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ANTICANCER EFFECTS OF TOW TRADITIONAL DRINKS HIBISCUS (*Hibiscus sabdariffa* LINN) AND LIQUORICE (*Glycyrrhiza glabra*)

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ABSTRACT: Breast cancer (MCF-7), as well as liver cancer (HepG2), were the leader among new cancer cases. The objective of this study is to assess the viability percentage of tow traditional drinks (hibiscus and liquorice extracts) toward human breast and liver cancer cell lines at different concentrates from 250 to 5000 µg/ml in hibiscus extract and from 50 to 3000 µg/ml in liquorice extract. The viability percentage of hibiscus on breast and liver cancer cell lines were 86.9% and 84.7% at 5000 µg/ml, respectively. The IC₅₀ of the hibiscus extract were 18478.6 µg/ml and 16159 µg/ml breast and liver cancer. In addition, the viability percentage of liquorice extract on breast and liver cancer cell lines were determined. Results revealed that liquorice extract has possessed high anti-carcinogenic properties it was zero (%) at 3000 µg/ml for breast cancer and 5% for liver cancer at same concentration, the IC₅₀ was 491.7 µg/ml in breast cancer and was 125 µg/ml in liver cancer cell line. DNA damage was determined to detect oxidative DNA damage in HepG2 cell line. This enhances the role of these traditional drinks especially liquorice as anti-cancer agents.

Key words: Breast cancer (MCF-7), liver cancer (HepG2), traditional drinks, *Hibiscus sabdariffa* linn, liquorice (*Glycyrrhiza glabra*).

INTRODUCTION

Hibiscus sabdariffa linn known as Roselle is a yearly bush ordinarily used to make jams and refreshments. The dried blossom of *H. sabdariffa* remove has indicated elevated amounts of polyphenol, flavonoid, and anthocyanin which was seen to be related to anticancer impacts as indicated by Naveen *et al.* (2012).

Licorice, the dry base of *Glycyrrhiza glabra*, has been utilized for therapeutic purposes for centuries; stores of licorice were found in the tombs of Egyptian pharaohs, including that of King Tut. Its history is painstakingly evaluated by Davis and Morris (1991).

Cancer growth advancement is a multi-step process where different oncogenic transformations offer ascent to disease cells with various hereditary imperfections, which can vary even inside individual tumors. This decent variety is a huge

impediment in disease treatment, so cell lines built up from human tumors tests can be a useful device for screening pertinent medications. For visualization of malignant growth affectability to specific therapeutics, coordinate cytotoxicity measures in disease cells can be joined with information in regards to the outflow of qualities fundamental for antitumor activity as per (Schilsky, 2010). The huge qualities of individual bosom malignancies are the declaration of estrogen, progesterone, and human epidermal development factor receptor 2 (HER2/CD340), and the proposed kind of treatment is situated to a limited extent on these attributes were surveyed by Rivenbark *et al.* (2013). Among them, the estrogen receptor (ER) status is more basic for foreseeing the reaction to hormonal treatment, while progesterone receptor (PGR) status has stayed disputable by Singhal *et al.* (2016).

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Essential liver malignant growth (counting hepatocellular carcinoma and intrahepatic cholangiocarcinoma) is the second reason for the disease-related demise and one of the tumors with as yet expanding incidence rate (**Kassebaum *et al.*, 2014**). Hepatocellular carcinoma, a noteworthy essential liver malignant growth obsessive sort, demonstrates low multiyear survival rate (<15%) attributable to its late finding and traded off hidden liver capacity (**Sapisochin *et al.*, 2016**). Albeit careful resection or transplantation enhances the survival rate of patients, there is still no successful treatment for cutting-edge patients who are ineligible for a medical procedure, bringing about just a middle in general survival of 6.6 months for these patients (**Cheng *et al.*, 2016**). Accordingly, it is basic to investigate powerful and safe prognostic biomarkers for early finding and helpful focuses to enhance treatment procedures.

In the present study, we expect to investigate the practicality level of tow conventional beverages (hibiscus and liquorice extracts) toward human breast and liver cancer cell lines at various concentrates, to improve the job of these customary beverages as hostile to malignancy specialists.

MATERIALS AND METHODS

Materials

Egyptian hibiscus *Hibiscus sabdariffa* Linn and liquorice *Glycyrrhiza glabra* were purchased from local market.

Reagents

Primary cells or established cell lines, Neutral red (3-amino-7-dimethylamino-2-methyl-phenazine hydrochloride) (Sigma, cat. No. N4638) (see REAGENT SETUP), Dulbecco's formulation PBS tablets (Bio-Whittaker, cat. no. 17-512F) (see REAGENT SETUP), Trypsin-EDTA (0.05% *W/V* trypsin, 0.02% *W/V* EDTA) (BioWhittaker, cat. no. BE17-161E), Medium adequate for cell requirements (*e.g.*, Eagle's minimum essential medium (EMEM)) (BioWhittaker, cat. No. BE12-125F), Medium additives adequate for cell requirements (*e.g.*, 10% fetal or newborn calf serum, heat inactivated) (GIBCO, cat. No.

10108-165); 200 mM L-Gin (BioWhittaker, cat. No. BE17-605E); 50 mg l₁ gentamycin sulfate (BioWhittaker, cat. No. 17- 518Z); 2 mg l₁ Fungizone (BioWhittaker, cat. No. BE17-636E); nonessential amino acids (BioWhittaker, cat. no. BE13-114E). m CRITICAL Complete medium with additives and serum should be stored at 41°C for no longer than 2 weeks, Glacial acetic acid (Sigma, cat. No. 537020), Ethanol 96% (Riedel-de Hae'n, cat. No. 32294), 0.4% (*W/V*) Trypan blue in 0.9% Nacl solution (Sigma, cat. No. T8154) and Glutaraldehyde 5% by dilution of the commercial 25% (Sigma, cat. No. G6257) (optional)

Samples Preparation

The plant materials hibiscus and liquorice were dried in an oven at 50±1°C then powdered using a lab grinder and stored in air- tight jars maintained at 4°C till use. Dried materials (10g) were extracted with distilled water by soaking at room temperature for 48 hr. The extract was centrifuged at 2000 rpm 15 min. (Jouan, MR 1822, France). Extraction and filtration were repeated until the residue was colorless. The solvent was removed under vacuum at 40°C using a rotary evaporator (Laborota 4000-efficient, Heildolph, Germany). Extracts were freeze-dried using lyophilizer. The obtained powder were kept in light- protected containers at -18°C until further use.

Neutral Red Uptake Assay for the Estimation of Cell Viability/Cytotoxicity

The neutral red uptake assay provides a quantitative estimation of the number of viable cells in a culture. It is one of the most used cytotoxicity tests with many biomedical and environmental applications according to **Guillermo *et al.* (2008)**.

Analysis of DNA Damage by Comet Assay

The comet assay was carried out by the method previously described by Collins and his collaborators (**Collins, 2004**) with some modification (**Yedjou and Tchounwou, 2007**). Cells were counted (10,000 cells/well) and aliquots of 100 µl of the cell suspension were placed in each well of 96 plates, treated with 100µl aliquot of either media or different concentrates from 250 to 5000 µg/ml in

hibiscus extract and from 50 to 3000 µg/ml in liquorice extract, respectively and incubated in a 5% CO₂ at 37°C for 2 hr. After incubation, the cells were centrifuged, washed with PBS free calcium and magnesium, and re-suspended in 100 µl PBS. In a 2 ml tube, 50 µl of the cells suspension and 500 µl of melted Agarose were mixed and 75 µl was pipetted onto a pre-warmed comet slide. The side of the pipette tip was used to spread completely agarose/cells over the sample area. The slides were placed flat in the dark at 4°C for 10 minutes to allow the mixture to solidify and then immersed in a prechilled lysis solution at 4°C for 40 minutes. The slides were removed from lysis solution, tapped, and immersed in alkaline solution for 40 minutes at room temperature in the dark. The slides were washed twice for 5 min with Tris-Borate-EDTA (TBE). The slides were electrophoresed at low voltage (300 mA, 25 V, 4°C) for 20 minutes. The slides were placed in 70% ethanol for 5 min, removed, tapped, and air-dried for overnight. The slides were stained with SYBR Green stain designed for the Comet Assay and allowed to air dry at room temperature for six hours. SYBR Green-stained comet slides were viewed with an Olympus fluorescence microscope and analyzed using LAI's Comet Assay Analysis System software (Loates Associates, Inc. Westminster, MD).

RESULTS AND DISCUSSION

Inhibition of Cell Viability

The viability percentage of hibiscus on breast and liver cancer cell lines were 86.9% and 84.7% at 5000 µg/ml, respectively. The IC₅₀ of the hibiscus and liquorice extract were 18478.6 µg/ml and 16159 µg/ml, against breast and liver cancer respectively. In addition, the viability percentage of liquorice extract on breast and liver cancer cell lines were determined and the results revealed that liquorice extract has possessed high anti-carcinogenic properties, it was zero (%) at 3000 µg/ml for breast cancer and 5% for liver cancer at the same concentration, The IC₅₀ of liquanice was 491.7 µg/ml against breast cancer and was 125 µg/ml against liver cancer cell line (Table 1 and Fig. 1). Licorice flavonoids have become one of the hotspots of pharmacological studies for their structural diversity and important

pharmacological activities of the isolated flavonoids, including chalcones, isoflavones, isoflavans, flavonones, flavanonols, 117 isoflavenes, and arylcoumarins as reported by **Fu et al. (2013)**. Liquiritin, isoliquiritin and liquiritigenin are considered to be the main flavonoids of licorice, exhibited a variety of biological properties including antitumor, antidepressant, neuroprotective, antioxidant and anti-inflammatory stated by **Zhou and Ho (2014)**. Licochalcone A, an oxygenated chalcone in licorice, was reported to possess antitumor, antimalarial, anti-metastatic, chemopreventive, antibacterial, and anti-spasmodic activity presented by **Kim et al. (2010)**. Glycycomarin, a representative coumarin in *G. uralensis*, showed antithrombotic and antispasmodic activities (**Qiao et al., 2014**). Some isoflavans derived flavonoids in licorice, such as glabridin, licoricidin and licorisoflavan A showed anti-inflammatory, antioxidative and antitumor activities (**Fu et al., 2013**).

According to **Duh and Yen (1997)**, the Roselle extract is an electron donor and can scaven free radicals to convert them into more stable products and terminate radical chain reactions. Besides demonstrating the potent antioxidant activity of the extract, they revealed that the extract does not only induce mutagenicity but possibly play an important role as dietary antioxidants after ingestion, in the chemical protection against oxidative damage of cell membranes. Thus, the extract may be a strong chemopreventive agent in carcinogenesis. Antioxidant activity of the Roselle extracts correlated strongly to its anthocyanin content (**Tsai et al., 2002**). Anthocyanins (extracted from the dried calyx of *H. sabdariffa*) are natural polyphenolic compounds in the red pigments of Roselle and several other intensely- colored plants. The Hibiscus anthocyanins were found to possess antioxidant bioactivity both *in vivo* and *in vitro* (**Wang et al., 2000**), in addition to this the anthocyanins in Roselle calyx are found to not only possess antioxidant activity (**Tsuda et al., 2000**), but also mediate other physiological functions related to cancer suppression (**Meiers et al., 2001**). Hibiscus anthocyanins (HAs) extracted from the Roselle calyx was found to have a concentration-dependent inhibitory effect on the growth of several cell lines, including MCF-7 cells (**Chang et al., 2005**).

Table 1. Percentage cell viability and IC₅₀ of Hibiscus and Liquorice of MCF-7 and HepG2 cell lines

Concentration (µg/ml)	Viability (%)	
	Breast cancer (MCF-7)	Liver cancer (HepG2)
	Hibiscus	
5000	86.9	84.7
3000	95	87.4
2000	97	92.4
1000	98	95.8
500	100	97
250	100	98.3
IC ₅₀	18478.6 µg/ml	16159 µg/ml
	Liquarice	
3000	0	5
2000	0	4.5
1000	0	8.8
500	51.6	12
250	65	29.8
50	100	73
IC ₅₀	491.7 µg/ml	125 µg/ml

IC₅₀: The half maximal inhibitory concentration is a measure of the effectiveness of a compound in inhibiting biological or biochemical function.

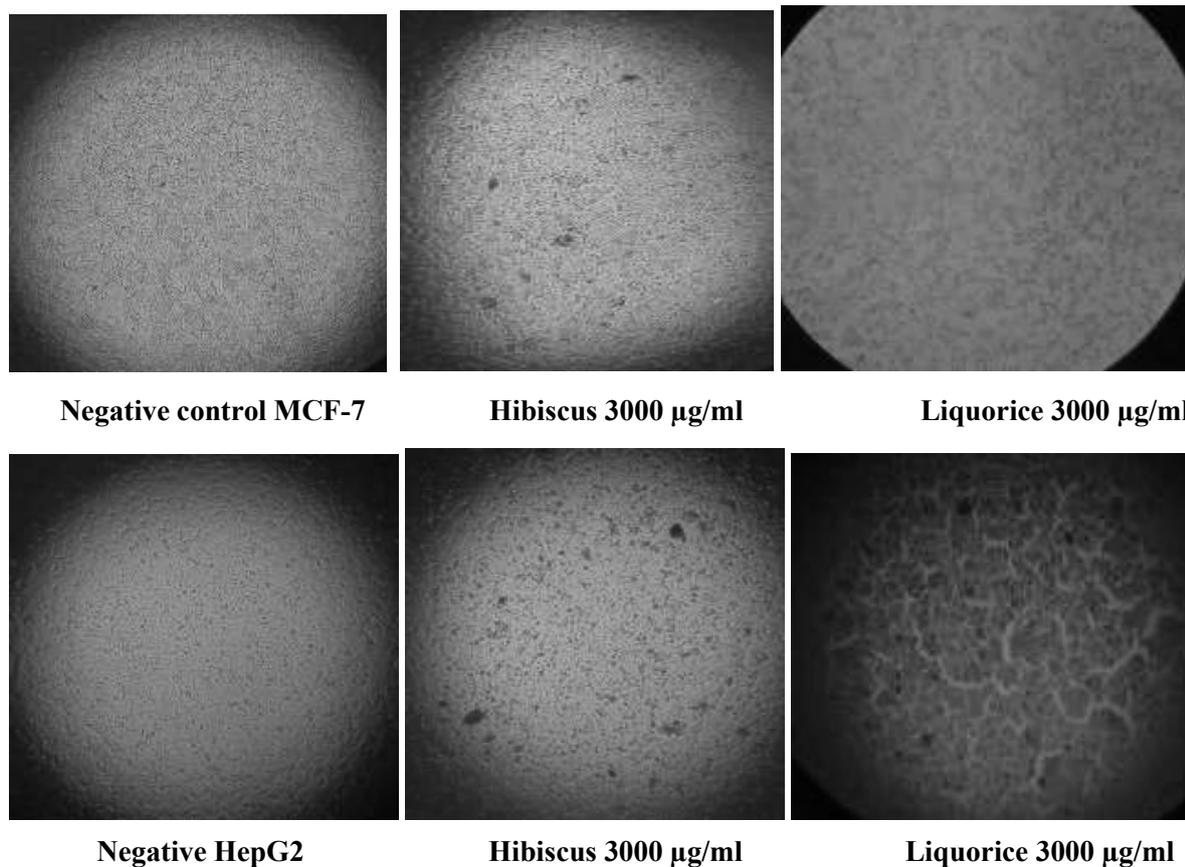


Fig. 1. Morphologically cytotoxic effects of negative, hibiscus and liquorice extracts on MCF-7 and HepG2 cells

Induction of DNA Damage

The adverse impacts of high liquorice *Glycyrrhiza glabra* extract (3000 µg/ml) on liver malignant growth (HepG2), there stays extensive uncertainty about the cell and molecular mechanisms of activity of liquorice remove in HepG2 cells.

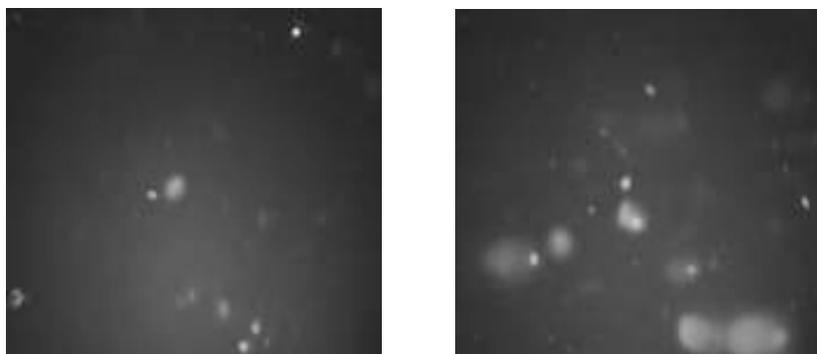
Table 2 and Figs. 2 and 3 demonstrated the related DNA harm in HepG2 cells; by the methods for soluble single cell gel electrophoresis (Comet) measure. It could be noticed that liquorice extract has a solid genotoxic potential and is capable of causing DNA harm in malignant growth cells. The study outcomes showed that liquorice extract actuates genotoxic impacts to HepG2 cells in a portion subordinate form, it was 24% suggestive clear proof that liquorice concentrate might be an intense DNA harming operator against bosom and liver malignant growth when utilized at high dosages (3000 µg/ml), these outcomes were compared by negative control (17%).

Cell passing is thought to happen at any rate by two different ways that incorporate apoptosis and necrosis. Apoptosis is a functioning and

physiological method of cell passing that is accepted to be intervened by dynamic characteristic instruments, albeit extraneous¹¹⁹ elements can contribute according to **Padanilam (2003)**. For the most part, it demonstrates no incendiary morphological changes of cell shrinkage which incorporate cytoplasmic and atomic buildup, chromatin buildup (pyknosis), requested DNA fracture (karyorrhexis), blebbing of the plasma layer, development of apoptotic bodies (cell discontinuity of film bound sections) and presentation of surface particles, for example, phosphatidylserine (PS) on the plasma film to encourage finish phagocytosis of apoptotic cells these were reported by **Wyllie (1994)**. Conversely, rot is an uncontrolled cell passing that is portrayed by the dynamic loss of cytoplasmic film respectability, the quick convergence of Na⁺, Ca²⁺, and water, bringing about cytoplasmic swelling and atomic pyknosis Barros stated by **Barros et al. (2001)**. The last component prompts cell fracture and arrival of lysosomal and granular substance into the encompassing extracellular space, with ensuing aggravation (**Majno and Joris, 1995**).

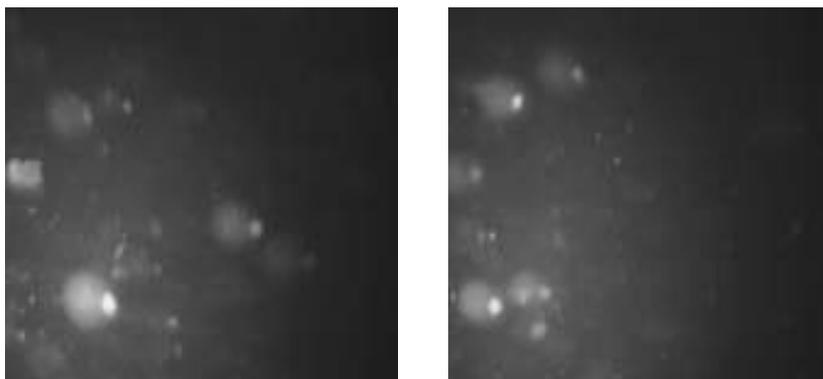
Table 2. DNA damage of control and liquorice extract on HepG2 cells

Sample	Comet (%)	Tail Length (px)	DNA in Tail (%)	Tail Moment	Olive tail moment
Control	17	4.909091	3.69653	0.247281	0.837201
liquorice extract	27	11.33333	3.784216	0.454482	0.949797



(a)

Fig. 2. DNA damage of control on HepG2 cells (a)



(b)

Fig. 3. DNA damage of liquorice extract on HepG2 cells (b)

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تأثير تناول المشروبات التقليدية مثل العرقسوس والكرديه على مقاومتها للخلايا السرطانية

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سرطان الكبد وسرطان الثدي من أكثر الأورام السرطانية شيوعا وأكثرها شراسة، يهدف البحث لتقدير إمكانية استخدام نوعين من المشروبات التقليدية (مستخلص العرقسوس ومستخلص الكركديه) على مقاومة الأورام السرطانية تحت الدراسة وتم استخدام تركيزات من ٢٥٠ إلى ٥٠٠٠ ميكروجرام لكل مللى من مستخلص الكركديه وتركيزات من ٥٠ إلى ٣٠٠٠ ميكروجرام لكل مللى من مستخلص العرقسوس وتم تقدير تأثير كل مستخلص على حدة على كلا من سرطان الكبد والثدى وجد ان تأثير مستخلص الكركديه على سرطان الثدي وسرطان الكبد كانت ٨٦,٩% و ٨٤,٧% على التوالي بينما كانت النسبة في مستخلص العرقسوس على سرطان الثدي والكبد (صفر %) و ٥ على التوالي، كانت قيمة IC_{50} لمستخلص الكركديه ١٨٤٧٨,٦ ميكرو جرام لكل مللى على سرطان الثدي وكانت ١٦١٥٩ ميكروجرام لكل مللى على سرطان الكبد بينما كانت لمستخلص العرقسوس ٤٩١,٧ ميكروجرام لكل مللى على سرطان الثدي ١٢٥ ميكرو جرام لكل مللى على سرطان الكبد، تم تقدير تلف الحامض النووى بطريقه الكوميت حيث يمكن تحديد تلف الحامض النووى فى خلايا الكبد المزروعه وكانت نسبة الكوميت فى خلايا الكونترول ١٧% بينما كانت ٢٧% فى حالة استخدام مستخلص العرقسوس مما سبق تبين إمكانية استخدام مشروب العرقسوس كواقى ضد سرطان الثدي والكبد لما يحويه من مركبات مضادة للأكسدة.

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