenic models in rodents (Miller, 1970) and mice (Currier *et al.*, 2005) experimentally.

Several models of radiation carcinogenesis (Wincewicz et al., 2010) and by chemical agents (Jee et al., 2011) have found through using different concentrations to induce tumors, like 3-methylcholanthrene (Shi et al., 2003), N-methyl- N-nitrosourea al.. 2008)(Maffini et and 7,12dimethylbenzanthracene (Medina, 1974). While, DMBA and N-methyl-Nnitrosourea are considered the most carcinogens recently used (Medina & Kittrell, 2005).

DMBA-treated Gadd45a-null mice possess noticeable multiples tumors than wild ones, with many developing multiple different malignant tumors (Hollander et al., 1999). The tumorigenicity was positively growth correlated with the and transformation characters observed for Gadd45-null cells in cell culture. Since Gadd45a-null MEF is growing fast and immortal. Then, it is transformed by a single oncogene (activated ras) and displays genomic instability. Therefore, carcinogenesis in a multistage way is susceptible more to malignant transformation by additional cellular events (Hollander et al., 2001).

Lagenaria siceraria (LS) joined to herbal plant; supplied an anticarcinogenic effect via its antioxidant role and cytotoxic activity. Lagenaria siceraria fruits possess a water-soluble polysaccharide composed of 3-O-acetyl methyl-á-d-galacturonate, methyl-á-dgalacturonate, and â-dgalactose. In vitro, its polysaccharide constituent has cytotoxic activity against the human adenocarcinoma (MCF-7) (Ghosh *et al.*, 2009).

"From a biological point of view, the chemistry of cellular structure and function is well established. Therefore. studying the chemical components in their natural locations in the cells and tissues, and tracking the changes that occur to them under conditions. abnormal whether pathological or experimental, is very important, as any change that occurs to these substances is often accompanied by some pathological manifestations" (Aref et al., 2021).

The aim of the present work, providing the answer to the question that in the research's title, by assessing the therapeutic efficacy role of LS against the carcinogenic effect induced by DMBA, through the present cell biological, histochemical, immunohistochemical, and histopathological studies.

MATERIALS AND METHODS Animals:

A total number of thirty adult male Swiss albino mice weighed 25 ± 2 gm and aged 90 ± 2 days were obtained from the Autoradiographic lab. of Cell Biology and Immunology studies. Faculty of Science, South Vallev University, under supervision of Dr. Abdelbaset Aref Mohamed. All male mice were subjected to the same conditions of an artificial light-dark cycle (12h-12h), temperature (23 \pm 2 c°), and humidity (37-40 %). They were supplied standard food and water ad libitum. Experiments of this research were conducted in the lab. achieve stability of environmental conditions, the separation between treated animals and control ones, and IACUC targets.

IACUC approval of project number: IACUC-SVU-EGYPT/ SVU/F. S.

/4-2016/2 (1).

Chemicals:

1. 9,10-dimethyl-1,2benzanthracene (DMBA)

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(Sigma Chemical Co., St. Louis, Mo) was dissolved in corn oil at a concentration of 50 mg/ml.

2. LS: special preparation of *Lagenaria Siceraria* (Aref1) was prepared via Dr. Abdelbaset Aref (Aref *et al.*, 2018 & Aref *et al.*, 2021).

Experimental Design:

For the study the short-term time exposure of DMBA for (121 days):

Thirty male Swiss albino mice were divided into 3 groups, each containing 10 males as following.

The control group (Group C): Animals were injected with a single injection of corn oil (0.2 ml/ 100 g b.w.) subcutaneously and received a daily dose of distilled water (0.2 ml/ 100 g b.w.).

DMBA group (Group D): Animals were injected with a single dose of DMBA (10 mg/ 100 g b.w.) subcutaneously and 91 days ago, male mice were received morning and night daily oral doses of distilled water (0.2ml/ 100 g b.w.) for 30 days.

DBMA-LS group (Group DL): Animals were injected with a single dose of DMBA (10 mg/ 100 g b.w.) subcutaneously and 91 days ago, male mice were received morning and night daily oral dose of LS (100 μ g/ 100 g b.w.) for 30 days.

The dose and number of doses of Lagenaria siceraria are detrained according to the animal therapeutic (Aref *et al.*, 2021) where the LS using as treatment gave good results by this dose, and according to human therapeutics based on the opinion of doctor's cancer oncology. The administrations of LS were by gavage.

All animals were anesthetized by Chloroform and were sacrificed the day following the last oral dose, ensuing an exposure time of 31 days.

Microscopic Examination (Cell Biology, Histochemistry, and Histopathology):

Tissue samples from the ileum of male Swiss albino mice were dissected from each animal of each group. Specimens of ileum were fixed in neutral formalin 10%, followed by dehydration in ascending series of ethanol (70-100%), cleared in xylene, embedded in paraffin wax, and sectioned at 5 microns thickness. The ileum sections were stained according to the microscopical examinations and techniques as the following:

1-Cell Biological & Histopathological Examinations:

The paraffin sections of the ileum were stained in Hematoxylin and eosin stain and used for cell biological and histopathological examinations.

2-Histochemical Examinations of Ileum Tissue:

The identification of histochemical materials was done as the following techniques;

- Feulgen reaction for DNA content change.
- RNA content by toluidine blue technique.
- Protein content by bromophenol blue technique.
- Collagen content by Masson's trichrome.
- Polysaccharides by the periodic acid-Schiff reaction

The paraffin sections of the ileum are examined using a light microscope (Bancroft and Gamble) for Cell biological, Histochemical, and Histopathological studies.

All histochemical and pathological methods were applied according to Carleton *et. al.* (1980).

Karyometric Studies:

The volumes of cell nuclei of the ileum were performed using a camera program (LAS ZA). The volume of a total number of (200) cell nuclei in H & E-stained ileum were measured/animal. According to nuclear shape, the measurements were carried out in two ways according to Lewinski, *et al.* (1984).

1. The first way concerned the oval nuclei of cells. The volume of the nucleus was obtained utilizing revolution spheroid: $V=\pi/6 \text{ AP}^2$

2. The second way concerned the rounded nuclei of cells where the formula of the sphere was applied: $V = \frac{4}{3} \pi r^3$. Where: A - longer diameter & P- short diameter.

3-Immunohistochemistry:

Paraffin sections of the small intestine were sectioned at 4µm, deparaffinized and stained with immunehistochemical reaction for either polyclonal rabbit anti-BAX protein (ready to use, AR347-5R) or rabbit antihuman BcL2 alpha (Ready to use, AN758-5M) according to (Chun Gao & Ai-Ying Wang, 2009; KOGA et al., 1999) and counterstained with Mayer's hematoxylin. the light microscopical examination was made using Olympus (CX21) and sections were photographed by Micro cam Ver 5.6. The results of the immunostaining were analyzed semi-quantitatively as previously described by KOGA et al. (1999). The intensity (amount of staining) of positive reaction of BAX and Bcl2 was estimated as follows: No reaction, mild, moderate, and strong (Chun Gao & Ai-Ying Wang, 2009; KOGA et al., 1999). The antibodies concentration used in the immunohistochemistry study: Bcl2: recommended dilution for

concentrated Ab: 1-50 in HK941-YAK. BAX: recommended dilution for concentrated Ab: 10-20 in HK 156-5k.

RESULTS Cell Biological Changes (Karyometric studies - Hematoxylin and eosin stain):

In the ileum of male mice of the control (C), DMBA group (D), and DMBA and LS group (DL), the values of the mean volume of nuclei of epithelial cells lining villi were $33.9 \pm 1.4 \mu m$, $157.9 \pm 3.4 \mu m$ and $72.2 \pm 4 \mu m$ respectively (Table. 1).

From the quantitative point of view, the short-term exposure time of DMBA for 120 days increased by 365 % the value of the mean volume of nuclei of epithelial cells lining villi in the ileum of mice (D) versus those of the control animals.

While the short-term exposure time of DMBA for 120 days and the treatment daily morning and night with LS for the last 30 days decreased by 54.3 % and increased by 113% the value of the mean volume of nuclei of epithelial cells lining villi in the ileum of mice (DL) versus those of the mice of groups (D) and (C), respectively.

From the cell biological point of view, DMBA had a high stimulatory effect on the cellular activities of the epithelial cells lining villi in the ileum of the male mice. In contrast, the treatment via LS had a high inhibitory effect on DMBA efficacy on cellular activities. LS has a high anti-effectcellular activity-induced-DMBA on the ileum of male mice.

Ileum	Experiment 1			
Groups Measurements	С	D	DL	
Mean volume nuclei (±S.E.) of epithelial cells lining villi	33.9 ± 1.4	157±3.4	72.2±3.7	
(S%) or (I%) of mean		S= 365 %		
volume nuclei of epithelial			1= 54.3 %	
cells lining villi			S=113 %	

Table 1: Mean volume of nuclei in epithelial cells lining villi of the ileum.

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Mean volume of nuclei in epithelial cells lining villi in the ileum of control male mice (**C**) and DMBAexported-male mice for 120 days (**D**) and DMBA exported-male mice for 120 days and treated daily morning and night with *Lagenaria siceraria* oil in last 30 days (**DL**). The percentage of mean volume stimulation (**S** %) or inhibition (**I** %) is given. S.E.: standard error of the mean.

Microsc	opic	Analysis	of				
Histochemical Components:							
1-DNA	Content	Changes	(Feulgen				
reaction	ı) :						

The epithelial cells lining villi of ileum of the mice in the group (C) exhibited remarkable stained coloration with moderate DNA content in their nuclei, while the ileum of mice in the group (D) and Group (DL) showed very deep and deep colorations with very high and high DNA contents, respectively (Figs. 1 a, b, c, d & Table 2).

Table 2: The score of histochemical special stains of Group C (control), Group D (9,10-dimethyl-1,2-benzanthracene, DMBA), Group DL (DMBA and lagenaria siceraria), in the ileum were classified according to severity into severe (+++), moderate (++), mild (+) and absent (-).

Groups						
Lesions	Group C	Group D	Group DL			
	-					
Mallory trichrome technique						
Interstitial fibrosis	-	+++	+			
Perivascular fibrosis	-	+++	+			
Blue stained coloration of dense collagen fibers	-	+++	+			
PAS technique						
Glycogen precipitation inside cells	+++	++	++			
Red stained coloration of glycogen content	+++	++	++			
Bromophenol technique						
Protein distribution inside cells	+++	+	+			
blue stained coloration of protein content	+++	+	+			
DNA stain						
DNA contents	+++	+	+			
Red stained coloration	+++	+	+			
RNA stain						
RNA contents	+++	+	+			
Blue stained coloration	+++	+	+			

Absent (-), Mild (+), Moderate (++), and severe (+++).

The DNA content revealed a very high increase in the mice of the group (D) versus that of the mice of the group (C), while the mice of the group (DL) revealed a high decrease in DNA content than with the mice of the group (D) and not yet reached almost to those of the control mice .

From the cell biological and histochemical points of view, DMBA had very high stimulatory effects on the cellular activity, DNA synthesis, DNA content, and cell proliferation of the epithelial cells lining villi of ileum of the male mice. Also, the treatment with LS had a high inhibitory effect on the DMBA efficacy, but this efficacy was not treated completely yet (Fig. 1). LS has a high anti-proliferative effect induced-DMBA on the ileum of the male mice.

2-RNA Content Changes (Toluidine blue technique):

The ileum of control male albino rats showed deeply stained RNA contents with normal bluish discoloration (Fig. 2 a & Table 2). The ileum of a group (2) showed moderate stained RNA with moderate blue coloration (Figs. 2 b & c & Table 2). Ileum of a group (3) showed deeply stained RNA contents with high blue color (Fig. 2 d & Table 2).

DMBA made increased RNA in the normal epithelial cell while the treatment with *Lagenaria siceraria* highly increased RNA in the ileum of DMBA-induced male mice. From a Histopathological point of view, the carcinogen DMBA has an inhibitory effect on RNA synthesis while the treatment with *Lagenaria siceraria* repair DMBA efficacy reached the normal case.

3-Protein Content (Bromophenol blue technique):

The ileum of the control male albino rats showed normally distributed protein (Fig. 3 a & Table 2). Ileum of the group (2) showed deeply infiltrated protein with deeply stained blue color (Figs. 3 b & c & Table 2). Ileum of the group (3) showed moderate protein with moderate stained blue color (Fig. 3 d & Table 2).

DMBA highly enhancement the proteins in the normal cell of the ileum, in contrast, *Lagenaria siceraria* decreased the protein in the ileum of DMBA-induced mice.

From the histochemical concept, the carcinogen DMBA stimulates the protein synthesis in normal ileum but the treatment with *Lagenaria siceraria* resistance this the carcinogenic DMB efficacy reached the normal case.

4-Collagen Content (Masson' s trichrome technique):

Masson trichrome stain showed normal collagen fibers infiltration of the control ileum (Fig. 4 a & Table 2). The ileum of the group (D) of DMBA injection showed a noticeable degree of collagen fibers infiltration (Figs. 4 b and 2 c & Table 2). The ileum of the group (DL) injected with DMBA and LS showed slight infiltration of the collagen fibers (Fig. 5 d & Table 2).

Histochemically, DMBA has a stimulatory effect on collagen fibers

infiltration. In contrast, LS has an inhibitory effect on DMBA efficacy.

5-Polysaccharide's Content (the periodic acid-Schiff reaction):

The ileum of control male albino rats showed normal distribution polysaccharides in the intestine with intense stained red color (Fig. 5 a & Table 2). Ileum of a group 9,10-dimethyl-1,2-(2)of injection benzanthracene (DMBA) polysaccharides showing weak distribution in between the intestinal villi (Figs. 5 b & c & Table 2). Ileum of group (3) recorded moderate а polysaccharides distribution with less intensely stained red color (Fig. 5 d & Table 2).

DMBA slightly decreased the normal polysaccharides and *Lagenaria siceraria* slightly increased DMBAinduced polysaccharides.

Histochemically, both the carcinogen DMBA and *Lagenaria siceraria* nearly have no efficacy polysaccharides formation in the ileum of male mice.

Histopathological Changes (Hematoxylin and eosin stain):

Histopathologically, the ileum of the control albino mice (C) showed a normally arranged ileum (Fig. 6 a & Table 3). The ileum of the group (D) detected cellular hyperplasia and abnormal cell proliferation with the disorganized mucosal structure which is characterized by irregular crypt and villi structure (dysplasia) (Figs. 6 b and 6 c & Table 3). The ileum of a group (DL) showed normal intestinal villi (Fig. 6 d & Table 3).

Table 3: The histopathological score of Group C (control), Group D (9,10-dimethyl-1,2-benzanthracene, DMBA), and Group DL (DMBA and *Lagenaria siceraria*), stained with Hematoxylin and eosin in ileum were classified according to severity into severe (+++), moderate (++), mild (+) and absent (-).

Groups	Group C	Group D	Group DL
Tumor cells	-	+++	+++
Necrosis of cells	-	+++	+++
Congestion of blood vessels	-	+++	+++

Absent (-), Mild (+), Moderate (++), and severe (+++).

From a histopathological point of view, DMBA had carcinogenic effects on the ileum of male mice. In contrast, the treatment via LS had a high inhibitory effect on the carcinogenic effects of DMBA.

Immunohistochemistry:

Immunohistochemically, normal mice ileum had a moderate positive staining reaction of BAX in the enterocytes, and the crypts (Fig. 7 a). While the mild positive reaction of Bcl2 in the enterocytes was observed in normal mice ileum (Fig. 7 b). Mice ileum treated with DMBA (Group D) showed a strong positive staining reaction of BAX in the enterocytes, crypts, submucosa, and serosa (Fig. 7 c). The mild positive reaction of Bcl2 in the ileum of mice treated with DMBA (Fig. 7 d). Treated mice's ileum with DMBA and special preparation of *Lagenaria Siceraria* (Group DL) showed a moderate staining reaction of both BAX (Fig. 7 e) and Bcl2 (Fig. 7 f).

Immunohistochemically, DMBA showed a strong and mild positive reaction of BAX and Bcl2 respectively in the ileum of mice in normal male mice. In contrast, *Lagenaria cesarian* moderate staining reaction of both BAX and Bcl2.

From the histopathological and Immunohistochemical points of view, DMBA has high carcinogenic effects and in contrast, LS has high anticarcinogenic effects induced-DMBA on the ileum of male mice.



Fig. 1. Photomicrographs of the DNA content in the ileum of male Swiss albino mice. (a) Ileum of control male albino mice showing normally stained DNA contents. (b and c) Ileum of DMBA group showing deeply stained DNA with an intense red coloration. (d) Ileum of DMBA and LS group showing moderately stained DNA contents with moderate red color. Arrows indicate the DNA content.



Fig. 2. Photomicrographs of the RNA content in the ileum of male Swiss albino mice. (a) Ileum of control male albino mice showing deeply stained RNA contents with normal bluish discoloration. (b and c) Ileum of DMBA group showing moderate stained RNA with a moderate blue coloration. (d) Ileum of DMBA and LS group showing deep RNA contents with high blue color. Arrows indicate the RNA content.



Fig. 3. Photomicrographs of the protein content in the ileum of male Swiss albino mice. (a) Ileum of control albino mice showing normal distribution of the protein content. (b and c) Ileum of DMBA group showing deep infiltration of protein with a deep blue coloration. (d) Ileum of DMBA-LS group showing moderate protein with moderate blue color. Arrows indicate the protein materials.





Fig. 4. Photomicrographs of the collagen content in the ileum of male Swiss albino mice. (a) Ileum of control albino mice showing normal infiltration of the collagen fibers. (b and c) Ileum of DMBA group showing noticeable infiltration of the collagen fibers. (d) Ileum of DMBA-LS group showing slight infiltration of the collagen fibers. Arrows indicate the collagen fibers.



Fig. 5. Photomicrographs of the polysaccharides content in the ileum of male Swiss albino mice. (a) Ileum of control male albino mice showing normal polysaccharides distribution with intense red coloration. (b and c) Ileum of DMBA group showing weak polysaccharides. (d) Ileum of DMBA and LS group showing moderate polysaccharides distribution with a less intensely red coloration. Arrows indicate the polysaccharide's content.



Fig. 6. Photomicrographs of the intestinal villi in the ileum of male Swiss albino mice. (a) Ileum of control albino mice showing normally arranged Intestinal villi. (b and c) Ileum of DMBA group showing excessive cell proliferation of the intestinal epithelium (dysplasia). (d) Ileum of DMBA-LS group showing normal intestinal villi.



Fig. 7. Immunohistochemically evaluation of BAX (a, c and e) or BCL2 (b, d, and F) in the ileum of male Swiss albino mice. (a) Normal mouse intestine showing strong positive staining reaction of BAX in the enterocytes (arrow), and the crypts (head arrow). (b) Normal mouse intestine showing mild positive staining reaction of Bcl2 in the enterocytes (arrow). (c) Mouse intestine treated with DMBI showing a strong positive staining reaction of BAX in the enterocytes (arrow), and the crypts (head arrow). (d) Mouse intestine treated with DMBI showing mild positive staining reaction of BcL2 in the enterocytes (arrow), and the crypts (head arrow). (d) Mouse intestine treated with DMBI showing mild positive staining reaction of BcL2 in the enterocytes (arrow), and the crypts (head arrow). (d) Mouse intestine treated with DMBI showing mild positive staining reaction of BcL2 in the enterocytes (arrow), and the crypts (head arrow). (d) Mouse intestine treated with DMBI showing mild positive staining reaction of BcL2 in the enterocytes (arrow), and the crypts (head arrow). (d) Mouse intestine treated with DMBI showing mild positive staining reaction of BcL2 in the enterocytes (arrow), and the crypts (head arrow). submucosa (s) and serosa (Se).

DISCUSSION

From the cell biological and histochemical points of view, the present results showed that DMBA has very high stimulatory effects on the cellular activity, DNA synthesis, DNA content, cell proliferation, protein and collagen fibers infiltration of the epithelial cells lining villi of ileum of the male mice. In contrast, the special preparation of *Lagenaria Siceraria* has a high inhibitory effect on DMBA efficacy.

From the Pathological and Immunohistochemical points of view, our data showed that DMBA detected cellular hyperplasia and dysplasia, and strong and mild positive reaction of BAX and Bcl2 respectively in the ileum of mice in normal male mice. In contrast, the special preparation of *Lagenaria Siceraria* showed normal intestinal villi and moderate staining reaction of both BAX and Bcl2 in the ileum of DMBA- induced male mice.

In a mature organism, every cellular community has special behavior in the process of cell proliferation that causes it to divide naturally at a rate proportional to the natural rate of loss of its cells by the programmed mortality, therefore each cellular community proliferates via its special behavior under abnormal the conditions, whether experimental stress or pathological events.

There is no or little published literature concerned of the biological effects of DMBA or/and *Lagenaria Siceraria* on the ileum of the male mice therefore, we could not compare our results with others.

Cancer has been termed as a genetic disease that has arisen by cellular genomic alterations that led to uncontrolled cell growth that is evident in tumors. DMBA induced marked cell proliferation with cellular disorganization in the ileum tissues with loss of glycogen content in the cell and increased collagen fibers distribution. The chemical carcinogen, 9,10-dimethylbenzylamine (a) anthracen (DMBA) exhibited а stimulatory effect on both the cell proliferation and rate of DNA (at intervals 60, 120, 150 days post-DMBA) (Carter et al., 1988). DMBA was previously used to induce breast cancer that initially appears at the 10th week from the first dose. Where, the peak majority of tumors appeared after the 16th week (Medina and Kittrell, 2005).

The higher cases of breast cancer were correlated with increased DMBA dose that generates cancers via activation of aryl hydrocarbon receptor/transcription factor (AhR), a member of the family Per- ARNT-Sim (PAS), of transcription factors, results in circadian rhythms and hypoxia responses (Trombino *et al.*, 2000), where

DMBA increase AhR activity and induction of some enzymes CYP1A1, CYP1A2 that involved in xenobiotic metabolism (Hoffman and Gay, 1981). Such enzymes participate role to convert the DMBA into epoxide mutagenic intermediates, the 7hydroxymethyl-12methylbezantracene (7-HMBA), 12-hydroxymethyl-7metilbezantracene (12-HMBA), and 7.12dimetilbezantracene that form DNA adducts. These metabolites activate the AhR, mainly that complex with the heat-shock protein 90 (hsp90), besides other non-mentioned proteins. Therefore. free the AhR is to translocate to the nucleus and dimerize with cofactor ARNT (aryl hydrocarbon receptor nuclear translocator), resulted in specific transcription regulatory sequences in DNA. Immediately, it induces the expression of genes encoding growth factors and protooncogenes, including c-erb-2, cmyc, c-fos, c-Jun, and H-ras (Trombino et al., 2000).

Lagenaria siceraria modulates the toxic effect induced by DMBA. It plays an important role in the elevation of the immunologic case through antioxidant, immunomodulatory, antiinflammatory, free radical scavenging, and cytotoxic, effect (Aslam and Najam, 2013). Lagenaria siceraria has also been isolated from LS seeds, where possesses antitumor, it immunoprotective, and antiproliferative properties. The fruits possess significant hepatoprotective activity. LS is traditionally used in systematic disorders and various free radicalinduced diseases (Saha et al., 2011).

Immunohistochemistry, the Bcl2 family is a group of proteins including BAX such as Bad, Bak, Bik/Nbk, Bid, Bag-1. These proteins have pro-apoptotic activity (activate intrinsic the apoptotic pathway in the mitochondria). While, BCL-2, BCL-xl, BCL-w, A1, MCL-1, inhibit apoptosis. The equilibrium of pro- versus antiapoptotic BCL-2 proteins offers appropriate regulation of programmed cell death and keeps on organism health. Since unbalanced, the BCL-2 family facilitates tumor development and prevents cell apoptosis and resistance to cancer therapy (Pluta *et al.*, 2011, Srinivas et al., 2000).

These gene protein family (both BAX and Bcl2) have been used as markers for several pathological entities such as pediatric acute lymphoblastic leukemia (Srinivas et al., 2000), breast cancer (Pluta et al., 2011), subacute thyroiditis (KOGA et al., 1999), (Koçak,2003), colorectal psoriasis cancer (Khodapasand et al., 2015), in and hyperplasia of normal the endometrium (Tommi et al., 2002), Small intestine adenocarcinoma (Gao and Ai-Ying Wang2009), in type 2 diabetes mellitus (Hasnan et al., 2010); in cartilage and bone cells after highdose corticosterone treatment in rats (Mocetti et al., 2001).

In the present work, from the cell biological point of view, DMBA had a high stimulatory effect on the cellular activities of the epithelial cells lining villi in the ileum of the male mice. In contrast, the treatment via LS had a high inhibitory effect on DMBA efficacy on cellular activities. LS has Anti-cellular activities-effect that were induced by DMBA in the ileum of male mice in

From the cell biological and histochemical points of view, DMBA had very high stimulatory effects on the cellular activity, DNA synthesis, DNA content, and cell proliferation of the epithelial cells lining villi of the ileum of the male mice. Also, the treatment with LS had a high inhibitory effect on the carcinogenic DMBA efficacy, but this efficacy was not treated completely yet. It is clear that LS has a high antiproliferative-effect that were induced by DMBA in the ileum of the male mice.

Histochemically, the carcinogen DMBA has a stimulatory effect on both protein and collagen fibers infiltration. In contrast, LS has an inhibitory effect on the carcinogenic DMBA efficacy.

From the histochemical concept, while the carcinogen DMBA has an efficacy RNA, protein synthesis, and collagen fibers, *Lagenaria siceraria* resistance and repair this the carcinogenic DMB efficacy reached the normal case.

From a histopathological point of view, DMBA had carcinogenic effects on the ileum of male mice. In contrast, the treatment via LS had a high inhibitory effect on the carcinogenic effects of DMBA.

From the histopathological and Immunohistochemical points of view, DMBA has high carcinogenic effects and in contrast, LS has high anticarcinogenic-effects that were induced by DMBA in the ileum of male mice.

In the present study, immunohistochemical analysis of both BAX and Bcl2 in mice ileum treated with DMBA (D), DMBA and Lagenaria siceraria (DL), and normal mice intestine (C) had shown strong, moderate, and mild intensity of both BAX and Bcl2. Overexpression of in the present study was BAX predominant over the BCl2 in the treated group with DMBA (D). These results may suggest that the possibility of apoptosis could occur in the enterocytes and may lower the incidence of growth abnormalities in the intestine such as hyperplasia and/ or dysplastic changes. Gao and Ai-Ying Wang (2009) stated that increased apoptosis and expression of BAX, not Bcl2 or the BAX /Bcl-2 ratio, may play some role in the relatively lower incidence of human small intestinal carcinomas.

While the groups treated with DMBA and LS (DL) showed a similar of immune reaction intensity (moderate) of both BAX and Bcl2. These results suggested that the special preparation of DL may have a role in keeping up the balance of both functions of the genes (up and downregulation), which in turn will keep the activation of the intrinsic pathway of apoptosis in response to cellular stresses such as DNA damage, g-irradiation, oncogene activation.

CONCLUSION

The Carcinogen DMBA has biological effects on cellular activities and a proliferative, and a carcinogenic efficacy on the ileum of the DMBAinduced male mice. In contrast, *Lagenaria siceraria* has anti-cellularactivities, anti-proliferative, and anticarcinogenic effects.

Additionally, Both BAX and BCL2 immune reactions could be a helpful tool in demonstrating the abnormalities such as hyperplasia /and or dysplasia, which may have occurred before the tumor development of the ileum of male mice were induced by DMBA.

An Optimistic View of The Future:

This manuscript is part of a preclinical research project to study the short and long-term biological effects of the carcinogen (DMBA) on different tissues of the organs of male and female mice and treat the carcinogenic DMBA efficacy with a special preparation of Lagenaria siceraria. Based encouraging on the and unprecedented preclinical results of this manuscript. we will continue publishing other scientific manuscripts for this project.

The encouraging unprecedented results of this manuscript make us complete the pre-clinical trial phases and move to clinical trials, finally, we may reach the completion of treatment for various carcinomas tumors.

Ethics Approval and Consent for Participation:

The authors verify careful study of the Journal directions on the ethical publication-associated issues and complete compliance with the stated instructions

Consent for Publication:

Consent for publishing the resulting draft of the present research has been confirmed by all co-authors.

Author Statement:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the

work reported in this paper. The authors declare that this paper is a part of a research project named (biological effect of DMBA and Lagenaria siceraria) was carried out via the first author Dr. Abdel-baset Aref Mohamed Aref, and all experiments protocols approved by IACUC-SVU, were Zoology department, Faculty of South Valley University, Science. Qena, Egypt. All methods were carried out under relevant guidelines and regulations. All authors have contributed to the writing of this article. There is no conflict of interest, and that all authors have read and approved of the manuscript being submitted.

Acknowledgment

The authors thank the Cell Biology and Histochemistry Division, zoology department, Faculty of Science, South Valley University for supporting and providing the required tools and instruments. Also, the Authors thank Aboel-hagag Hossen Mohamed, the worker of Zoology Depart, SVU, Egypt.

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