Biological Effect of DMBA and Lagenaria Siceraria:3: The Potential Extent of Lagenaria Siceraria to Counteract the Motivator Effect of DMBA on the Testes of Male Swiss Mice

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Original Article

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ABSTRACT

Introduction: Undoubtedly, cancer is distinguished in an incidence of a critical fatality in the world community. There are several models of carcinogenesis by chemical materials utilized in the experimental models. Regarding DMBA, it was validated to be an ideal selective inductor of cancer employed for several purposes in tumor researches.

Aim of the Work: The current consideration was planned to explore the potential alleviating effect of Lagenaria siceraria in conquering detrimental changes induced by 9,10 dimethyl-1, 2-benzanthracene (DMBA) in testes of males Swiss mice.

Materials and Methods: In the existing study, 30 adult male Swiss mice within 90 days of age were evenly distributed into 3 groups as follows; control, DMBA, and DMBA plus Lagenaria siceraria (DMBA + LS). After the experiment timed out which extended for 4 months, the biopsies from testes tissues were dissected and passed the histological, histochemical, and immunohistochemical investigations.

Results: By cross-sectional screening, DMBA is implying a prominent proliferative effect on the spermatocytes and Leydig cells of male mice inferred through dense DNA and RNA synthesis. Furthermore, DMBA rummaged apparent decrement in its protein and polysaccharides. As well, it prompted a lucid infiltration of the collagen fibers around vasculatures. However, Lagenaria siceraria could efficiently suppress stimulatory impact induced by DMBA in epithelial lining seminiferous tubules.

Conclusion: Depending on these findings, it could be concluded that DMBA verily elicited a powerful model of carcinogenesis that progressed by the time distinctly. However, Lagenaria siceraria could potentially ameliorate the deleterious effect disclosed in the testicular cells by carcinogen DMBA.

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INTRODUCTION

Assuredly, carcinogenesis is a process inferred as a genetic turmoil resulting from somatic cells deterioration. It plainly describes abnormal cell growth which may be spread to other body parts[1].

Polycyclic aromatic hydrocarbons (PAHs) are no longer recognized as ubiquitous contaminants wide-ranged in the environment. It is graded as an important class of organic compounds made up of carbon and hydrogen atoms during natural carbonization[2].

DMBA is the most familiar compound being included among the polycyclic aromatic hydrocarbons group. It acts as a potent tumor initiator capably to experimentally induce diverse tumors. Since these constitute a potential carcinogen threat to humans. It is commonly regarded as an environmental pollutant in the air, water, and soil. Additionally, it is frequently emitted through the exhaustion of wood and fossil fuel. Also, it much formed after automobile combustion, smoke, and industrial waste by-products. In consequence, it directly exerted a damaging role in various tissues in laboratory animals and humans[3].

Accordingly to scientific investigations, DMBA concerned more potent neoplastic and tumorigenic subscribe to develop heritable genetic damage and malformations distinguished by abnormal cells proliferation. DMBA could be virtually absorbed through the skin resulted in reproductive damage. Furthermore,

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the toxic effect of DMBA was associated with liver and kidneys damage[4]. Overwhelming evidence proved that DMBA induced female reproductive disturb out of the way male overview[5].

A variety of therapy is ultimately approached to treat cancer like surgery, chemotherapy, immunotherapy, and radiation. Otherwise, these conventional therapies are still cost-effective. Up to date, scientific trials on the herbal plants are employed to be assessed as alternative medicine; by its wide spectrum of chemical compounds[6].

"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 the abnormal conditions, whether experimental stress or pathological events"[7].

Substantially, Lagenaria siceraria is a fruit that supplied an influential anticancer activity[8]. A growing concern has been conducted on the extent to which Lagenaria siceraria possesses variable biological activities, like other medicinal herbs with the predominant antioxidant role[9]. Where, a novel protein is known as lagenin isolated from Lagenaria siceraria gives a share in counteracting the carcinogenesis[10]. Worth mentioning, its component from alkaloids arrests the cell cycle in the metaphase stage by hindering the formation of the microtubules; subsequently destruct tumor cells[11]. The measure of that, phenols in Lagenaria siceraria attack tumor cells posteriorly antagonize proliferation processes and angiogenesis, these impacts the routes of phosphoinositide 3-kinase and protein kinase B[12].

MATERIALS AND METHODS

Materials

Animals

Thirty adult male Swiss mice at 90 days of age, with an initial weight of 25 ± 2 gm were used in the current study. They were brought from the Autoradiography Lab. of Cell Biology and Immunology belonging to the Faculty of Science, South Valley University, Qena, Egypt. Mice were kept in a well-ventilated animal house. Animals were adequately allowed for two weeks of acclimatization under suitable environmental conditions before the experimentation. All animals were subjected to the same experimental conditions of temperature (23 ± 2 co), an artificial light-dark cycle (12h-12h), humidity (37-40 %). Water and food were 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 as in[13].

IACUC approval of project number

IACUC-SVU-EYGPT SVU/ F S 4/2016	0	0	2 (3)

Chemicals

DMBA (dimethyl-1, 2-benzanthracene)

9, 10-dimethyl-1, 2-benzanthracene (DMBA), a carcinogenic material belonging to the class of polyaromatic hydrocarbons (PAH) compounds, was purchased from Sigma-Aldrich Co., Louis, Mo. The United States. This material has been widely used in the induction of carcinogenesis in scientific research[14,15,7,16].

Lagenaria siceraria

The fruits of Lagenaria siceraria were obtained from a local market in Qena Governorate, Egypt.

Preparation of plant:

A processed method for special preparation of the extract of Lagenaria siceraria was made and identified by[17,7,13,16].

Methods

Experimental Design

Thirty sufficiently acclimatized Swiss mice were used in this study. It was evenly differentiated into 3 groups of 10 mice each as follows:

Group (1): It was exploited as a control, where all mice subcutaneously injected with one dose of corn oil at 0.2 ml/ 100 gm.

Group (2): Mice were subcutaneously injected with a single dose of DMBA at 10 mg/100 gm. After 3 months, mice received 0.2 ml/ 100 g b.wt. of distilled water, twice per day, at both morning and night for 30 days

Group (3): After 90 days, animals injected with 10 mg/100 gm DMBA were given, twice per day, daily oral doses of Lagenaria siceraria at 100 µg/ 100 g b.wt. for 30 days.

After experimentation time is completed which was prolonged for 4 months, all animals from each group were sacrificed under anesthetized control via chloroform. Any gross lesions in animals were daily noted.

The administrations of Lagenaria siceraria were by gavage. The number of doses of Lagenaria siceraria was detrained according to the animal therapeutic where Lagenaria siceraria used as treatment gave good results by this dose, and according to human therapeutics based on the opinion of doctor's cancer oncology[13,16].

Organ collection

Once mice were sacrificed, testes were immediately inspected for any gross changes then dissected out and preserved in formalin 10% for 24 hr. for the histological and Histochemical examinations.

Histochemical and histopathological examination

As for the qualitative analysis of histology, specimens of testes from mice of different groups were dissected

out and fixed in 10 % neutral buffered formalin. Paraffin sections of about 4 μm thicknesses were cut, then stained with hematoxylin and eosin (H&E) and examined under the light microscope[18].

Further histological sections within 4-5 μm thicknesses were subjected to different histochemical techniques; such as 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.

RESULTS

Histochemical screening

For DNA

Histochemically, DNA expression by Feulgen reaction indicated that nuclei of the seminiferous tubules with impotent DNA contents embedded inside nuclei (Figure 1 a). However, DMBA-induced mice cells of the epithelial lining seminiferous tubules exhibited pointedly stained DNA materials, whereas it featured with sharp red coloration inside the nuclei of the cells (Figure 1 b,c). In parallel to the control mice, LS- treated group showed less distinguished DNA contents inside nuclei of the seminiferous tubules (Figure 1 d).

For RNA

For RNA content, testes of the control mice inferred normal allocation of RNA contents inside the epithelial cells lining the seminiferous tubules that were achieved through toluidine blue stain (Figure 2 a). In toluidine blue preparations of DMBA-subjected group, RNA content was expressed by intense bluish within the cells (Figures 2 b,c). As for the Lagenaria siceraria group, RNA contents of the seminiferous tubules appeared normally stained in the target cells (Figure 2 d).

For protein

Regarding the protein contents, bromophenol reaction exhibited heavily positive staining in the cells of the control mice expressed by marked existence of protein inside most of the spermatocytes (Figure 3 a). On the other hand, the cells of DMBA-injected animals exhibited decrement in their protein content and most of the cells of the seminiferous tubules showed pale stain color (Figures 3 b,c). Lagenaria siceraria recorded a sharp blue stain of the protein content intensely-stained in the form of deep blue color (Figure 3 d).

For collagen fibers

Concerning collagen fibers, the testicular tissues of the mice from the control revealed the normal distribution of the collagen appeared as blue fibers in dense bundles infiltrated around blood vessels (Figure 4 a). Contrary, DMBA exposed group displayed an obvious amount of collagen fibers demonstrated all over vasculature (Figures 4 b,c). Lagenaria siceraria treated group showed normally infiltrated collagen fibers among blood vessels (Figure 4 d).

For polysaccharides

From the histochemical aspect via PAS, control animals were distinguished with significant polysaccharides distribution inside spermatocytes through PAS reactions, whereas its content from polysaccharides was elucidated as intensively red-colored materials in the cytoplasm at the pole of the cells (Figure 5 a). On the other hand, the testes sections of DMBA group showed marked depletion of polysaccharides in most cells (Figures 5 b,c). Where spermatocytes appeared weakly red-colored. Inversely to the DMBA group, the Lagenaria siceraria group revealed the piercing distribution of polysaccharides content within the spermatocytes (Figure 5 d).

Histopathological finding

Testes of the control mice shoed normal architecture with a normal arrangement of the seminiferous tubules comprising an appropriate amount of the spermatocytes (Figure 6 a).

In DMBA-treated animals, histopathological assessment of the tumor revealed to implantation of carcinogenic cells inside the seminiferous tubules and Leydig cells in the interstitial tissues of the testes characterized by larger cells with more condensed chromatin (Figure 6 b). Furthermore, other morphological lesions graded from edema to inflammatory changes at the interstitial tissues were also seen (Figure 6 c).

As in the control group, LS-treated mice demonstrated normal histological arrangement of the seminiferous tubules and interstitial tissues (Figure 6 d).

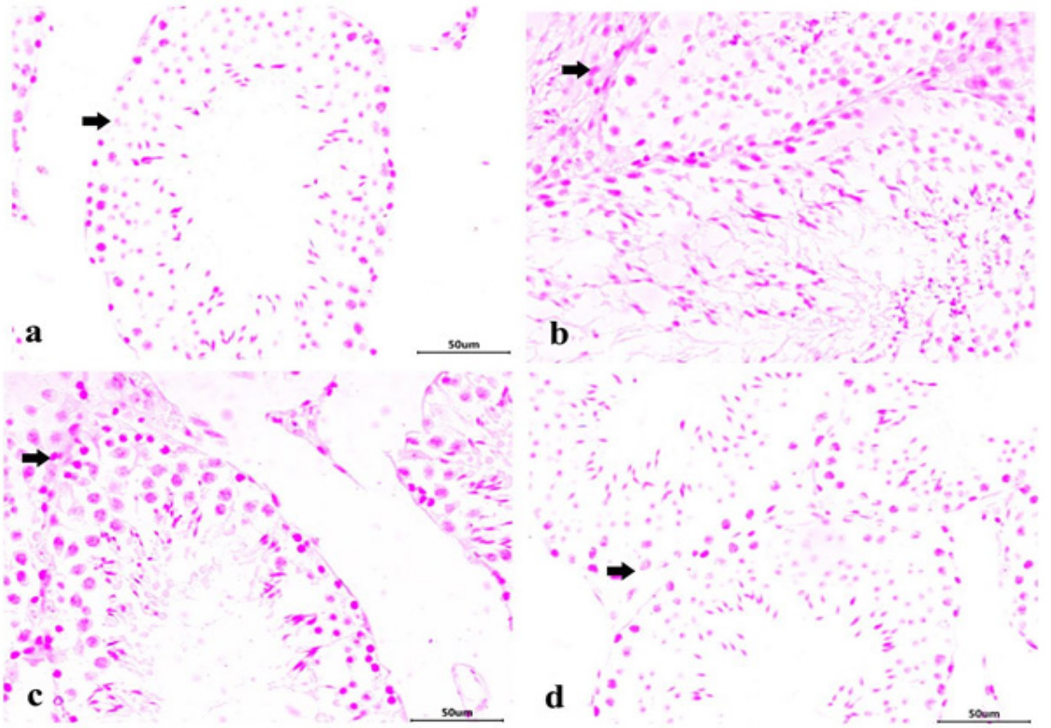

Fig. 1 (a-d): Testes of the control mice showing normal expression of the DNA content inside spermatocytes (a). Testes of DMBA received mice showing potent infiltration of DNA content with darkish red coloration (b & c). LS treated animals showed mild DNA materials within the spermatocytes (d). (Feulgen reaction, bar= 50µm)

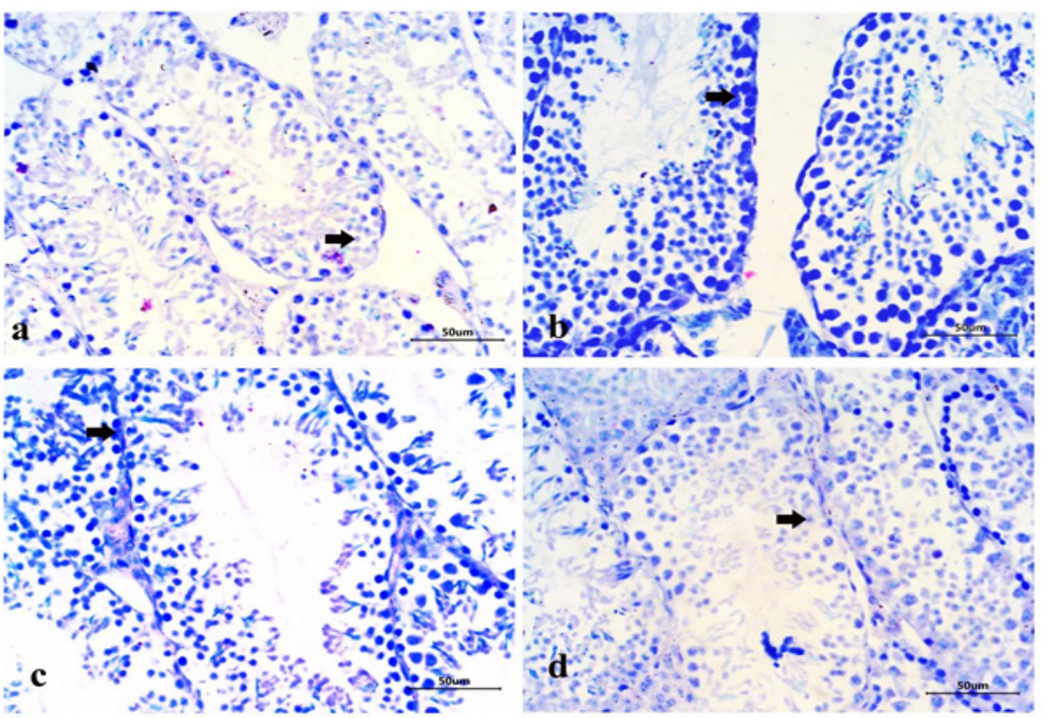

Fig. 2 (a-d): Control testes showing normally distributed RNA content inside spermatocytes of the seminiferous tubules (a). Testes of the DMBA group showed an intensive bluish discoloration of RNA within the cells (b & c). LS administrated animals showing a faint discoloration of RNA materials within the spermatocytes (d). (Toluidine blue, bar= 50µm)

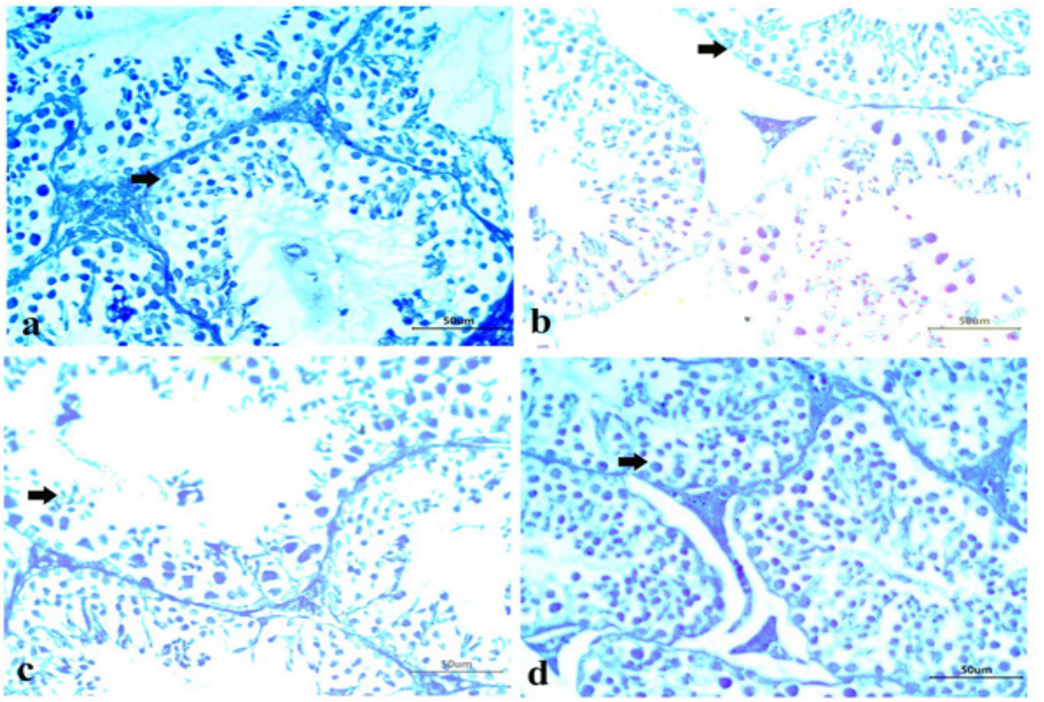

Fig. 3 (a-d): Testes of the control mice showing marked stained protein in the seminiferous tubules (a). Testes of DMBA group showing weakly stained protein (b & c). Testes of LS received mice showing deeply stained protein within the spermatocytes (d). (Bromophenol blue, bar= 50µm)

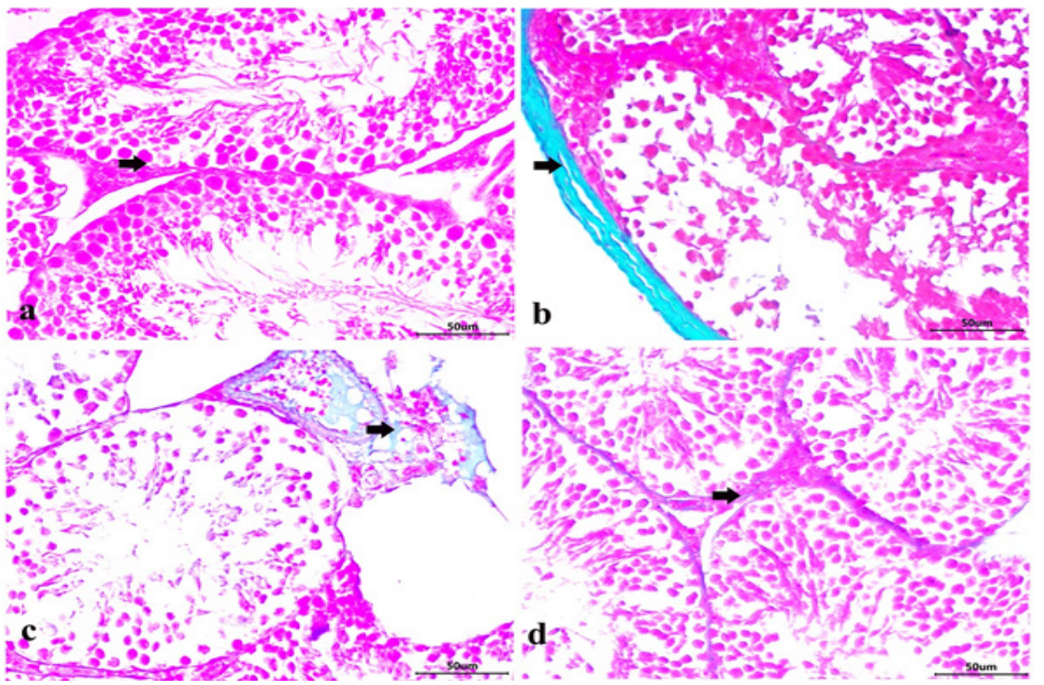

Fig. 4 (a-d): Testes from the control mice showing minimally infiltrated collagen fibers (a). DMBA-intoxicated mice showing heavily infiltrated collagen fibers around the vasculature (b & c). LS received a group showing weak distributions of the collagen fibers (d). (Masson's trichrome, bar= 50µm)

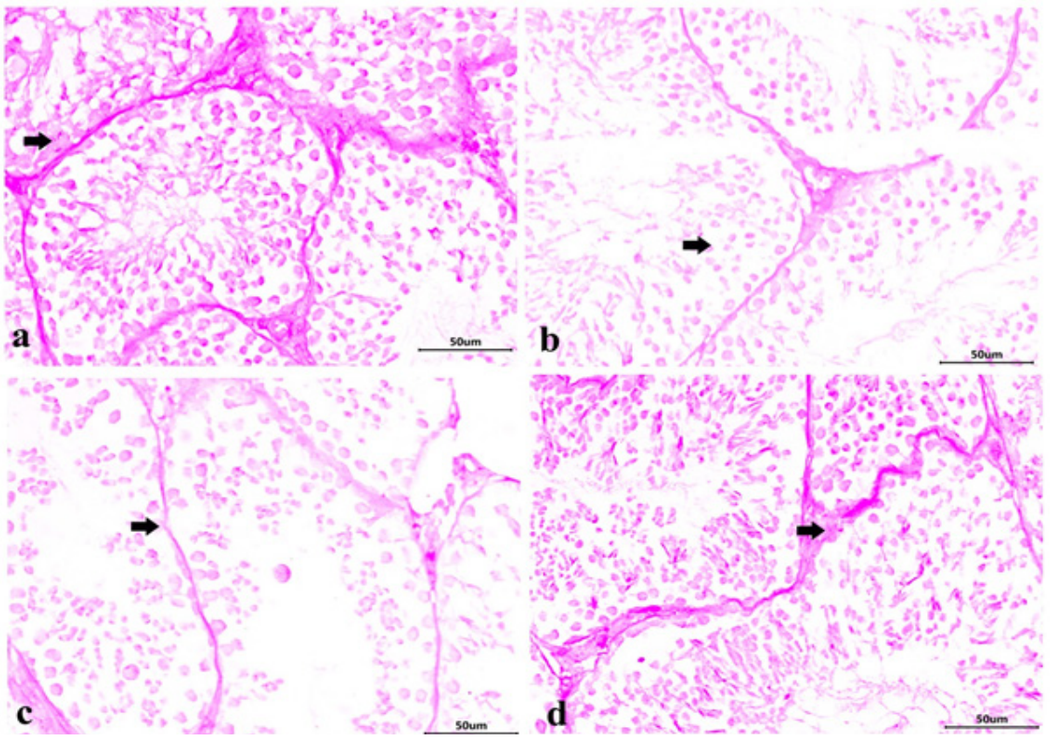

Fig. 5 (a-d): Control mice showing appropriate existence of the polysaccharides content inside spermatocytes (a). The DMBA-exposed group showed a faint red discoloration of the spermatocytes indicating significant depletion in the polysaccharides (b & c). LS-treated group showing the well-defined distribution of the polysaccharides content within the cells of the seminiferous tubules (d). (PAS, bar= 50µm)

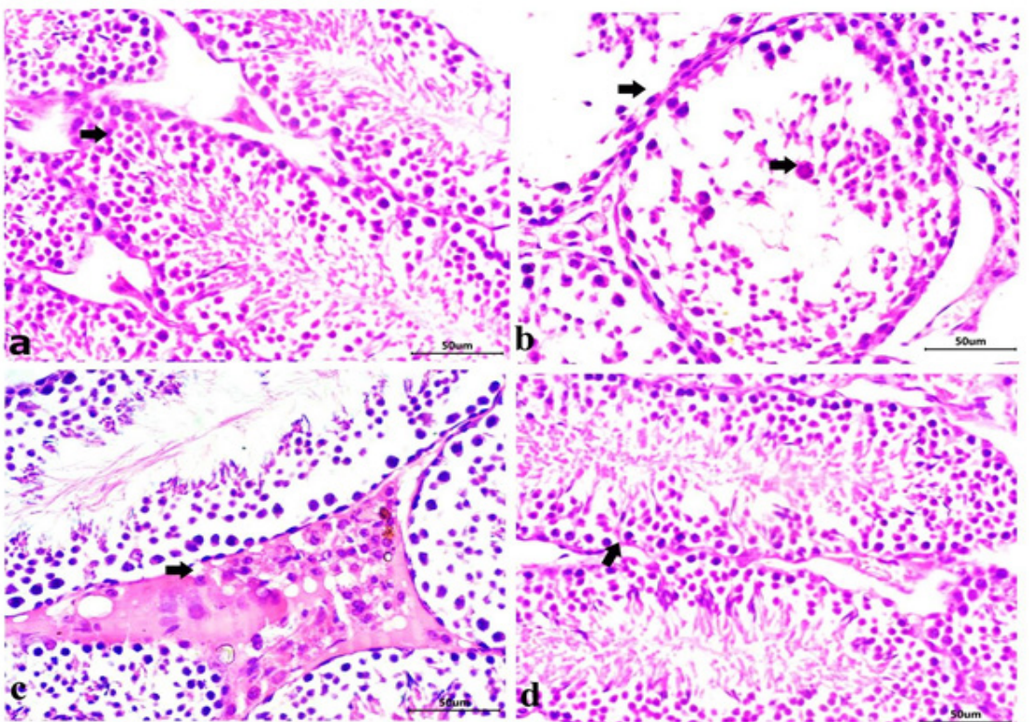

Fig. 6 (a-d): Control testes showing normal histological criteria of the seminiferous tubules (a). Testes of DMBA group showing large neoplastic cells with more condensed chromatin implanted inside the seminiferous tubules and interstitial tissues (Leydig cells) (b), besides edematous changes with distension of the interstitial tissues (c). LS-treated group showing restoration of the normal architecture of the seminiferous tubules (d). (H&E., bar= 50µm).

DISCUSSION

The present work showed that, from the histochemical points of view, the carcinogen DMBA highly increased the examined chemical components, DNA, RNA, and collagen except for total protein and polysaccharides, which were decreased in the cells of testes. In contrast, in the Lagenaria siceraria-treated group, DNA, RNA, and collagen contents were decreased, while protein and polysaccharides contents were increased

The present data revealed that, From the histopathological point of view, the carcinogen DMBA showed large neoplastic cells with more condensed chromatin implanted inside the seminiferous tubules and interstitial tissues (Leydig cells), besides edematous changes with distension of the interstitial tissues. Lagenaria siceraria showed restoration of the normal architecture of the seminiferous tubules.

"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 in these substances is often accompanied by some pathological manifestations"[13]

To date, cancer has become a real reason for the sensitive upset among human-being; it is targeted for high mortality worldwide. Carcinogens are chemical material's own ability to fulminate cancer; it substantially assessed in *vivo* in experimental animals[19]. DMBA, one of which is a chemical motivator was chosen as a target carcinogen that could be able to induce cancer. It's was recognized as one of the most potent carcinogens joined to class members of polycyclic aromatic hydrocarbons[20]. Based on the medical records, exposure to DMBA induces various parenchymal damages including tumors and cancer risks. Moreover, pronounced histological deteriorations following DMBA administration have been listed[21].

Our histochemical results showed that the carcinogen DMBA has a highly stimulatory effect on the normal cell proliferation in cells of the seminiferous tubules. In contrast, special preparation of LS ameliorates the carcinogenic effect of DMBA.

From the histochemical view, Feulgen reaction expounded pointedly stained DNA materials with darkish red coloration inside the nuclei of the cells. This is up to DMBA disclosed carcinogenesis by increasing cell proliferation activity and upset apoptosis evidenced by regulation of p53[22].

Also, the present data, revered that, the carcinogen DMBA has an inhibitory effect on protein synthesis. Contrary, the Lagenaria siceraria resists the effect of carcinogen DMBA.

As for protein content, DMBA induced depletion in the protein content; this was clearly expressed via a stain of bromophenol blue. DMBA induced ischemia-reperfusion injury and fibrosis to the cells[23]. Oxidative stress plays a vital role in cells injury resulting in ROS that is generated in the mitochondria. Consequently, it induced serious damage to macromolecules of the cellular system as proteins[24].

Histochemically, the present data showed that DMBA has a stimulatory effect on collagen synthesis in testes tissues. In contrast, the Lagenaria siceraria ameliorates the action of DMBA.

Concerning Masson's trichrome for collagen, administration of DMBA caused distinct fibrosis. It has been revealed that DMBA is manifested by increasing collagen synthesis in the tumor capsule after DMBA exposure[25].

From the histochemical point of view, our results showed the carcinogen DMBA has a highly stimulatory effect on RNA synthesis in cells of the seminiferous tubules, but the Lagenaria siceraria resists the action of carcinogen DMBA on RNA synthesis.

For RNA content, toluidine blue elucidated intensive bluish color of the cells. It is attributed to DMBA activated RNA at a greater grade associated with chromosomal instability in tumor promotion[26].

From the histochemical point of view, our data revered that the carcinogen DMBA has an inhibitory effect on the formation of polysaccharides content. In contrast, the Lagenaria siceraria resists the action of carcinogen DMBA on the formation of polysaccharides content.

Respecting polysaccharides, a marked decrement of polysaccharides content was determined in most of the cells of DMBA induced group utilizing PAS. Depletion in polysaccharides content was distinguished with cytoplasmic vacuolization. Such decrease could be linked with increased stress on tissues or to loss of the stored glycogen in cells resulting from DMBA toxicity[27].

According to our light microscopy histopathological results, DMBA led to induce damage to the carcinogenic cells within the seminiferous tubules of the testes which appeared with larger cells with more condensed chromatin. In addition, other morphologic changes such as edema and inflammation were detected. Since, DMBA-mediated carcinogenesis resulted in DNA damage with genes deregulation of that is critical for cell proliferation and survival[28]. DMBA compound is metabolized promptly into reactive carcinogens, consequently creating DNA adducts. Resultant free radicals led to cross-linking of DNA, protein, and lipids to each other or oxidatively damaged functional groups on these important macromolecules causing molecular cell injury and damage[29]. The following stage of carcinogenesis has been included initiation, promotion until progression. Variant enzymes involved in the hepatic microsomal and hydroxylation are participating in interactions between metabolites of carcinogens with DNA

to form DNA adducts[30]. Published studies announced that receptors called the arylhydrocarbon (AhR) are shared with a high success rate in developing carcinogenesis using DMBA compounds, particularly in the reproductive system.

Most likely, administration of Lagenaria siceraria has improved the deleterious influences which emerged due to DMBA administration. Lagenaria siceraria exerted anticancer effects attributed to its contents from phenolic and alkaloid compounds[31]. Since, its phenolic constituent interferes with cell proliferation processes through the arrest of the G2/M cell cycle by removal of free radicals, cell cycle arrest, apoptosis induction, and inhibition of angiogenesis[12]. In addition to methanolic extract of Lagenaria siceraria offered significant scarcity in aberration cells in chromosomal aberration assay[32].

CONCLUSION

From the cell biological and histochemical points of view, the carcinogen DMBA had stimulatory effects on the synthesis of the examined chemical components, DNA, RNA, and collagen, except for total protein and polysaccharides, where it had an inhibitory effect on them in the cells of testes. In contrast, the Lagenaria siceraria induced a resistant effect on the action of carcinogen DMBA. From the histopathological and immunological points of view, the carcinogen DMBA had a carcinogenic effect on the tissue of the testes of mice and special preparation of the Lagenaria siceraria treats the carcinogenic action of DMBA.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

التأثير البيولوجي لـ DMBA و: ٣ : Lagenaria Siceraria المدى المحتمل لـ Lagenaria Siceraria لمواجهة التأثير المحفز لـ DMBA على خصيات الفئران السويسرية الذكور

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الخلفية: لا شك أن مرض السرطان يتميز بأنه السبب فى حوادث الوفاة الخطيرة في المجتمع العالمي. ومن المعروف أن المادة المسرطنة (DMBA) والفئران نموذج ثابت لدراسة الأورام. وقد وضح المؤلفون أنه في الكائن الحي الناضج ، لكل مجتمع خلوي سلوك خاص في عملية تكاثر الخلايا الذي يؤدي إلى انقسامها بشكل طبيعي بمعدل يتناسب مع المعدل الطبيعي لفقدان خلاياها من خلال الموت المبرمج, لذلك يتكاثر كل مجتمع خلوي عبر سلوكه الخاص في ظل الظروف غير الطبيعية ، سواء كانت مؤثرات تجريبيَّة أو أحداثًا مرضية.

الهدف من العمل: تم إجراء هذا البحث الأكاديمى التطبيقى القبل سريرى لمحاولة قمع تكاثر الخلايا فى أنسجة خصى ذكور الفئران السويسرية, المستحث بتأثير جرعة واحدة من المادة المسرطنة (DMBA) لمدة ١٢٠, باستخدام جرعة فموية مزدوجة يوميا من مستحضر خاص من Lagenaria Siceraria لمدة ٣٠ يوم.

المواد والطرق: في الدراسة الحالية ، تم تقسيم ٣٠ فأرًا سويسريًا بالغًا (٩٠ يومًا من العمر) بالتساوي إلى ٣ مجموعات على النحو التالي ؛ ضابطة و DMBA و DMBA + Lagenaria siceraria . بعد انتهاء فترة التجربة التي امتدت لمدة ٤ أشهر ، تم تشريح الفئران وإستخلاص الخصي وإعدادها واجراء الفحوصات النسيجية والكيميائية النسيجية والكيميائية المناعية.

النتائج: من وجهة النظر البيولوجية الخلوية والهستوكيميائية والهستوكيمياء المناعية، أن المادة المسرطنة (DMBA) لها تأثير تحفيزي على التكاثر الخلوي فى الحويصلات المنوية وخلايا Leydig فى خصى ذكور الفئران من خلال تخليق DNA و RNA الكثيف.، علاوة على ذلك ، DMBA أدى إلى تناقص واضح في المحتوى الكيميائى البروتينات والسكريات وحدوث تسلل واضح لألياف الكولاجين حول الأوعية الدموية. إلا أن العلاج بواسطة Lagenaria Siceraria قد عالج تقريبا هذا التأثير لـ DMBA.

الخلاصة: اعتمادًا على هذه النتائج ، يمكن الاستنتاج أن DMBA يمثل حقًا نموذجًا قويًا للتسرطن والذي تطور بمرور الوقت بشكل واضح. ومع ذلك ، يمكن أن يخفف Lagenaria siceraria من التأثير الضار الذي تم الكشف عنه في خلايا الخصية بواسطة مادة DMBA المسرطنة.