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Rutin a Citrus Flavonoid Attenuates Sodium Arsenate Induced Cytotoxicity in CHO Cells

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Abstract

The potential role of oxidative stress in the pathogenesis induced by arsenic proposes that antioxidants can be deliberated as an alternative approach in alleviating arsenic-induced toxicity. The aim of this work was to assay in vitro the potential advantageous effects of plant flavonoid rutin against arsenate exposure, by studying lipid peroxidation and the stress-related markers. Rutin, a flavanol, has been exhibiting anti-cancer property, mitigating, hostile to diabetic and anti-inflammatory properties. CHO cells were selected to study the effect of sodiumarsenate and its amelioration by rutin a citrus flavonoid glycoside. Sodium arsenate at low concentration induced cytotoxicity and genotoxicity. MTT assay, Catalase, Superoxide dismutase, GSH, GST and lipid peroxidation was measured in CHO cell. Pre-treatment of CHO cells with rutin attenuated arsenate induced toxicity. DNA damage was also measured

Keywords: CHO, GSH, GST, SOD, Sodium Arsenate

1. Introduction

Phenolic compounds act as natural antioxidants and assist to neutralize the free radicals. Natural flavonoids are known for their significant scavenging properties on oxygen radicals in vivo and in vitro. Many studies show the importance of the free radical scavenging activity of flavonoids. Flavonoids are known to possess numerous biological activities such as antioxidant, anti-inflammatory, oestrogenic, cytotoxic antitumoural, antiviral and others. Their application in improving human health has been the subject of extensive research[1]. Rutin (quercetin-3-rhamnosyl glucoside), a characteristic flavone derivative, was first found in buckwheat in the nineteenth century. It is a low molecular weight polyphenolic aggravate that is broadly circulated in vegetables and organic products[2]. The name 'rutin' comes from the plant *Ruta graveolens,* which also contains rutin. Chemically it is a glycoside comprising of flavonolic aglycone quercetin along with disaccharide rutinose[3]. Moreover, hypolipidaemic, cytoprotective, antispasmodic and anticarcinogenic. It has likewise been found to forestall gastric mucosal ulceration, including restraint stress in experimental models. Rutin is a sort of flavonoid glycoside also known as P Vitamin, has shown antiviral, antiplatelet and antihypertensive properties, just as strengthening the vessels of veins, which are the consequences of its high radical quenching action and cell reinforcement limit[4]. This is a common dietary citrus flavonoid which is found in fruits, vegetables and plant-derived concoctions such as tea and wine. In another experiment, it was built up that incessant utilization of flavonoids like rutin could be good for cardiovascular wellbeing[5].

In the current study, CHO cells were selected as a model in order to study the attenuating property of rutin a citrus flavonoid. Chronic arsenic poisoning is caused due to drinking of contaminated potable water. It is also associated with many health issues. Since rutin is found in many vegetables and fruits, consumption of that in diet

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should help to combat the free radicals generated due to the activity of arsenic metabolism. The study gives an insight into the protective effect of rutin against arsenic-induced cytotoxicity in CHO cells.

2. Materials and Methods

2.1 Cell Culture

CHO (Chinese Hamster Ovary) cell line was procured from National Centre for Cell Sciences-Pune, India. The cells were grown in medium T-25 flasks (Falcon, Becton Dickinson, USA), provided with DMEM including 10% FBS, 1% antibiotic, at 37°C in a carbon dioxide incubator for known period (NuAire, Plymouth, MN, USA) with 5% carbon dioxide in 95% air. The cells were maintained at 85% confluence in flasks and utilized. Trypan blue assay was used to evaluate the viability of CHO cells.

2.2 MTT Assay

Cytotoxicity of the arsenate was estimated with MTT assay. 85% confluent cells were harvested through trypsinisation and used at a density of 1 x 10^4 cells per chamber and supplied with arsenate, rutin and in combination estimate the toxicity. The CHO cells were washed with PBS (200 µl pH 7.4). Incubated with 100 µl MTT reagent (1 mg/ml in HBSS) at 37°C with 5% CO_2 in 95% air (24 h). After the incubation the supernatant was discarded and 100 µl of DMSO was added to dissolve formazancrystals. Absorbance was recorded at 550 nm[6]. The relative percentage cell viabilities were calculated with the use optical densities obtained from microplate reader. Cells were cultured without arsenate was considered as 100% cell viability and compared with other treated groups. Calculation of % Cell viability = [(test OD – blank OD)/(control OD – blank OD)]*100.

2.3 Estimation of Catalase

Briefly, 3 x 10^5 cells were used per unit in a 6 well plate. The plates were kept overnight in an incubator at 37°C, in 5% CO_2, later supplied with sodium arsenate, Rutin and mixed, kept for 24 h, then cells were harvested and taken in PBS. The catalase assay was performed as described by Abei[7]. 50 mM KH_2PO_4 and 50 mM Na_2HPO_4 in a 1:1.5 v/v ratio, pH 7.0 mixed with 30 mM H_2O_2. Absorbance was as blank. The decomposition of H_2O_2 was read at 240 nm. The difference in absorbance (A240) per unit time is measured as catalase activity.

2.4 Estimation of Superoxide Dismutase (SOD)

3 x 10^5 cells were aliquoted in a single well (6 well plate) kept overnight in carbon dioxide incubator at 37°C, then provided with sodium arsenate, rutin and combination, allowed to stand for 24 h, later cells were harvested in PBS. 1.85 mL of sodium carbonate buffer was mixed with 50 µL of the sample. Further 0.1 mL of epinephrine was mixed and the increase in the absorbance was recorded immediately at 480 nm using a colorimeter[8].

2.5 Estimation of Glutathione (GSH)

GSH contents were estimated as total non-protein sulfhydryl group. Proteins were precipitated with 25% TCA, centrifuged and the supernatant was removed and supplied with 0.2 M sodium phosphate buffer saline (pH 8.0), 0.06 mM DTNB and incubated for 10 min at room temperature[9]. The absorbance was recorded at 412 nm using a spectrometer (Shimadzu UV-260, Shimadzu Corp, Tokyo, Japan).

2.6 Estimation of Glutathione-S-transferase (GST)

GST was determined according to the procedure[10]. The specific activity of GST is expressed as mMol of reduced CDNB conjugate formed per min per mg protein of the tissue. The absorbance was recorded against blank at 340 nm.

2.7 Estimation of Lipid Peroxidation

Cells were cultured in medium flasks and collected in PBS. 0.1 ml of cell extract was mixed with harvested solution containing KCL and Tris HCl. Incubated for 1 h at 37°C. 2 ml of TBA, 1 ml of TCA added at the end. Again mixture was incubated for an hour, the supernatant was separated and absorbance was recorded at 540 nm[11].

2.8 Comet Assay

Single strand DNA breaks were quantified by comet assay in alkaline medium. 7x10^5 cells were seeded in 6 cm^2 plates. Cells were kept for 24 h with arsenate and rutin and in combination, post incubation cells were trypsinized and collected in PBS. Again cells washed and collected with PBS and suspended in low melting agarose. Roughly 2 x 10^4 cells were suspended in low melting point agarose. Agarose covered slides were layered with low melting agarose along with cells maintained at 37oC and recoated with normal melting agarose. The slides were immersed in the lysing solution maintained at 4oC overnight; slides were transferred to an electrophoretic chamber containing an alkaline buffer (pH 13). The lysed cells were subjected to electrophoresis in a horizontal tank for 28 mins (300 mA, 20 V). The slides were washed with neutralizing buffer. Ethidium bromide stained slides were immediately observed under the fluorescence microscope and photographed, analyzed with software[12].

3. Results and Discussion

Biochemical assays were performed and all the data is expressed as Mean±SE. All the experiments were carried out in triplicates. To determine the nontoxic concentrations of rutin, CHO cells were incubated with various concentrations of rutin in dulbecco's modified eagle medium. MTT assay results showed that the rutin had no cytotoxic behavior. MTT assay resulted in IC_{50} of 46 µM for sodium arsenate. 30 µM dose was selected to evaluate the antioxidant activity and anticytotoxic activity of arsenate in CHO cells. SOD levels were altered significantly ($p<0.001$) in rutin treated cells compared to the control group. Catalase levels increased in rutin treated group ($p<0.01$) compared to arsenate treated group. Catalase enzyme is ubiquitously present in cells containing a cytochrome system. Ferrihemoprotein groups are present in these enzymes, around four groups per molecule (240 kDa). Catalase activity varies greatly between tissues[13]. Hydrogen peroxide is produced in the cell as the part of leakage in mitochondrial oxidative phosphorylation as well as terminal oxidation. Hydrogen peroxide can create other harmful free radical which in turn can induce cell death or apoptosis. Free radicles also induce DNA damage[14].

GSH and GST activity was also restored in CHO cells compared to arsenate treated group significantly ($p<0.001$). %TDNA increased in arsenate treated group compared to the control and rutin ameliorated arsenic-induced DNA damage significantly ($p<0.001$). Lipid peroxidation is a result of peroxidative damage. Hydroxyl and other free radical in cells are responsible for the formation of lipid peroxidation. Single initiation can affect hundreds of lipid moieties which basic units of membranes. They can also attack the membrane proteins.Lipid peroxidation is one of the oxidative stress marker increased significantly in arsenate treated CHO cells and rutin attenuated the level of peroxidation in cells. Results were significant ($p<0.001$)[15].

Our data indicates that rutin, a citrus flavonoid ameliorated arsenic induced toxicity in CHO cells. Here in, the results are discussed. Diminished antioxidant capacity of the cell results in increased oxidative stress and associated with a disease initiation and progression. Catalase removes hydrogen peroxide generated by superoxide dismutase in cells. Depletion of catalase and SOD activity supports the arsenic induced oxidative injury[16]. Decreased catalase activity (Figure 1) and SOD (Figure 2) is the reason for the accumulation of peroxide in cells in the arsenate exposed cells. Pre-treatment of CHO cells with rutinharmonized the adverse effect of arsenate significantly. SOD and CAT enzymes act together to break the reactive oxygen types.

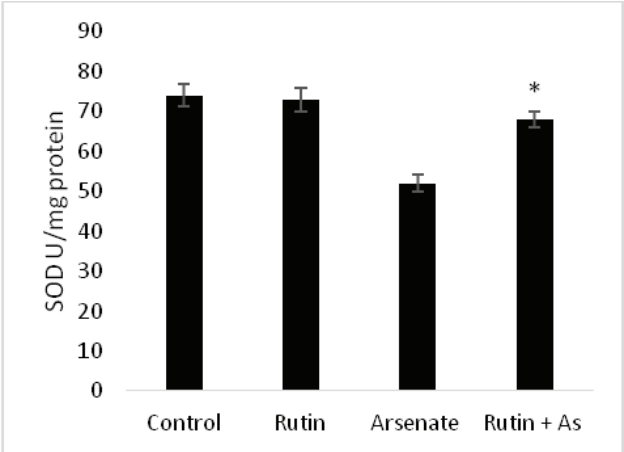


Figure 1. Changes in SOD activity before and after rutin treatment along with sodium arsenate (30 µM). *p<0.001 with respect to arsenate vs. rutin.

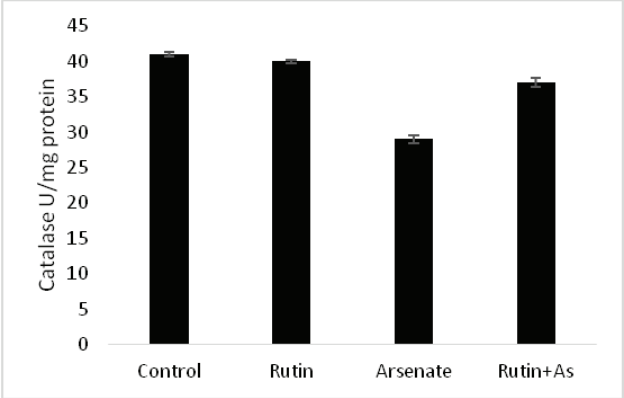


Figure 2. Changes in catalase activity in control, arsenate (30 µM) and rutin treated groups. *p<0.001 with respect to arsenate vs. rutin.

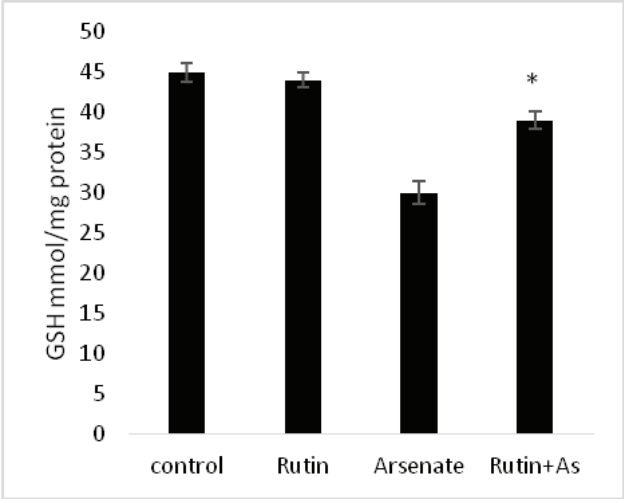


Figure 3. Changes in GSH content of the CHO cells in control, arsenate (30 µM) and rutin treated groups. *p<0.001 with respect to arsenate vs. rutin.

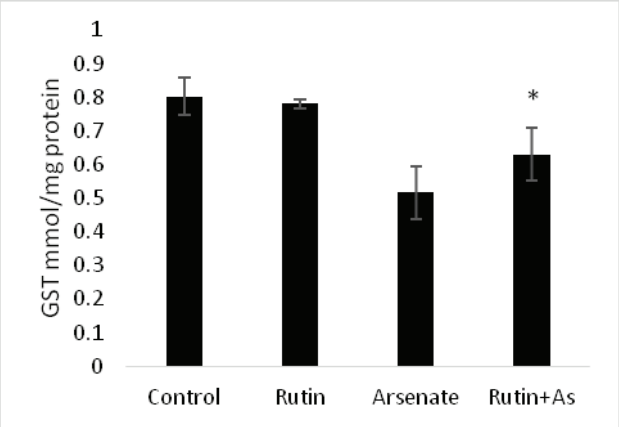


Figure 4. GST levels in CHO cells, in control, arsenate (30 μM) and rutin treated groups. *p<0.001 with respect to arsenate vs. rutin.

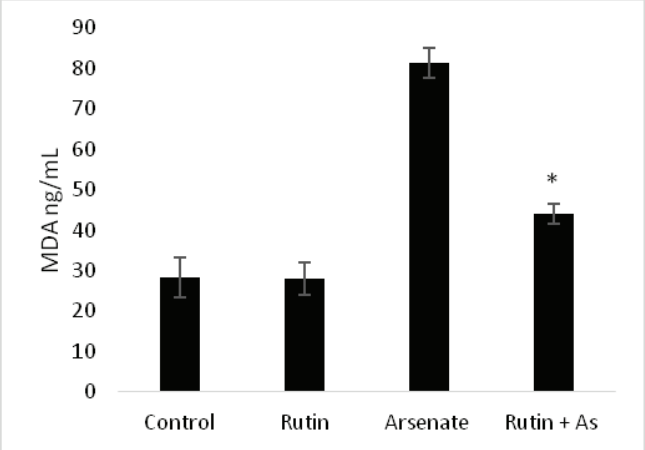


Figure 5. MDA levels in control, arsenate (30 μM) and rutin treated groups. *p<0.001 with respect to arsenate vs. rutin.

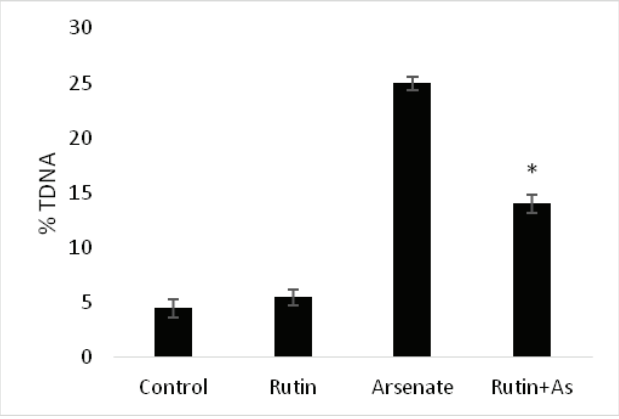


Figure 6. Comet assay indicating the %TDNA in control, arsenate (30 μM) and rutin treated groups. *p<0.001 with respect to arsenate vs. rutin.

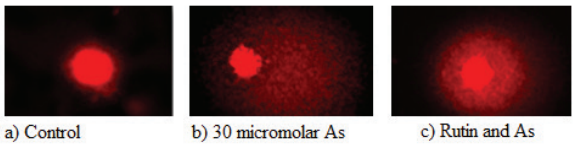


Figure 7. Representative images of comet assay.

GSTs are enzymes which play important role in the detoxification process of several xenobiotics in humans as well as in animals. Thiol group is important in defending cells against carcinogenic and toxic effects of the compounds. They do so by conjugation of thiol group of GSH to xenobiotics. GSTs also conjugate with glutathione and various electrophilic compounds. Rutin accumulated in cells protected from adverse effect of the metalloid and GST levels (Figure 3) increased significantly in CHO cells.

GSH is non-enzymatic antioxidant that plays a very important role in protecting cells from ROS. GSH levels decreased in the current study indicating the possible arsenic–GSH complex formation. Arsenic exposure after the treatment of rutin exhibited a significant increase in the GSH (Figure 4). MDA levels are used as a marker for free radical-mediated lipid peroxidation. Serum levels of arsenic and MDA were positively correlated in multiple sclerosis patients. It is also linked with various diseases related to chronic and acute arsenic exposure[17]. In the current study CHO cells exposed to arsenate showed a significant increase in MDA levels. Pre-treatment of rutin altered the MDA levels and a significant decrease was noted (Figure 5).

Arsenic acts indirectly by inhibiting DNA repair. The comet assay was first described by Ostling and Johansson, who described a neutral comet assay. It was later modified to be performed under alkaline conditions. The alkaline (pH > 13) SCGE assay, developed by N.P. Singh, combines the simplicity of biochemical techniques for detecting DNA single strand breaks (frank strand breaks and incomplete excision repair sites), alkali-labile sites and crosslinking with the single cell approach typical of cytogenetic assays. The advantages of the SCGE technique include: the collection of data at the level of the individual cell, allowing for more robust types of statistical analyses; the need for small numbers of cells per sample. Single strand DNA breaks were quantified by comet assay in alkaline medium. Comet assay has been considered a rapid, simple and sensitive technique for measuring DNA damage[18]. Sodium arsenate induced DNA damage in CHO cells. Decreased damage was observed in rutin treated groups (Figure 6) and change was significant.

4. Conclusion

Collectively these data argue a compelling case for exploring rutin further as a useful antioxidant in improving human health. Present experimental work showed a significant increase in SOD and catalase levels in rutin supplemented CHO cells. Enhancement of GSH and GST activities were also observed. MDA levels and DNA damage decreased in treated groups. Rutin ameliorates in vitro genotoxic effects of arsenic and this can be attributed to its immense antioxidant capacity.

5. Conflict of Interest

None

6. Acknowledgement

The authors acknowledge MAHE, Manipal for the facilities.

7. References

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