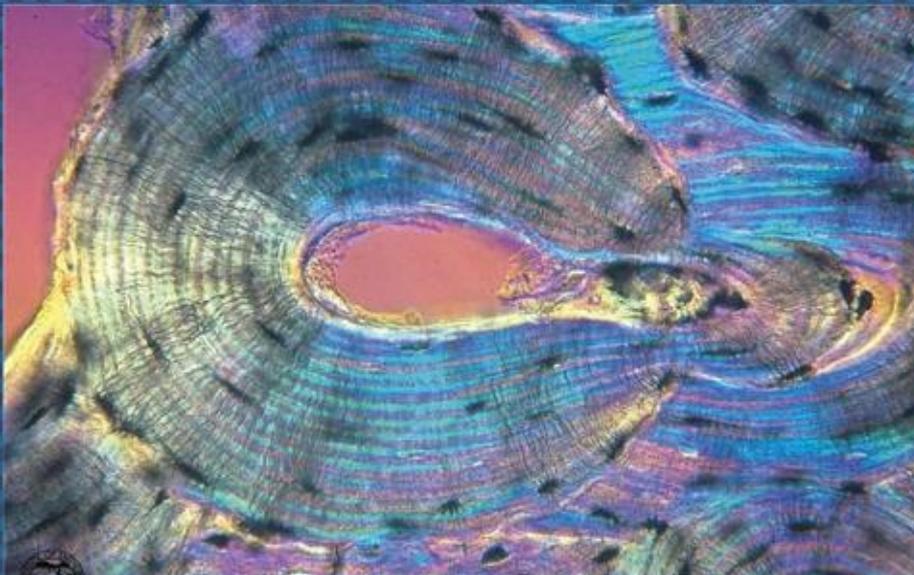




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Biological Effects of DMBA and *Lagenaria siceraria*:2: Modulatory Role of *Lagenaria siceraria* on The Ileum of Females Swiss Albino Mice Induced by The Possible carcinogenic effect of DMBA

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ABSTRACT

Background: Cancer is constituted as the most famous fatal disease among human-being. DMBA is a common carcinogen and immunosuppressant and experimentally used for the induction of mutations via the creation of DNA adducts.

Aim: Evaluation of the therapeutic effect of *Lagenaria siceraria* on 9,10 dimethyl-1, 2-benzanthracene (DMBA)-induced changes.

Materials and Methods: A total number of thirty adult female albino rats within average age 3 months classified into 3 groups as follow: Group1 is designated as control. Group 2 was subcutaneously injected with a single dose of DMBA (10 mg/ 100 g b. wt) and after 90 days, animals have received a daily injection of distilled water for 30 days. Group 3 was subcutaneously mice were injected with a single dose of DMBA and after 90 days, mice were received morning and night daily injection of *Lagenaria siceraria* (100 µg/ 100 g b.w) for 30 days. After scarifying the mice, ileum samples were taken and passed the histological examination, also Histochemical stains were done.

Results: From the cell biological, histochemical, and histopathological points of view, the carcinogen DMBA has a highly stimulatory effect on the cellular activities, DNA synthesis, RNA synthesis, and cell proliferative in the normal epithelial cells lining villi of the ileum of female mice, while *Lagenaria siceraria* has a highly inhibitory effect on the volume of the nuclei, DNA synthesist, and the cell proliferation of the DMBA-induced cells lining villi of the ileum of female mice.

Conclusion: Finally, it could be said that the carcinogen DMBA has a proliferative and/or possibly a carcinogenic effect on the ileum of female mice. Also, It could be concluded that *Lagenaria siceraria* could be offered an antiproliferative and/or possibly an anti-carcinogenic effect against DMBA.

INTRODUCTION

Malignancy is one of the most frequent causes of mortalities world widely recorded in humans. World Health Organization (WHO) expected that cancer in 2030 tends to increase. Malignant neoplasm is a disease; since a crowd of cells has undergone abnormal growth sometimes termed metastasis (Samudral *et al.*, 2015). This disease once attacks the body; it begins to grow without control (Jones and Johnson, 2012). Carcinogens that accelerate cancer have many precursors (Tomasetti *et al.*, 2017). Multiple exogenous and endogenous factors shared cancer development (Gutiérrez and Salsamendi, 2001) like nutritional habits associated with food processing, social conditions, life activity, physical agents as radiation with all types, chemical compounds, and biological agents as infected virus (Lutzwk, 2002). DMBA is a prevalent carcinogen experimentally induced mutation through the creation of DNA adducts (Chatterjee *et al.*, 2010).

Carcinogens are compounds that can be used to induce cause cancer. Carcinogenesis is defined as multiple processes of developing cancer due to exposure to carcinogenic substances. It passed many stages including initiation, promotion, and progression. The process of carcinogenesis is dose and times of carcinogenic material-dependent (Wibowo *et al.*, 2010).

The carcinogenicity enhanced by DMBA is due to its capability to occur imbalance between cytochrome P4501A1 (CYP1A1) and quinone oxidoreductase 1 (NQO1) genes (Dias *et al.*, 1999).

DMBA is considered one of the common polycyclic aromatic hydrocarbons; known as a potent, immunosuppressant, and teratogen which is more familiar in rodents. It can exert major toxic effects as an imitation of cancer in variant tissues (Matikainen *et al.*, 2001).

In the 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 the abnormal conditions, whether experimental stress or pathological events (Aref *et al.*, 2020).

Medicinal plants possess anticancer character attributed to antioxidant properties due to phenol constituents. Seriously, it exerts its anticancer effects with antioxidant activity by scavenging function (Pahari *et al.*, 2012). Moreover, are capable of modulating oxidative stress (Nasri and Rafieian-Kopaei, 2013).

Phenolic compounds provided anticancer activity through acting on cell proliferation processes via arrest of G2/M cell cycle and inhibition of topoisomerase II). Also, it has a role to control apoptosis and upset angiogenesis mechanisms (Memmott and Dennis, 2009).

Lagenaria siceraria is a large herb cultivated in many countries as Africa, Asia and recently cultivated in Egypt. This plant fruit is rich in some vitamins especially vitamin-B complex along with β -carotene and vitamin-C (Kirtikar and Basu, 2001). The fruits offered antimicrobial, cytotoxicity, anticancer, and antihepatotoxicity activities (Shiwaikar and Sreenivasan, 1996). Lagenin is an active principal substance isolated from *Lagenaria siceraria* possesses immune protective, antitumor, and anti-proliferative characters (Wang and Ng, 2000).

Methanolic extract of *Lagenaria siceraria* fruit exhibited a significant decrease in cells associated with chromosomal aberration assay. The significant antimutagenic activity of selected plants against Cyclophosphamide is attributed to antioxidant components as a poly-

phenolic compound, flavonoids, and other micronutrients (Hasmukhlal *et al.*, 2016).

The objective, the based work aimed to study the biological effects of DMBA and/or *Lagenaria siceraria* on the ileum of the female mice. Also, study the modulatory and antiproliferative, and/or antineoplastic functions of *Lagenaria siceraria* on DMBA.

MATERIALS AND METHODS

Materials:

1. Animals:

Thirty adult female mice within the average age of 90±3 days and weigh of 25 ± 2 gm were used. It was obtained from the Autoradiographic lab. of Cell Biology and Immunology studies, Faculty of Science, South Valley University. All animals were subjected to the same experimental conditions of temperature (23 ± 2 c°), an artificial light-dark cycle (12h-12h), humidity (37-40 %), and feed supplied *ad libitum*. The experiments were conducted in the lab. achieve stability of environmental conditions, the separation between treated animals and control ones, and IACUC goals.

IACUC approval of project number:

IACUC-SVU-EYGPT				
SVU/FS		4/2016	0	0
			2	(2)

Chemicals:

1- **DMBA (dimethyl-1, 2-benzanthracene):**9,10-dimethyl-1,2-benzanthracene (DMBA) purchased from Sigma Company for Chemicals, Louis, Mo.

2- ***Lagenaria siceraria*:** special preparation of *Lagenaria Siceraria* (Aref1) was prepared via Dr. Abdel-baset Aref (Aref *et al.*, 2018 & Aref *et al.*, 2020 & Aref *et al.*, 2021).

Methods:

1. Experimental Design:

Thirty female mice were classified into 3 groups; each one was containing 10 as following.

Group (1): Animals administrated a single injection of corn

oil at 0.2 ml/ 100 gm subcutaneously, and served as a control group.

Group (2): Mice subcutaneously received one dosage of DMBA at 10 mg/ 100 gm. Mice after 3 months, received daily dose orally morning and night of distilled water (0.2 ml/ 100 g b.wt.) for 30 days.

Group (3): female mice subcutaneously injected a single dose of DMBA; then after 90 days, received daily dose orally morning and night *Lagenaria siceraria* at 100 µg/ 100 g b.wt. for 30 days.

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

All animals from each group were anesthetized by Chloroform and sacrificed after the last oral dose by a day. Any clinical signs and abnormalities in animals were daily noted.

2. Organ Collection:

Ileum was dissected from all groups then preserved in 10 % neutral buffered for histological examinations.

3. Cell Biological, Histochemical and Histopathological Examination:

Specimens from the ileum were collected and fixed in 10% formalin solution followed by dehydration in serial grades of ethyl alcohol. After samples were dehydrated, they were cleared in xylene and paraffin blocks were made. Serial sections of about 5 microns were prepared and subjected to hematoxylin and eosin (H&E) (Bancroft and Gamble, 2002). and histochemical techniques: the Feulgen reaction for DNA, toluidine blue for RNA, bromophenol blue for protein, Masson's trichrome for collagen, and Periodic Acid Schiff (PAS,) for polysaccharides.

Karyometric Studies: According to nuclear shape, the measurements were

carried out in two ways (Lewinski, *et al.* (1984).

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

RESULTS

Cell Biological Study:

The mean volume of the nuclei in the epithelial cells lining villi of the ileum of the female mice in the three groups C, D, and DL was $83 \pm 3.7 \mu$,

$211 \pm 4.5 \mu$, and $165 \pm 4.6 \mu$ respectively (Table 1).

The short-term exposure time (120 days) of DMBA highly increased by 154% volume of the nuclei of the cells versus the control mice, while the treatment with *Lagenaria siceraria* decreased by 22% volume of the nuclei of the cells comparing with that of DMBA-induced mice.

From the cell biological point of view, the carcinogen DMBA has a highly stimulatory effect on the cellular activities of the epithelial cells of the ileum in the female mice, while the treatment via *Lagenaria siceraria* resistant this the carcinogenic effect of DMA.

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

Ileum	Experiment		
	C	D	DL
Groups			
Measurements			
Mean volume nuclei (\pm S.E.) of the epithelial cells lining the villi	$83 \pm 3.7 \mu$	$211 \pm 4.5 \mu$	$165 \pm 4.6 \mu$
(S%) or (I%) of mean volume nuclei of the epithelial cells lining the villi	←	S=154%	I=22%
	←	←	S=99%

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

Histochemical Special Staining:

DNA:

Control mice showed normally stained DNA contents of the epithelial cell in the villi of the ileum (Fig. 1 a). Group (2) showed highly stained DNA content with the deep red coloration in the cells lining the ileum of DMBA-induced female mice (Figs. 1 b & c). While group (3) showed slight stained DNA contents with faint red color in the cells of ileum villi of *Lagenaria siceraria*-treated female mice (Fig. 1 d).

The short-term exposure time of DMBA highly increased DNA content in the cells of ileum of mice versus of control animals, while, treatment with *Lagenaria siceraria* highly decreased DNA content versus DMBA-induced female mice.

From the histochemical point of view, DMBA carcinogen has a highly stimulatory effect on DNA synthesis and cell proliferation of the lining epithelial cells of the normal ileum. of female mice, while the treatment via

Lagenaria siceraria resistant this carcinogenic effect of DMBA.

RNA:

Usage of RNA stain exhibited normally stained RNA contents in the epithelial cells of the ileum of female control animals (Fig. 2 a). the ileum of the group (2) showed deeply stained RNA content with sharp blue coloration in epithelial cells (Figs. 2 b & c). *Lagenaria siceraria* group showed moderately stained RNA contents with moderate blue color in the target cells (Fig. 2 d).

120 days of exposure time of carcinogen DMBA pronounced increased RNA content in the cells of villi compared with the normal animal, while the *Lagenaria siceraria* slightly decreased RNA in the cells versus the DMBA-induced female mice.

Histochemically, the carcinogen DMBA has a stimulatory effect on RNA synthesis in the epithelial cells of the ileum.

Bromophenol Technique for Protein:

The ileum of control mice showed deep protein distribution with deep blue discoloration in the epithelial cells (Fig. 3 a). DMBA induced mice showed weakly infiltrated protein content with faint stained blue color in the target cells (Figs. 3 b & c). Group 3 showed moderate protein content with moderate stained blue color (Fig. 3 d).

DMBA moderately decreased the total protein content in cells of the ileum versus the normal ileum, while *Lagenaria siceraria* slightly increased protein content versus DMBA-induced cells.

Histochemically, carcinogen DMBA has an inhibitory effect on protein synthesis.

Masson Trichrome Stain for Collagen:

Control mice showed few collagen fibers (Fig. 4 a). Group 2 showed moderate infiltrated collagen fibers (Figs. 4 b & c). Group 3 showed few infiltrated collagen fibers (Fig. 4 d).

The carcinogen DMBA increased collagen content in the ileum of female mice versus control animals, *Lagenaria siceraria* decreased collagen content in the ileum of DMBA-induced mice.

From the histochemical point of view, the carcinogen DMBA has a stimulatory effect on normal collagen synthesis, while *Lagenaria siceraria* has an inhibitory effect on DMBA-induced collagen synthesis.

PAS Technique for Polysaccharides:

Deeply infiltrated glycogen with intense stained red color detected in control mice (Fig. 5 a). While groups 2 and 3 showed a moderate degree of glycogen distribution (Figs. 5; b & c & d).

Both DMBA and *Lagenaria siceraria* did not change the collagen content in the epithelial cells of the ileum of both normal and DMBA-induced mice respectively.

Both DMBA and *Lagenaria siceraria* do not affect both normal and DMBA-induced collagen synthesis respectively.

Histopathological Findings by Light Microscopy:

Ileum of control showed normal intestinal structure (Fig. 6 a). Mice that received DMBA detected a high proliferation of the lining epithelial cells of the ileum (Figs. 6 b & c). Group 3 showed mild proliferation of the epithelial cells in the ileum (Fig. 6 d).

The carcinogen DMBA highly increased the normal cell proliferation of the epithelial cells in the ileum of female mice, while *Lagenaria siceraria* highly decreased the DMBA-induced cell proliferation.

From the histopathological point of view, the carcinogen DMBA has a stimulatory effect on normal cell proliferation, while *Lagenaria siceraria* treatment has an inhibitory effect on the DMBA-induced cell proliferation of the epithelial cells in the ileum of the female mice.

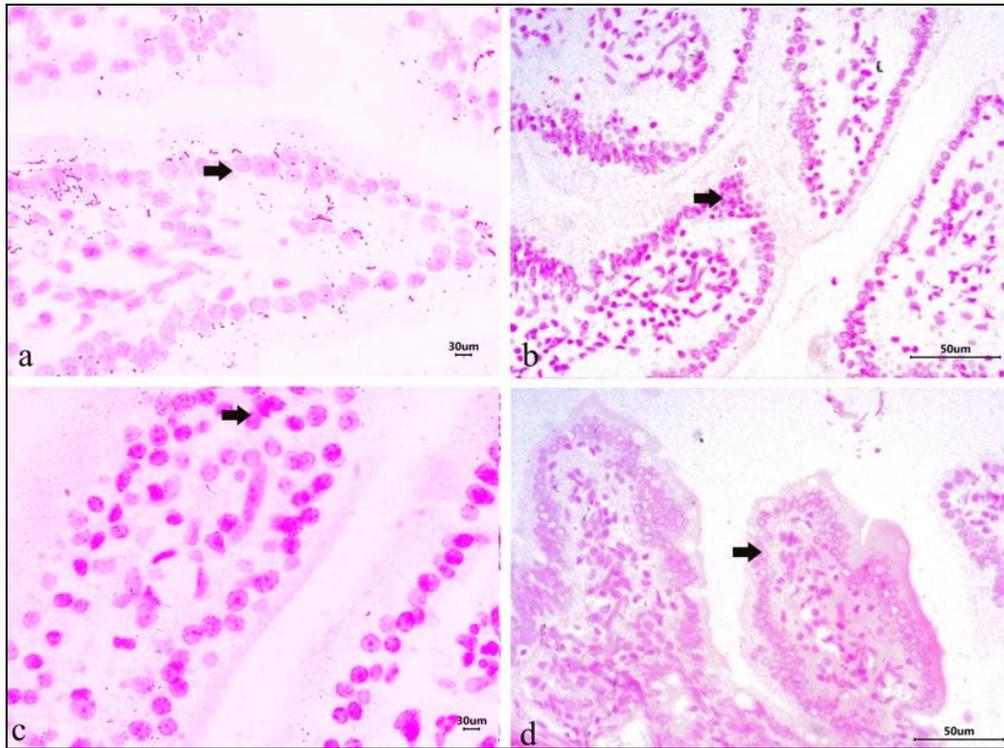


Fig. 1 (a-d): Photomicrograph of ileum of control female mice showing normally stained DNA contents (a). Ileum of the group (2) of 9,10-dimethyl-1,2-benzanthracene (DMBA) injection for 30 days showing highly stained DNA with deeply red coloration (b & c). Ileum of the group (3) of 9,10-dimethyl-1,2-benzanthracene (DMBA) injection and *Lagenaria siceraria* for 30 days showing mild stained DNA contents with faint red color (d). (DNA stain, bar=50 μ m).

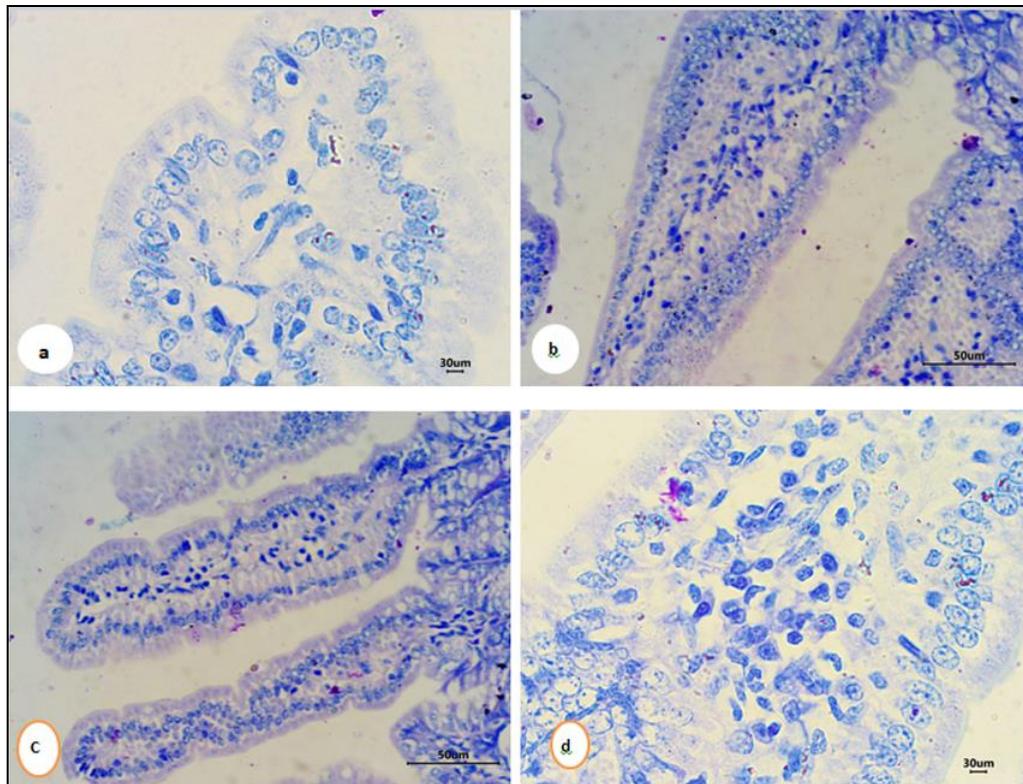


Fig. 2 (a-d): Ileum of control mice showing normally stained RNA contents (a). Ileum of DMBA exposed group showing deeply stained RNA with marked blue coloration (b & c). Ileum of DMBA and *Lagenaria siceraria* showing moderately stained RNA contents with moderate blue color (d). (RNA stain, bar=50 μ m).

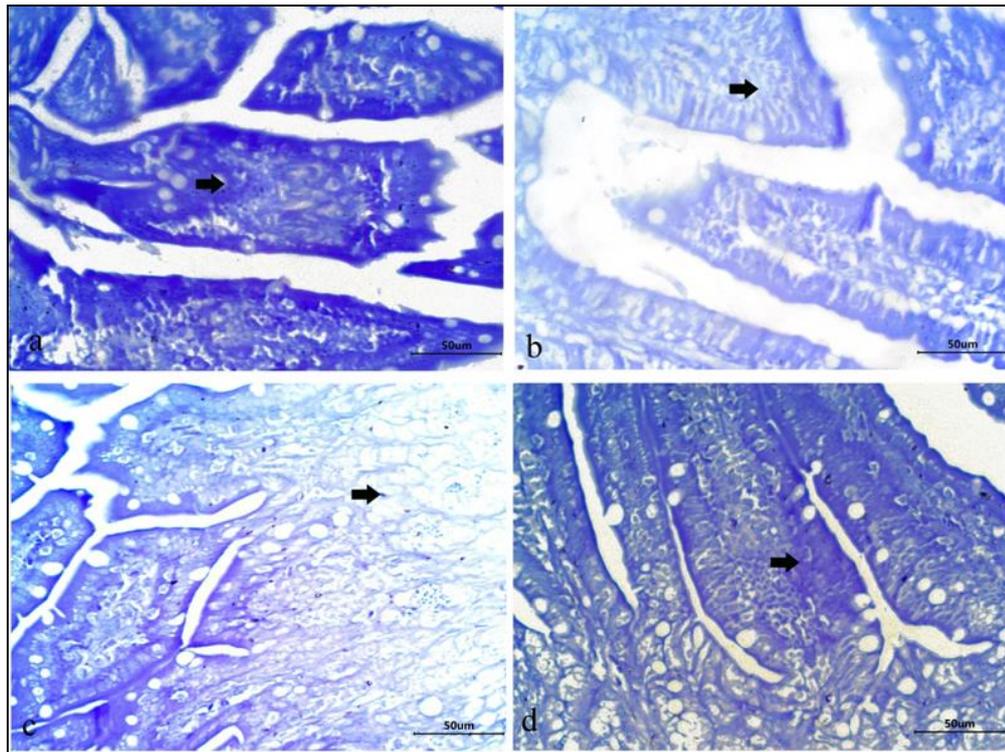


Fig. 3(a-d): Control female mice showing deep blue discoloration (a). Ileum of DMBA group days showing weakly infiltrated protein (b & c). Group 9,10-dimethyl-1,2-benzanthracene (DMBA) and *Lagenaria siceraria* showing moderate protein with moderate stained blue color (d). (Bromophenol technique, bar=50 µm).

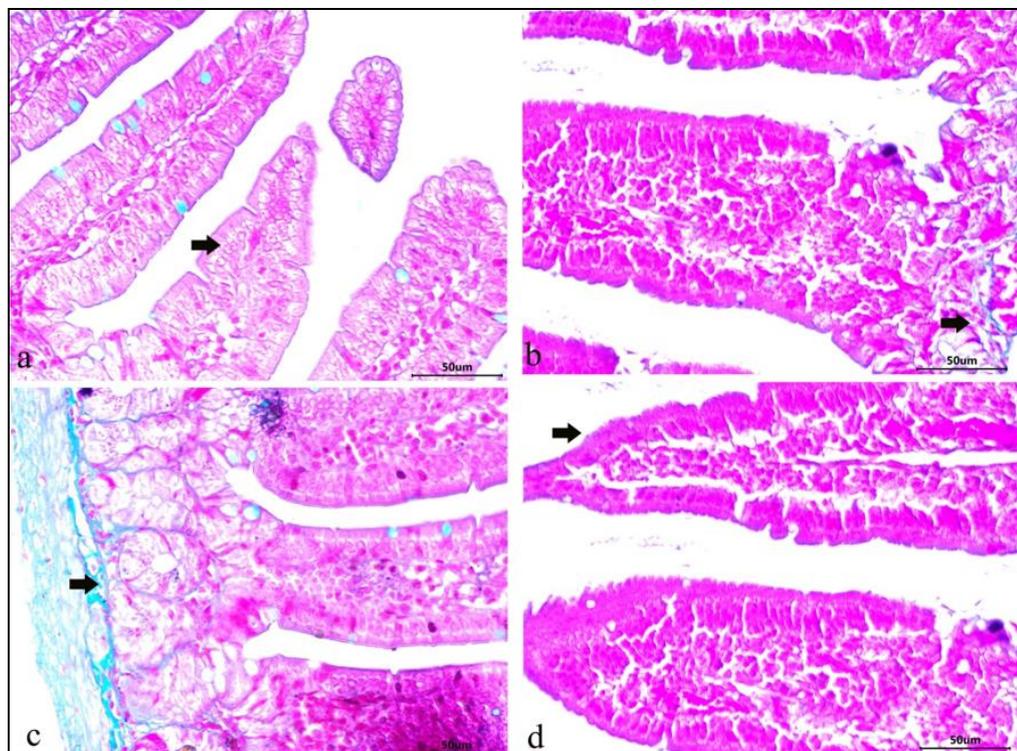


Fig. 4 (a-d): Ileum of control mice showing few collagen fibers (a). Ileum of 9,10-dimethyl-1,2-benzanthracene (DMBA) group showing moderate infiltrated collagen fibers (b & c). Ileum of DMBA and *Lagenaria siceraria* group showing few infiltrated collagen fibers (d). (Mallory trichrome technique, bar=50 µm).

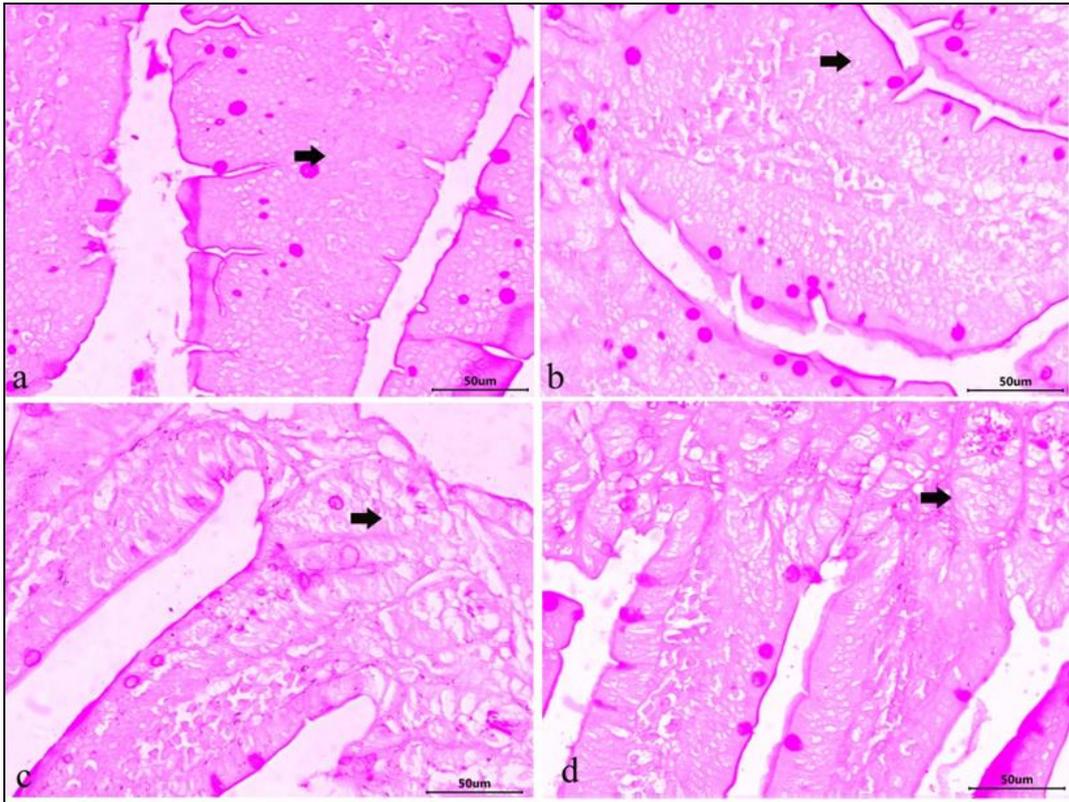


Fig. 5(a-d): Control female mice showing deeply infiltrated glycogen with intense stained red color (a). Group 2 (9,10-dimethyl-1,2-benzanthracene) showing moderate glycogen content (b & c). Group 3 (9,10-dimethyl-1,2-benzanthracene plus *Lagenaria siceraria*) showing moderate glycogen content (d). (PAS, bar=50 µm) .

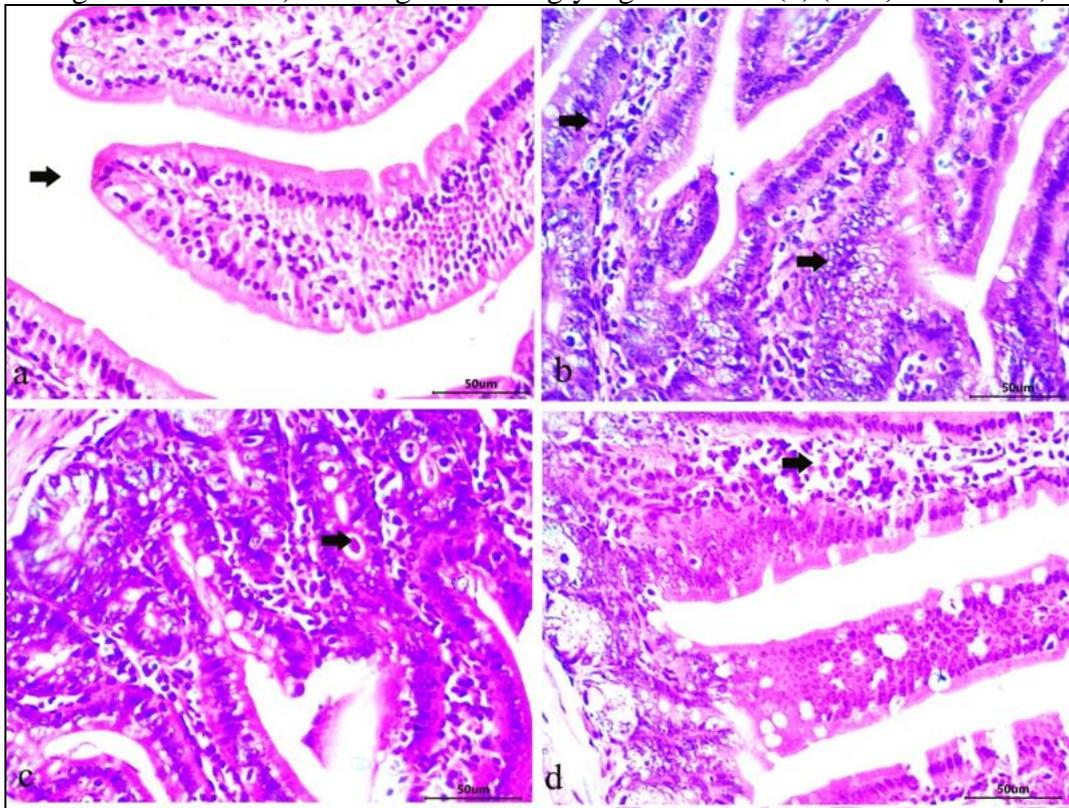


Fig. 6 (a-d): Ileum of control mice showing normal histological structure (a). DMBA group showing the obvious proliferative degree of malignant cells (b & c). DMBA and *Lagenaria siceraria* group showing mild proliferation of malignant cells (d). (H&E., bar=50 µm).

DISCUSSION

The present data showed that the carcinogen DMBA highly increased the volume of the nuclei, DNA content, RNA content, and cell proliferation in the normal epithelial cells lining villi of the ileum of the female mice.

While *Lagenaria siceraria* highly decreased the volume of the nuclei, DNA content, and cell proliferation and changed the contents of RNA, protein, and collagen in the epithelial cells lining villi of the ileum of the DMBA-induced female mice.

"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 abnormal conditions, 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).

One well-established model system for the study of tumor development involves the use of a single dose of 7,12-dimethylbenz(a)anthracene (DMBA) to initiate carcinogenesis in many tissues and organs of mammals, example includes intestinal tumors in the Japanese house musk shrew (Tsubura *et al.*, 1992), ileum carcinogenesis in male mice (Aref *et al.*, 2020), mammary cancer (Nesaretnam *et al.*, 1998), liver carcinogenesis (Nesaaretnam *et al.*, 1998), lymphoma (Qing *et al.*, 1997), skin tumors in mice (Satomi, 1993). Husain *et al.*, (1991) has been reported a single dose of DMBA did not produce melanoma in mice.

Every cellular community, in the mature organism, 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 the abnormal conditions, whether experimental stress or pathological events (Aref *et al.*, 2020).

There is little or no published literature concerning to study of the biological effect of carcinogen DMBA or/and *Lagenaria siceraria* on the ileum of female mice, therefore may it is difficult to discuss the results of the present work with the other published works previously.

From the cell biological, histochemical, and histopathological points of view, our present results showed the carcinogen DMBA has a highly stimulatory effect on cellular activities, DNA synthesis, and cell proliferation, and a stimulatory effect on RNA synthesis and collagen synthesis, while *Lagenaria siceraria* has an inhibitory effect on the carcinogenic effect of DMBA in the epithelial cells lining villi of the ileum of the female mice. These present results agree with the results of Aref *et al.*, (2020) who showed that the Carcinogen DMBA has cellular activities, a proliferative, and a carcinogenic efficacy on the ileum of the DMBA-induced male mice. In contrast, *Lagenaria siceraria* has anti-cellular-activities, anti-proliferative, and anti-carcinogenic effects via usage, 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 induced by DMBA.

Cancer is a fatal disease that demonstrated higher distribution among the human population. Nowadays, there were synthetic and natural sources are used alone or in combination to manage cancer. Different allopathic medicine of natural sources is preferred mainly to control the development of cancer due to resistance. Where, herbal plants exhibited various and vital chemical

moiety involving flavonoids, terpenoids, and steroids (Gangwal, 2010).

DMBA is a procarcinogen act as an active metabolite that performs a crucial effect in the formation of malignant via covalently arise DNA adducts (Lenoir *et al.*, 2005). Interaction between DNA with carcinogenic hydrocarbon materials catalyzed by the enzyme 7.12-DMBA-3,4-diol and to 7.12-DMBA-3,4-diol-1,2-epoxide by the enzyme CYP1A1 and epoxide hydrolase which will convert the compound benzo[a] pyrene into active intermediates that can bind covalently with DNA (Androutsopoulos *et al.*, 2009).

DMBA was capable of initiation of carcinogenesis via the release of a group of pro-inflammatory cytokines such as tumor necrosis factor α (TNF- α), interleukin-1 β , 16 & 18, and cyclooxygenase 2 (COX2). Such inflammation-related cytokines are potential in inflammation progression (Lofrumento *et al.*, 2014). NF- κ B phosphorylation is crucial for the release of inflammation-associated, (Yuan *et al.*, 2014 where resultant NF- κ B is shared in skin damage and evident in various stages of carcinogenesis (Kim *et al.*, 2015). subsequently induced TNF- α , IL-1 β , and IL-6cytokines, that are participating in the inflammation process.

Recently, there was the direction for herbal plants as alternative medicine. It turned to its pharmacological functions comprising cytotoxic and anti-carcinogenic effects (Kundu Sen *et al.*, 2011). Since, herbs are rich in active natural products like flavonoids, terpenoids, alkaloids, and steroids.

Lagenaria siceraria remarkably suppressed the proliferative action induced in mice by DMBA; *Lagenaria siceraria* possesses a multifunctional role as an antioxidant, cytoprotective, and anti-cancer. The antimutagenic agents of *Lagenaria siceraria* are generated from the antioxidant

property. So that, due to the genotoxicity of free radicals plays the function as stabilizers of homeostasis (Amit *et al.*, 2012). Sharma *et al.*, (2013) assessed the antioxidant role of methanolic extract of *Lagenaria siceraria* leaves (MELS). It was assured that the extract possesses variant phenols to exceed 99.09 ± 0.10 $\mu\text{g}/\text{mg}$ that are considered as a potential and natural level of antioxidant against reactive tissues species-mediated diseases. Traditionally, methanolic extract of *Lagenaria siceraria* possesses antitumor activity. Moreover, *Lagenaria siceraria* is known for its antimutagenic potential and supplied chemopreventive pharmacological importance. For this reason, we could encourage its usage as a functional food (Thakkar *et al.*, 2013).

Conclusion:

From the cell biological, histochemical, and histopathological points of view, the carcinogen DMBA has a highly stimulatory effect on the cellular activities, DNA synthesis, RNA synthesis, and cell proliferative in the normal epithelial cells lining villi of the ileum of female mice, while *Lagenaria siceraria* has a highly inhibitory effect on the volume of the nuclei, DNA synthesis, and the cell proliferation of the DMBA-induced cells lining villi of the ileum of female mice. Finally, it could be said that the carcinogen DMBA has a proliferative and/or maybe a carcinogenic effect on the ileum of female mice. Also, It could be concluded that *Lagenaria siceraria* could be offered an antiproliferative and/or an anti-carcinogenic effect against DMBA.

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