Journal of Pharmaceutical and Applied Chemistry

ISSN: 2357-0210 VOLUME 6, ISSUES 2, 2020 www.egyptfuture.org/ojs/

Toxic/protective effects of some antioxidants on the copper sulfate induced toxicity in *Allium cepa*

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Received: 15 Feb.2020, Revised: 16 Mar. 2020, Accepted: 17 Apr. 2020.
Published online: 1 May 2020.

Abstract: Certain substance can exert toxicogenetic effects in our body. Antioxidants can counteract these effects due to their protective effects. This study aims at evaluating the toxic/protective effects of some antioxidants on the copper sulfate ($CuSO_4$) induced toxicity in a eukaryotic test system. For this, ascorbic acid (AA), quercetin (QUR) and phytol (PHY) at 25, 40 and 75 25 µg/mL were exposed in *Allium cepa* up to 72 h by taking distilled water and $CuSO_4$ (0.6 µg/mL) as a vehicle (Veh) and positive control, respectively. To see the protective capacity, each antioxidant was combinedly treated with the 0.6 µg/mL of $CuSO_4$. After 24, 48 and 72 h of exposure of test sample and/or control groups, the root length was measured in mm. The results suggest that AA, QUR and PHY decreased the root length in *A. cepa*, but the values were lower than the $CuSO_4$. All the antioxidant co-treated with the $CuSO_4$ have been augmented %root growth profile in the test system. The highest %increased root growth was observed in AA + $CuSO_4$ group at 72 h. QUR increased root growth in a time-dependent manner, suggesting an adaptive effect of this antioxidant in *A. cepa* meristem. PHY also exhibited a time-dependent protective effect up to 48 h, however, it did not augment % root growth at 72 h inspect of 48 h. Taken together, this study suggests antioxidant-mediated protective effects against toxicogenetic agent in eukaryotic system.
Keywords: *Allium cepa*, Antioxidants, Pro-oxidative effect, Toxicity

1 Introduction

Certain substance, including drugs or other chemicals may exert toxic effects on our body [1]. Our body displays several mechanisms to encounter or repair the toxic effects caused by external or internal toxicants [2]. However, if failed, it may cause serious health complications, including oxidative damage and inflammation leading to organ damage, and even certain types of cancers [3].

Antioxidants are substances that are known to neutralize free radicals or their actions [4]. An excessive production of free radicals, and depletion of antioxidants may lead to chronic inflammation in our body [5]. It is evident that the neural cells are more susceptible to the oxidative stress in comparison to the other tissues [6]. Scientific reports suggest that antioxidant therapy can be used in hyperglycemia [7], obesity [8], cardiovascular diseases [9], neurological diseases and disorders [10], immunological diseases [11], cancer [12] and so on.

Our body contains enzymatic or non-enzymatic antioxidants, such as tocopherols, glutathione, superoxide dismutase, catalase, and ascorbic acid that are involved in the above-mentioned process. Among internal antioxidants, it plays an important role in fighting against ROS as well as in the maintenance of normal oxidative balance [13]. On the other hand, quercetin inhibits the oxidation of model phospholipid bilayers exposed to aqueous oxygen radicals and the uptake of modified low density lipoprotein (LDL) by macrophage scavenger receptors [14]. Phytol (3,7,11,15-tetramethylhexadec-2-en-1-ol), a chlorophyll-derived diterpenoid is a member of the group of branched-chain unsaturated alcohols [15]. Recently phytol has come out in the spotlight due to its promising biological effects, including antioxidant activity [16]. It can act through multi-dimensional ways to counteract oxidative stress [17]. Moreover, phytol is non-mutagenic in nature [18].

Allium cepa is an important test eukaryotic system [19]. By using this test model, we can screen toxic, cytotoxic, genotoxic and mutagenic effects of wide varieties of substances, including crude extracts or their fractions, isolated compounds, other biochemicals, laboratory synthetic compounds, drugs, environmental toxins and so. It is easy to perform and can be routinely

used to evaluate the toxicogenetic potential of substances due to its sensitivity and good correlation with mammalian test systems [20]. Although, cupper sulphate is extensively used as a standard toxic agent in this test model [21], but other substances as standards can be used such as hydrogen peroxide [22], maleic hydrazide [23], and so on.

This study evaluates the toxic or protective effects of the ascorbic acid, quercetin and phytol on the copper sulfate induced toxicity in *Allium cepa*.

2 Experimental Section

2.1 Reagents and chemicals

Quercetin (QUR) and phytol (PHY) were purchased from Sigma-Aldrich (Chem Ex. Co. St. Louis, Missouri, USA), while ascorbic acid (AA) and copper sulphate were purchased from Merck (India). Purified water (Veh) and other necessary tools were purchased from the local market of Gopalganj, Bangladesh.

2.2 Source of onions (A. cepa)

The medium size onions (*A. cepa*) were purchased from the local market of Gopalganj, Bangladesh, in 2019.

2.3 Selection of contentration and preparation of the test samples

The concentrations of the test antioxidants were selected through literature citation. Test concentration of PHY was coined from Santos et al. [24], while for AA and QUR from Bleilevens et al. [25] and Isnaini et al. [26] respectively. PHY was emulsified with 0.05% tween-80 dissolved in saline (0.9% NaCl) solution, while QUR, AA and $CuSO_4$ were dissolved in distilled water (DW) to attain the required concentration of each sample.

2.4 Evaluation of toxic/protective effects of test samples in A. cepa

The outer layers and budding parenchyma of the onions were carefully removed by making a small circular incision to facilitate root growth (RG). The bulbs were then rinsed with tap water for 20 min and the root portion was soaked in DW in previously washed and cleaned plastic containers (capacity: 8-10 mL) for the first 24 h at 25 ± 1 °C in the dark. Only, the bulbs with satisfactory RG were transferred to the containers (five for each concentration) containing sample/control for 24, 48 and 72 h of exposure. After the exposure period, the roots were counted and measured in mm [27]. To determine the toxic/protective effect of the test antioxidants, fresh onoins with satisfactory RG (after 24 h in DW) were transferred to the containers (five for each concentration) containing the sample and $CuSO_4$ for 24, 48 and 72 h of exposure and the RG profile was calculated similarly.

2.5 Stistical analysis

Results are presented as mean ± standard error of mean (SEM) or percentage. The data were analyzed by means of analysis of variance (ANOVA) followed by Tukey post test by using Graph Pad Prism (version 6.0), considering $p < 0.05$ at a confidence level of 95%.

3 Results

Table 1 suggests the average root length in mm of the treatment groups exposed at 24, 48, 72 h. The highest RG was seen in the Veh group at 24, 48, 72 h. The standard $CuSO_4$ suppressed the RG at all exposure time in comparison to the Veh group. A low RG profile was seen in QUR treated group than the AA and PHY group. However, AA (25 µg/mL) when co-treated with $CuSO_4$ (0.6 µg/mL), it increased RG up to 24 and 48 h, but reduced at 72 h. On the other hand QUR (40 µg/mL) and PHY (75 µg/mL) co-treated with $CuSO_4$ group reduced RG at all exposure time (**Figure 1**).

Table 1. Average root length in mm of the controls/treatment groups at different exposure time

Treatment groups	Root length (mm)		
	24 h	***48 h***	***72 h***
Veh	20.20 ± 1.98	26.4 ± 1.96	33.4 ± 1.68
AA (25 µg/mL)	10.8 ± 0.82*	20.6 ± 1.52*	33.0 ± 2.76
QUR (40 µg/mL)	12.8 ± 0.96*	18.6 ± 3.01*	25.8 ± 2.16*
PHY (75 µg/mL)	14.6 ± 1.48*	25.4 ± 1.72	32.0 ± 2.40
$CuSO_4$ (0.6 µg/mL)	7.4 ± 1.60*	14.4 ± 1.25*	21.8 ± 1.78*
AA + $CuSO_4$	14.2 ± 1.47*[a]	22.2 ± 1.82*[a]	27.0 ± 2.32*
QUR + $CuSO_4$	9.8 ± 1.19*	14.6 ± 1.64*	20.4 ± 3.29*
PHY + $CuSO_4$	13.4 ± 1.35*	19.6 ± 1.44*	30.8 ± 3.02*

Values are Mean ± SEM (n = 5); *p <0.05 when compared to the Veh group; [a]p<0.05 when compared to the AA group; Veh: Vehicle (distilled water); AA: Ascorbic acid; QUR: Quercetin; PHY: Phytol

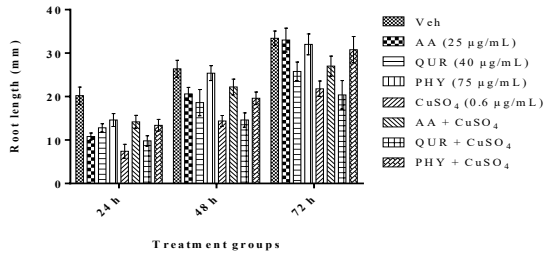

Figure 1. Root length of the *Allium cepa* exposed to the test samples and controls at different exposure time. [Values are mean ± SEM (n = 5); ANOVA followed by Tukey post test, considering $p < 0.05$ at a confidence level of 95%]

Table 2 suggests the percentage of RG of the treatment groups at 24, 48, 72 h of exposure time. Highest %RG was observed for all test samples and the positive control group at exposure time 24 h, then followed by a reduction from 48 to 72 h of exposure. However, there was an increased %RG in the PHY (75 µg/mL) and AA + $CuSO_4$ groups at 72 h when compared with the 48 h exposure time of the respective treatment group.

QUR and PHY, co-treated with $CuSO_4$, significantly ($p < 0.05$) increased %RG in all exposure time when compared to their individually treated group inspect of the respective exposure time. On the other hand, AA co-treated with $CuSO_4$ increased the %RG profile significantly ($p < 0.05$) only at 72 h exposure time (**Figure 2**).

Table 2. Percentage root growth in the control/treatment groups at different exposure time

Treatment groups	%RG		
	24 h	***48 h***	***72 h***
Veh	100	100	100
AA (25 µg/mL)	46.54*	21.97*	1.20*
QUR (40 µg/mL)	36.64*	29.55*	22.75*
PHY (75 µg/mL)	27.73*	3.79*	4.19*
$CuSO_4$ (0.6 µg/mL)	63.37*	45.45*	34.73*
AA + $CuSO_4$	29.70*	15.91*	19.16*[a]
QUR + $CuSO_4$	51.49*[b]	44.70*[b]	38.93*[b]
PHY + $CuSO_4$	33.66*[c]	25.76*[c]	7.78*[c]

Values are expressed in percentage inspect of the Veh group; *$p < 0.05$ when compared to the Veh group; [a]$p<0.05$, [b]$p<0.05$ and [c]$p<0.05$ when compared to the AA, QUR and PHY group, respectively; Veh: Vehicle (distilled water); AA: Ascorbic acid; QUR: Quercetin; PHY: Phytol; RG: Root growth

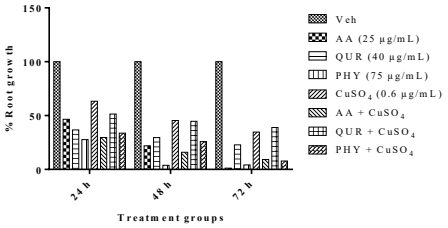

Figure 2. Percentage root growth profile of the *Allium cepa* exposed to the test samples and controls at different exposure time. [Values are percentage compared to the Veh group]

According to the **Table 3**, AA co-treated with the $CuSO_4$ did not increase in RG at 24 and 48 h, but it augmented RG at 72 h of exposure time. QUR co-treated with the $CuSO_4$ increased RG profile at a time-dependent manner. On the other hand, PHY showed maximum IRG at 48 h, then it slightly reduced at 72 h. However, %IRG calculated in the PHY + $CuSO_4$ group at 72 h was higher than that was observed at 24 h (**Figure 3**).

Table 3. Percentage increase in root growth profile in the combined treatment groups

Combined treatment groups	%IRG		
	24 h	***48 h***	***72 h***
AA + $CuSO_4$	-	-	93.74
QUR + $CuSO_4$	28.84	33.89	41.56
PHY + $CuSO_4$	17.62	85.29	46.14

Values are expressed in percentage inspect of the respective exposure time; AA: Ascorbic acid; QUR: Quercetin; PHY: Phytol; IRG: Increase in root growth

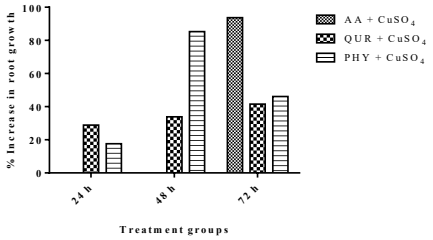

Figure 3. Percentage in hibition of root growth of the *Allium cepa* exposed to the test samples combined with the standard at different exposure time. [Values are percentage inspect of the respective exposure time]

4 Discussion

Onion (*A. cepa*) test is popularly used as a test modality for the investigation of pro-oxidative or toxicogenetic profiles of wide varieties of substances, including crude extracts, isolated compounds, other biochemicals, and heavy metals antioxidant and anti-cancer agent [28,29]. *A. cepa* test is

also used for the determination of lethality calculation of biochemicals and synthetic chemicals, as it is a eukaryotic setup [30]. However, *Allium* test is commonly used for the risk assessment of a wide varieties of substances, including crude extracts, drugs, antioxidants and chemicals [31]. It is due to the fact that *A. cepa* test is easy to perform and it is sensitive; requires no expensive or complex laboratory setup [17].

The RG profile is an important parameter to understand the toxic effect of a substance in *A. cepa* test model [32]. A low root length reflects the toxic effects of the test substance exposed in this model [33]. Standards such as Cu used in this model, are evident to accumulate in roots of *A. cepa* and inhibit RG, result in chromosomal aberrations (e.g.- C-mitosis, chromosomal bridges, chromosomal tack and micronuclei) [21, 34]. However, an inhibition of RG is also related to cell cycle elongation [35], apical meristematic activity [36], and inhibition of protein synthesis [37]. In our study, $CuSO_4$ at 0.6 µg/mL was found to decrease in %RG in a time-dependent manner. All the antioxidants were seen to reduce %RG at 24, 48 and 72 h of exposure time in comparison to the Veh group, suggesting a toxic effect on *A. cepa*. However, these antioxidants when co-treated with the toxicogentic agent $CuSO_4$, significantly increased in %RG.

Antioxidants at low concentrations are protective, while at high concentrations they may act as a pro-oxidative agent [38]. However, their effects may vary dependent upon the test systems, frequency of usage and exposure time [17,39]. By going through the root length profile, it is clear that the $CuSO_4$ reduced root length of *A. cepa* better than the test antioxidants. Furthermore, an increased in %IRG by the test antioxidants in the combined treatment groups, suggesting a protective effect in *A. cepa* test system.

In some studies in *A. cepa*, it has been reported that a certain substance can cause DNA damage in high concentration, but can show an adaptive response at low concentration, probably through their genomic protective capacity [40,41]. Therefore, in this study, AA, QUR and PHY mediated protective effects inspect of exposure time 48/72 h may link to their adaptive responses.

4 Conclusions

Test antioxidants, AA (25 µg/mL), QUR (40 µg/mL) and PHY (75 µg/mL) decreased the root length in *A. cepa*, but the values were lower than the standard toxicogenetic agent $CuSO_4$ (0.6 µg/mL). All the antioxidants co-treated with the $CuSO_4$ have been augmented %RG profile in *A. cepa*. The highest %IRG was observed in AA + $CuSO_4$ group at 72 h. QUR exhibited a time-dependent protective effect in *A. cepa*. It may be due to an adaptive effect in this test system. PHY exhibited time-dependent protective effect up to 48 h, however, it reduced %IRG at 72 h inspect of 48 h. Taken together, this study suggests antioxidant-mediated protective effects against the toxicogenetic agent in the eukaryotic test system. Of note, antioxidants can be used to counteract toxic effects of certain substance in a biological system.

Acknowledgement

We are very much thankful to the Pharmacy Department of BSMRSTU, Gopalganj (Bangladesh) for the laboratory facilities to conduct this project.

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