Cytotoxicity Activity Of Green Tea and Mint Formulation

Running Title: Cytotoxicity activity of green tea and mint formulation

ABSTRACT:

Background:

Cytotoxicity refers to a substance or process which results in cell damage or cell death. The prefix 'cyto' refers to a cell and 'toxic' to poison. The term is often used to describe chemotherapy drugs that kill cancer cells, but it may also be used to describe toxins, such as venom. Within our own immune system , we have cells that are considered cytotoxic, such as the T cell that kills bacteria, viruses, and cancer cells.

Materials and Methods:

100ml of distilled water, 1 gram of green tea leaf and 1 gram of powdered mint is added and the mixture is heated for 15 to 20 minutes and then filtered with the help of filtered paper. The mixture was again heated and concentrated from 70ml to 20ml. 2 grams of iodine free salt was weighed and dissolved in 200ml of distilled water. 6 well ELISA plates were taken and 10-12ml of saline water was filled. To that 10 nauplii were added to each well(5µl, 10µl, 20µl, 40µl, 80µl). Then the nanoparticles were added according to the concentration level. The plates were incubated for 24 hours. After 24 hours, the elisa plates were observed and noted for the number of live nauplii's present.

Results and Discussion:

The cytotoxicity activity of green tea with mint formulation shows that all the introduced shrimps were alive in the control whereas it is shows that the lethality of 5µl is 60%, 10µl is 70%, 20µl is 70%, 40µl is 70% and 80µl is 100%.

Conclusion:

Based on the result of the study it is finally concluded that cytotoxic activity of green tea and mint formulation has better effect.

Keyword: cytotoxicity, mint, green tea, antioxidant, green synthesis.

Introduction:

Cytotoxicity (1) refers to a substance or process which results in cell damage or cell death. The prefix ‘cyto’ refers to a cell and ‘toxic’ to poison. The term is often used to describe chemotherapy drugs that kill cancer (2)(3)(2,4) cells, but it may also be used to describe toxins, such as venom. Within our own immune system , we have cells that are considered cytotoxic, such as the T cell that kills bacteria, viruses, and cancer cells. And it also kills any cell harboring such pathogens (5) by recognizing foreign peptides that are transported to the cell surface bound to MHC class I molecules. Oxygen radicals, organic hydroperoxides, and other types of free radicals are produced by certain chemical carcinogens and appear to play a role in the carcinogenic process(6)(7). We now control many tumors with antitumor agents. However, the need for new antitumor agents is pressing, especially against solid tumors, especially since antitumor agents need to be used for long durations. It seems that edible plants, food spices, and daily beverages that have been continuously consumed by many people for many years may be a superior source of antitumor agent(8).

Many aromatic plants used in medicine, food, and pharmaceutical industries belong to the lamiaceae family. In this family, mentha is a well known genus that includes 25-30 species that are generally grown in temperate areas around the world(9). Mentha also acts as a good expectorant. The most cited activities of the plants are its antiviral, antibacterial(10)(11), antifungal and cytotoxicity(12). The widespread use of M.piperita in traditional medicines has inspired us to explore its potential biological activities, knowing that there are few previous studies reporting the cytotoxicity. 120 edible plants for antitumor promoting activities against the non-12-O-tetradecanoylphorbol- 13-acetate (TPA)-type promoter, okadaic acid (OA), which promotes tumor formation by inhibiting protein phosphatase-2A. Peppermint was one of only eight plants that showed strong activity (86–100%) in suppressing the effect of OA.

Menthol derived from M. piperita appears to affect cytosolic arylamine N- acetyltransferase (NAT) activity in the human liver tumor cell line J5 differentially dependent on dose (Lin et al., 2001); higher doses (32 and 3.2 mM) inhibited NAT, a more moderate dose (0.32 mM) had no effect, and lower doses (0.032 and 0.0032 mM) promoted NAT relative to controls(13). Herbal(14) teas are not true teas, true teas like green tea, black tea, oolong tea. Green tea has been considered a medicine and a healthful beverage since ancient times. Traditional Chinese medicine has recommended this plant for headaches, body aches and pains, digestion, depression, detoxification, as an energizer and, in general, to prolong life. Green tea leaves contain three main components which act upon human health: xanthic bases (caf- feine and theophylline), essential oils and especially, polyphe- nolic compounds. Caffeine acts mainly upon the central ner- vous system, stimulating wakefulness, facilitating ideas association and decreasing the sensation of fatigue(15).

Green tea polyphenols- namely catechin, epicatechin, epigallocatechin, and epigallocatechin-3-gallate are important constituents of green tea. They are known to possess chemopreventive and therapeutic properties against various diseases, including cancer(16)(16,17). Several studies using cancer cell lines(18) and animal models of cariogenesis have shown that green tea polyphenol anti cancer and apoptosis(19) inducing properties. An important, well noted attribute of the chemopreventive action of green tea polyphenols is their differential activity in selectively targeting cancer cells and sparing normal cells(20).

Our team has extensive knowledge and research experience that has translate into high quality publications(21–25),(26),(27),(28),(29),(30),(31),((23,32,33),(34–38) .,(39),(40)

The aim of the study is to evaluate the cytotoxic effect of green tea and mint formulation.

Materials and Methods:

Preparation of Herbal Formulation:

100ml of distilled water, 1 gram of green tea leaf and 1 gram of powdered mint is added and the mixture is heated for 15 to 20 minutes and then filtered with the help of filtered paper. The mixture was again heated and concentrated from 70ml to 20ml.

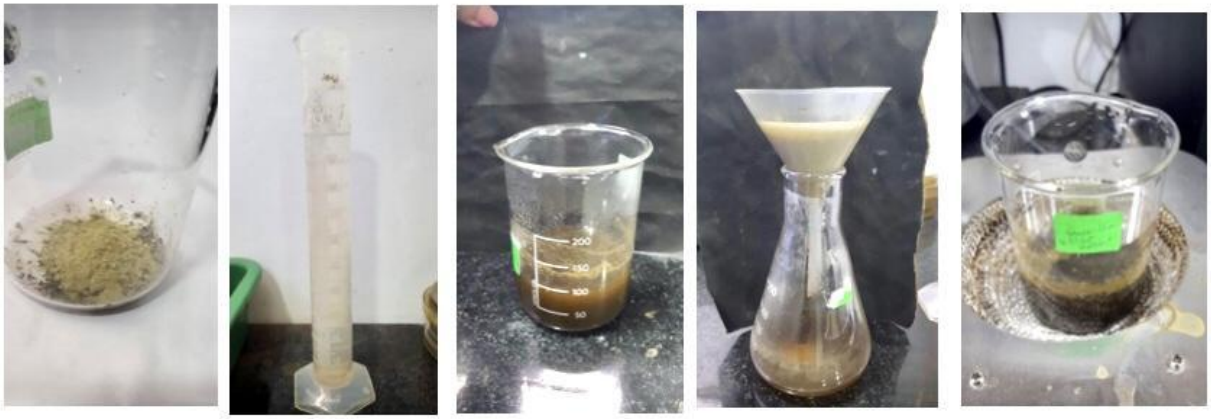
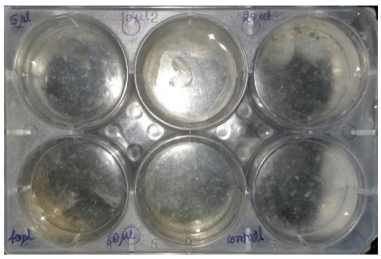


Figure 1: Shows the preparation of herbal formulation



Brine shrimp lethality assay:

Figure 2: Shows the Brine shrimp lethality assay.

Determination of cytotoxicity effect: 2 grams of iodine free salt was weighed and dissolved in 200ml of distilled water. 6 well ELISA plates were taken and 10-12ml of saline water was filled. To that 10 nauplii were added to each well(5µl, 10µl, 20µl, 40µl, 80µl). Then the nanoparticles were added according to the concentration level. The plates were incubated for 24 hours. After 24 hours, the elisa plates were observed and noted for the number of live nauplii's present.

Results and Discussion:

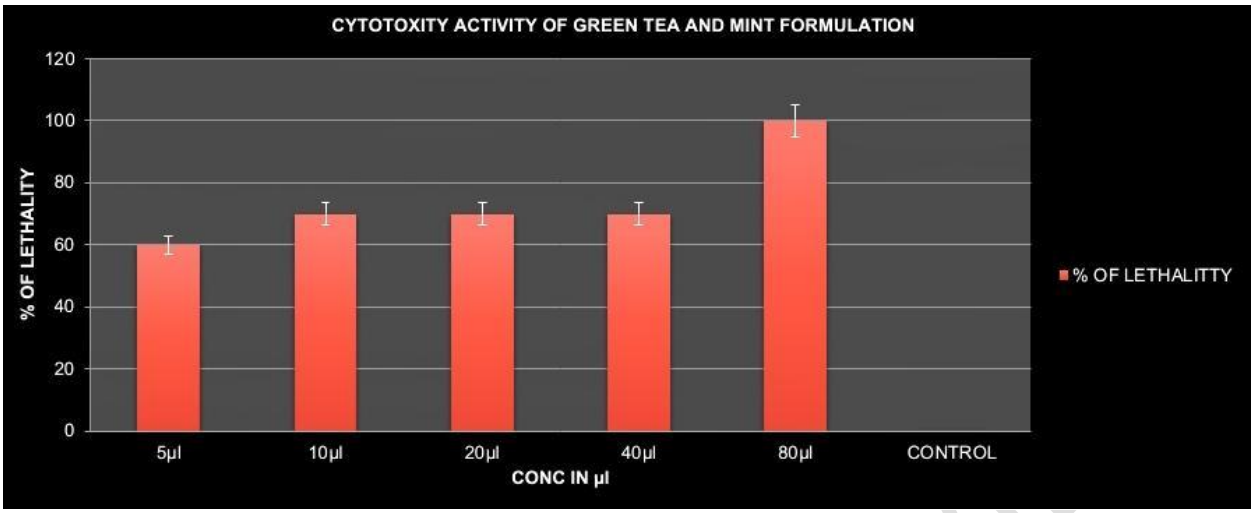


Figure 3: Shows the count of alive shrimp after 24 hours in Brine shrimp assay

The cytotoxicity activity of green tea with mint formulation shows that all the introduced shrimps were alive in the control whereas it is shows that the lethality of 5µl is 60%, 10µl is 70%, 20µl is 70%, 40µl is 70% and 80µl is 100% (Figure 3). The cytotoxicity of the volatile may not be potent enough to use them as leading compounds for drug design, but they are worthy of further investigation as natural products isolated from daily beverages. Importantly, these volatile compounds are identified in many edible plants, food spices and beverages are frequently used for fragrance and flavor. There is no doubt that these volatile compounds have long been consumed by many people(41). For example, the most potent cytotoxic volatile green tea flavour, nerolidol, is also a common component of many essential oils.

Cell death and the inhibition of cellular communication may occur in vivo following exposure to free radical- generating tumor promoters and may be mechanisms of tumor promotion by such compounds. Such promoters might include TPA which enhances chemiluminescence in epidermal cells and phagocytes(42)(42,43). On the other side, when assessing cytotoxicity, factors including size, shape, physicochemical surface property, concentration, exposure time, and cell type should be considered.

In addition to that, cytotoxic mechanism including disruption of the cell membrane, oxidative stress, destruction of the cytoskeleton and loss of mitochondrial function is important(44)(44,45)Similarly, in previous study, the protective effect of green tea extract and its

constituent polyphenols on the nephrotoxicity induced by the immunosuppressant FK506 in porcine renal proximal tubular cell line, LLC-PK1 cells, was evaluated. A significant increase in apoptotic cells but the addition of green tea extract, and particularly its major polyphenolic component epigallocatechin- gallate, suppress the cell death(46).

Conclusion:

The allopath based anticancer drugs are expensive and also show adverse effects via alteration in molecular pathways(37) (47) (48) (49) (50) (51) (52) (53) (54) (55) (56) (57) (58) (59) (60). Green tea has been widely used in traditional medicine since ancient times due to being inexpensive, efficacy, and fewer side effect properties. Based on the result of the study, it is finally concluded that cytotoxic activity of green tea and mint formulation has better lethality effects in 80µl is 100%.

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