Chrysin and rutin protect against hydrogen peroxide and tert-butyl hydroperoxide induced oxidative cell damage

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Abstract

Objective: Chrysin and rutin are two dietary flavonoids lying in fruits or honey bee’s products. Their pharmacological properties include antioxidant, anti-inflammatory, anticancer, neuroprotection and immunomodulatory. In the current study, the potentiality of chrysin and rutin to protect human gingival fibroblasts against oxidative cell damage has been investigated in vitro.

Method: Human gingival fibroblasts, passage 3, were concomitantly put in contact with the cytotoxic compounds and chrysin or rutin for 24 h at 37 °C, 5% CO2 atmosphere, and 96% humidity. The amount of viable cell after the incubated time was recorded by using the thiazolyl blue tetrazolium bromide (MTT) assay.

Results: Chrysin in all tested concentration didn’t exhibit any cytoprotective effect against the tert-butyl hydroperoxide-induced oxidative cell damage. Moreover, chrysin in a low concentration (5 and 10 µg/mL) didn’t protect the fibroblasts against oxidative cell damage induced by the hydrogen peroxide. However, chrysin in a concentration of 20 µg/mL showed a significant cytoprotective activity in the hydrogen peroxide-induced cell damage (p < 0.05). Rutin in all tested concentrations protected fibroblasts against hydrogen peroxide and tert-butyl hydroperoxide-induced oxidative cell damage. The cytoprotective effect of rutin didn’t increase with the increase of the concentration when hydrogen peroxide is used to induce oxidative cell damage. However, rutin has protected cells against the tert-butyl hydroperoxide cytotoxicity in a concentration dependent manner.

Conclusion: Given to the interesting cytoprotective activities exhibited by chrysin and rutin, further investigations to highlight their cytoprotective involved mechanisms are justified.

Keywords: Chrysin, Cytoprotective, Fibroblasts, Rutin.

INTRODUCTION

The oxidative stress is due to the imbalance between the production of reactive oxygen species (ROS) and their elimination by the intracellular antioxidant system. ROS generation is not only limited to determine deleterious effects but also involved in the extraction of energy from organic molecules, in immune defense, and in the signaling process. Dietary flavonoids have been widely used for the prevention of the stress oxidative related diseases because of their free radicals scavenging properties. Two of these dietary flavonoids are chrysin and rutin.

The flavonoid chrysin (Figure 1) is a bioactive compound found mainly in honey bee’s products, propolis and grapefruits. Previous studies were investigated aiming to establish the structure-activity relationship of chrysin. The biological activities of chrysin such as its antioxidant ability has been largely attributed to the number and position of hydroxyls, the number of the double bounds on the heterocyclic and aromatic rings.

Figure 1: Structure of chrysin

Chrysin possesses several beneficial pharmacological properties including antioxidant, anti-inflammatory, enzymes inhibition, anticancer and antiviral activities. The antioxidant activities of chrysin include free radical quenching, reduction of the disturbances of intracellular antioxidant enzymes such as catalases, superoxide dismutase...
and glutathione peroxidase, metal ions chelation involved in free radicals generation \(^4\), and inhibition of pro-oxidative enzymes \(^3\). Others studies have screened the anticancer property of chrysin and indicated that chrysin exercises inhibitory action of cancer cells proliferation and invasion by targeting multiple cells signaling pathways such as nuclear factor-kappaB (NF\(_\text{-kB}\)), PI3K/Akt and MAPK signaling pathways \(^5\). Others anticancer mechanisms of chrysin include ROS generation, inflammation induction and ubiquitous proteins activation \(^6\). Despite chrysin possesses cytotoxic effects on cancer cells, previous studies demonstrated that chrysin is less toxic on normal cells like fibroblasts and epithelial cells. Others studies demonstrated that chrysin inhibit highly the proliferation of colon cancer cells without exercise any cytotoxic effects on colon epithelial normal cells \(^9\).

**Rutin** (3,30 ,40 ,5,7-rhamnoglucoside, Figure 2) is mainly found in apple, green tea and passion flower \(^7\). Pharmacological properties of rutin include anti-inflammation \(^8\), antioxidant \(^9\), anti-physical fatigue \(^10\), antidiabetic and anticancer \(^7\) as well as neuroprotective \(^11\) and cardioprotective agent \(^12\).

![Figure 2: Structure of rutin](image)

Previous biological investigations demonstrated that rutin increases antioxidant enzymes, catalase, superoxide dismutase, glutathione peroxidase, and glutathione level in rat PC-12 cells and prevents cells damages induced by a neurotoxic agent, 6-hydroxydopamine \(^16\). Moreover, rutin restored memory in doxorubicin-induced memory deficits in rat. The effectiveness of chrysin and rutin to protect human fibroblasts against oxidative stress induced-human fibroblasts damages was evaluated in this study *in vitro* \(^13\).

**METHODS**

**Chemicals**

Chrysin (CAS number 480-40-0; purity >99%), rutin (CAS number 153-18-1; purity >99%), hydrogen peroxide solution, tert-butyl hydroperoxide solution, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Hanks’ Balanced Salt Solution (HBSS), gentamycin, penicillin, streptomycin, Dulbecco’s Modified Eagle Medium (DMEM), glutamine, fetal bovine serum, trypsin-EDTA solution 10X and dimethyl sulfoxide (DMSO) were acquired from Sigma-Aldrich, Germany. All chemicals were an analytical grade.

**Cells culture**

Human gingival fibroblasts, passage 3, were used to evaluate the cytoprotection potenti of rutin and chrysin. Homogenous population of cells had been obtained using the explant culture method as performed previously \(^14\). The study has been approved by the ethical comity of the odontostomatologie department of the Bogodogo’s University Hospital Center (Ouagadougou, Burkina Faso). Gingival tissues collection of patients was in accordance with the declaration of Helsinki as revised in 64th WMA General Assembly, Fortaleza, Brazil \(^15\). Gingival tissues of voluntary patients who have given verbally their consent were seeded into petri dish containing the growth medium DMEM supplemented with 10% fetal bovine serum, 1% penicillin/Gentamycin and 10mM of glutamine. The petri dish was placed in an incubator (37 °C, 5%CO\(_2\)). The growth medium was replaced every 24h until confluence and the gingival tissues were removed, the fibroblasts population was harvested by using trypsin-EDTA solution 0.025%. Cells were re-suspended in the growth medium and seeded in 96-plate wells (5000 cells/well) for the cytoprotection study.

**Cytoprotection study**

In 96-plate wells containing 100µL of cells suspension, 100µL of rutin or chrysin at different concentrations (5 10 20µg/mL) were added followed to the addition of 50µL of hydrogen peroxide or tert-butylhydroperoxide. Plate was incubated at 37 °C, 5%CO\(_2\) for 24h and the cells viability was measured by using the MTT assay as described previously \(^20\). The yellow-colored MTT was reduced by the mitochondrial succinate dehydrogenase of active living cells to purple formazan which the optical density can be measured at 570 nm \(^16\). At the end of the incubation period, the supernatant was aspirated from the wells of the plate and 100 µL of the MTT solution (5 mg / mL in HBSS buffer) was added. The plate was incubated in an incubator (37 °C, 5%CO\(_2\)) for 3 h. The excess MTT was aspirated and 100 µL of DMSO was added to dissolve the formazan blue metabolized by living cells. Absorbance was measured at 570 nm with a UV-Visible spectrophotometer and the percentage of viable cells was calculated.

**Statistical analysis**

Statistical analysis of the data was performed by analysis of variance (one-way ANOVA) followed by Newman Keuls test using Graph Pad Prism software, version 5.0. The values are expressed as mean values ± standard deviation (n = 3 independent experiments). A statistical difference was observed for \(p < 0.05\).

**RESULTS**

Cells were simultaneously exposed with different concentrations of chrysin (5 – 20 µg/mL) and 100 mM of hydrogen peroxide for 24h, and the cells viability was recorded by using the MTT essay. As data showed in the figure 3, chrysin in a low concentration (5 10 µg/mL) didn’t protect the fibroblasts against hydrogen peroxide induced oxidative cell damage. However, chrysin in a concentration of 20 µg/mL showed a significant cytoprotective activity in the hydrogen peroxide induced oxidative cell damage (\(p < 0.05\)).
Figure 3: Chrysin protects fibroblasts against hydrogen peroxide induced oxidative cell damage. Values are expressed as the mean ± std of the mean from three independent repetitive experiments (n = 3). Values with different superscripted letter a,b,c are statically different at p < 0.05 (analyzed by means of one-way ANOVA).

Regarding to the data as showed in the figure 4, chrysin in all tested concentration didn’t exhibit any cytoprotective effect against the tert-butyl hydroperoxide induced oxidative cell damage when the viability of cells exposed to the tert-butyl hydroperoxide alone was compared to those of the chrysin addition (p > 0.05). These finding suggested that chrysin doesn’t interfere with the cytotoxic mechanism of the tert-butyl hydroperoxide.

Figure 4: Chrysin protects fibroblasts against tert-butyl hydroperoxide induced oxidative cell damage. Values are expressed as the mean ± std of the mean from three independent repetitive experiments (n = 3). Values with different superscripted letter ab are statically different at p < 0.05 (analyzed by means of one-way ANOVA).
Moreover, cells were concomitantly treated with different concentrations of rutin (5–20 µg/mL) and 100 mM of hydrogen peroxide for 24h, and the cells viability was recorded by using the MTT essay. As data showed in the figure 5, rutin in all tested concentrations protected fibroblasts against hydrogen peroxide induced oxidative cell damage when the viability of cells exposed to hydrogen peroxide alone was compared to those of the rutin addition (p < 0.05). The cytoprotective effect of rutin didn’t increase with the increase of the concentration.

When cells were concomitantly treated with different concentrations of rutin (5–20 µg/mL) and 100 mM of tert-butyl hydroperoxide for 24h, the cells viability was enhanced compared to the tert-butyl hydroperoxide treatment alone (Figure 6). All tested concentrations protected fibroblasts against the tert-butyl hydroperoxide induced oxidative cell damage. Moreover, the cytoprotective effect of rutin was increased with the increase of the concentration.
DISCUSSION

Oxidative stress corresponds to an aggression on cells by free radicals including reactive oxygen species and derives from oxygen in the cell, especially in the mitochondria through the respiratory chain. ROS are reactive and very toxic substances. Oxidative stress is caused by an imbalance between pro-oxidant free radicals and antioxidants. When ROS build up in the cell, they can be neutralized by antioxidant molecules, like vitamins E and C, or enzymes such as superoxide dismutase, catalase and glutathione peroxidase. The high production of free radicals can be linked to inflammation, smoking, a diet too rich in fat, alcohol. The accumulation of attacks by free radicals could promote cells malignant transformation or cells death due to biomolecules oxidative damages like phospholipids of cells membrane, proteins and DNA. Carcinogenesis or cells death are associated to the development of numerous stress oxidative related diseases, like cancer, diabetes, neurodegenerative disorders, cardiovascular diseases and aging. Epidemiological studies have shown that the consumption of certain foods or drinks (juice, fruit, vegetables and oils) rich in flavonoids (particularly flavonols and anthocyanins), is inversely correlated with the development of the stress oxidative related diseases.

In this study, the flavonoids used are chrysin and rutin in order to assess their capacity to protect cells from oxidative damages. Oxidative cell damages were induced by hydrogen peroxide and tert-butyl hydroperoxide. Hydrogen peroxide in contact with iron via the reaction of Fenton generates ROS in cell living environment. Hydroxyl radical is the most toxic ROS produced because it is extremely reactive and interacts with cells membrane phospholipids initiating lipid peroxidation. It also reacts with sugar of DNA and promote DNA single or double breakage. Hydroxyl radical can lead to the production of toxic aldehyde MDA by degrading glycoproteins in cells. The two dietary flavonoids chrysin and rutin have protected in this investigations human fibroblasts against hydrogen peroxide induced oxidative cell damage. Several mechanisms could justify the cytoprotective effects of chrysin and rutin observed in this study: i) the direct quenching of hydroxyl radical generate by hydrogen peroxide, ii) the reduction of hydrogen peroxide, iii) the stopping of lipid peroxidation initiation and propagation and iv) the induction of intracellular antioxidant enzymes. Tert-butyl hydroperoxide induces oxidative stress by an enzymatic and non-enzymatic process. In an enzymatic process, tert-butyl hydroperoxide requires metabolic activation, dependent on cytochrome P450, in butoxyl radicals and ROS responsible for its cytotoxicity. Tert-butyl hydroperoxide can also decompose spontaneously in the presence of metal ions into butoxyl radicals capable of cells oxidative damage. Rutin has been, the only flavonoid that has protected human fibroblasts against tert-butyl hydroperoxide induced oxidative cell damages. The cytoprotective activity of rutin could be due to its potent to scavenging directly butoxyl radical issue of the tert-butyl hydroperoxide metabolism or the inhibition of the cytochrome P450 enzymatic activity impeaching the enzymatic metabolism of tert-butyl hydroperoxide to its cytotoxic components. Previous studies have demonstrated that the cytoprotective activities of rutin and chrysin are associated with the reduction of the level on intracellular oxidative stress. Others studies demonstrated that chrysin protects against tert-butyl hydroperoxide-induced oxidative stress in rat primary hepatocytes. The mechanisms involved include the up-regulation of the protein expression of heme oxygenase 1 and the increase of the intracellular glutathione level. Other studies demonstrated that chrysin protects against cisplatin-induced rat jejunum oxidative damages through the induction of antioxidant enzymes (catalases, superoxide dismutase and glutathione peroxidase) activities and the improvement of the p53 gene expression. The cytoprotective mechanisms of rutin include the reduction of intracellular ROS production and the cell membrane lipids peroxidation. Moreover, rutin inhibited cell apoptotic death through the inhibition of the pro-apoptotic genes expression such as Bax, caspase-3 and 9.

CONCLUSION

Chrysin and rutin are two dietary flavonoids found in fruits, honey and vegetables. In the current investigation, chrysin and rutin have exhibited interesting cytoprotective activities against the stress oxidative induced oxidative cell damages. Rutin has protected cells both against hydrogen peroxide and tert-butyl hydroperoxide oxidative damages but chrysin has protected cells only against hydrogen peroxide oxidative damages. The dietary intake of food rich in chrysin and rutin could prevent the prevalence of stress oxidative related chronic diseases.

Conflict of interest

All authors state that none conflict of interest exists.

Author’s contribution

Ablassé Rouamba and Raoul Bationo conducted the experiments, Ablassé Rouamba, Vincent Ouedraogo and Moussa Compaoré wrote the first draft of the manuscript. Maurice Ouedraogo and Martin Kiendrebeogo read and corrected the grammar. All authors read and approved the final paper.

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