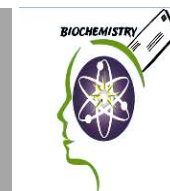




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Protective Effect Of Hesperidin And Tiger Nut Against DMBA Carcinogenicity In Female Rats

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ABSTRACT

Nutritional studies recommend the regular consumption of fruits and vegetables to favor a healthy quality of life. This study was carried out to evaluate the efficacy of hesperidin and tiger nut against the carcinogenic activity of DMBA in female rats. 72 adult Sprague Dawley female rats were divided equally into six groups: control group (I); hesperidin (HES) treated group (II); tiger nut (TN) treated group (III); DMBA treated group (IV); HES-DMBA treated group (V); and TN-DMBA treated group (VI). There was a significant increase in the levels of serum total sialic acid (TSA), progesterone (Prog) and estradiol (E2), and significant decreases of body weights and reduced glutathione (GSH) level and superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activities of DMBA treated group compared with the control. Histopathological observations of mammary gland showed that only female rats of DMBA and TN-DMBA treated groups showed breast cancer, while there was no evidence of malignancy in HES-DMBA treated group. In conclusion, our results suggested that supplementation of a diet with hesperidin provided antioxidant defense with chemoprotective activity more significant than tiger nut against the toxicity of DMBA in breast tissue.

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INTRODUCTION

7, 12-Dimethylbenz (a) anthracene is a polycyclic aromatic hydrocarbon (PAH) produced during the incomplete combustion of carbon-containing compounds [1], and predominantly found in tobacco smoke, woodsmoke and motor vehicle exhaust emissions [2]. It is an

immunosuppressor and a powerful organ-specific laboratory carcinogen [3,4]. The main target sites for the potent carcinogenicity of DMBA are the skin and the mammary gland [5]. It has been reported that DMBA induces substantive nephrotoxicity that is characterized by

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renal tubular necrosis, increased number of lysosomes, dilated mitochondria and chromatin condensation in the nucleus [6,7].

Hesperidin (4-methoxy-7-O-rutinosyl-30,5-dihydroxyflavanone), a naturally occurring flavanone glycoside, is predominantly found in citrus fruits [8]. It has several biological functions such as anti-inflammatory effects [9], prostaglandin-synthesis inhibition, neuroprotective [10] and modulation of drug-metabolizing enzymes and it does not present toxicity [11]. It exhibits antioxidative properties by several different mechanisms, such as scavenging of free radicals, chelation of metal ions such as iron and copper which are of major importance for the initiation of radical reactions, inhibition of enzymes responsible for free radical generation and facilitation of the endogenous antioxidative defense system [8,12,13]. Also, dietary hesperidin exerts anticarcinogenic actions in the tongue, colon, esophagus, and urinary bladder in rat models of carcinogenesis [14-16].

Tiger nut tubers (*Cyperus esculentus*) are edible, with a slightly sweet and nutty flavor. The tubers are used as a foodstuff, particularly in Africa, where it's an important food crop with certain tribes. Tiger nuts have excellent nutritional qualities with a fat composition similar to olives [17] and they are gluten and cholesterol free [18]. Tiger nuts are rich in vitamins E and C and rich in minerals such as phosphorus, potassium, calcium, magnesium and iron which are necessary for bones, tissue repair, muscles, blood stream, body growth and development [19]. Also they contain high percentage of alkaloids, saponins and tannins which are known to have antimicrobial activity, as well as other physiological activities [20]. Moreover, they exhibit anti-inflammatory properties upon inflammation, and immunostimulatory effects [21].

The objective of the present study is to evaluate the efficacy of hesperidin and tiger nut in modulation of oxidative stress, tumorigenicity and endocrine derangement in rats treated with DMBA. Histological examinations of the breast tissue were assessed to highlight the protective effects of these natural products.

Materials and methods

Healthy adult female virgin albino rats (Sprague dawley) (70±10) g, average 6 weeks old, purchased from the Egyptian Holding Company for Biological Products and Vaccines (Cairo, Egypt) were used as experimental animals. The animals were maintained under standard conditions of light, ventilation, temperature, and humidity and allowed to free access to standard pellet diet and tap water. All animal procedures were carried out in accordance with the Ethics Committee of the National Research Centre conformed to the "Guide for the care and use of Laboratory Animals" published by the US National Institutes of Health (NIH publication No. 85-23, 1996).

Animal groups

After an acclimation period of 2 weeks, the rats were randomly divided into 6 groups (12 rats/group). Group I: control; Group II: rats received HES (50mg/Kg/day) via oral gavage during the experimental period [22]; Group III: rats fed on a standard diet mixed with 25 % whole powder of tiger nut, TN [21]; Group IV: DMBA treated group were received 10 mg DMBA /rat [5] via oral gavage at 1 week intervals on days 15, 22, and 29; Group V: rats treated with HES + DMBA where rats were received HES (50mg/Kg/day) during 14 successive days before treating with neoplastic agent and during 105 days after treatment; and Group VI: rats treated with TN + DMBA where rats were received TN (25% of diet) during 14 successive days before treating with neoplastic agent and during 105 days after treatment.

Animals were sacrificed, under anesthesia of ether, after 90 days post the

last dose of DMBA administration, and blood was collected by heart puncture and centrifuged at 3000 rpm for 15 minutes to separate the serum and stored at -20°C . Serum was used for the determination of total sialic acid (TSA) as markers for tumorigenicity, progesterone and estradiol as markers of endocrine derangement, protein carbonyl (CO), malondialdehyde (MDA) as markers for oxidative stress and the level of reduced glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) activities as markers for antioxidant status. Mammary, liver and kidney tissues of each animal were excised and preserved in 10% neutral buffer formalin for histopathological examination.

Biochemical analysis

Tumorigenicity Biomarkers assays: Assay of TSA, TSA was determined according to the method described by **Plucinskyet al.**[23].

Hormonal assays: ELISA procedure was used for quantitative determination of Estradiol (E2) concentration in rat serum using a kit purchased from Sigma Chemical Company (St Louis, MO, USA) according to **Tietz**[24] and also progesterone using a kit purchased from Sigma Chemical Company (St Louis, MO, USA) according to **Tietz** [25].

Oxidative stress biomarkers assays: Lipid peroxidation was evaluated by measuring MDA levels according to the method of **Yoshioka et al.** [26]. Protein oxidation was evaluated by measuring protein carbonyl (CO) content as described by **Levine et al.** [27]. The activity of SOD was determined according to the method of **Minami and Yoshikawa** [28]. The activity of CAT was determined according to the method of **Aebi**[29]. The activity of GSH-Px was determined according to the method of **Paglia and Valentine** [30]. Reduced glutathione content was estimated according to **Beutler et al.** [31].

Histopathological investigation:

The mammary tissues were taken and fixed in 10% buffered formalin,

embedded in pure paraffin wax (melting point of 58°C) and then mounted into blocks and left at 4°C until the time of use. The paraffin blocks were sectioned on the microtome at thickness of 5 mm and mounted on clean glass slides then left in the oven at 40°C for dryness. The slides were deparaffinized in xylol then immersed in descending series of alcohol concentration (90–50%). The ordinary hematoxylin and eosin stain were used [32].

Statistical analysis:

The Statistical Package for the Social Sciences (SPSS/PC) computer program was used for statistical analysis of the results. Data were analyzed using one way analysis of variance (ANOVA) followed by Post Hoc to determine significant differences between means. The data were expressed as mean \pm standard deviation (SD). Differences were considered significant at $P \leq 0.05$.

Results

Biochemical Results

No rats died in groups I,II and III, 6 of 12 rats died in Group IV. In Group V, 3 of 12 rats died, while in Group VI, 5 of 12 rats died. There was an increase in the body weight in all groups at the time of killing compared to at the start of experiment, with percentage changes 69.3%, 122%, 87.4%, 18.7%, 57.6% and 38.5% for groups I-VI, respectively, (**Table 1**). At the end of the experiment, groups IV, V and VI were significantly decreased in the body weight compared to control group. There was a significant increase in the body weight on rats treated with HES and DMBA (Group V) compared with rats treated with DMBA only (Group IV). On the other hand, there was no significant change in the body weight between rats treated with TN and DMBA (Group IV) and rats in group IV (**Table 1**). **Table 2** showing that serum levels of TSA, progesterone and estradiol were significantly increased in groups IV-VI when compared with control (Group I). There were no significant changes in the

levels of TSA, progesterone and estradiol between group IV and group VI, while they decreased significantly in Group V when compared to DMBA-treated group (Group IV).

Serum levels of both protein carbonyl and MDA were significantly increased in groups IV-VI when compared with control rats. They were significantly decreased in group V with p value <0.001 when compared to DMBA-treated group, while there were no significant differences between groups IV and VI. Serum SOD, CAT, GSH-Px activities and the level of GSH were significantly decreased in groups IV-VI when compared to control group, while they were significantly increased in group V when compared to DMBA-treated group (**Table 3**) and there were no significant differences between groups IV and VI.

Histopathological Examinations of Rat Mammary Glands

Histopathological examinations of the control mammary glands showed that mammary glands were made up of scattered tubular branching ducts and glandular alveoli; both lined by one to two layers of low cuboidal epithelium and had well defined lumen. The epithelium rests on a basement membrane was surrounded by a layer of myoepithelial cells. The lumen of the alveoli of the mammary glands appeared clear without any cell debris or any secretion. The breast tissue of female rat of group II and III is more or less similar to those of control group (**Fig. 1A,B&C**).

In DMBA-treated group (group IV), the studied paraffin sections showed well developed breast carcinoma with wide areas of necrosis and tumor breakdown indicating the high proliferation of tumor cells with surrounding residual viable proliferating malignant cells (**Fig. 2A**). The cell showed nucleomegaly, pleomorphism, increased

mitosis and moderate overall anaplasia. The mammary glands also showed atypical proliferative changes characterized by a progressive elongation of ducts as well as proliferation and expansion of the terminal lobular units with compression of the surrounding fat pad, progressing to cystic papillary carcinoma (**Fig. 2B**).

Female rat protected with HES against the carcinogenicity of DMBA showed no appearance of malignancy with very mild histopathological alterations as shown in **figure 3**. In other instances hyperplasia still present, vacuolization in many cells was commonly occurred and decreased numbers of alveoli with hypertrophic and hyperplastic epithelium forming small lobules of glandular tissue surrounding ducts. While, the mammary alveoli of group VI exhibited well defined mass of breast carcinoma that surrounded by fibrous tissue and embedded in adipose connective tissue with adjacent enlarged activated lymph node. (**Fig. 4**).

Discussion

It is vital to enhance our understanding of the role of naturally occurring antioxidants in cancer prevention and their possible use in intervention trials for the prevention of cancer in humans. DMBA-induced experimental carcinogenesis might therefore be used as an ideal model to study the chemopreventive potential of natural entities [33]. Frequent production of ROS cause free radicals and electrophiles mediated oxidative stress favouring the progression of carcinogenesis. These highly reactive species causes oxidative modification of DNA, proteins, lipids and small intracellular molecules [34]. In our study, we administered DMBA at the age of 56 days due to susceptibility of the mammary gland to DMBA carcinogenesis is strongly age-dependent, being maximal when the drug is administered to rats between the age of 45 and 60 days, the age of the beginning of sexual maturity[35].

In the present study, the total body weights of the DMBA and TN-DMBA treated groups were declined with p value: <0.001 when compared to control group. Our findings desperately agree with the previous findings of **Cheng *et al.*** [36] and **Anbuselvam *et al.***[37] who reported that tumor growth elicited marked loss of body weight in animals with breast cancer. The decreased body weight might be due to the changes in energy metabolism during tumor formation. It was well known that in cancer condition excessive energy expenditure of the host, ultimately contributing to mechanisms that promote weight loss [38]. Additionally, our results were in accordance with the result of **Davis and Kuttan**[39]and **Moselhy *et al.*** [40] who stated that the generation of ROS and the peroxidation of membrane lipids were associated with the initiation and progression of cancer affecting the normal biochemical process which further leading to the reduction of body weight.

In our results, upon treatment with each of hesperidin and tiger nut, the body weights increased significantly in HES-DMBA treated group versus DMBA treated group ($p=0.001$) while there was no significant difference between DMBA and TN-DMBA treated groups. Additionally, there was a significant increase in HES-DMBA treated group with p value: 0.029 when compared to TN-DMBA treated group.

Our findings of the increased body weights in the groups protected with hesperidin were in parallel with the studies of **Kamaraj *et al.***[41] and **Al-Jasabi and Abdullah** [42]who reported that while the total body weight was decreased in tumor-bearing mice (lung cancer); pre and post treatment with hesperidin resulted in a significant increase in the body weight. Also, **Nandakumaret al.** [43] reported that daily oral administration of hesperidin to breast cancer bearing rats showed a significant improvement in the body weight when compared with cancer bearing animals and this might be due the

antioxidant activity exhibited by the bioflavonoid hesperidin.

In our study, serum level of total sialic acid (TSA) significantly elevated in the DMBA and TN-DMBA treated groups ($p<0.001$) versus control group, and there was no significant difference between TN-DMBA and DMBA treated groups. These findings were in agreement with our histopathological results that showed the formation of breast cancer in these two groups. The increased TSA levels here were similar with the findings of **Raval *et al.*** [44], **Hamdy *et al.*** [45] and **Oto *et al.*** [46]who reported that administration of DMBA increased the level of serum TSA, which is a sensitive marker for detecting the toxic effects of DMBA and this increase might be explained by enhanced activity of sialidase enzyme in breast cancer [47,48].

Conversely, our results showed a highly significant reduction in serum TSA level in HES-DMBA treated group ($p<0.001$) when compared with DMBA and TN-DMBA-treated groups. Our findings were in agreement with the results obtained by **Nandakumar *et al.*** [43,49] who reported that administration of hesperidin controlled the nucleic acid biosynthesis and exhibited the tumor inhibitory effect during the treatment of breast cancer induced by DMBA toxicity. Moreover, our results showed a significantly increase ($P = 0.001$) in the levels of serum TSA in HES-DMBA group as compared with the control group (no malignancy was recorded in our histopathological results). This increase in the TSA level might be due to the presence of inflammation in any part of animal body and not due to breast cancer. In this respect, **Suer and Kazwzoglu**[50]and **Hamzah *et al.*** [51] reported that the biochemical alternation in total sialic acid concentrations was associated positively with the presence of inflammation and could be suggested as a potential inflammatory marker for several diseases including myocardial infarction,

inflammatory disease and bacterial infections.

Moreover, there was a significant increase in the estradiol and progesterone levels in DMBA and TN-DMBA treated groups ($p < 0.001$) when compared with the control. Similar results were observed by **Hamdy et al.** [45] who reported that the injection of single dose of (10 mg/rat) DMBA resulted in a significant increase in the marker levels of endocrine derangement, prolactin and estradiol. In this respect, **Vondráček et al.** [52] and **Hussein and Ismael** [53] proved that the endocrine derangement that occurred in the human breast had an important impact on the development of breast cancer.

In the present study, hesperidin supplementation in HES-DMBA treated group significantly decreased serum levels of estradiol and progesterone ($p < 0.001$) when compared to DMBA and TN-DMBA treated groups, while there were no significant changes in their levels between DMBA and TN-DMBA treated groups.

Our study showed elevated levels of serum MDA and CO in DMBA and TN-DMBA treated groups when compared to the control ($p < 0.001$) and there was no significant difference in their levels between DMBA and TN-DMBA treated groups. This was consistent with previous reports that DMBA induced critical oxidative damage in the liver *in vivo* [54,55]. Moreover, our results were in agreement with the data obtained by **Tabaczaret al.** [56] who reported that the development of DMBA-induced mammary tumors in rats was associated with a significant increase in protein and lipid oxidation markers in blood plasma. Furthermore, **Rajakumar et al.** [57] reported that the levels of MDA and lipid hydroperoxides in plasma and mammary tissues were significantly increased in DMBA-induced breast cancer rats via the overproduction and diffusion of free radicals from damaged tumor tissues compared with normal rats.

Our results indicated that hesperidin supplementation was significantly decreased the levels of MDA in HES-DMBA treated group ($p < 0.001$) when compared with DMBA and TN-DMBA treated groups. Additionally, serum CO level decreased significantly in HES-DMBA group ($p < 0.001$) versus DMBA and TN-DMBA treated groups. Our results were in agreement with **Wang et al.** [58] who reported that chronic administration of hesperidin attenuated oxidative damage by decreasing lipid peroxidation, restoring mitochondrial enzyme activity, and increasing oxidative defense. Moreover, **Javed et al.** [59] reported that pretreatment with hesperidin attenuated the elevated level of MDA content in streptozotocin injected mice.

The enzymatic antioxidants SOD, CAT and GSH-Px and GSH content play a vital role during the process of scavenging reactive oxygen [60]. Our results demonstrated significant decreases of SOD, CAT and GSH-Px activities and GSH content in DMBA and TN-DMBA treated animals with p value: < 0.001 when compared to control group and there was no significant difference between DMBA and TN-DMBA treated animals. The decrease of SOD, CAT and GSH-Px activities and GSH content was probably might result from their increased utilization to neutralize the excess of free radicals generated in the body after DMBA induction and might indicated the weak free radical defense system against oxidative stress [37]. The observed lower level of glutathione in mammary carcinoma rats might be resulted in enhanced lipid peroxidation and excess utilization of this antioxidant for tumor cell proliferation, which was in line with earlier reports [61].

In our results, there was statistically significant increase in serum GSH level and SOD, CAT and GSH-Px activities in HES-DMBA treated group with p value < 0.001 when compared with

both DMBA and TN-DMBA treated groups, while there were no significant differences between TN-DMBA and DMBA treated groups for these levels. The improvement in oxidant/antioxidant status might support the role of HES in scavenging free radicals. Similarly, **Bentli et al.** [62] reported that the use of HES in combination with 2,3,7,8-Tetrachlorodibenzo-p-dioxin, minimized its toxicity as revealed by increased antioxidant enzyme activity (SOD, CAT) and GSH levels. Moreover, **Khan and Parvez**[63] reported that hesperidin treatment attenuated the altered levels of oxidative stress and neurotoxicity biomarkers against oxidative damage in cadmium treated rat model.

Our histopathological results showed that administration of three successive doses of DMBA (10 mg/rat/week) induced breast carcinoma in only DMBA and TN-DMBA treated animals. The studied paraffin sections of DMBA treated animals showed well developed breast carcinoma with wide areas of necrosis and atypical proliferative changes characterized by a progressive elongation of ducts as well as proliferation and expansion of the terminal lobular units with compression of the surrounding fat pad, progressing to cystic papillary carcinoma. The cell showed nucleomegaly, pleomorphism, increased mitosis and moderate overall anaplasia. These findings agree with our previously reported higher breast cancer markers (TSA, estradiol and progesterone).

Similar observations were obtained by **Pandi et al.** [64] who reported that administration of DMBA caused rat mammary gland carcinoma characterized by extensive solid areas, tumor necrosis exhibiting the appearance of a comedocarcinoma and clumping of chromatids. Also, **Bishayeea and Mandal** [65] investigated that administration of DMBA manifested ductal hyperplasia, marked alteration and enlargement of the alveolus, uniformly neoplastic ductal

epithelial cells with nuclear pleomorphism and growth variation of epithelial cells in nuclear size and irregular chromatin. Moreover, **Minari and Okeke**[66] showed that the intragastrically treatment of 20 mg/ml/week of DMBA by gavage for six weeks induced lobular alveolar hyperplasia, fibroadenomatoid hyperplasia in breast tissues suggesting a neoplastic transformation which is an indication of DMBA-induced cell proliferation.

On the other hand, no malignancy appeared in animals of HES-DMBA treated group. Protection of DMBA treated animals by HES administration (50 mg/kg body weight) provided a significant improvement with very mild histopathological alterations such as hyperplasia; vacuolization in many cells and decreased numbers of alveoli with hypertrophic and hyperplastic epithelium. Our results agree with the results obtained by **Nandakumar et al.**[49] who reported that administration of hesperidin after mammary cancer induction by a single dose of 20mg/kg DMBA showed that almost normal cytoplasm with undisturbed, protected number of mitochondria and reduced neoplastically transformed cells, which inevitably proved the antineoplastic activity of hesperidin. Moreover, it was shown that HES modifies the production of cytokines and enzymes that are involved in inflammation which play a crucial role in the anti-cancer effects of HES [67].

As mentioned above the administration of tiger nut didn't prevent the appearance of the breast cancer in the female rats of TN-DMBA treated group but in less number of animals and with less degree where a well-defined mass of breast carcinoma that surrounded by fibrous tissue with adjacent enlarged activated lymph node were noted. The activation of lymph node and fibrosis around the cancerous tissue in TN-DMBA group might be due to the nutritional benefits of tiger nut on the immune cell. In this respect, **Hassan** [68] showed that tiger

nut exhibits selective effects on T lymphocytes, which inhibits the proliferation of the inflammatory lymphocytes and increases the proliferation of naive lymphocytes. Moreover, tiger nut is a good source of fatty hydroxamic acid. Suberoylanilidehydroxamic acid, specific derivatives of hydroxamic acid, has been reported to be a potent inhibitor of histone deacetylases, and possesses anticancer and apoptosis effect against several tumor types [69,70]. Furthermore, fibrosis is a common, persistent and potentially debilitating complication of chemotherapy and radiation regimens used for the treatment of cancer [71]. Breast fibrosis is a common occurrence following anti-cancer radiation therapy [72].

In conclusion, the present study proved that the oxidative stress resulted from DMBA played an important role in inducing breast cancer. The histopathological changes that occurred in breast tissue of female rats as well as the disturbance of the biochemical parameters recorded in animals treated with DMBA were counteracted after HES administration causing a protective effect for DMBA toxicity. Here, hesperidin was more potent as chemoprotective agent than tiger nut.

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Table 1. Mean \pm SD of body weight at the start and end of the experiment in different groups

| | | Control (Group I) | HES (Group II) | TN (Group III) | DMBA (Group IV) | HES-DMBA (Group V) | TN-DMBA (Group VI) | |
|------------------------|--------------------------|----------------------------------|-------------------|-------------------|--------------------|-----------------------|-----------------------|-------------------|
| Body weight (g) | At start | N | 12 | 12 | 12 | 12 | 12 | |
| | | Range | 105 - 154 | 89 – 130 | 95 - 138 | 100 – 146 | 94 - 147 | 96 - 140 |
| | | Mean \pm S.D | 129.3 \pm 16.16 | 108.6 \pm 13.29 | 117.8 \pm 13.37 | 122.8 \pm 14.18 | 114.6 \pm 14.67 | 115.3 \pm 13.76 |
| | At sacrifice | N | 12 | 12 | 12 | 6 | 9 | 7 |
| | | Range | 170 – 260 | 194 – 284 | 186 – 260 | 122 – 180 | 145 - 215 | 124 – 200 |
| | | Mean + S.D | 218.9 \pm 28.5 | 241.4 \pm 29.74 | 220.8 \pm 24.19 | 145.8 \pm 21.5 | 181 \pm 25.29 | 159.7 \pm 26.11 |
| | | P^(a) value | | 0.005 | 0.819 | < 0.001 | < 0.001 | < 0.001 |
| | | P^(b) value | | < 0.001 | < 0.001 | | 0.001 | 0.204 |
| | | P^(c) value | | < 0.001 | < 0.001 | | | 0.029 |
| | Percentage change | | \uparrow 69.3% | \uparrow 122% | \uparrow 87.4% | \uparrow 18.7% | \uparrow 57.6% | \uparrow 38.5% |

• Significant at P value \leq 0.05

• $P^{(a)}$ value versus control group (group I)

• $P^{(b)}$ value versus DMBA treated group (group IV)

• $P^{(c)}$ value versus HES-DMBA treated group (group V)

(TN)-----Tiger nut - (HES)-----Hesperidin - (DMBA)---7, 12-Dimethylbenz (a) anthracene

Table 2. Mean + S.D. for rat serum TSA, progesterone and estradiol levels in the different groups

| Groups Parameter | Group I n=12) | Group II)n=12(| Group III (n=12) | Group IV (n=6) | Group V (n=9) | Group VI (n=7) |
|--|------------------|---|---|-------------------------------------|--|---------------------------------------|
| TSA (ng/dl) (percentage change) | 110 ± 11.75 | 112 ± 18.39 ^{b,c} (↑1.8%) | 109 ± 16.10 ^{b,c} (↓1.87%) | 317 ± 30.13 ^a (↑188%) | 139± 14.94 ^{a,b} (↑26.36 %) | 326± 23.13 ^{a,c} (↑196 %) |
| Estradiol (pg/ml) (percentage change) | 44.08 ± 11.08 | 42.17 ± 10.14 ^{b,c} (↓4.3%) | 41.67 ± 7.56 ^{b,c} (↓5.46%) | 105 ± 18.7 ^a (↑138%) | 59.1± 11.24 ^{a,b} (↑34.07%) | 102± 21.06 ^{a,c} (↑131%) |
| progesterone (ng/ml) (percentage change) | 15.73 ± 2.43 | 15.3 ± 2.39 ^{b,c} (↓2.7 %) | 16.14 ± 2.51 ^{b,c} (↑2.6 %) | 33.70±6.78 ^a (↑114 %) | 22.06± 4.25 ^{a,b} (↑40.24 %) | 31.31± 3.85 ^{a,c} (↑99 %) |

Significant at P value ≤ 0.05 , $p > 0.05$ is considered non-significant (NS).

- ^aSignificant P value versus control group (group I)
- ^bSignificant P value versus DMBA treated group (group IV)
- ^c Significant P value versus HES-DMBA treated group (group V)

Table 3. Mean + S.D. for the levels of rat serum MDA, CO and GSH and the activity of SOD, CAT and GSH-Px in the different groups

| Groups Parameter | Group I n=12)(| Group II n=12)(| Group III (n=12) | Group IV (n=6) | Group V (n=9) | Group VI (n=7) |
|--|-------------------|--|---|--|--|--|
| MDA (nmol/ml) (percentage change) | 0.171±0.038 | 0.174±0.033 ^{b,c} (↑1.8 %) | 0.177±0.048 ^{b,c} (↑3.5 %) | 0.579±0.061 ^a (↑238 %) | 0.259±0.072 ^{a,b} (↑51.5 %) | 0.544±0.095 ^{a,c} (↑218 %) |
| CO (nmol/ml) (percentage change) | 2.31 ± 0.681 | 2.39 ± 0.757 ^{b,c} (↑3.5 %) | 2.20 ± 0.684 ^{b,c} (↓4.8 %) | 5.69 ± 0.531 ^a (↑146 %) | 3.32 ± 0.438 ^{a,b} (↑43.7 %) | 5.41 ± 1.01 ^{a,c} (↑134 %) |
| SOD (U/ml) (percentage change) | 129.8 ± 14.5 | 134.7 ± 17.8 ^{b,c} (↑3.7%) | 135.6 ± 14.06 ^{b,c} (↑4.5%) | 20.7 ± 5.46 ^a (↓84%) | 100± 14.36 ^{a,b} (↓23%) | 21.6± 5.65 ^{a,c} (↓83.4%) |
| CAT (U/ml) (percentage change) | 5.46 ± 0.667 | 5.63 ± 0.568 ^{b,c} (↑3.11%) | 5.77 ± 0.779 ^{b,c} (↑5.7%) | 2.04 ± 0.473 ^a (↓62.64%) | 4.88± 0.546 ^{a,b} (↓10.62%) | 2.47 ± 0.369 ^{a,c} (↓54.76%) |
| GSH-Px (mg consumed glutathione/min/ml) (percentage change) | 4.90 ± 0.673 | 4.92 ± 0.606 ^{b,c} (↑0.41 %) | 4.68 ± 0.906 ^{b,c} (↓4.5 %) | 0.014 ± 0.0039 ^a (↓99.7 %) | 3.56 ± 0.564 ^{a,b} (↓27.3 %) | 0.017 ± 0.0036 ^{a,c} (↓99.7 %) |
| GSH (mg/ml) (percentage change) | 17.31 ± 1.91 | 17.37 ± 2.28 ^{b,c} (↑0.35%) | 17.37 ± 1.74 ^{b,c} (↑0.35%) | 2.14 ± 0.328 ^a (↓87.64%) | 10.58 ± 1.98 ^{a,b} (↓38.88%) | 1.38 ± 0.295 ^{a,c} (↓92.03%) |

Significant at P value ≤ 0.05 , $p > 0.05$ is considered non-significant (NS).

- ^aSignificant P value versus control group (group I)
- ^bSignificant P value versus DMBA treated group (group IV)
- ^c Significant P value versus HES-DMBA treated group (group V)

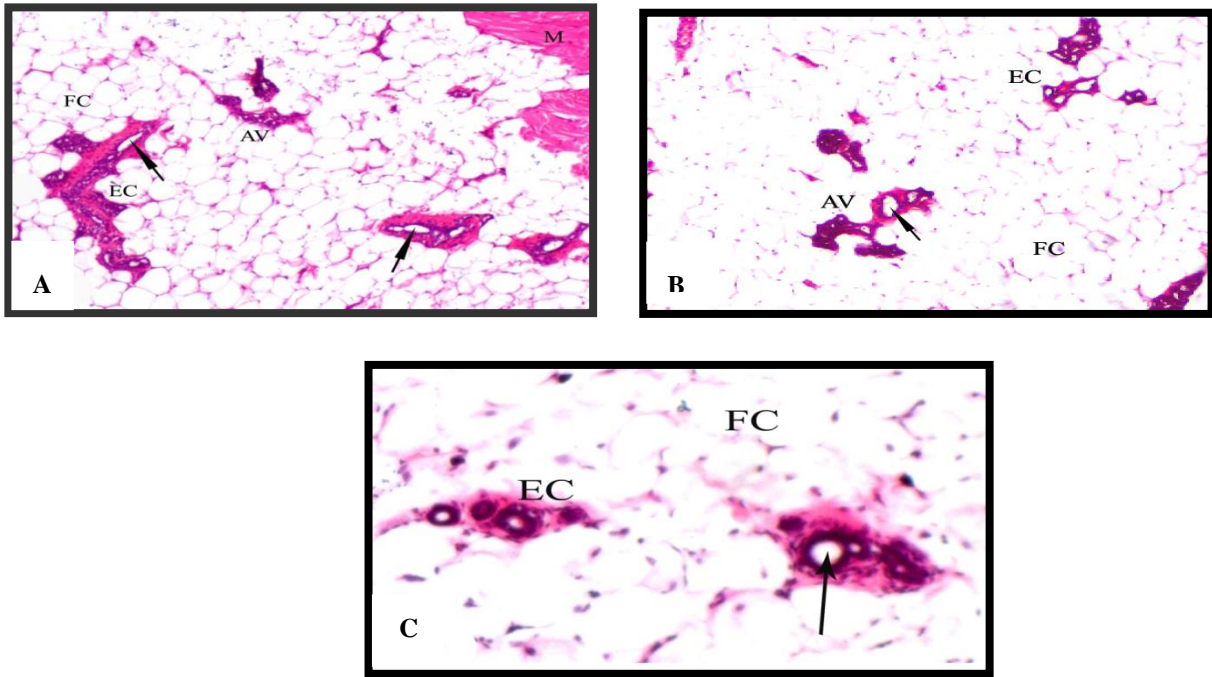


Figure 1(A): Light micrograph showing mammary gland epithelia of control rat that consisting of two layers of epithelial cells (EC) surrounding the lumen (arrow) of the alveoli (AV) embedding in adipose tissue (fat cell FC). Note the presence of muscle part (M). Haematoxylin and eosin (H&E X 200).**(B):** Light micrograph showing mammary gland epithelia of HES treated group (H&E X 200).**(C):** Light micrograph showing mammary gland epithelia of TN receiving rat (H&E X 400).

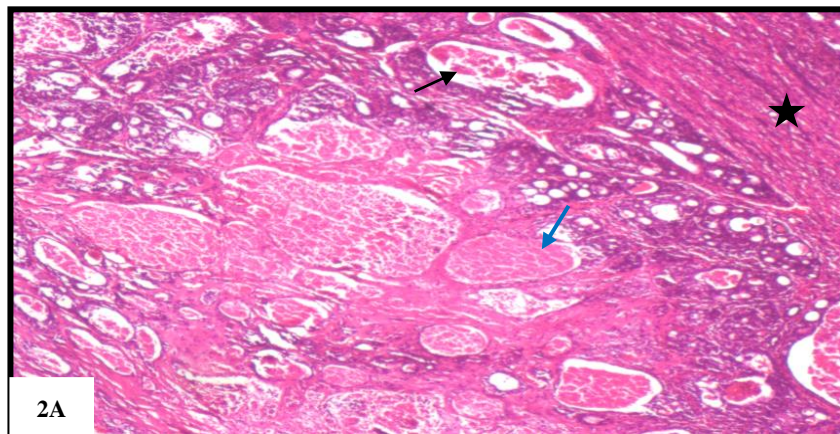


Figure 2 (A): Light micrograph of breast cancer tissue showing wide areas of necrosis and tumor breakdown indicating the high proliferation of tumor cells with surrounding residual viable proliferating malignant cells (black arrow), Note the presence of dead cells (blue arrow) and neoplastic cells invasive feature (★). (H&E X 200).

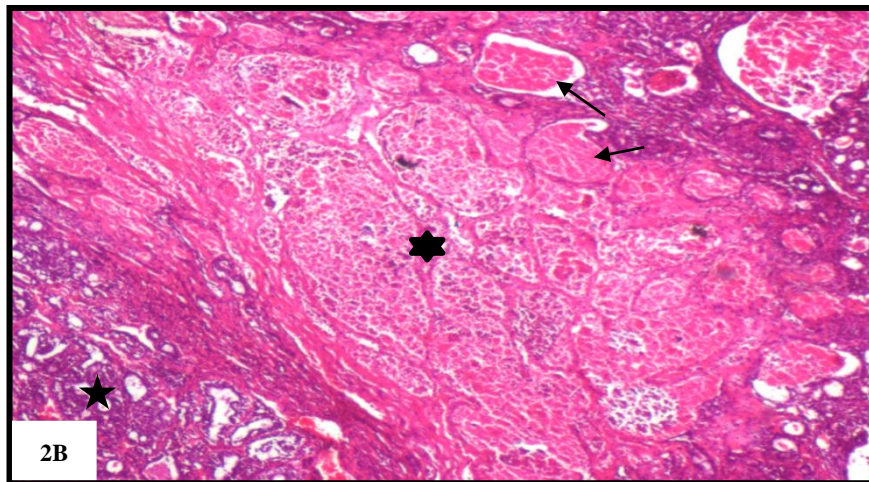


Figure 2 (B): Light micrograph of a section of breast tumor arising in rat breast receiving DMBA showing atypical proliferative changes characterized by a progressive elongation of ducts as well as proliferation and expansion of the terminal lobular units (HLU)() with compression of the surrounding fat pad, progressing to cystic papillary carcinoma (CA)(★) (H&E X 200). Note the presence of dead cells (arrow).

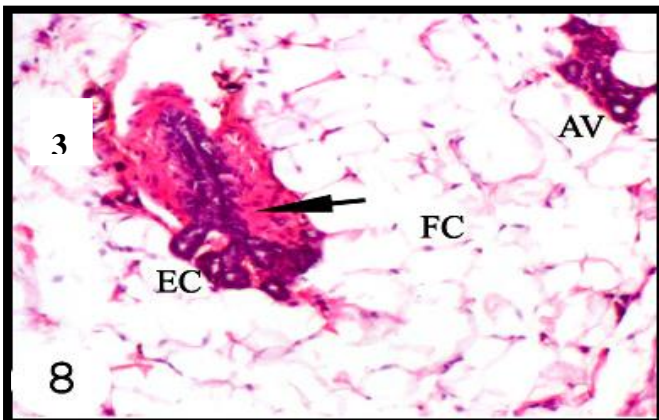


Figure 3: Light micrograph showing mammary gland epithelia of HES-DMBA treated rat that consisting of two layers of epithelial cells (EC) surrounding the lumen (L) of the alveoli (AV), surrounded by thick basement membrane (arrow) and embedded in adipose tissue (fat cell FC). (H&E X 400).

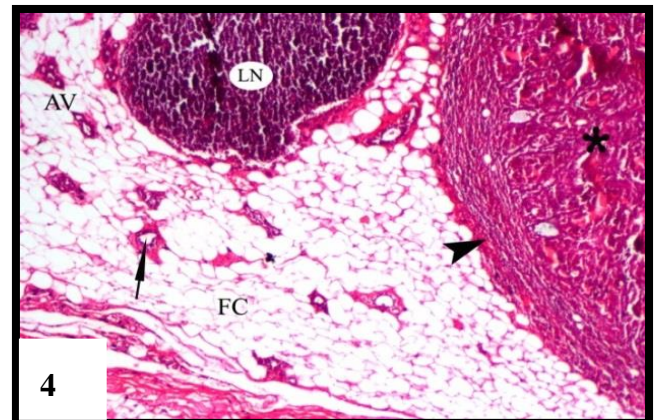


Figure 4:Light micrograph showing mammary gland of TN-DMBA treated rat that showing well defined mass of breast carcinoma that surrounded by fibrous tissue (arrowhead) embedded in fat cells (FC) with adjacent enlarged activated lymph node (LN). (H&E X100).