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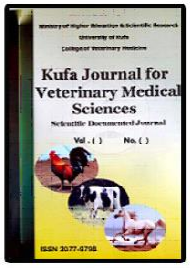

Histopathological Effect of Annona Muricata Fruit Extract on Methyl nitrosourea -Induced Mammary Gland Carcinoma in Female Albino Mice

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Abstract

The Annona muricata (Family-Annonaceae) fruit contain annonaceous acetogenins which have an effect on cancer cell as reported in many research studies. This study was aimed to evaluate the effect of methanolic fruit extract of Annona muricata (MFEAM) on breast cancer histology and invasive cell of MNU-induced breast cancer mice. Fifty female albino mice were divided into five groups (of ten each): control; MNU-induced breast cancer; MNU-induced breast cancer + (MFEAM) 200 mg/kg, MNU-induced breast cancer + (MFEAM) 300 mg/kg and MNU-induced breast cancer + (MFEAM) 500 mg/kg. The research indicates that breast cancer invasive ductal carcnoma(IDC) developed in the MNU-induced breast cancer group. The control group showed normal duct structure. The (MFEAM) -treated group showed lower invasive (DCIS) compared with the untreated cancer group. We concluded that (MFEAM), that improved the histological changes of breast cancer-induced MNU. Also (MFEAM) reduced proliferative epitheal cell of breast cancer-induced MNU and the most effective dose was 300 mg/kg. The present study concluded that the methanol extracts derived from Annona muricata fruit potentially exhibit antitumor activity.

Keywords: Annona muricata, breast cancer, methyl nitrosourea –induced breast cancer, mice

Introduction

Breast cancer is the most common malignancy among females affecting approximately one out of ten women. [1]. Mortality impacts are somewhat ambiguous and often linked to socioeconomic and lifestyle status [2,3]. It is a very heterogeneous disease with variation in histological grade, proliferative index, immunohistochemistry and clinical presentation [4]. Graviola (Annona muricata) is a tropical fruit tree of the family Annonaceae. The fruit is of economic value and hence cultivated and used widely as an edible food. The plant possess the major pharmacological activities includes cytotoxic, antileishmanial, wound healing, antimicrobial activity. It also has the anticarcinogenic and genotoxic effect. Phytochemical analysis of the plant revealed the presence of tannins, steroids and cardiac glycosides which are the major phytochemical compounds [5]. Annonaceous acetogenins are a unique set of derivatives of C35 or C37 long chain fatty acids derived from the polyketide pathway 1 and characteristic of family Annonaceae. These phytochemicals have been reported to possess significant antitumor properties [6].

Materials and Methods

Ethics approval:

The experimental processes protocols were approved by Research Centre's Ethics Committee's suggestion, of the of Kufa University, Council on guidelines to ensure that experimental animals have properly cared. authorization from the Animal Experimentation Ethics Agency; Ethic no. (20576).

Experimental design:

Fifty (8 weeks-old) female albino mice of body weight range between 20-26g

were obtained from college of Science, University ot Al Kufa. the mice were placed in polypropylene plastic cages where 10 mice were placed in each cage with wood chips for bedding and housed in an animal room with controlled conditions involving these parameters namely; temperature (25 +2 °C). humidity (55 +10%) and lighting (12 hours light/dark) in the animal house at the college of Science, University of Kufa, Najaf Governorate The mice were provided with tap water and commercial chow daily (ad libitum) and allowed to acclimatize for four weeks. Methyl nitrosourea was obtained from local supplie and used to induced mammary cancer in albino mice. Mice were received two s/c injection of of MNU at dose of 60 mg/kg of body weight, one –week during/ two weeks pre-experimental period. MNU was prepared according to the method described by Thompson and Adlakha in 1991 [7]. Preparation of fruit extract of Annona muricat. Annona muricat fruit option from local market and thoroughly washed in water, cut into small pieces and shade dried at 35°C–40°C then convert in to powder form using fine grinder, two hundred (200) mg of of fruit powder were crushed with 400ml of mixture methanol 95% and distilled water (9:1), mixed for18h in magnetic stirrer at room temperature, then filtered under vacuum using whatman No. (1). Fifty female albino mice were assigned into five groups (ten mice in each group), namely C-, C+, T1, T2 andT3.control negative (C-) group mice were received normal saline daily for 16 weeks. Control positive group mice were received four S.C injected of MNU at dose of 60 mg/kg of body weight during first two week of experimental period.T1group mice were received four SC injected of MNU at dose of 60 mg/kg of body weight during first two week of experimental period and received (AMFME)at the dose of 200 mg/kg of body weight by gavage daily for16 week .T2group mice were received four SC injected of MNU at dose of 60 mg/kg of body weight during first two week of experimental period and received (AMFME)at the dose of 300 mg/kg of body weight by gavage daily for16 week. T2 group mice were received four S.C injected of MNU at dose of 60 mg/kg of body weight during first two weeks of experimental period and received (AMFME)at the dose of 500 mg/kg of body weight by gavage daily for16 week.

Histopathological Examination:

The cross-sectional full-thickness skin sample was isolated from each group of mice were collected at (24hrs, 48hrs, 72hrs) for the histopathological alterations. The samples were fixed in 10% buffered formalin, processed and blocked with paraffin, and then sectioned into 5 μm sections and stained with hematoxylin and eosin.

Statistical Analysis:

The data were analyzed using the ANOVA computer program, with a P value of less than 0.05 considered significant.

Results:

According to the histopathological findings. A tumor lesion was observed in all mice, except for the normal group, however, the severity of the tumor lesion varied depending on the group. As illustrated in tables 1, 2, and 3. Mammary gland carcinoma lesions were observed in all mice, that received MNU included MNU, T1, T2 and T3 groups, except for the normal group.

Table 1: Mammary gland carcinoma grading.

Invasive ductal carcinoma	Ductal carcinoma *in situ*			Group
	High	Intermediate	Low	
Nell	Nell	Nell	Nell	C-
5	2	/	/	MNU
3	3	1	/	T1
/	/	1	6	T2
/	/	2	5	T3

Table 2: Descriptive means of Mammary glands carcinoma score.

Groups	**Mammary glands carcinoma score**	
	Mean	**±S.D**
MNU	3.7143 [z]	±0.48
T1	3.2857 [b]	±0.75
T2	1.1429	±0.37
T3	1.2857	±0.48

a: Significant difference between group MNU compared with groups T2 and T3.
b: Significant difference between group T1 compared with groups T2 and T3.

Table 3: Non-parametric mean ranks of Mammary glands carcinoma score.

GROUPS	**Mammary glands carcinoma score**
	Mean Rank
MNU	22.64 [a]
T1	20.14 [b]
T2	7.07
T3	8.14

a: Significant difference between group MNU compared with groups T2 and T3.
b: Significant difference between group T1 compared with groups T2 and T3.

According to the Mammary gland carcinoma scoring results (Table 2) the mammary gland carcinoma lesion severity showed a different grade within these groups. where the invasive ductal Mammary gland carcinoma was observed (71%) in MNU group and (43%) in T1 group. An invasive ductal mammary gland carcinoma was characterized by massive proliferation of undifferentiated pleomorphic neoplastic epithelial cell, which, these neoplastic cell formed a large mass, also shown sever angiogenisis with presence high of mitotic index in these cell (figure 1).

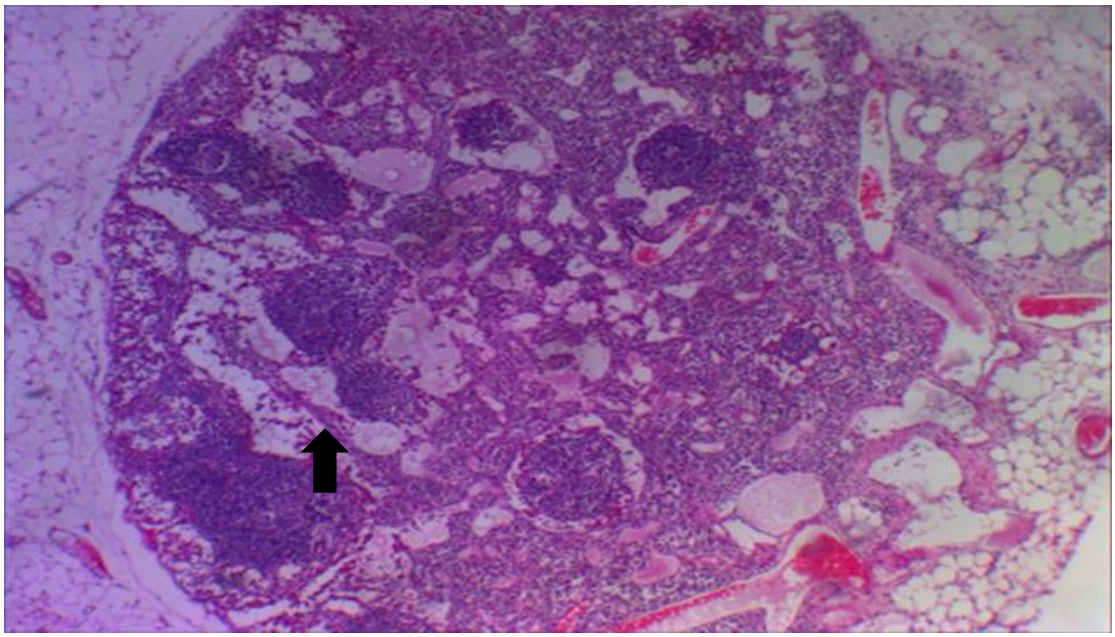

Figure 1. Photomicrograph of mammary gland of (MNU group) mouse. Massive proliferation of undifferentiated pleomorphic neoplastic epithelial cell, which, these neoplastic cells formed a large mass occupied most of the fatty tissue that lies under nipples area (black arrow). **H&E 40x.**

The high grade mammary gland carcinoma was observed (29%) in MNU group and (43%) in T1 group and this high grade carcinoma was characterized by proliferation of neoplastic epithelial cell form cribriform pattern in affected area and nuclei are of high grade, markedly pleomorphic as shown in figure 2.

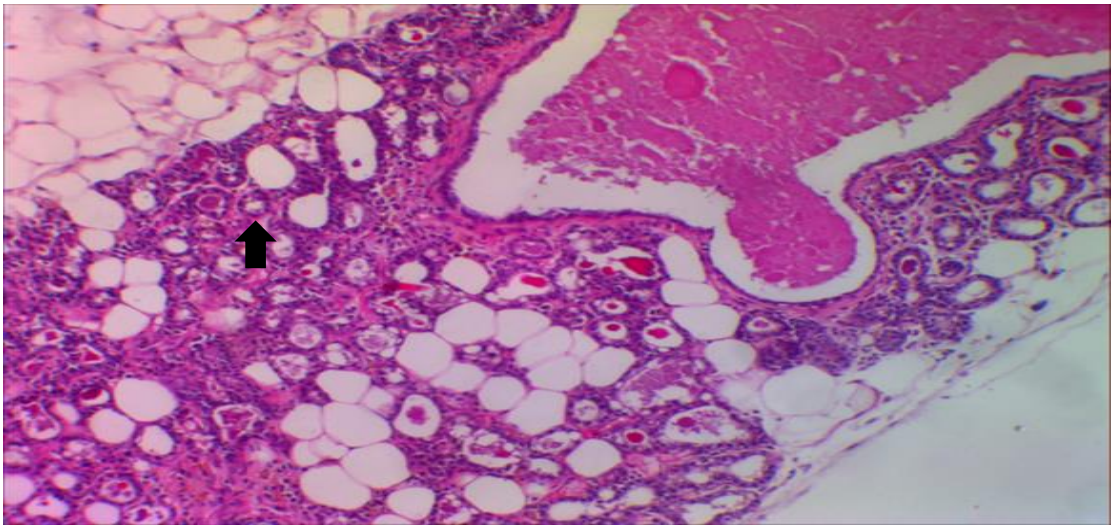

Figure 2. Photomicrograph of mammary gland of (T1 group) mouse. Proliferation of neoplastic epithelial cell form cribriform pattern in affected area. Not the newly ducts formed by neoplastic epithelial cell (black arrows). **H&E 100x.**

The intermediate grade mammary gland carcinoma was observed (14%)in T1 group and (14%) in T2 group this intermediate grad carcinoma was characterized by cells that are cytologically similar to those of low-grade .The low grade mammary gland carcinoma was observed (86%) in T2 group and (71%) in group this low grad carcinoma was characteristic by small monomorphic cell patterns forming micropapillary patterns, as presented in figures 3 & 4. The Statistical Analysis of mammary

gland carcinoma score results showed a significant ($p < 0.05$) differences in MNU group compare with others groups also showed a significant differences inT1 group compared the T2 and T3. While the scoring result of T2 and T3 did not showed any significant($p > 0.05$) difference between them.

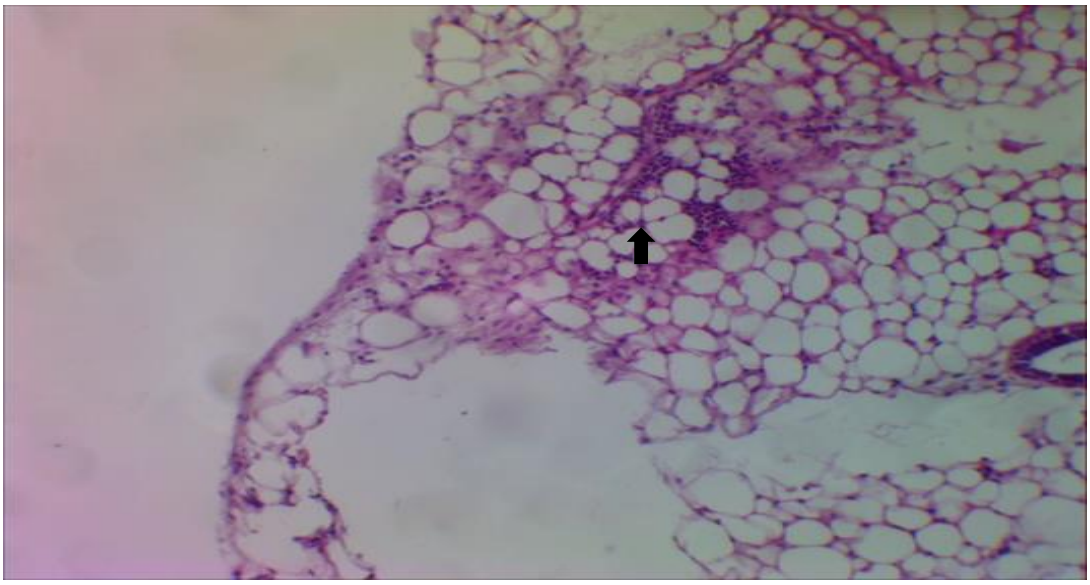

Figure 3. Photomicrograph of mammary gland of (T2 group) mouse. Small monomorphic cell patterns forming micropapillary patterns (black arrow). H&E 40x.

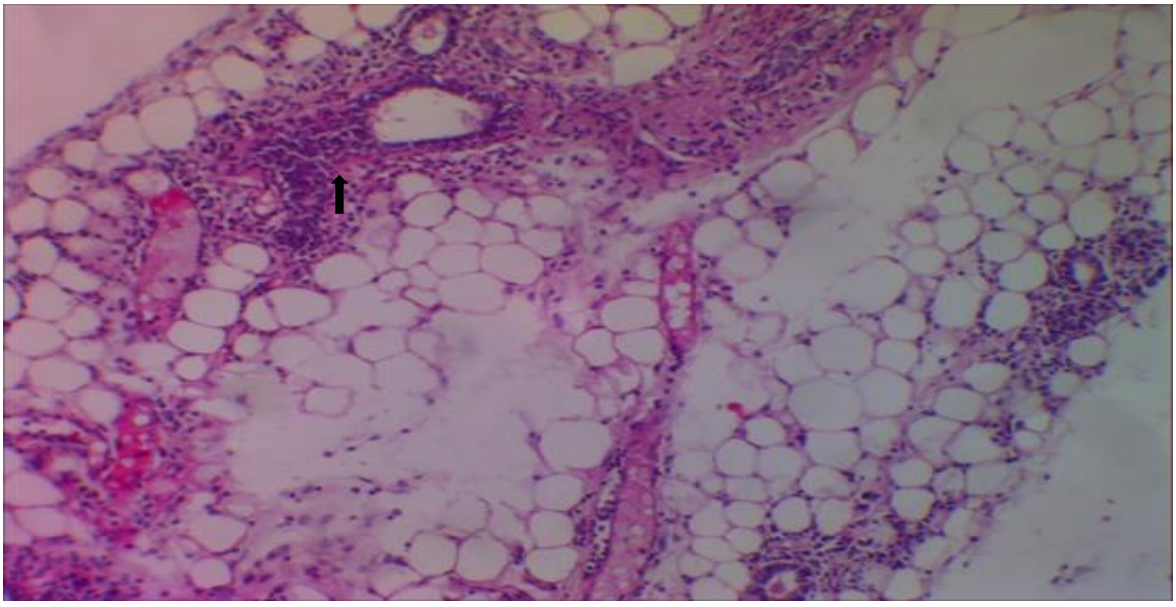

Figure 4. Photomicrograph of mammary gland of (T3 group) mouse. Proliferation neoplastic epithelial cells forming micropapillary patterns, also newly ducts forming. (black arrow). H&E. 100x.

Discussion

Breast cancer has become one of the diseases with a high mortality rate. [8]. Since the new cancer treatment that has a selective treatment effects and low cost required to improve patient quality life. For this recent advances in understanding the mechanistic roles of phytochemicals that make up whole foods continue to reveal the importance of their synergistic interactions in their complex natural environment [9]. Therefore, this research indicates that (Annona muricata) fruit

extract improves the tissue appearance of the breast cancer MNU-induced mice. A. muricata is widely cultivated and possesses substantial therapeutic value and to no surprise, used in many traditions to treat multiple ailments, together with cancer primarily contributed to its cytotoxic efficacy. [10]. Methylnitrosurea (MNU) is a well-known potent carcinogen which has been used to induce carcinogenesis in the mammary gland of experimental rodents such as rats and mice [11]. Our study reveals that mice induced by MNU exhibit breast nodules with various sizes starting at about four weeks after MNU administration. The preventive effect of a methanol extract of A muricata fruit against MNU- induced DNA damage could be due to the presence of the various secondary metabolites (tannins terpenoids, cardiacglycosides, and flavonoids) discovered from the phytochemical screening of the extract of A. Muricata [12]. The gross pathology result showed a variation in tumor in MNU induced mammary gland in mice. Where the administration of MNU S.C had the ability to induced tumor within 8 weeks of induction. [13]. However, the MNU group mice showed a tumor in mammary gland area, where the control group is considered normal these resuts agree with [14].

N-nitroso compounds are a broad class of chemical compounds that can be easily synthesized by the reaction of nitrogen oxides. Nitroso compounds are carcinogenic agents that stimulate tumor formation in many experimental animal models, and they may also be linked to the development of various human malignancies. Some of these nitroso-compounds, namely N-methyl-Nnitrosourea (MNU) [15]. Annona muricata extract had no effect on mortality it is certainly safe [16]. Nature is nontoxic,and Annona muricata extract used in T1andT2 groups mice is reduce the mass compared with control positive groups. However, the tumor index result showed a significant protection effect Annona muricata extract treated mice, this result supported by [17]. As anti-cancer drugs, natural plant compounds have showed promising outcomes When compared to anti-cancer medicines, their effectiveness is described as lower toxicity, safety, and fewer recurring resistances [18]. Annona muricata, according to reports It possesses anti-cancer properties. It has typically been used to shrink tumors and has little negative effects. [19]. In histopathology result the MNU only treated mice showed destruction in tissue of mammary gland that involve all architecture features and malignant abnormal proliferation of neoplastic cells in the breast tissue, which has penetrated through the duct wall into stroma and sever angiogenesis compare with other groups this result supported by [20].while T1,T2andT3 treated mice carcinoma was involve DCIS.,is noninvasive this generally divided in to three type low-grade DCIS and high-grade DCIS [21]. However, the T2 treated mice showed type low-grade DCIS compared with T1and T3. The histopathology result indicated that the optimum protective effect was observed in Annona muricata extract treated mice. However, T1 treated mice showed a less protective effect compare with T2. This result is highly effective and the current results indicate an effect Annona muricata extract, on the tumor will reduce the cancer aggressiveness. This result agreed with [22].

Conclusion

A. muricata fruit methanol extract was found to have significant antitumor activity. This suggests that the fruit might potentially generate improved bioactive chemicals with significant anti-proliferative properties that could be beneficial in primary care. The administration of A. muricata fruit methanol extracts at a dose of 300 mg/kg of body weight is more efficacy in the reduction of tumor in female albino mice compared with 200 mg/kg of body weight dose. The histopathology results indicated that A. muricata fruit methanol extracts reduce the invasion of tumor in the mammary gland of female albino mice to adjacent tissue.

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