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Oolong tea is a potential prophylactic anti-cancer herbal modality against mice bearing mammary tumor

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Abstract:

Background: It is prepared from crushing and fermentation of the rims of the leaves of Camellia sinensis. It contained a plenty quantity of polyphenols that differed from that in the green or black tea.
Objectives: This study aimed to demonstrate the anticancer effect of oolong tea extract against in situ mammary tumor in mice ex vivo experimental model, and to compare its effect with the gallic acid as antioxidant substance.
Materials and methods: Mice bearing mammary tumor are used as a source of mammary tumor cells. The animals are treated intraperitoneally with 0.2 ml of distilled water (Group I) or gallic acid (1% w/v) or oolong tea-extract prepared with microwave assisted method (10% w/v) twice weekly. The animals were sacrificed by cervical dislocation after two weeks and the tumor masses were excised and processed for determination of total protein, malondialdehyde and histopathological findings.
Results: Oolong tea extract and gallic acid induced changes in the morphology and texture of tumor mass compared with distilled water treatment. Oolong tea non-significantly increased the tumor mass-protein and malondialdehyde levels compared with Group I and Group II. The histopathological findings in term of giant multi-nucleated cell, necrosis, hemorrhage, and fibroblast cells infiltration that observed in Group (I) treated animals are improved in Group (III) animals
Conclusions: Oolong tea acts as anti-cancer substance in a mechanism differed from that proposed by the gallic acid as antioxidant.
Keywords: *Oolong tea, Mammary tumors, histopathology*

Introduction:

Oolong tea or black dragon (Oo means black and Long means Dragon) appeared firstly in in Qing dynasty publications (1644 to 1911) (http://www.amazing-green-tea.com/oolong-tea-history.html) (2014). It is prepared from the rims of the leaves of Camellia sinensis that crushed and subjected to fermentation process for short time period that gives the tea's specific flavor and taste (Sang et al., 2011). Its leaves contained catchins polyphenols, theaflavins and thearubigins. Other substances are also identified in oolong tea leaves such as epigallocatechin esters, theasinensins, dimeric catechins, and dimeric proanthocyanidins (Sang et al., 2011). Theasinensins are a group of polyphenols found in oolong tea which differed from green tea catechins (in green tea) and black tea theaflavins (in black tea) characterized by having antiinflammatory effects by the evidence of reducing the proinflammatory markers and nitrative oxygen species (nitric oxide) (Hisanaga et al., 2014). The other bioactive polyphenol that isolated from oolong tea is chafuroside B which ameliorate the keratinocyte damage induced by ultraviolet radiation by increasing the synthesis of interleukin 12 (Hasegawa et al., 2013). High molecular weight polyphenols that isolated from oolong tea are able to increase the mitochondrial membrane potential and they referred as mitochondrial activation factors. These factors are found to be heterogeneous polymers of flavan-3-ols and flavan-3-ol gallates (Fujihara et al., 2007). There is no specific mechanism that tea polyphenols exert their anti-cancer effects. In brief, Thakur et al (2012) in their review, included 68 references, summarized the pathway by which the tea polyphenols de-or-under-regulated the growth of cancer. And Kanwar et al (2012) summarized, in their review included 149 references, the changes in the biomarkers that linked to the cancer and the effects of tea polyphenols. The aim of this study is to demonstrate the anticancer effect

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of oolong tea extract administered intraperitoneally rather than orally against in situ mammary tumor in mice ex vivo experimental model, and to compare its effect with the effect of gallic acid as antioxidant substance.

Materials and Methods:

This study is conducted in The Iraqi Center for Cancer and Medical Genetic in cooperation with the Department of Pharmacology, College of Medicine at Al-Mustisiriya University in Baghdad, Iraq during 2014.

a. Oolong tea extraction

In brief, a known weight of oolong tea is dissolved in known volume of distilled water to get 10% w/v herbal tea beverage and then exposed to microwave irradiation. The specifications of microwave irradiation are the power was 600 Watts and the temperature adjusted at 84ºC. The procedures of extraction by microwave is as following: each extract was preheated for 45 sec. followed by a cycle of 10 sec. turn off, then a three sec. irradiation by microwaves up to three cycles (Tatke p and Jaiswal, 2011)

b. Animal preparation

Mice bearing mammary tumor are used as a source of mammary tumor cells. The mammary tumor cells (AM3) were aspirated from the tumor mass using a wide bevel, gauge 18 needle in sterile condition. The aspirated materials washed frequently using phosphate buffer solution contained antibiotics (amoxicillin and streptomycin) at a concentration higher than that used with in vitro cell line study by five times. Then, the suspended tumors left to settle down for fifteen minutes. A volume of 0.2 ml suspension of tumor cells were injected (subcutaneously once) to each mouse of the following treated three groups, each of five animals:

Group I (n=5): injected intraperitoneally with an equivalent volume 0.2 ml of distilled water

Group II (n=5): injected intraperitoneally with an equivalent volume of gallic acid (1% w/v distillled water).

Group III (n=5): injected intraperitoneally with an equivalent volume of oolong tea extract (10% w/v) prepared by microwave assisted method.

Animals were housed in stainless cages (five per cage),in the Iraqi Center for Cancer and Medical Genetic Research, under standard environmental conditions (28 ± 2°C, 12 h light/dark cycle). The animals were allowed a standard diet and water ad libitum. The animals kept under observation during the period of experiment reporting the evolution of tumor mass and the mice lethality

At the end of two weeks period, the survival mice were sacrificed by cervical dislocation and the tumor mass that located beyond the scruff of neck exposed and inspected for its size, shape, color, texture, matted and muscle invasion. The tumor mass excised and divided into two pieces , the first immersed immediately in 10% formalin for histopathological study and the second freezed and kept at - 4ºC for biochemical determination of tissue protein and malondialdehyde (a marker of lipid peroxidation).

c. Determination of total tissue protein

The frozen pieces of tumor mass allowed to melt, dried by filter paper, weighed and homogenized in 5 ml isotonic saline solution (0.9% NaCl) using tissue homogenizer. Total protein was assayed using Bradford method (1976). 100µl of homogenate solution was added to 5 ml of Bradford's solution. (The compositions of Bradford's solution are 100 mg Comossie reagent 250G dissolved in 50 ml ethanol and 100 ml of phosphoric acid (85%) and the volume was completed with distilled water up to 1L. The protein content was calculated using the linear equation based on the standard diluted human albumin (22%) calibration curve (0.022 x concentration of protein (µg) + 0.046) and it expressed per mg dry weight.

d. Determination of tissue malondialdehyde

Malondialdehyde was determined in tumor tissue mass using the method prescribed by Bird and Draper (1984). Two volumes of cold trichloroacetic acid (10% w/v) was added to one volume supernatant of homogenized heart tissue, centrifuged 10 minutes to precipitate protein. Then an equal volumes of supernatant and thiobarbituric acid (0.67% w/v) were mixed, incubated in boiling water bath for 30 minutes. The absorbance was recorded at 532 nm using UV-visible spectrophotometer. The concentration of malondialdehyde was calculated using the extinction coefficient (ε) 1.56 X 10-5 M-1.cm-1 and is expressed as nmol/mg dry weight mass tissue and as nmol/mg protein content. The preserved tumor mass tissues in formalin (10%) were manually processed in different alcoholic and organic solutions and slides were prepared and stained by eosin and hemotoxylene stains. Several sections were processed. These slides then examined by taking different fields randomly and comparing the frequency of occurring of abnormal nucleated malignant cells, inflammatory cells, fibroblast cells, fibrosis and necrosis among groups of different treatments.

e. Statistical analysis

The results are expressed as number and mean ± SE. The data are analyzed using Student's t test (unpaired, two tailed) taking the probability of ≤ 0.05 is the lowest limit of significance.

Results:

All the animals are survived over the two weeks from the treatment. Table 1 shows that the macrospical findings of tumor masses that related to each treatment showed variation even in each treated group. Tumors obtained from gallic acid and oolong treated groups are less likely to extended into the adjacent muscles.

Table 1: The macroscopical findings of the tumor masses obtained from mice treated with distilled water, gallic acid and oolong tea extract.

Tumor features	Control group	Gallic acid group	Oolong tea group
Size	1 cm, 1*9*9 ml, 6*9 mm, 7*9 mm and 1 cm.	5*2, 5*6, 1*1*1, 6*9 and 9*11*8 mm	7*5, 15*7*5, 4*4, 2*2 and 14*7 mm
Shape	All oval	All oval and one circular.	Two circular, two ovals and one longitudinal.
Texture	All hard	All hard and one soft	All hard
Matted	All single and one scattered	All single and one scattered	Two sngle mass and three with two masses.
Color	All gray and one with black spots.	All pink and one white grayish color.	All pink
Muscle invasion	All invade the muscle.	No invasion	Three invade and two no invasion

The total mass protein of animals treated with distilled water is (37.4 ± 7.9 µg /mg tissue) non significantly less than that of gallic acid (43.4 ± 10.9) µg /mg tissue) or oolong (44.3 ± 16.1 µg /mg tissue) treated groups. as shown in figure 1.

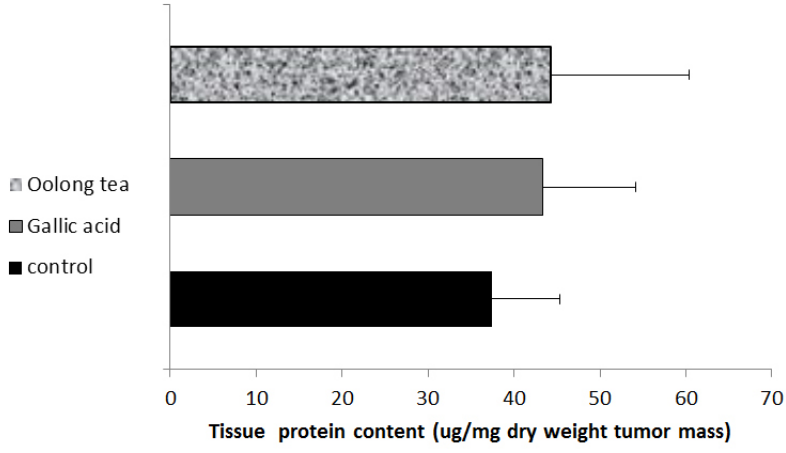

Figure 1: Total protein content in tumor mass of mice treated with distilled water (control), gallic acid or oolong tea extract. The results are expressed as mean ±SE

The tissue malondialdehyde level of animals treated with distilled water is (4.65 ± 1.3 nmol/µg protein) significantly less than that of gallic acid (4.92 ± 1.91 nmol/µg protein) or oolong (5.74 ± 2.15 nmol/µg protein) treated groups, as shown in figure 2.

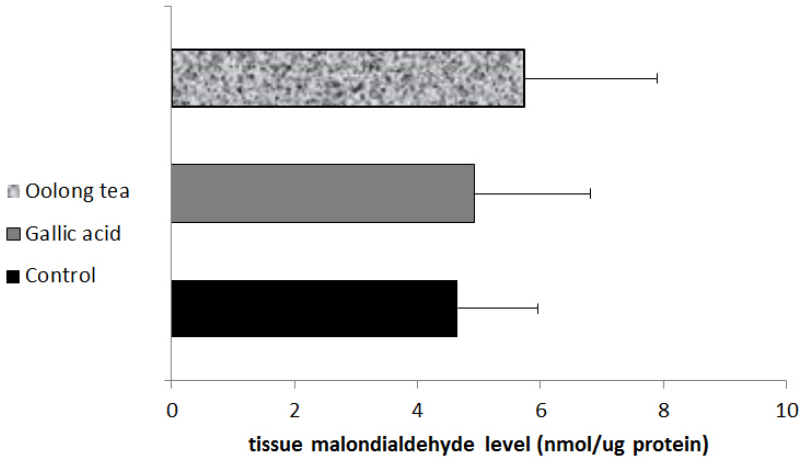

Figure 2: Malondialdehye level in tumor mass of mice treated with distilled water (control), gallic acid or oolong tea extract. The results are expressed as mean ±SE

Histopathology results

Mice treated with distilled water showed extensive giant multinucleated cancer cells, few inflammatory cells infiltration, slight hemorrhage by the evidence of red cells, tissue fragmentation and slight cellular necrosis. Few fibroblasts are also observed (Figure 3). This picture is more pronounced in mice treated with gallic acid (Figure 4). Oolong tea attenuated the histopathological findings that observed in distilled water or gallic acid treatment groups. A patchy multinucleated cells infiltration associated with cellular necrosis were observed (Figure 5).

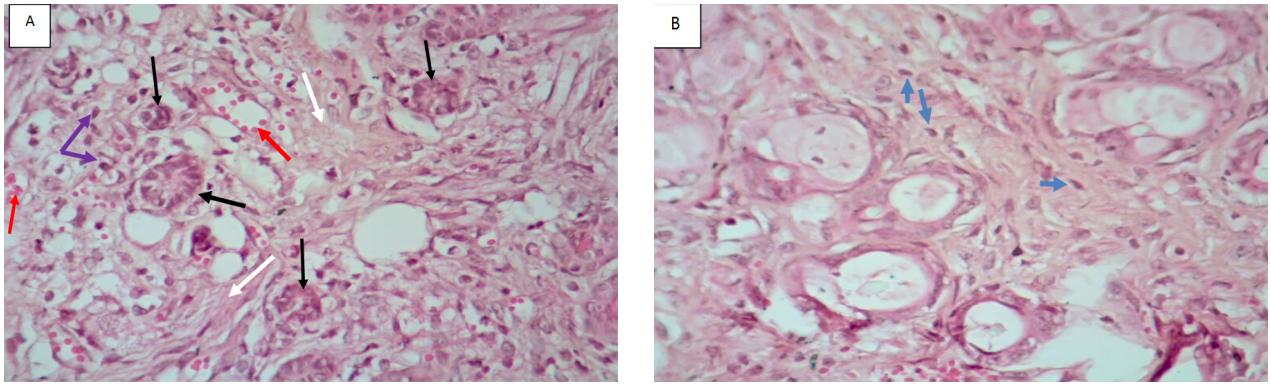

Figure 3: Sections obtained from masses excised from mice treated with distilled water (serve as control. Section [A] shows giant multinucleated cells (black arrows), few inflammatory cells infiltration (purple arrows), hemorrhage by the evidence of red cells (red arrows), tissue fragmentation and slight cellular necrosis (white arrow). Section [B] shows the appearance of fibroblasts (blue arrows). The magnification of these slides is 40 X.

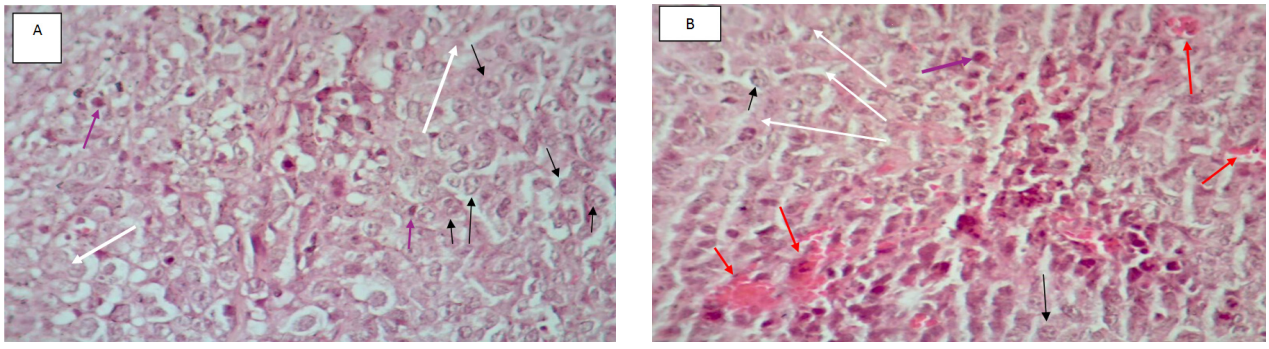

Figure 4: Sections obtained from masses excised from mice treated with gallic acid. Heavy giant multinucleated cancer cells (black arrows) (sections A, B), few inflammatory cells infiltration (purple arrows), hemorrhage by the evidence of red cells (red arrows), tissue fragmentation and prominent cell necrosis (white arrows). The magnification of these slides is 40 X.

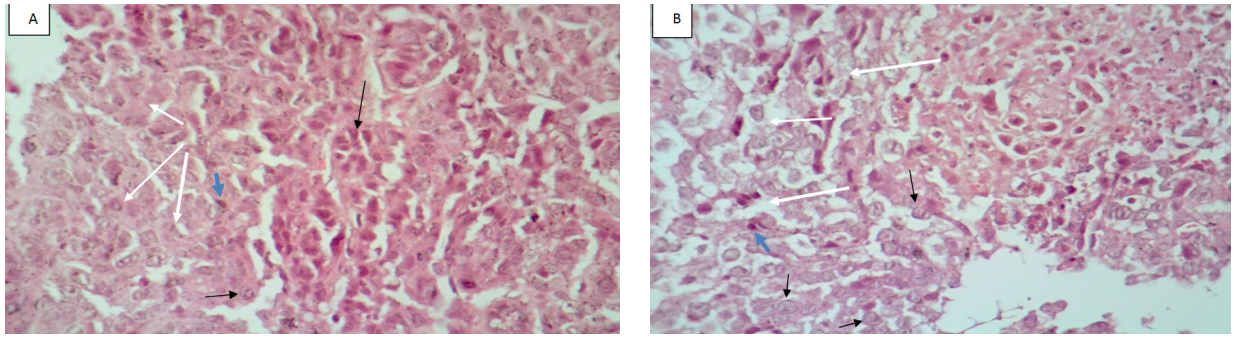

Figure 5: Sections obtained from masses excised from mice treated with oolong tea extract. Giant multinucleated cancer cells (black arrows), infiltration accompanied with cellular necrosis (white arrows) and few fibroblasts (blue arrows). (sections A, B)

Discussion:

The results of this study show that the oolong extract attenuate the histopathological findings of in situ mammary tumor in a mechanism related to the generation of free radicals by the evidence of increased level of tissue malondialdehyde level. In this study, the volume, shape, consistency and the other macroscopical characteristics of the evolution masses are used as indices of the anti-tumor effect of herbal tea extract. Such assessment carried pitfall as well it does not indicate that herbal tea extracts exhibit anti-tumor effect. Luo et al (2014) assessed the metastasis of breast cancer cell after using oral administration of Camellia sinensis plant extracts in BALB/c mice bearing 4T1 tumors and found that this extract inhibits the metastasis to the other organs. In this study, three variables are considered to assess the antitumor effect of herbal tea extracts. The histopathological findings show that oolong tea-extract clearly attenuate but not completely obviate the development of breast cancer. The mechanism of action of oolong seems to be related to the suppression key enzymes that involved in carcinogenesis. In hepatoma cell line, incubation of oolong tea with hepatoma cell line resulted in inhibition a number of enzymes and nuclear factors and such effect not observed when the hepatoma cell line incubated with low- molecular weight catechins such as (-)-epigallocatechin gallate (Yasui et al., 2011). The other mechanism is related to the induction of apoptosis in the malignant cells as reported with rat hepatoma cell line (AH109A) and murine B16 melanoma cells but not normal rat mesothelial (M) cells (Zhang et al., 2000). The histopathological findings are associated with the activation of lipid peroxidation and increase protein content in tumor masses that confirmed the previous studies that generation reactive oxygen species by tea extract is the mechanism of inducing apoptosis in cancer cells. In vitro experimental studies, the oolong extract exerts antioxidant activity by scavenging the activity of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and 2,2'-azo-bis(2-amidinopropane) dihydrochloride (AAPH)-induced lipid oxidation in erythrocyte membranes (Satoh et al., 2005). The difference in the methodology can explain the discrepancy between our results and others. Our results are in agreement with others who found that administration of green tea extract orally in rats treated with a potent oral carcinogen (4-Nitroquinoline 1-oxide) resulted in a significant improvement of catalase, superoxide dismutase, thiol….etc except the malondialdehyde level which still remained high (Pandurangan et al., 2012).

We conclude that oolong tea protects the mice from bearing mammary tumor by the histopathological evidence and through the mechanism beyond the antioxidant property by the evidence that its effect is more pronounced than that observed with gallic acid.

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شاي الاولونج شاي ذو خاصية وقائية ضد سرطان الثدي في الفئران المختبرية

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الخلاصة:

مقدمة تأريخية: شاي اولونج و يسمى (التنين الاسود) لشكل الاوراق المتعرج و لونها الغامق, يحضر بسحق و تخمير غير كامل لاوراق نبتة الشاي المعروفة Camellia sinensis, ويحوي كمية كبيرة من مركبات البولي فينول حيث تختلف عن تلك الموجودة في الشاي الاخضر و الاسود.

اهداف الدراسة: هدفت الدراسة الى التحري عن مدى تأثير المستخلص المائي لعشبة شاي اولونج على نمو سرطان الثدي المغروس في الفئران المختبرية و مقارنة هذا التأثير بتأثير مادة ال gallic acidكمادة مضادة للاكسدة.

مواد و طرق العمل: استعملت فئران مختبرية حاملة لورم سرطان الثدي كمصدر لخلايا سرطان الثدي. تم تعريض الفئران الحاملة للورم للحقن داخل الصفاق intraperitoneally مرتين اسبوعيا بكميات متساوية (0.2 ml) من الماء المقطر للمجموعة الاولى , و نفس الكمية من مادة gallic acid (1% w/v) لحيوانات المجموعة الثانية ، و نفس الكمية من المستخلص المائي لشاي اولونج(10% w/v) المحضر بطريقة الاستخلاص بالمايكروويف , لمدة اسبوعين , بعدها تم التضحية الفئران بفصل الرقبة ثم استئصال الورم و تفحص و ملاحظة الورم من حيث الحجم و الشكل و القوام و غيرها من الملاحظات اضافة الى قياس مستوى malondialdehyde كمؤشر على وجود و مدى حدوث بيروكسدة الشحوم، كما قيست الكمية الكلية للبروتين في الاورام اضافة الى دراسة انسجة الاورام مختبريا.

النتائج: اظهرت النتائج ان المعالجة بمستخلص شاي اولونج و مادة ال gallic acid احدثت تغيرات في كتلة الورم من حيث الشكل والقوام و الحجم مقارنة بالمعالجة بالماء المقطر, كما ان مستخلص شاي اولونج قد تسبب في زيادة مستوى البروتين في الورم واحدث زيادة في مستوى ال MDA مقارنة بالمجموعتين الباقيتين كما و اظهرت دراسة الانسجة تحسنا في مجموعة مستخلص شاي اولونج مقارنة بالمجموعتين الباقيتين.

الاستنتاجات: يعمل شاي اولونج كمادة مضادة لنمو السرطان بطريقة مختلفة عن طريقة عمل مادة gallic acid كمادة مضادة للتأكسد.