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Cytotoxicity of 1, 2-Dihydroxybenzene Towards Hep-2 and AMGM5 Cancer Cell Lines in Presence of SuperOxidase Dismutase and Peroxidase

Marwa I. Salman

Department of Biotechnology, College of Science, University of Baghdad, Iraq

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Abstract: The cytotoxic effect of 1,2-Dihydroxybenzene(catechol) was studied in human cancer cell lines, Epidermoid larynx carcinoma (Hep-2) and Cerebral glioblastoma multiforme (AMGM5), catechol undergo autoxidizes in physiological buffer to quinones. The results showed that catechol has a fatal effect to these cells after 72h of exposure .This toxicity was connected to the creation of quinones. There was a noticeable defeat of cell viability in a dose reliant manner in both cell type. Cytotoxicity was vetoed by the adding of 100ul SOD, while the addion of 500 ul POD or SOD combined with POD did not upturn the inhibition encouraged by SOD alone in both cells type.

Key words: AMGM5, Hep-2, SOD, 1, 2-dihydroxybenzen

Corresponding author: should be addressed (Email: marwa-i-salman@yahoo.com)

Introduction

The 1, 2-dihydroxybenzene (Catechol) is organic compound widely distributed in nature. It is found in trace amount in fruit and vegetable, as a solid substance, colorless with a weak odour of phenol.This compound was first discovered by destructive distillation of the plant extract catechin(1).Catechol itself is an environmental cocarcinogen present in tobacco smoke(2).The catechol functionality existent in the catechines is accountable for the protecting properties applied by green tea against a varied sort of human diseases(3).The anti- carcinogenic activity of tea catechins is measured to be associated to their protection of DNA from Reaction Oxygen Species (ROS-induced) harms by assuaging ROS stress(4). ROS are a group of chemically reactive molecules comprising superoxide anion radical, hydrogen peroxide, hydroxyl radicals and single oxygen, which are complicated in manifold steps of carcinogenesis (5) . To equilibrium oxidative state, animals and plants reservation multidimensional system of overlying antioxidant , such as enzymes and glutathione e.g superoxide dismutases (SOD) which responsible of the breakdown of superoxide anion into oxygen and hydrogen peroxide (6). Catalase which catalyze the conversation of hydrogen peroxide to oxygen and water, using one or the other an manganese cofactor or iron and peroxiredoxins which catalyze the tradable of hydrogen peroxide, organic hydroperoxides other than peroxynitrite (7,8). The mechanism for the mitigation of ROS stress by catechins comprises their cumulative of the activity of anti-oxidase such as catalase,superoxide

dismutase (SOD), and glutathione peroxidase (GHS-PX)and directly scavenging ROS(9,10). Catechol reduced glutathione depletion after 24 h, which induce nuclear fragmentation and apoptosis of neuroblastoma cells after 72h of exposure (11). Furthermore catechol was have cytotoxic effect on glioblastoma cell(12,13). Oxidation of catechol to semiquinones and quinones have a role in mechanism of tumor initiation (14).Since catechol autoxidizes in physiological phosphate buffer to ROS and quinones so this study was designed to evaluate the cytotoxicity and protection of catechol in Hep-2 and AMGM5 cell lines .

Materials and Methods

Cell Lines and Culture

The human cancer cell lines, Epidermoid larynx carcinoma (Hep-2) and Cerebral glioblastoma multiforme (AMGM5), were obtained from the Iraqi Center for Cancer and Medical Genetic Research (ICCMGR)/ Cell Bank Unit and maintained in RPMI 1640 media (Sigma - Aldrich, Taufkirchen, Germany) 100 units/mL penicillin, and 100 µg/mL streptomycin (Sigma-Aldrich, Taufkirchen, Germany),and supplemented with 5% calf bovine serum Preparing of cells Cultures were clarify as study pronounced by planchenault, *et al.*(15) .

Measurement of cell viability and quinone formation

Catechol solution supplied from powder of catechol (fluka/Germany) freshly prepared stock solution at 5000 µg/ml in $1*10^{-5}$ M HCl and 50 mM PBS at the serial concentrations (2000, 1000, 500, 250,125 ,62.5,31.2) µg /ml in the serum free medium (SFM) RPMI 1640 media for exposure time of cells (72 h). The percentage of cell viability assess according to Kamuhabwa and *et al*. (16) and the autoxidation rates were measured by ELISA micoplate spectrophotometer (fecil /France).The catechol oxidation was estimated in cell cultures after 72h of exposure by measuring quinone formation at 405 nm. Then the inhibitory concentration for 50% availably of cells (IC**50**) was calculated in both cancer cell lines according to Takimoto (17).

Effect of anti-oxidant enzyme on quinone formations

Influence of superoxide on catechol induced cytotoxicity towards HEP-2,AMGM5 cell line which were incubated for 72h with three different concentration of catechol 62.5,125 and 250µM. To study the protective of cytotoxicity induced by these concentration of catechol were estimated in the presence of 100 UI SOD, 500 UI POD and their combination of 100 UI SOD with 500 UI peroxidase (SOD/POD) in the same cell cultures.

Results and Discussion

Cytotoxicity of catechol

To determine the cytotoxicity of catechol, Hep-2 and AMGM5 cell line cultures were treated for 72h at several concentration of this compound, catechol effect resulted in a noticeable loss of cell viability in a dependent dose in both cell type (figure 1,2) . The concentration of chatechol that reflect lethal effect on 50% of cells (IC50) was

430 µg/ml for HEP-2 cells, while it was 210 µg/ml for AMGM5 cells. The toxic effects of catechol appeared to companion by way of creation of quinones in cultured cells (figures 1,2). Cell viability decreased when quinones formation increased in both cell line types.

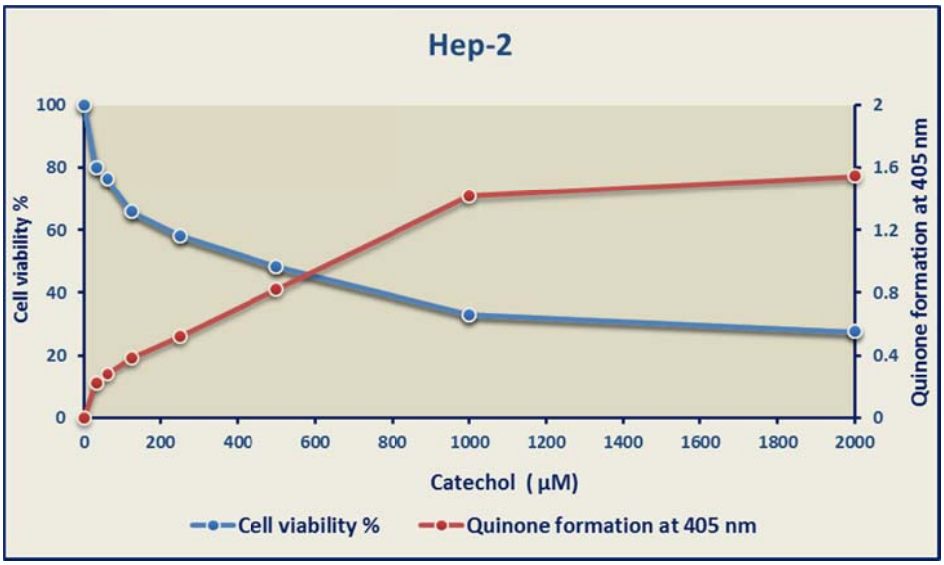

Figure (1): cytotoxicity of Catechol (cell viability%) and formation of quinones in Hep-2cells line after (72 h) of exposure.

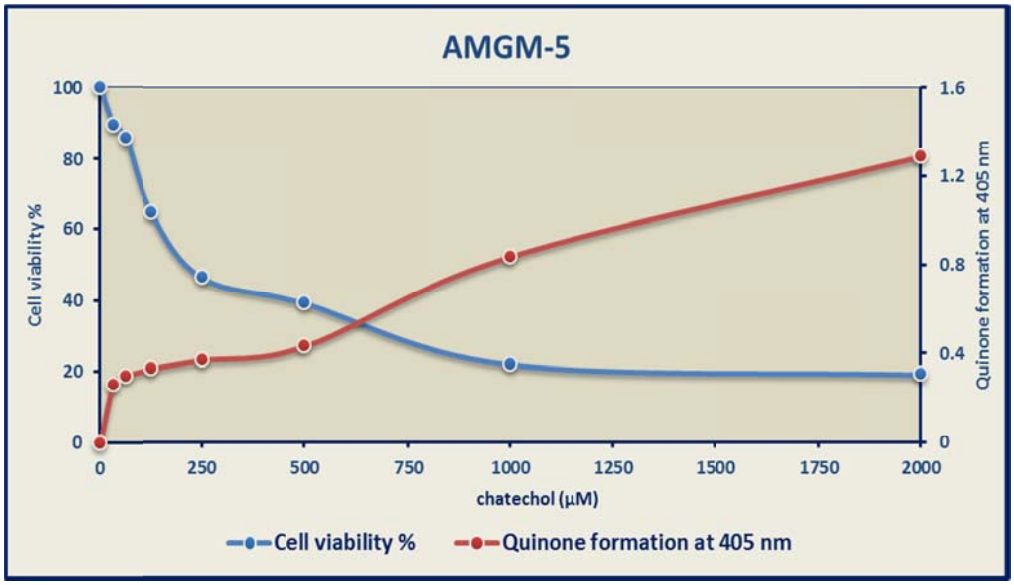

Figure (2): cytotoxicity effect of Catechol (cell viability %) and formation of quinones in AMGM5 cell line at (72h) of exposure.

Effect of anti-oxidant enzyme on the quinones formation

The adding of 100 UI SOD to Hep-2 treated with 62.5,125 and 250 µg/ml of catechol for 72h inhibited the formation of quinones to 0.167, 0.206 and 0.281, respectively (Figure 3). On the other hand, the cells survival increased to 80.8, 71.9 and 64.1 respectively (Figure 4). The addition of 500 UI of POD or 100µ SOD shared with POD did not intensification the inhibition induced by SOD alone (Figure 3) and did intensification the defensive effect to cell of SOD alone (Figure 4). The adding of 100 UI SOD to AMGM5 treated with the same above concentration of catechol for the same period also inhibited the quinones formation to 0.132, 0.22 and 0.26, respectively (Figure 5). Cell viability chanced to 89.5,78.4 and 71.2, respectively (Figure 6). While the addition of 500 UI of POD or 100 UI SOD combined with 500 UI POD once again did not escalation the inhibition effect by SOD unaided (Figure 5),or increase the cell viability when the incubated with SOD alone (Figure 6).

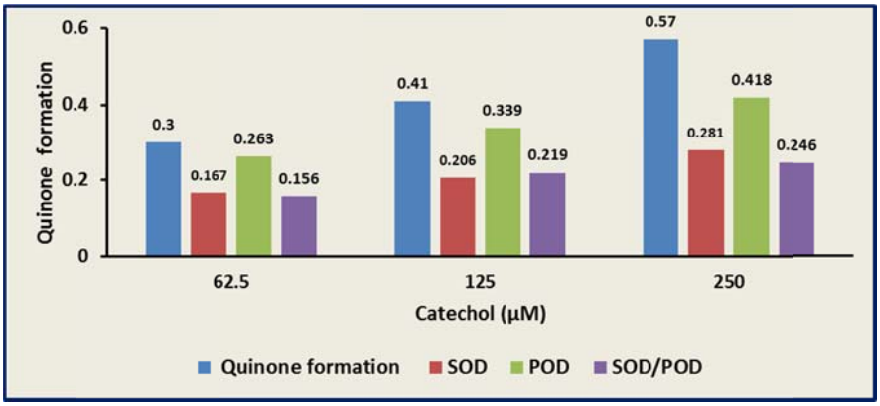

Figure (3): effect of superoxide SOD, POD and SOD/ POD on quinone formation towards (Hep-2) cells at (250,125 and 62.5) catechol concentrations after (72 h) of exposure.

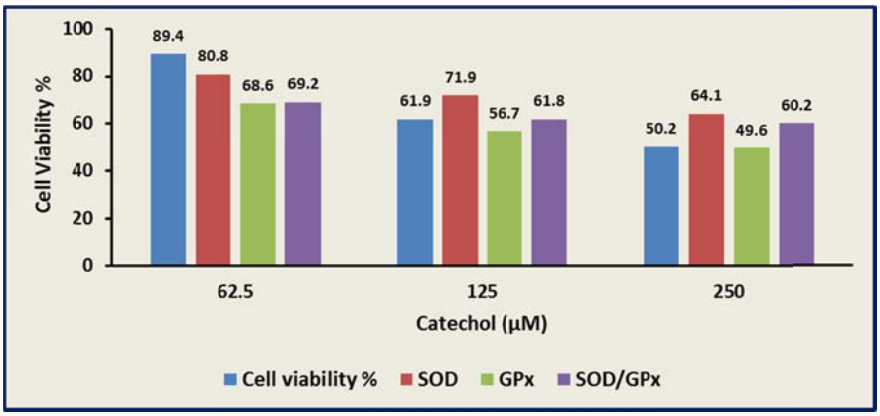

Figure (4): effect of superoxide SOD, POD and SOD/ POD on chatechol indused cytotoxicity (cell viability %) towards (Hep-2) cells at (250,125 and 62.5) catechol concentrations after (72h) of exposure.

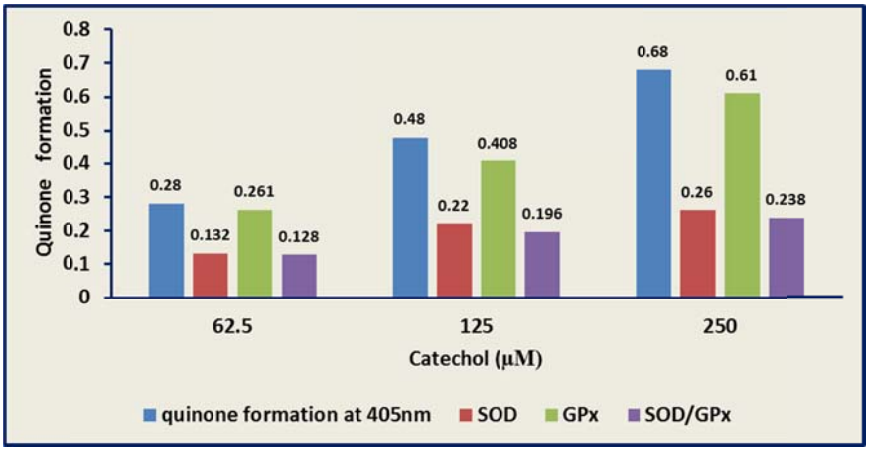

Figure (5): effect of superoxide SOD,POD and SOD/POD on quinone formation towards (AMGM5) cells at (250,125 and 62.5) catechol concentrations after(72h) of exposure.

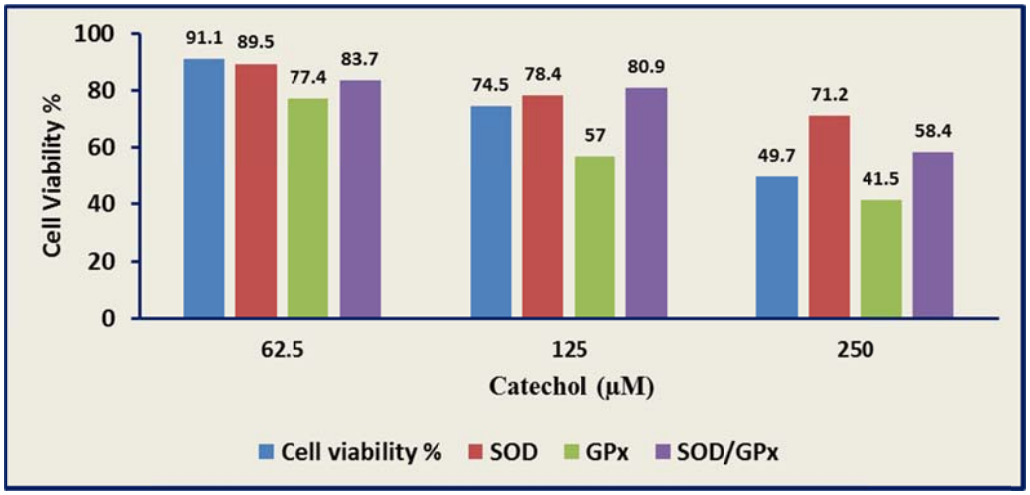

Figure (6): effect of superoxide SOD,POD and SOD/POD cytotoxicity of catechol (cell viability%) on the (AMGM5) cells at (250,125 and 62.5) catechol concentrations after (72h) of exposure.

The present results indicated the lethal effect of catechol to Hep2 and AMGM5 at 72hours. And that explain correlated to the creation of quinines. Quinones can undergo either an intracellular two-electron reduction to the hydroquinone or a one-electron reduction to the semiquinone(18,19). Quinones signify a class of intermediates toxicological which can generate a variety of risky special effects *in vivo*, as well as severe cytotoxicity, carcinogenesis, and immunotoxicity quinoines or Michael acceptor and cellular harm canister arise through alkylation of decisive cellular proteins moreover DNA. Otherwise ,quinones are extremely redox energetic particles which can redox cycle with their semiquinone radicals foremost to creation of reactive oxygen species (ROS), excluding superoxide hydrogen peroxide and ultimately the hydroxyl radical , fabrication of (ROS) can source of separate oxidative stress in the interior cells concluded the formation of oxidized cellular macromolecules including DNA, proteins and lipids

(20,21).Quinones are found in many medications, comprising mitoxantrones, mitomycin, doxorubicin , daunorubicin and saintopin, entirely of which are utilized in the medical treatment of solid tumors (22).The cytotoxic special effects of these quinones are predominantly due to inhibition of DNA topoisomerase-II (23,24). Current study it has been exposed that SOD defend cells alongside catechol affects as well prevent creation of quinones, one of twofold phenolic hydroxyl groups of the catechol molecule is ionized in alkaline solutions then electron transmission from groups that ionized to a dissolve Oxygen happens effortlessly this outcomes in superoxide realization during the catechol autoxidation superoxide is the most collective intracellular free radicle foremost to the creation of other reactive cell damaging species , when present in excessive amount(25). It is healthy acknowledged that SOD characterizes as a chief line of resistance against oxygen toxicity (13). Since superoxidase (POD) did not inhibit quinone formation in contrast to SOD this means that supeoxide and not peroxide was the core for formation of ROS in the autoxidation of catechol.

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