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STUDY THE ANTICANCER EFFECT OF CURCUMIN, PARAQUAT AND BRUSATOL COMBINATIONS AGAINST THE PULMONARY CARCINOMA (NCI-H292) (ATCC ® CRL-1848™)

Dilla Jose* & P. Senthilkumaar**

* Department of Zoology, St. George's College, Aruvithura, Erattupetta, Kerala
** School of Enzymology and Environmental Toxicology, P.G and Research Department of Zoology, Sir Theagaraya College, Chennai, Tamilnadu

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Abstract:

The present study scrutinized the anticancer activity of curcumin, paraquat and brusatol combinations against the pulmonary carcinoma (NCI-H292) (ATCC® CRL-1848™) by two different methods such as colony forming and apoptosis assay. Significant increase in colony formation compared to the control was observable in 25 µg/ml dose of curcumin. In colony forming assay at both concentration study (50 and 100 µg/ml), high increase in colony formation resulted in 50 µg/ml dose of curcumin. Likewise in apoptosis study, high increase in apoptosis resulted in 50 µg/ml dose of curcumin. This study is witnessed that combination of these things were support the anticancer activity in large extend.

Key Words: Curcumin, Paraquat, Brusatol, Pulmonary carcinoma, Colony formation & Apoptosis

Introduction:

Many plants have been identified to possess anti-cancer properties that can be used as herbal remedy for various types of cancer. One of the potential plants in that category is the Indian turmeric (Curcuma longa). It is a rhizomatous herbaceous perennial plant of the ginger family, Zingiberaceae bearing many rhizomes on its root system which are the source of its culinary spice known as turmeric and its medicinal extract called Curcumin. The plant belongs to the genus: Curcuma and species: longa. Its scientific name is Curcuma longa Linnaeus. It is native in southeast India, and needs temperatures between 20^0C and 30^0C and a considerable amount of annual rainfall to thrive (Chan et al., 2003). It is a potent antioxidant, anti-inflammatory, analgesic and anticancer agent used in traditional medicine. It has been shown to suppress cellular transformation, proliferation, invasion, angiogenesis, and metastasis through a mechanism not fully understood. A lipooxygenase and cyclooxygenase inhibitor, Blood-brain barrier permeable substance and it exhibits a diverse range of actions including free radical scavenging activity in vitro and in vivo with cardio and neuroprotective effects (Aggarwal et al., 2005).

Curcumin inhibits the activation of NF-KB and the expressions of oncogenes including c-jun, c-fos, c-myc, NIK, MAPKs, ERK, ELK, PI3K, Akt, CDKs, and iNOS. It is considered that PKC, mTOR, and EGFR tyrosine kinase are the major upstream molecular targest for curcumin intervention, whereas the nuclear oncogenes such as c-jun, c-fos, c-myc, CDKs, FAS, and iNOS might act as downstream molecular targets for curcumin actions. It is proposed that curcumin might suppress tumor promotion through blocking signal transduction pathways in the target cells. The oxidant tumor promoter TPA activates PKC by reacting with zinc thiolates present within the regulatory domain, whereas the oxidized form of cancer chemopreventive agent such as curcumin can inactivate PKC by oxidizing the vicinal thiols present within the catalytic domain. Recent studies indicated that proteasome-mediated degradation of cell proteins play a pivotal role in the regulation of several basic cellular processes, including differentiation, proliferation, cell cycling, and apoptosis. It has been demonstrated that curcumin-induced apoptosis is mediated through the impairment of the ubiquitin-proteasome pathway (Lin, 2007). The main aim of the present study is to find the efficacy of anticancer activity of curcumin and combination of curcumin with some products.

Materials and Methods:

Curcumin:

Curcumin is a derivative from *Curcuma longa*. Curcumin powder was obtained from Sigma chemicals (St. Louis, MO, USA) and stored at room temperature. It was purchased as a crystalline solid. A stock solution was made by dissolving the curcumin in acetone. Further dilutions of stock solution into aqueous solution were made prior to performing experiments. Different doses of Curcumin were used (1, 5, 10, 25, 50 and 100 µg/ml).

Paraquat:

The herbicide paraquat (Paraquat Dichloride 24% SL) was purchased from HPM Chemicals & Fertilizers Ltd., Coimbatore and Tamil Nadu. Different doses of paraquat were used (1, 5, 10, 25, 50 and 100 µg/ml). Serial dilutions of the test herbicide were made as per the requirements in the present investigation.

Brusatol:

Brusatol(13,20-Epoxy-3,11β,12α-trihydroxy-15β-[(3-methyl-1-oxo-2-butenyl)oxy]-2,16-72dioxopicras-3-en-21-oic acid methyl ester) was purchased from Sigma chemicals (St. Louis, MO, USA). Required dilution

was prepared using appropriate solvent. 1µg/ml dilution of brusatol was made as per the requirements in the present investigation.

Cancer Cell Lines:

The test cancer lines of Pulmonary Carcinoma (NCI-H292) (ATCC® CRL-1848™) were procured from American Type Culture Collection (ATCC), Manassas, VA 20108 USA.

Complete Growth Medium:

The following culture methods were followed for growth, sub-culturing, cryopreserving and thawing of Pulmonary Carcinoma (NCI-H292) (ATCC® CRL-1848™). The base medium for this cell line is ATCC-formulated RPMI-1640 Medium, ATCC 30-2001. To make the complete growth medium, add the following components to the base medium: fetal bovine serum (ATCC 30-2020) to a final concentration of 10%.

Volumes are given for a 75 cm^2 flask. Increase or decrease the amount of dissociation medium needed proportionally for culture vessels of other sizes. Remove and discard culture medium. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mMEDTA solution to remove all traces of serum that contains trypsin inhibitor. Add 2.0 to 3.0 mL of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting. Add appropriate aliquots of the cell suspension to new culture vessels. Incubate cultures at 37°C. Sub-cultivation Ratio: A sub-cultivation ratio of 1:3 to 1:8 is recommended. Medium Renewal: Every 2 to 3 days.

Clonogenic Assay:

The cancer cells were plated in six-well plates overnight and treated with 40 µM curcumin for a period of 3 or 6 h. After the removal of the drug-containing medium, the cells were washed using PBS, trypsinized and plated at a low density (2000 cells/ well in six-well plates). The cells were then incubated with an equivalent amount of DMSO without curcumin, which served as a control. The cells were cultivated for 7 or 12 d and the medium was refreshed every two days. The colonies were stained with crystal violet (Sigma Chemical Co, St. Louis, MO). The number of clones in a given area was counted for each condition.

Apoptosis:

Apoptosis induction was determined by measuring Annexin V activity using Annexin V apoptosis kit. The kit was sourced from Promega corporation, Southapton, UK and consisted of Annexin V buffer and lyophilized Annexin V substrate. Cells were seeded at the concentration of 10^4 cells per well in 96 well microtitre plate and incubated for 48 hours. Aliquot of 100 µL of fresh media containing appropriate concentration of extracts were transferred to the assigned respective wells. Stauroporine 0.1 µg/mL was used as positive control and untreated wells were treated as negative control. Total of six wells were assigned for each treatment. The plate was allowed to equilibrate to room temperature after 24 hours of incubation prior to performing the assay. 100 µL of Annexin V reagent was added to each well and mixed for 60 seconds and incubated for further 1 hour at room temperature. A aliquot of 100 µL of contents from each well was transferred to white-walled 96 well plate. The light emitted was measured by Packard lumicount microplate luminometer and measurement was recorded using THERMOmax™ plate reader linked to a computer using SoftMax Pro software (Muñoz-Alonso *et al*., 2008). The results are depicted in the tabular form.

Statistical Analysis:

The mean and standard deviation were performed for the observed results by ORIGIN 8.0 software.

Results and Discussions:

Curcumin has been previously evaluated by JECFA in 1982, 1987, 1996 and 2004, the EU Scientific Committee for Food (SCF) in 1975 and Tema Nord in 2002. The present opinion briefly reports the major studies evaluated in these reports and describes additional newly reported literature data in some more detail. For the current evaluation, only studies with curcumin or purified turmeric extract in which the curcumin concentration is high and specified are taken into account, since otherwise, the test material in the study is not comparable in composition to curcumin used as a colour additive in food. In the present study, the selected cancer line is Pulmonary Carcinoma (NCI-H292) (ATCC® CRL-1848™). Curcumin, brusatol and Paraquat were used to study Colony forming assay and Apoptosis.

Colony Forming Assay Using Pulmonary Carcinoma (NCI-H292) with Curcumin 25 and 50 µg/ml, Brusatol (1µg/ml) and 50 µg/ml of Paraquat:

Two separate doses of curcumin (25 and 50 µg/ml) were used. Two separate doses of curcumin were used along with 1µg/ml of brusatol and 50 µg/ml of paraquat. Increase in colony formation compared to the control was observable in 25 µg/ml dose of curcumin. Only a slight increase in colony formation resulted in 50 µg/ml dose of curcumin. Three culture plates were used in the experiment for each dose of curcumin. Mean and SD were calculated for statistical analysis (Figure 1).

Colony Forming Assay Using Pulmonary Carcinoma (NCI-H292) with Curcumin 25 and 50 µg/ml, Brusatol (1µg/ml) and 100 µg/ml of Paraquat:

Two separate doses of curcumin (25 and 50 µg/ml) were used. Two separate doses of curcumin were used along with 1µg/ml of brusatol and 100 µg/ml of paraquat. Significant increase in colony formation compared to the control was observable in 25 µg/ml dose of curcumin. High increase in colony formation resulted in 50 µg/ml dose of curcumin. Three culture plates were used in the experiment for each dose of curcumin. Mean and SD were calculated for statistical analysis (Figure 2).

Apoptosis Using Pulmonary Carcinoma (NCI-H292) with Curcumin 25 and 50 µg/ml, Brusatol (1µg/ml) and 50 µg/ml of Paraquat:

Two separate doses of curcumin (25 and 50 µg/ml) were used. Two separate doses of curcumin were used along with 1µg/ml of brusatol and 50 µg/ml of paraquat. Increase in apoptosis compared to the control was observable in 25 µg/ml dose of curcumin. Only a slight increase in apoptosis resulted in 50 µg/ml dose of curcumin. Three culture plates were used in the experiment for each dose of curcumin. Mean and SD were calculated for statistical analysis (Figure 3).

Apoptosis Using Pulmonary Carcinoma (NCI-H292) with Curcumin 25 and 50 µg/ml, Brusatol (1µg/ml) and 100 µg/ml of Paraquat:

Two separate doses of curcumin (25 and 50 µg/ml) were used. Two separate doses of curcumin were used along with 1µg/ml of brusatol and 100 µg/ml of paraquat. Significant increase in apoptosis compared to the control was observable in 25 µg/ml dose of curcumin. High increase in apoptosis resulted in 50 µg/ml dose of curcumin. Three culture plates were used in the experiment for each dose of curcumin. Mean and SD were calculated for statistical analysis (Figure 4).

Five male Sprague-Dawley rats were given by gavage a dose of 1 g/kg of curcumin suspended in arachis oil. Between 67 - 87% of the dose was eliminated in the faeces within 72 hours and excretion was highest in the initial 48 hours. Urinary excretion was negligible. Three hours after gavage, curcumin was detected in the plasma of one of four animals. Biliary concentration of curcumin was 1 µg/ml after 30 minutes and remained stable throughout the experiment. The amount collected in the bile during 3 hours was less than 0.0006% of the dose. After 3 hours, about 0.015% of the administered curcumin had accumulated in the liver, kidneys and body fat. Perfusion of curcumin through the liver resulted in a transitory increase in bile flow; 10% of the dose was excreted in the bile within 3 hours after administration. Of the curcumin excreted in the bile, 49% was in the conjugated form. Because of the poor absorption, rapid metabolism and excretion of curcumin it is unlikely that substantial concentrations of curcumin occur in the body after ingestion (Jiang et al., 2013).

In rats receiving a single oral dose of 0.6 mg curcumin, 89% of the dose was excreted in the faeces and 6% in the urine within 72 hours (Holder et al., 1978). When labelled curcumin was administered to cannulated rats by i.v. injection, 85% of the dose was recovered in the bile after 6 hours. Major metabolites included the glucuronides of tetrahydrocurcumin and hexahydrocurcumin, with dihydroferulic acid and ferulic acid present as minor metabolites. Based on the three studies with human volunteers described above, for dose levels up to 12 000 mg/day, only short-term and semi-chronic adverse effects, such as gastrointestinal effects, headache and rash were observed, but without clear dose-relationships. I

Jia et al., (2014) described that nine healthy volunteers between 20 and 33 years of age were tested for haemoglobin, blood counts, liver and kidney functions, bleeding and clotting time and serum electrolytes initially and at 1 and 3 months of treatment. They were administered 0.6 ml of turmeric oil (TO) three times a day for 1 month and 1 ml in 3 divided doses for 2 months. The acute tolerability study on Day 1 was conducted in a Clinical Pharmacology Day Care Unit. Blood pressure and pulse were recorded frequently on Day 1 and at 24, 48, 72 and 96 hours and fortnightly till 12 weeks. Volunteers were daily supervised for TO intake as well as for any side effects throughout the study period. Of the nine volunteers enrolled for the study, one discontinued on the 3rd day for allergic skin rashes which, on discontinuation of TO, gradually disappeared after two weeks. Another discontinued on the 7thday for inter current fever requiring antibiotic treatment. Seven volunteers completed the study. There was no effect of TO, in two doses, on pulse and blood pressure and no side-effects in acute tolerability study on Day 1. There was no effect of TO intake on weight, blood pressure, symptoms and signs up to 12 weeks. There was no clinical, haematological, renal or hepatic toxicity of TO at 1 month and 3 months. Serum lipids did not show significant change except in one volunteer (reversible) (Huang et al., 2011). According to the present study, we confirmed that the curcumin and combination of other materials with curcumin gave better anticancer activity with different concentrations.

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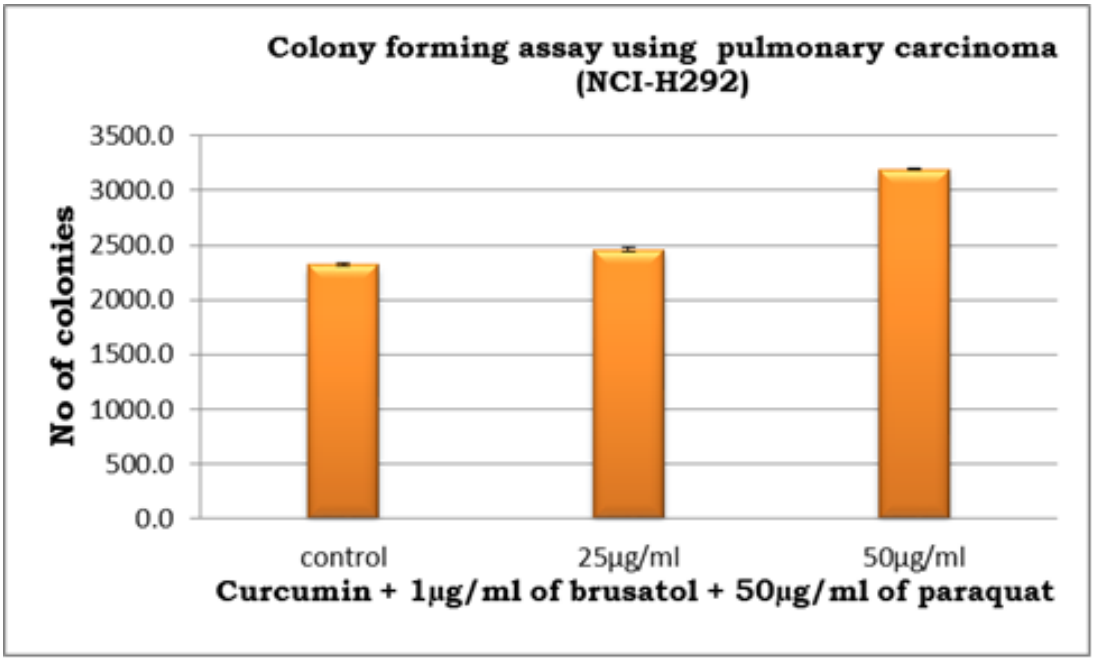

Figure 1: Colony forming assay using Pulmonary Carcinoma (NCI-H292) with curcumin 25 and 50 µg/ml, brusatol (1µg/ml) and 50 µg/ml of paraquat

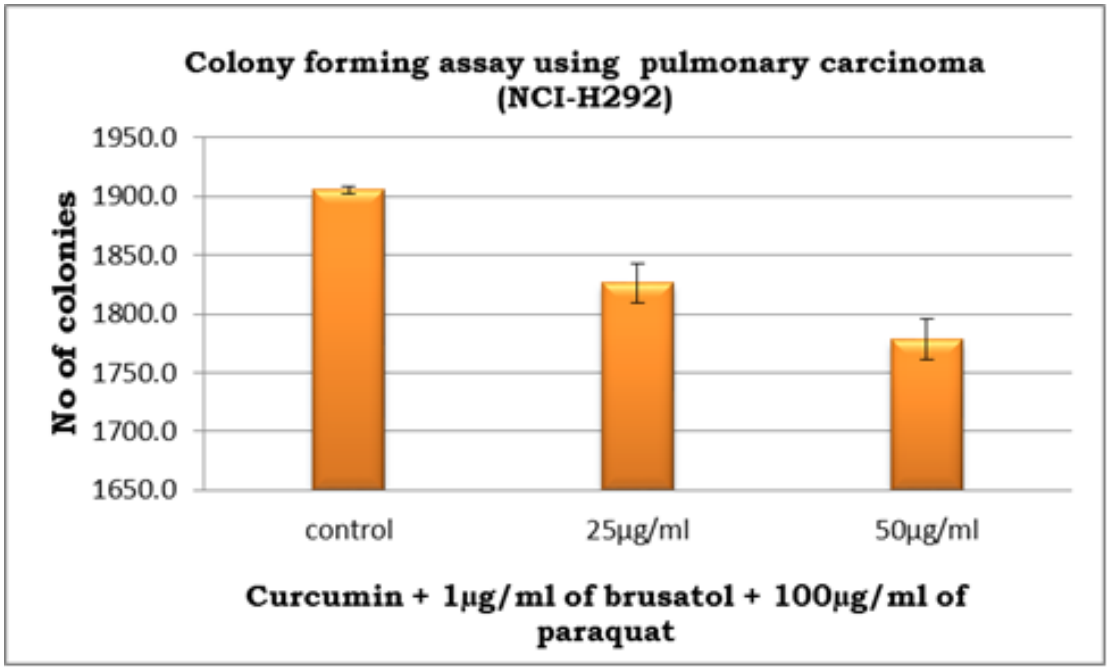

Figure 2: Colony forming assay using Pulmonary Carcinoma (NCI-H292) with curcumin 25 and 50 µg/ml, brusatol (1µg/ml) and 100 µg/ml of paraquat

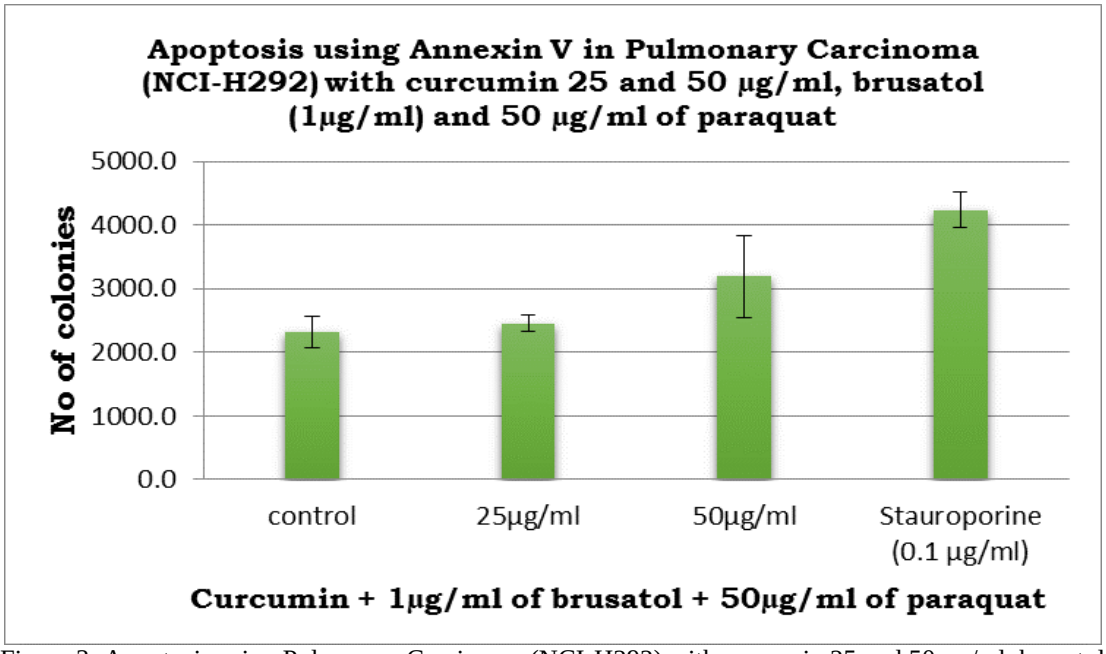

Figure 3: Apoptosis using Pulmonary Carcinoma (NCI-H292) with curcumin 25 and 50 µg/ml, brusatol (1µg/ml) and 50 µg/ml of paraquat

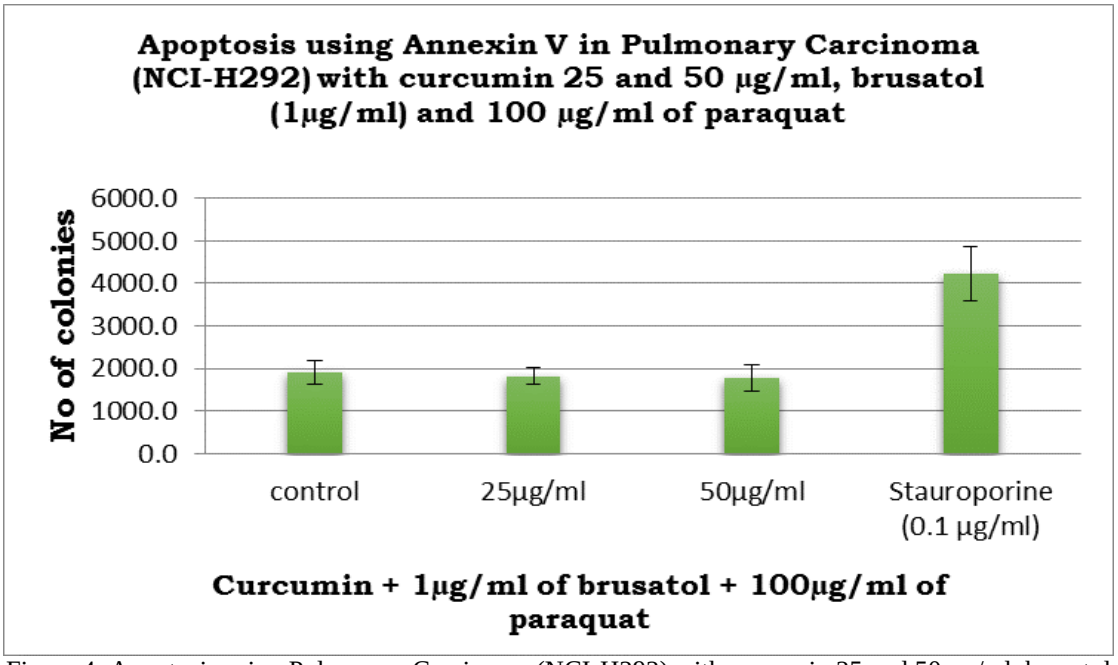

Figure 4: Apoptosis using Pulmonary Carcinoma (NCI-H292) with curcumin 25 and 50 µg/ml, brusatol (1µg/ml) and 100 µg/ml of paraquat