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Effect of combined Hesperidin, Piperine, Bee venom and Tamoxifen on oxidative stress and hepato-nephrotoxicity amelioration against breast cancer in rats

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KEY WORDS

Breast cancer;
Tamoxifen;
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Hepato-nephrotoxicity

ABSTRACT

Breast cancer is the most commonly diagnosed cancer and the major cause of cancer-related death among females. Studies on natural compounds used alone or in combination with chemotherapeutic agents proved their efficacy to treat and prevent cancer. Hesperidin, Piperine, and Bee venom displayed anti-oxidant and anti-cancer activity against various types of cancers and cell lines and can be used in combination with Tamoxifen with the aim to decrease cytotoxicity profile. In-vivo study of Piperine (Pip), Bee venom (BV) and hesperidin (Hes) as a single treatment or in combination with/without tamoxifen indicated low hepato-nephrotoxicity and hepato-nephroprotective against tamoxifen-treated and untreated xenograft MCF-7 induced breast cancer. Serum ALT, AST, ALP activities and creatinine, and urea level were elevated. MDA level was increased with a marked reduction in SOD activity in the untreated xenograft MCF-7 induced breast cancer. Treatments for these natural products improve these parameters. It could be concluded that alone or in combination with/ without Tamoxifen clarified a modulatory role against breast cancer.

1. Introduction

Breast cancer prevalence is substantially serious. The incidence and mortality of breast cancer are 43.3 and 12.9 per million in women respectively, which is in the top of all female related cancers [1]. Breast cancer is controlled by surgery, chemotherapy, radiotherapy and frequently supported by hormone therapies [2].

Even though treatment strategies of breast cancer have been developed, current treatments of breast cancer are still not enough to fundamentally treat this disease due to; side effects, drug resistance and non-specificity [3]. This situation pushes researcher to keep moving to discover new promising anticancer drugs.

Numerous modalities for treatment of cancer are available for cancer such as radiation therapy, chemotherapy, immunotherapy surgery, and hormonal therapy. Tamoxifen (TAM), a nonsteroidal anti-estrogen drug, is widely used for the treatment of patients with all stages of estrogen-dependent breast cancer, and the long-term single use of tamoxifen has been applied to treat patients during the premenstrual period [4]. For women with ER-positive breast cancer, treatment for 5 years with adjuvant tamoxifen substantially reduces the rate of recurrence [5]. Recent trials have shown that continuing tamoxifen for 10 years rather than stopping at 5 years produces a further reduction in recurrence and mortality [6]. However, many ER-positive patients are low reactive or resistant to tamoxifen and such long treatment with tamoxifen causes serious side-effects such as increases in endometrial hyperplasia and carcinomas, an excess of venothrombotic episodes, and the development of de novo or acquired tamoxifen resistance [5]. Thus, there is a need for more effective therapy with fewer side-effects. Several natural products including hesperidin, piperine, and bee venom have served as anti-cancer where they used alone or in combination with anticancer drugs hesperidin (Hes) is one of the most abundant natural flavonoids and is present in many fruits and vegetables.

Some authors reported that Hes prevents oxidant injury and cell death by several mechanisms, such as scavenging oxygen radicals and protecting against lipid peroxidation and chelating metal ions [10]. Hesperidin has a non-toxic effect on normal cells, but it can suppress cell proliferation in several cancer types and so it acts as an anti-carcinogenic agent in some tumors. In addition, hesperidin as a radio-protective and the chemo-protective therapeutic agent is expected to prevent invasion or metastasis of human cancers [11]. Anti-cancer effects of hesperidin were studied in tumor-implanted animal models or culture cell lines of several cancer types, including colon cancer, bladder cancer, hepatocarcinoma cancer, and breast cancer [11].

Piperine (Pip) is the main phenolic ingredient of black pepper [17], exhibits various activities

including anti-tumor, anti-inflammatory, immunomodulatory, anti-metastatic and hepatoprotective activities. It possesses anti-oxidative, and chemo-protective property [18, 19]. It also exerts anti-cancer effects and anti-invasion activity with less toxicity [20]. Piperine inhibits lung metastasis induced by B16F-10 melanoma cells and also inhibits fibrosarcoma cells invasion [21].

Bee venom (BV) treatment is the therapeutic application of honeybee venom to treat the various diseases. BV has been used in Oriental medicine to treat a variety of conditions, such as arthritis, rheumatism, back pain, cancerous tumors, and skin diseases. BV contains at least 18 active components, including melittin, apamin, adolapin, the mast-cell-degranulating (MCD) peptide, enzymes (i.e., phospholipase [PL] A2), biologically active amines (i.e., histamine and epinephrine), and nonpeptide components which have a variety of pharmaceutical properties [22]. Many studies investigated its anti-inflammatory, anti-rheumatoid arthritis and immune modulatory effects [23, 24]. Bee venom has also been reported to enhance the apoptotic signaling pathway in experimental osteosarcoma, breast cancer, and lung cancer cell lines [24, 25]. Induction of apoptosis by melittin or Bee venom has been reported in human lung cancer [23], rheumatoid arthritis [24], hepatocellular carcinoma [26], human leukemic cells [25], human breast cancer [27], prostate [28] and ovarian cancer cells [29]. Modulation of Tamoxifen sensitivity that results in lessening of its side-effects, resistance and maximizes its therapeutic efficiency is a desirable goal. The combination therapy with potent chemotherapeutic agents from natural compounds may achieve synergistic therapeutic effect, dose and toxicity reduction, and to minimize or delay the induction of drug resistance.

However, to date, there has been no study on oxidative stress and hepato-nephrotoxicity modulation effects of tamoxifen in combination with the three natural products Hes, Pip, and BV. Therefore, this study has focused on evaluating the oxidative stress and hepato-nephrotoxicity amelioration of tamoxifen and the three natural products against breast cancer MCF7 cells.

2. Materials and Methods

2.1. Animals

One hundred and thirty-two healthy female albino rats aged 4 weeks and weighted approx. 150- 170 gm were housed in a temperature-controlled and light-controlled room (12 h light/dark cycle) with free access to food and water. All experimental procedures followed the guidelines of the Institutional Animal Care and Use Committee of the Faculty of Science, Tanta University. Rats were acclimatized to laboratory conditions for two weeks prior to experiments [30].

2.2. Induction of breast cancer model by xenografting of MCF7 cells

The rats were immunosuppressed by intraperitoneal injection of cyclophosphamide (CTX) at a dose of 40 mg/kg for 4 successive days. To establish the breast cancer model, a human breast cancer cell line cells (MCF7) were counted and injected intradermally around the teat of the mammary gland at a dose of 1×10^7 cells in a volume of 1 ml [31]. To initiate tumor formation, rats were injected intra muscularly by estrogen (Folon, 0.01 ml/kg body weight) one day before MCF7 injection, and continued daily till tumor formation (approximately 4 weeks).

2.3. Groups

The 132 rats were equally divided (12 per group) into the following groups: **Group 1:** Healthy control group was orally administered with 1ml 0.9% saline and intraperitoneally injected with 0.5ml saline. **Group 2:** Breast cancer control group. **Group 3:** Breast cancer rats orally (by gastric tube) administrated tamoxifen (Nolvadex®, Astra Zenca Cambridge, UK) with a dose equal to 5mg/kg once daily [32]. **Group 4:** Breast cancer rats treated before and with induction of breast cancer with hesperidin (Acros Organics, USA; Cat no. 123460050), piperine (Acros Organics, USA; Cat no. 381450050) and bee venom (Apis Injeel™, Heel GMBH, Germany) and divided into 3 sub-groups: **Sub-group 4A:** Breast cancer rats orally (by gastric tube) administrated hesperidin with a dose 200mg/kg once daily [33]. **Sub-group 4B:** Breast cancer rats orally (by gastric tube) administrated piperine with a dose equal to 50mg/kg once daily [34]. **Sub group 4C:** Breast cancer rats intraperitoneally injected by

bee venom (BV) with a dose equal to 0.5mg/k.g once daily [35, 36]. **Group 5 and group 6:** Breast cancer rats treated before and with induction of breast cancer with combination of Hes, Pip and BV alone or in combination with Tam with the best selected ratio in vitro study as the following: **Group 5:** divided into 2 sub-groups **Sub-group 5A:** Breast cancer rats treated by Tam and Pip with a ratio of 1:4, respectively. **Sub-group 5B:** Breast cancer rats treated by Hes, Pip, and BV with a ratio of 1:2:1, respectively. **Group 6:** divided into 2 sub-groups: **Sub group 6A:** Breast cancer rats treated by Tam and Pip with a ratio of 1:4, respectively. **Sub-group 6B:** Breast cancer rats treated by Tam, Pip, and BV with a ratio of 1:2:1, respectively. **Sub-group 6C:** Breast cancer rats treated by Tam, Hes, Pip, and BV with a ratio of 1:1:4:1, respectively.

2.4. Sample collection

Blood samples were collected from the medial canthus venous plexus into dry clean centrifuge tubes. The tubes were kept at room temperature for 15 minutes for blood clotting and centrifuged at 3000 rpm for 10 minutes. The sera were separated and were kept at -20°C until analyzed for biochemical estimations. Mammary glands were immediately excised under sterilized conditions and snap-frozen in liquid nitrogen and stored at -80°C for further use.

2.5. Biochemical analysis

In the normal and tumorous mammary gland homogenate, Serum ALT and AST activities were determined by a colorimetric method which described by [39]. Serum ALP activity was measured according to the method of [40]. Serum creatinine level was determined by a colorimetric method which described by [41] was used. Serum urea level was determined by [42]. SOD activity was determined by the colorimetric method described by [37] and determination of MDA level was carried out according to the method of [38] was used.

2.6. Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) with Tukey-Kramer multiple comparisons test as a

post ANOVA test. Significant differences among means were estimated at $p < 0.05$. The results were expressed as mean \pm SEM (standard error of the mean). Values were analyzed using Graph Pad Prism 5 (Graph Pad Software, Inc., LaJolla, CA, USA).

3. Results

3.1. Effect of treatments with tamoxifen, hesperidin, piperine, and bee venom on liver function (ALT, AST, ALP)

The serum activities of liver enzymes (AST, ALT and ALP) were found to be significantly higher in xerographic MCF-7 injected rats when compared to normal control animals (Table 1, Fig. 1). These elevated enzymes were significantly reduced following treatment by tamoxifen, hesperidin, piperine, and bee

venom alone or in combination with the best improvement in combined groups especially those including the four compounds.

3.2. Effect of treatments with tamoxifen, hesperidin, piperine, and bee venom on kidney function (urea and creatinine)

Urea and creatinine levels in serum of xerographic MCF-7 injected rat's groups showed a highly significant increase at when compared with the normal group. Those findings recorded in (Table 2, Fig. 2). Treated groups tamoxifen, hesperidin, piperine, and bee venom alone or in combination showed significant decreases at in serum creatinine and urea levels comparing to control animals with the best improvement in combined groups especially those including the four compounds.

Table 1: Effect of Tam, Hes, Pip, and BV on serum activities of liver enzymes.

Groups	ALT(U/L)	AST(U/L)	ALP(U/L)
G1: healthy control	38.33 ^g \pm 0.88	36 ^f \pm 1	114.3 ^f \pm 2.6
G2: breast cancer control	106 ^a \pm 1.53	88.67 ^a \pm 1.5	223.3 ^a \pm 2
G3: Tam	101.83 ^a \pm 2	82 ^a \pm 1.42	216.7 ^a \pm 3
G4A: Hes	81.50 ^b \pm 0.67	59 ^{b,c} \pm 2.6	183.3 ^b \pm 2.3
G4B: Pip	74.67 ^c \pm 1.45	53.67 ^{c,d} \pm 2.2	177.7 ^b \pm 2
G4C: BV	81.73 ^b \pm 0.67	62 ^b \pm 2.1	183.3 ^b \pm 2.3
G5A: Hes+Pip	67.17 ^d \pm 1.01	48 ^d \pm 2.4	156.3 ^c \pm 1.9
G5B: Hes+Pip+BV	55.67 ^e \pm 1.31	45.67 ^{d,e} \pm 1.2	142.3 ^d \pm 1.5
G6A: Tam -pip	64.67 ^d \pm 1.45	49.6 ^d \pm 0.9	156.7 ^c \pm 1.9
G6B: Tam+Pip-BV	53.13 ^e \pm 1.04	46.57 ^{d,e} \pm 1.5	140.7 ^d \pm 1.2
G6C: Tam+Hes+Pip+BV	46.23 ^f \pm 1.18	39 ^{e,f} \pm 1.2	128.7 ^e \pm 0.9

N.B. Values in the same column with different superscript letters are significantly different p values. Data are presented as mean \pm S.E.M ($n = 12$).

Table 2: Effect of tamoxifen, hesperidin, piperine, and bee venom on serum kidney parameters.

Group	Urea (mg/dl)	Creatinine (mg/dl)
G1: healthy control	20.27 ^e \pm 0.39	0.87 ^g \pm 0.01
G2: breast cancer control	49.68 ^a \pm 0.93	2.73 ^a \pm 0.03
G3: Tam	47.14 ^a \pm 1.01	2.36 ^b \pm 0.09
G4A: Hes	32.34 ^b \pm 0.48	1.55 ^{c,d} \pm 0.03
G4B: Pip	31.83 ^b \pm 0.44	1.3 ^e \pm 0.06
G4C: BV	32.23 ^b \pm 0.54	1.62 ^c \pm 0.04
G5A: Hes+Pip	30.97 ^b \pm 0.75	1.23 ^e \pm 0.02
G5B: Hes+Pip+BV	26.03 ^d \pm 0.38	1.16 ^{e,f} \pm 0.01
G6A: Tam -pip	30.07 ^{b,c} \pm 0.58	1.36 ^{d,e} \pm 0.02
G6B: Tam+Pip-BV	27.47 ^{c,d} \pm 0.27	1.26 ^e \pm 0.02
G6C: Tam+Hes+Pip+BV	23 ^e \pm 0.53	1 ^{f,g} \pm 0.06

Values in the same column with different superscript letters are significantly different p values. Data are presented as mean \pm S.E.M ($n = 12$).

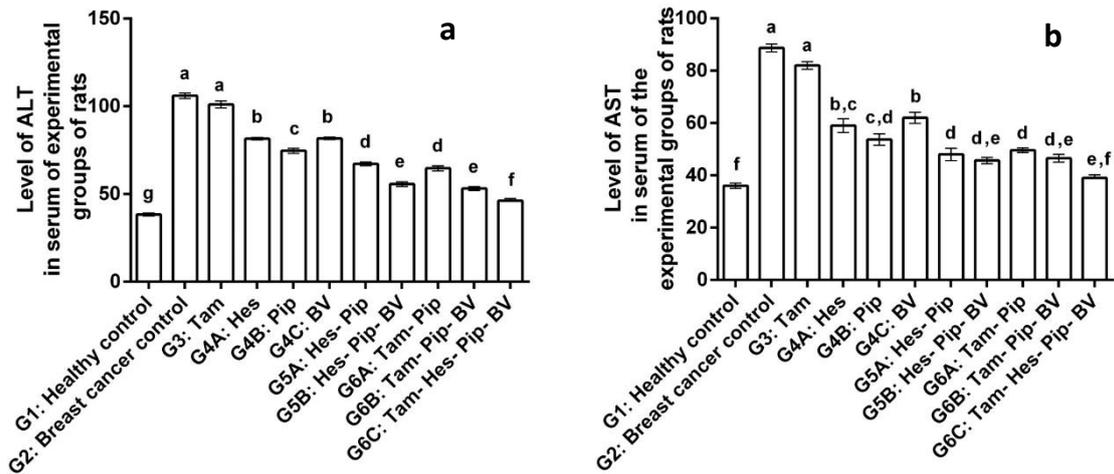


Figure 1. a: Serum ALT level in experimental groups of rats. a, b, c, d, e, f = $P < 0.001$ if compared with each other groups except (G4A α G4B) = $P < 0.05$, groups with the same superscript letter mean no significant difference. **b:** Serum AST level in experimental groups of rats. a, b, c, d, e, f = $P < 0.001$ if compared with each other groups except (G4A α G6A, G4B α G4C and G5A α G6C) = $P < 0.05$. Groups with the same superscript letter mean no significant difference. **c:** Serum ALP level in experimental groups of rats. a, b, c, d, e, f = $P < 0.001$ if compared with each other groups. Groups with the same superscript letter mean no significant difference.

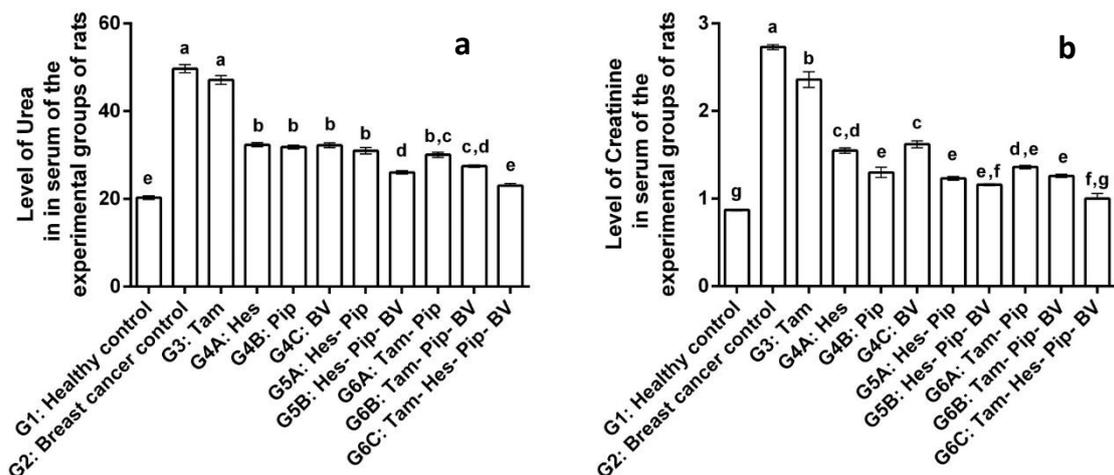
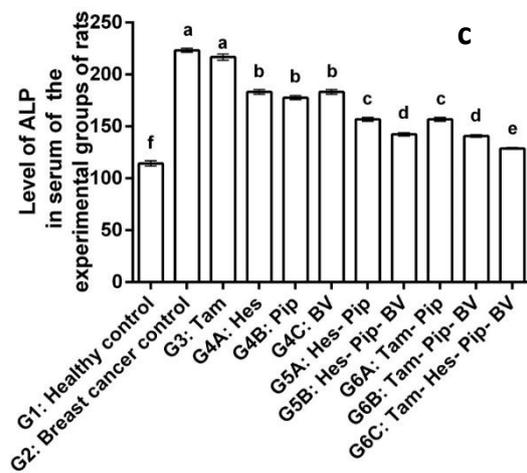


Figure 2. a: Serum Urea level in experimental groups of rats a, b, c, d, e = $P < 0.001$ if compared with each other groups except (G5B α G6C) = $P < 0.05$. Groups with the same superscript letter mean no significant difference. **b:** Serum creatinine level in experimental groups of rats a, b, c, d, e, f = $P < 0.001$ if compared with each other groups except (G5A α G6C) = $P < 0.05$. Groups with the same superscript letter mean no significant difference.

3.3. Effect of treatments with tamoxifen, hesperidin, piperine, and bee venom on MDA and SOD levels in mammary glands

The mammary glands of xenographic MCF-7-injected rats had a significantly higher MDA level and a significant lower SOD activity as

compared to normal control animals (Table 3, Fig. 3). Administration of tamoxifen, hesperidin, piperine, and bee venom alone or in combination improved the disrupted level of MDA and SOD with the best improvement in combined groups especially those including the four compounds.

Table 3: Effect of Tam, Hes, Pip, and BV on the MDA and SOD levels in normal and tumorous mammary glands.

Group	MDA (nM/g protein)	SOD (IU/g protein)
G1: healthy control	30.67 ^f ± 0.88	0.95 ^a ± 0.03
G2: breast cancer control	110.8 ^a ± 5.42	0.04 ^g ± 0.00
G3: Tam	66.5 ^{b,c} ± 2.90	0.3 ^e ± 0.01
G4A: Hes	69.7 ^b ± 2.93	0.22 ^f ± 0.02
G4B: Pip	68.63 ^b ± 2	0.32 ^e ± 0.01
G4C: BV	70.33 ^b ± 2.03	0.2 ^f ± 0.01
G5A: Hes+Pip	58.03 ^{c,d} ± 1.25	0.48 ^d ± 0.01
G5B: Hes+Pip+BV	46.5 ^e ± 0.89	0.60 ^c ± 0.01
G6A: Tam -pip	59.05 ^{b,d} ± 1.75	0.45 ^d ± 0.01
G6B: Tam+Pip-BV	51.47 ^{d,e} ± 0.87	0.62 ^c ± 0.03
G6C: Tam+Hes+Pip+BV	40.6 ^{e,f} ± 0.92	0.78 ^b ± 0.01

Values in the same column with different superscript letters are significantly different *p* values. Data are presented as mean ± S.E.M (*n* = 12).

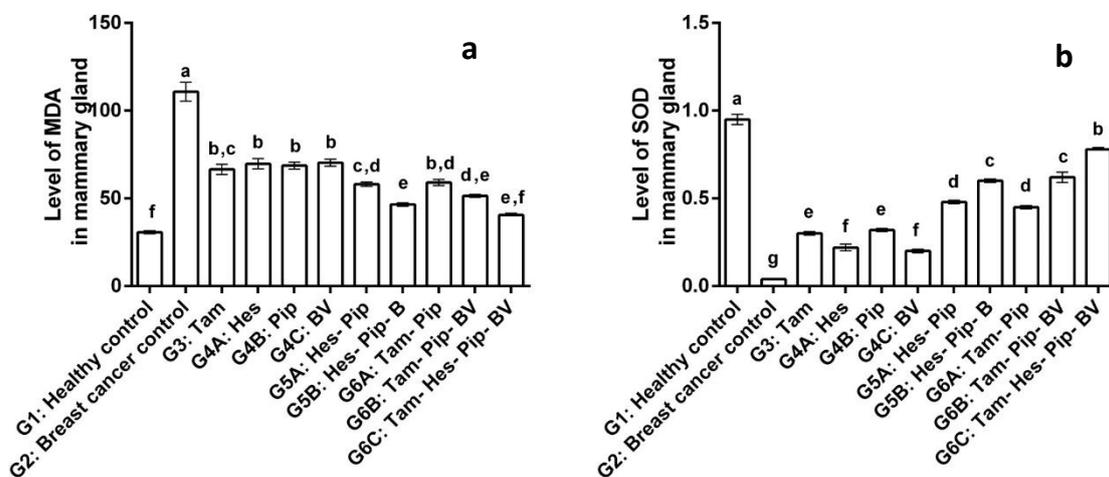


Figure 3. a: MDA level in tumorous mammary gland of rats a, b, c, d, e = $P < 0.001$ if compared with each other groups except (G5Ba G6C) = $P < 0.05$. Groups with the same superscript letter mean no significant difference except (G4Aα G5A, G4Cα G5A, G5Aα G5B and G5Ba G6A) = $P < 0.05$. **b:** SOD level in tumorous mammary gland of rats a, b, c, d, e, f = $P < 0.001$ if compared with each other groups except and (G3α G4A) = $P < 0.05$. Groups with the same superscript letter mean no significant difference.

4. Discussion

Induction of xenograft MCF-7 estradiol causes hepatotoxicity in an animal model and the main cause of this toxicity is estradiol [39]. Toxicity study revealed a low level of or improvement of hepatic and nephrotoxicity of

pip, BV and Hes the in case of single or in combination therapy with/without as compared with untreated breast cancer group which indicated by lower levels of AST, ALT, ALP, urea, and creatinine, especially in four

combination treatment. Tam has a nonsignificant change in the level of liver enzymes as compared with untreated group. These results are agreed with the previous investigation as the following:

The investigation that to cast more light on the toxic effect of tamoxifen on some liver enzymes in mice representing metabolic pathways is glycolysis, gluconeogenesis and glycogenolysis and the role of vitamin C in minimizing the toxicity induced by tamoxifen. Results showed that a significant increase in alkaline phosphatase (ALP) in treated groups, while AST and ALT enzymes activities, urea, and creatinine showed a significant reduction in treated mice as compared to the normal healthy control group [48].

Another study showed that Tamoxifen induces hepatotoxicity and changes to hepatocyte morphology at the early stage of endocrine therapy in mice, and this is known to occur mainly due to the leakage of these enzymes from the damaged hepatocytes into the bloodstream [49].

Previous studies of piperine as the study investigated the effect of piperine on liver damage and bone changes caused by bile duct ligation in rats showed Piperine administered at 20, 40 or 80 mg/kg to bile duct-ligated (BDL) rats reduced elevated plasma ALT, AST and ALP this may due to piperine a legend for the vanilloid receptor (TRPV1) reduced hepatic injury [50]. Also, another study was to investigate the antineoplastic activity of a combination of thymoquinone (TQ) and piperine against breast cancer implanted in mice and the results showed that normal serum levels of AST, ALT, and creatinine were observed in tumor-bearing mice treated with the combination therapy [51]. This might be through its direct action on free radicals of lead acetate, protecting the kidney from cellular damage by maintaining its membrane integrity [52].

The effect BV on liver and kidney parameters investigated in the previous studies as that to evaluate the tumor growth inhibiting effects of bee venom in rats (in vivo) and in tumor cell cultures (in vitro), the level of AST, ALT, ALP significantly ($p \leq 0.05$) decreased in Ehrlich ascites carcinoma bearing rats treated

groups hepato-protective effect of BV, Previous investigations of hesperidin were concomitant with this study as the following: Study was designed to explore the hepatoprotective and antioxidant effect of hesperidin, against gamma-irradiation which induced oxidative damage in the liver of rats. This may due to hesperidin tends to prevent liver damage, suppresses the leakage of enzymes through cellular membranes, preserves the integrity of the plasma membranes and hence restores these enzymes levels, Also ability to scavenge reactive oxygen species thereby preventing further peroxidation of membrane lipids, ability to enhance glutathione production by providing more substrate for reactive intermediates that promote detoxification mechanisms, restored the activities of the antioxidant enzymes, Thus, hesperidin contributes significantly to the intracellular antioxidant defense system by acting as a powerful consumer of superoxide anion and hydroxyl radicals [53]. a study designed to investigate the possible protective effects of multiple doses of hesperidin against cisplatin-induced nephrotoxicity induced by single i.p injection of cisplatin showed significantly reduced cisplatin-induced elevations in serum creatinine and BUN levels [54]. In this study induction of breast cancer causes a significant increase in the MDA and decrease in SOD enzyme. The most commonly used assay for the evaluation of the intensity of lipid peroxidation is the analysis of the content of TBARS represented by MDA. Higher MDA content was also detected in breast cancer tissue when compared with healthy surrounding tissue, thus suggesting that ROS are produced in tumor tissues [40]. SOD activity measured in experimental animals in the present study may suggest that an excess in ROS production appeared in rats with mammary gland tumors. The increase of antioxidative enzyme activities is the organism's response to oxidative stress [41]. At present, it is difficult to determine whether the ROS increase was a primary factor in relation to the carcinogenic process, or it was the result of ROS generation in tumor cells. It is known that some cancer cells can produce ROS [40]. SOD catalyzes the dismutation of superoxide anion radical to oxygen and hydrogen peroxide [42], resulting in the increase of hydrogen peroxide. Thus,

protection against ROS requires GSH-Px and catalase activities which neutralize hydrogen peroxide.

In the present study, there was a depletion of SOD and elevation of MDA in tumorous mammary gland as compared to the normal group. Administration of tam and other three natural products alter the concentration of SOD and MDA as compared to untreated group. Results of this study were agreed with previous research such as the following:

A study that detected the effects of the combination between tamoxifen and thymoquinone in patients with breast cancer. This study showed that Tam or thymoquinone alone or in combination induced a significant increase in tumor SOD with a significant decrease in the relapse rate, tumor tissue MDA. This effect may due to TAM has an antioxidant effect through increasing the activities of the antioxidant enzymes and decreasing lipid peroxidation products [43].

The study was to determine the protective effect of *Piper aduncum* capsules on DMBA (dimethylbenz[α]anthracene)-induced breast cancer in rats. Results showed significantly low level of MDA and elevated level of SOD implicates potent antioxidant effect. *P. aduncum* capsule contains saponins, polyphenols, tannins, alkaloids, and flavonoids in significant quantities. The effects of the capsule could be due to its ability to reduce oxidative stress and free-radical formation since it contains flavonoids. Also, flavonoids have effects on aromatase, a mitochondrial cytochrome P450 family enzyme produced at high levels in breast tissues that catalyzes the conversion of androgens to estrogens [44].

Previous investigations of hesperidin such as one that evaluated the therapeutic potential of hesperidin by assaying the activities of antioxidant enzymes, lipid peroxidation in anthracene- induced breast cancer. Daily oral administration of hesperidin (30 mg/kg body wt) to breast cancer-bearing rats. The result showed a significant decrease in the level of SOD and MDA [45]. Hesperidin significantly protects from DNA, lipids, and proteins against free radical damage. The antioxidant mechanism of hesperidin was planned by hydrogen donation to free radicals and the

formation of a complex between the lipid radical and the antioxidant radical. The presences of 3-o-hydroxy, 4-o-methoxy system in the B ring are necessary for radical scavenging properties of flavonoids, which possess electron donating properties and are a radical target. Also, electron delocalization from the B ring occurred by the C4-C8 double bond conjugated with a 4-keto group. The presence of both 3-OH and 5-OH groups, in combination with a 4-carbonyl function and C40 -C80 double bond, increases the radical scavenging activity. Flavonols that include more hydroxyl groups (1 to 6 OH groups) are potent free radical and superoxide anion radical scavengers, such as hesperidin. Hesperidin has a 2-hydroxyl group on the A and B rings and on methoxy group on the B ring, in addition to possessing the 4', 8' -double bond in conjugation with a 4-oxo function of the C ring, which is responsible for potent radical scavenging activities of hesperidin [46].

Previous investigations of hesperidin such as one that was designed to investigate the therapeutic effects of bee venom (BV) on high-fat diet (HFD)-induced nonalcoholic fatty liver (NAFL) in rats at different levels. The results of this study, which indicated that NAFL rats treated with BV showed significantly lower hepatic levels of MDA compared to untreated rats in a dose-dependent manner [47].

5. Conclusion

The obtained *in vivo* results on xenograft MCF7 induced breast cancer in rats establish the efficacy of new drugs combination that improve the oxidative stress and hepatonephroprotective which a mean to fight breast cancer with low toxicity.

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