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Full Length Research paper

Toxicity assessment of some tea labels from supermarkets in Ilorin, Nigeria using brine shrimp (*Artemia salina*) lethality assay

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Brine shrimp lethality assay is a useful tool for preliminary assessment of toxicity. Food items and material safety are of necessity a regular exercise for routine check. Tea, a widely consumed drink after water therefore, needs to be assessed periodically and brine shrimp lethality assay is a suitable tool for this assessment. Toxicity assessment was conducted for various tea brands obtained from supermarkets in Ilorin, Nigeria using the brine shrimp lethality assay. Solutions of the different tea brands and aqueous extract of *Cymbopogon citratus* (1000, 100, 10 ppm) were prepared and the effect of these tea solutions on brine shrimp larvae, *Artemia salina* was investigated. All the tea solutions were toxic at high concentration with caffeinated Tetley tea, decaffeinated Tetley tea and raw lemon grass being the most toxic against the brine shrimps. None of the tea brands constitute health hazard at the concentration level of usual human consumption. Processed and refined teas generally have lower toxicity and sweet aroma.

Key words: C*ymbopogon citratus,* green tea, black tea, caffeinated tea, decaffeinated tea, toxicity, brine shrimps.

INTRODUCTION

Tea drinks are produced from a wide variety of plants but majority comes from the young tender leaves and leaf bud of *Camellia sinensis.* In general term, tea is a hot water extract of plants of pharmacological properties (Hara et al., 1995; Hamilton-Miller, 1995). Tea is the most widely consumed drink after water due to its refreshing and mildly stimulant effects (Harbowy and Balentine, 1997). Alkaloids, caffeine (1,3,5-trimethylxanthine), polyphenols (e.g. catechin) and two minor isomeric dimethylxanthines, theobromine and theophyline are responsible for the mildly stimulant effects of tea (Stagg and Millin, 1975). Briskness and other taste characteristics are tea qualities attributed to caffeine (Choudhury et al., 1991).

C. sinensis is a shrub of Theaceae family. White tea, green tea, oolong, puerh tea and black tea are all harvested from this species but are prepared differently to attain different levels of oxidation (fermentation). Green tea is produced from leaves without oxidation, Oolong tea is semi-fermented tea while black tea (or red tea) is fully oxidized tea. White tea is produced from the young leaves covered with short white hair without any oxidation. Brick tea is made from old leaves which are high in fluoride content since fluoride accumulates with age whereas all other teas are made from fresh leaves, though they contain enough fluoride to prevent dental caries (Funk et al., 1999; Cao et al., 2004). Green and black tea have been found to have cancer preventive activity in a variety of animal models of cancer, including cancer of the skin, lung, mouth, esophagus, stomach, colon, pancreas and bladder (Lambert and Yang, 2003; Yang et al., 2002). Additionally, white and green teas were shown to suppress intestinal polyps in mice. Flavonoids contribute substantially to the cancer preventing

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effects of tea.

Literature reports on the pharmacological activities of various tea brands abound and these include anti-inflammatory (Stagg and Millin, 1975), anti - carcinogenic, anti-oxidant and angiotension inhibitory (Zongmao, 1991), anti-bacterial and anti-viral (Toda et al., 1989) effects. Green tea inhibition of lipid peroxidation in the kidneys, liver and testes of pre-treated animals as well as superoxide dismutase and catalase activities are reported by Soussi et al. (2006). Green tea is also reported to possess antidepressant property (Singal et al., 2006). Black tea play important role in maintaining cardio-vascular health by producing nitric oxide, a compound that promotes arterial relaxation (Vita, 2003).

Lemon grass, *Cymbogon citratus* (DC) Stapf and other species of Cymbogon are used in preparing tea drinks all over the world apart from utilization as spices. Tea from *C. citratus* leaves is used in Brazil as antispasmodic, analgesic, anti-inflammatory, antipyretic, diuretic and sedative (Carlini et al., 1986). The volatile oil obtained from the fresh leaves of this plant is widely used by the perfumes and cosmetic industries (Ferreira and Fonteles, 1989). Studies on the antimicrobial, especially anti-bacterial and antifungal activities of lemon grass oil and its components were reported (Wannisson et al., 1996; Schuck et al., 2001; Paranagama et al., 2003).

Eyebright is a parasitic plant that must attach its roots to the roots of other plants in order to survive. It has been used to treat respiratory conditions including bronchitis, allergies, cold and sinusitis. Green tea boost metabolism and help burn fat by catechin polyphenols reacting with chemical transmitter in the nervous system, known as norepinephrine. Slimming tea is an herbal tea and it promotes cleaning of gastrointestinal system by dis-lodging, breaking down and washing away accumulated wastes consequently, keeping one slim and healthy.

In Nigeria, the quantity of tea consumed daily is not yet determined and documented but an estimated 2.5 million cups are consumed daily worldwide (Kirk and Othmer, 1980). The high rates of tea consumption worldwide require that the safety in terms of toxicity should be determined. The brine shrimp lethality assay is considered a useful and simple tool for preliminary assessment of toxicity. This study therefore was undertaken to determine the brine shrimp toxicity of different brands of tea found in supermarkets in Ilorin city, Nigeria.

MATERIALS AND METHODS

Tea label collection

Tea brands were purchased from different supermarkets in Yoruba road area of Ilorin, Nigeria in October 2008. They were kept in a dry place at room temperature prior to analysis in the food and phytochemical laboratory in the department of chemistry, university of Ilorin, Nigeria. The brands include; Eyebright tea (EB), Lipton tea, a black tea (LP), Tetley caffeinated tea (TC), Tetley decaffeinated tea (TDC), Toptea (TT), Slimming tea, herbal tea (ST), Dara-Dara tea (DD) from lemon grass, Green tea (GT) and *Cymbopogon citratus* (LG), raw lemon grass.

Tea preparation

Four (4) tea bags (2 g each) were placed flatly onto the bottom of 100 ml beaker. 80 ml of hot sterile artificial sea water (prepared using sea salt 38 g/L and adjusted to pH 8.5 using 1N NaOH) was poured into the beaker and allowed to steep for 15 min, shaken gently at intervals of 5 min. The teabags were squeezed between two spoons and discarded. The resultant tea solution is 0.1 g/ml (1000 µg/ml). Serial dilution was done by diluting 2 ml of this solution to 20 ml with sea water giving a solution of 0.01 g/ml (100 µg/ml). Further dilution of this gave 0.001 g/ml (10 µg/ml). These three concentrations of tea solutions were used for the brine shrimp toxicity determination. The lemon grass, *C. citratus* was obtained from University of Ilorin senior staff quarters. 8 g of the room dried lemon grass was extracted with 80 ml of the artificial sea water also, and the dilution was carried out as described above.

Brine shrimp lethality assay

Modified method of Krishnaraju et al. (2005) was employed in this study. Brine shrimps (*Artemia salina*) were hatched from its eggs in an improvised hatchery made of plastic dish filled with natural sea water from Bar Beach, Lagos, Nigeria. The hatchery with the eggs was placed in light for 48 h. Active brine shrimp larvae were separated, from which those used for the experiment were chosen.

Ten nauplii were introduced into 5 ml of the various tea solutions in separate vials. Control experiments was set up simultaneously and this contain ten nauplii in 5 ml sea water and 10 µg/ml solution of cyclophosphamide respectively. Tests for each concentration were conducted in triplicate. The experiments were maintained at room temperature for 24 h under light, after which the dead nauplii in each vial was counted.

Statistical analysis

The percentage lethality was calculated from the mean of larvae that survived in the extracts treated vials and controls. The numbers of dead nauplii was divided by initial number of nauplii (10) and multiply by 100, using the arithmetic graphic method of Reish and Oshida (1986). Finney's probit analysis was used to determine the LC_{50} of each extract at 95% confidence interval.

RESULTS AND DISCUSSION

The results of this study are presented in Table 1. All the tea brands displayed high LC_{50} values which depict overall non-toxicity except Tetley caffeinated tea and the unrefined dried lemon grass (LG) that are of medium toxicity with LC_{50} of 23.67 and 35.58 µg/ml respectively. All the tea brands were toxic at high concentration with mortality of brine shrimp being over 80% but as concentration reduced, the mortality decreased for instance at 10 µg/ml, the mortality went below 10% in most cases. TC, TDC and LG showed a different pattern in toxicity reduction. At 1000 µg/ml, the toxicity of these three tea labels was very high and this was expressed by the high mortality of 100, 96.67 and 96.67% displayed, respectively.

Table 1. Brine shrimp assay of some tea labels from supermarkets in Ilorin, Nigeria.

Tea label	Conc. 0.1 g/ml	%mortality	Conc. 0.01 g/ml	%mortality	Conc. 0.001 g/ml	%mortality	LC_{50} µg/ml, 24h
LP	10, 9, 9	93.33	1, 1, 1	10	1, 0, 0	3.33	252.94[a]
TT	10, 10,10	100	2, 1, 2	16.67	1, 0, 1	6.67	168.38[a]
EB	9, 6, 10	83.33	2, 1, 3	20	0, 2, 1	10	235.67[a]
SL	5, 9, 10	80	2, 2, 3	23.33	0, 0, 0	0	266.86[a]
GT	7, 9, 9	83.33	5, 5, 6	36.67	2, 2, 2	20	83.42[a]
TC	10, 10, 10	100	10, 8, 9	90	2, 2, 3	23.33	23.67[b]
TDC	10, 10, 9	96.67	5, 6, 4	50	4, 1, 1	30	64.38[b]
DD	9, 6, 10	83.33	0, 2, 1	10	0, 0, 1	3.33	333.26[a]
LG	10, 10, 9	96.67	5, 4, 7	53.33	5, 3, 3	36.67	35.58[b]
CP	-	-	-	-	10, 9, 10	96.67	ND

LP=Lipton tea, TT=Toptea, EB=Eye bright tea, SL=Slimmy tea, GT=Green tea, TC=Tetley caffeinated tea, TDC=Tetley decaffeinated tea, DD=Dara dara tea, LG=Lemon grass, CP=Cyclophosphamide, ND=Not determined. LC_{50} values followed by the same letter are not significantly different at 5% level of significance.

Though the mortality dropped as the concentration reduced, the mortality drop was not as sharp as that of other tea brands. For instance at 100 µg/ml the tea brands are still very toxic with 90.0, 50.0 and 53.33% mortality respectively. At 10 µg/ml these three tea labels are still mildly toxic with percentage mortalities of 23.73, 30.0 and 36.67 respectively leading to LC_{50} of 23.67, 64.38 and 35.58% µg/ml respectively. Caffeine content of the Tetley caffeinated tea (TC) may be responsible for its higher toxicity compared to decaffeinated labels. High caffeine content intake has been associated with increased risk of miscarriage and low birth weight in some epidemiological studies (Caffeine, 2005). Caffeinated green tea causes gastrointestinal side effects including nausea, vomiting, abdominal pain and diarrhea (Jatoi et al., 2003; Pisters et al., 2001). Central nervous system symptoms, including agitation, restlessness, insomnia, tremors, dizziness and confusion have also been reported (Jatoi et al., 2003). Dara-Dara is a refined and packed tea made from *C. citratus,* lemon grass. A comparison of this tea brand with the unrefined lemon grass showed great mortality difference. Dara-Dara showed a mortality of 83.33% at 1000 µg/ml, 10% at 100 µg/ml and 3.33% at 10 µg/ml while the unrefined lemon grass has mortalities of 96.67, 53.33 and 36.67% at the various corresponding concentrations respectively. Mortality and LC_{50} value showed Dara-Dara label to be far less toxic compared to unrefined lemon grass (LG), though they are both made from lemon grass and *C. citratus*. Refining, oxidation and proper packaging might be responsible for low toxicity of Dara-Dara tea. Tea is generally considered to be safe, even in large amounts. However, cases of hypokalemia, a potentially life-threatening condition in the elderly has been associated with caffeine toxicity which is acquired by excessive consumption of black and oolong tea, 3-14 liters/day (Aizaki et al., 1999).

The high mortality shown by 10 µg/ml of cyclophosphamide, the positive control is not surprising since cyclophosphamide is an antitumor drug with high cytotoxic property. Mortality of 96.67% was shown by cyclophosphamide at 10 µg/ml and none of the tea solutions gave such a high mortality at this concentration. Also, none of the tea labels used in this study was found to show any cytotoxic property as deduced from their LC_{50} values.

Conclusion

All the tea labels used in this study were found to be safe with reference to brine shrimp toxicity assay and since tea drinks are produced and consumed at relatively low concentration, they are therefore safe. It is however, advisable that tea solutions of brands that showed low LC_{50} values, that is, the caffeinated, decaffeinated teas and the raw lemon grass should be well diluted before consumption to forestall abdominal discomfort which results from excess assimilation of caffeine.

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