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Protective Effect of *Borassus flabellifer* Haustorium Extract against Alkoxyl Radical-Induced Cytotoxicity by Improving Glutathione Metabolism by Modulating Nrf2/Haeme Oxygenase-1 Expression

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ABSTRACT

Aim: The study was aimed to assess the ability of *Borassus flabellifer* haustorium methanolic extract (BHE) on de novo glutathione biosynthesis in normal and pro-oxidant exposed cells via Nuclear factor erythroid 2-related factor 2 (Nrf2) and haeme oxygenase-1 (HO1) signaling in 2,2′-Azobis(2 -methylpropionamidine) di-hydrochloride (AAPH) induced cytotoxicity in normal intestinal epithelial cells (IEC-6 cells).

Methods: The in vitro antioxidant activity was determined in terms of radical scavenging and ex vivo hemolysis. The cytoprotective effect was studied using AAP H as the alkoxyl radical inducer in IEC-6 cell model. The mechanistic basis of protection is determined by Nrf2/HO1 expression using qPCR.

Results: *In vitro* screening observed DPPH, hydrogen peroxide and ABTS radical scavenging activity for the BHE; further, BHE also protected the oxidative hemolysis in the erythrocytes induced by AAPH. In IEC-6 cells, AAPH treatment significantly reduced the cell viability ($p < 0.001$) by inducing lipid peroxidation. Further, there observed a significant reduction in the activities of enzymes involved in the de novo glutathione biosynthesis ($p < 0.01$) and glutathione reductase in these cells. However, pretreatment with BHE (10, 25 and 50 µg/mL) dose-dependently protected from the cytotoxicity of AAPH-derived alkoxyl radicals ($p < 0.05$); besides, the de novo glutathione biosynthesis and regeneration of GSH from oxidized form was also increased in these cells. In corroboration with the biochemical parameters, the Nrf2/HO1 expression was upregulated by the BHE pretreatment concomitantly reducing the cellular lipid peroxidation products. The improvement glutathione biosynthesis was also observed in BHE alone treated cells.

Conclusion: The study indicated the potential of methanolic extract of *Borassus flabellifer* haustorium in enhancing the de novo glutathione biosynthesis in normal and pro-oxidant exposed cells by Nrf2/HO1 dependent manner, concomitantly mitigating the toxicity of AAPH-derived alkoxyl radicals in intestinal epithelial cells.

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Introduction

The components of *Borassus flabellifer*, commonly known as palmyra palm, are widely used as food components. The various portions of the plant such as haustorium, tender water and kernel are edible and having high nutritional values. Limited studies are available on the pharmacological effects of Borassus; among these the earlier studies were by,[1] where the hypoglycemic and anti-diabetic effects of Borassus male flower extract. Further, antidiabetic properties have also reported for the root[2] and fruit extracts[3] of *Borassus flabellifer*. One of the bioactive compounds of the plant include a dammarane triterpenoid, which is shown to inhibit prostate cancer cell proliferation by inducing apoptosis in them.[4] Further, antibacterial activities are also reported for the seed coat extract[5] and sirup[6] of the plant. Recent study from our lab has indicated the cytoprotective property of borassus haustorium extract against hydrogen peroxide and malondialdehyde; further, anti-inflammatory effects are also reported on cultured macrophages.[7] However, there is no clear information on the role of glutathione metabolism on cytoprotection by BHE.

The present study has been evaluated in cell culture models of cytotoxicity using AAPH induced cell death. It has been reported that the AAPH generated alkoxyl radicals in aqueous conditions. The generated alkoxyl radicals induce cell membrane damage and results in lipid peroxidation. Subsequently, the generated radicals induce oxidative imbalance and thereby cause death by mediated through apoptosis in cells. In addition, the alkoxyl radicals are usually formed during the oxidation of polyunsaturated fatty acids;[8] thus,

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the alkoxyl radicals forms a model for dietary source of free radicals. It has also been reported that the alkoxyl radical induces oxidative damages in intestinal cells.[9] Several antioxidants act as chain breaking antioxidants; therefore, it is expected that the BHE may have roles in alleviating the AAPH induced cell death and glutathione-dependent antioxidant systems may have significant roles in the cytoprotective properties.

Materials and methods

Chemicals and cell lines

The free radical generator AAPH (2, 2′-Azobis (2-amidinopropane) dihydrochloride), Sodium dodecyl sulfate (SDS), acetic acid and thiobarbituric acid (TBA) were purchased from Sisco Research Laboratories Pvt. Ltd. (Maharastra, India). Fetal bovine serum, RPMI-1640, HEPES buffer, sodium carbonate, L-glutamine, sodium pyruvate, antibiotics and other reagents required for the cell culture were collected from Sigma Aldrich (St. Louis, MO, United States).

Rat Normal immortalized colon epithelial cell line (IEC-6) was obtained from National Center for Cell Science, Pune. The cells were supplemented with complete growth media and passaged every third day and were maintained in 5% CO_2 at 37 °C in an incubator.

Collection, extraction and phenolic profiling of Borassus flabellifer haustorium

Mature borassus fruits were collected and allowed to germinate in wet soil. The germinated fruits were de-husked to collect haustorium, which was dried and powdered. The powdered haustorium was then extracted in HPLC grade methanol using Soxhlet (Borosil, Mumbai, India). Total phenol content[10] and total flavonoid content[11] were determined according to the standard methods available. The composition of phenolic acids and flavonoids were done by using LC/MS technique as described previously Malayil, House.[7]

The proximate composition was estimated according to the methods as described by official methods of AOAC.[12] The parameters assessed include the moisture content (AOAC 934.01), crude fat (AOAC 920.58), crude protein (AOAC 2001.11), ash (AOAC 938.08), and fiber content (AOAC 985.29). Trace elements present in the haustorium of *Borassus flabellifer* was determined using atomic absorption spectroscopy.

In vitro antioxidant and anti-hemolytic assay

In vitro antioxidant activity of BHE was carried out in terms of DPPH, ABTS, hydroxyl radical scavenging and AAPH induced hemolysis inhibition.

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

The DPPH radical scavenging assay was carried out using a stock solution of DPPH in methanol (30 µg/mL). The reaction mixture contained different concentration of BHE extracts and DPPH solution to a final volume of 1 mL and incubated for 20 minutes in dark. The optical density was measured spectrophometrically at 517 nm.

ABTS radical scavenging activity

Briefly, the 7 mM ABTS solution was mixed with 2.5 mM potassium persulfate to yield ABTS radicals. The radical solution was diluted in methanol and by adding different concentrations of BHE extract and change in absorbance was measured from initial and 10th minute, the percentage inhibition and LC50 was calculated by comparing with the control.

Hydrogen peroxide radical scavenging activity

The hydrogen peroxide inhibition was carried out by mixing of different concentrations of BHE and 200 mM hydrogen peroxide (dissolved in 50 mM, pH 7.4 phosphate buffer). The change in absorbance at 240 nm was recorded spectrophotometrically for 10 min. The percentage inhibition and LC50 values was calculated as described below.

Anti-hemolytic activity against AAPH induced damages

The blood that is close to the expiry date and that are about to be removed was collected from blood bank. The packed cells were washed in 50 mM, pH 7.4 phosphate buffer and lysis free cells were collected. The cells were diluted in phosphate buffer to a concentration of 24%; about 1 mL of blood was mixed with 0.5 mL of different concentrations of BHE for 1 hour at room temperature. Further, 0.1 M AAPH solution 0.5 mL was added and again incubated for 60 min. The mixture was centrifuged at 1000 g for 5 min and the supernatant was collected. The supernatant collected was diluted 1 in 10 and the optical density of the sample was estimated at 450 nm and percentage inhibition was calculated.

Cytotoxicity analysis of Borassus haustorium and AAPH and determination of cytoprotective effect of BHE

Cytotoxicity of both *Borassus flabellifer* haustorium extract and AAPH radical was carried out using the MTT assay.[13] The biologically safer concentration of Borassus was estimated to be the concentration which causes a toxicity of less than 2% over 48 hours. The percentage cell death was determined from the absorbance (Ab.) values, as follows.

$$\%\,Cell\,death = \frac{Absorbance\,of\,control - Absorbance\,of\,concentration}{Absorbance\,of\,Control} \times 100$$

The cytoprotective efficacy of BHE was determined as per the previous procedures described in our previous publication Malayil et al. [10]. Briefly, the cells were plated at a density of 1×10^6 cells/mL and allowed to attach for overnight. The cells were then pretreated with the different doses of BHE alone or in combination with BSO (50 µM). After 24 hours, all the cells were treated with AAPH (400 µM) and further incubated for 24 hours and cell viability was

evaluated by MTT assay as per the standard protcols mentioned earlier.

Activity of glutathione metabolizing enzymes

The cellular non-enzymatic antioxidant- reduced glutathione (GSH) was estimated according to the methods using Ellman's reagent.[14] The activities of *de novo* glutathione biosynthetic enzymes γ-glutamyl cysteine synthetase and glutathione synthetase were determined according to the methods described according to the methods described by Volohonsky, Tuby.[15] The activities of glutathione utilizing antioxidant enzymes-glutathione-s-transferase[16] and glutathione peroxidase[17] were determined according to previously described methods as cited. The activity of glutathione reductase (GR), an enzyme associated with glutathione salvage pathway, is determined according to the standard methods.[18] The enzyme activities were finally expressed as IU per milligram of protein.

Estimation of lipid peroxidation status

The extent of intracellular lipid peroxidation was estimated in terms of thiobarbituric acid reactive substance (TBARS) according to our previously published standard methods.[19] Apart from the TBARS, the early lipid peroxidation product- conjugated dienes was determined as per the methods of Narayanankutty, Mukesh.[20] All these parameters were expressed as nmoles per mg of protein.

Gene expression analysis by qPCR

The cells were treated as per previously described under different treatment regimen; the cells were collected by mechanical methods. The cDNA synthesis from the cells was carried out using cell to cDNA kit (CellAmp™, Takara Bio, India). The gene expression was analyzed using Applied Biosystem 7500 real-time PCR (Applied Biosystems, California, United States) using SYBR™ Select Master Mix according to the manufacturers instructions (Thermo Scientific, Massachusetts, United States). The gene expression was estimated in terms of fold change with respect to the house keeping gene beta actin.[21] The primers used in the study were as follows; Nrf-2 forward 5′-CAC AGT GCT CCT ATG CGT GA-3′, reverse 5′-TCT GGG CGG CGA CTT TAT T-3′, Haeme oxygenase-1 forward 5′-GGT GAT GGC CTC CTT GTA CC-3′, reverse5′-GTG GGG CAT AGA CTG GGT TC-3′, β-actin forward 5′-AAG ATC CTG ACC GAG CGT GG-3′, reverse 5′-CAG CAC TGT GTT GGC ATA GAG G-3′ .

Statistical analysis

The data of in vitro antioxidant assays were expressed as mean ± SD of five independent experiments, each conducted in triplicate. Besides, the data of cytoprotective study was also expressed in a similar manner with each concentration which was carried out in triplicate. The statistical analysis has been carried out using analysis of variance (ANOVA) and subsequently by Tukey Kramer multiple comparison test (Graph pad Prism 7.0 version, La Jolla, USA).

Results

Total phenolic content, phenolic composition and proximate composition

The phenolic content was quantitatively determined by reaction with Folin's reagent. In methanolic extract of Borassus haustorium, total polyphenol content was 41.22 ± 2.89 mg gallic acid equivalent. Similarly, the flavonoids content was estimated by aluminum chloride method, and was found to be 9.44 ± 0.37 mg quercetin equivalent. LCMS analysis revealed the presence of polyphenolic compounds such as ferulic acid, *p*- coumaric acid, protocatechuic acid etc and flavonoids including catechin and quercetin (Supplementary Material 1). Apart from these the study also observed the presence of various nutritional compounds including carbohydrates, fiber, potassium, manganese, and sodium (Table 1).

In vitro antioxidant activity of BHE

The in vitro antioxidant activity of BHE was determined in terms of radical scavenging and hemolysis inhibition assays (Figure 1). The half maximal inhibition concentration (IC50) value for DPPH radical scavenging activity was found to be 20.95 ± 1.21 µg/mL. The hydrogen peroxide scavenging and ABTS radical scavenging was also high with respective IC50 values 32.23 ± 3.94 and 28.43 ± 1.46 µg/mL, respectively. Anti-hemolytic activity was also observed for the BHE against AAPH induced damages with an IC50 value of 63.17 ± 2.24 µg/mL.

Cytoprotective effect of BHE in IEC-6 cells against AAPH-induced damages

The cytotoxicity of BHE was found to be less than 2% up to a concentration 60 µg/mL in IEC-6 cells (Figure 2a); on contrary, cytotoxicity analysis by means of MTT assay indicated the cytotoxic effects of AAPH is induced via alkoxyl radical-mediated lipid peroxidation with an IC50 value of 395.7 ± 3. 2 µM (Figure 2b).

Table 1. Proximate and mineral composition of haustorium from *Borassus flabellifer* as per official methods of Association of Official Analytical Chemists (AOAC).

Content	Borassus haustorium
Moisture content (%)	84.2 ± 1.87
Ash content (%)	1.19 ± 0.09
Crude fat (%)	2.08 ± 0.13
Protein (%)	4.8 ± 0.21
Carbohydrate (%)	69.2 ± 2.7
Dietary soluble Fiber	5.11 ± 0.26
Dietary insoluble Fiber	22.6 ± 1.67
Sodium	76.0 ± 5.4
Potassium	144.9 ± 12.7
Calcium	48.3 ± 3.0
Zinc	1.1 ± 0.20
Magnesium	132.4 ± 6.1
Phosphorus	46.1 ± 3.9
Copper	0.22 ± 0.06

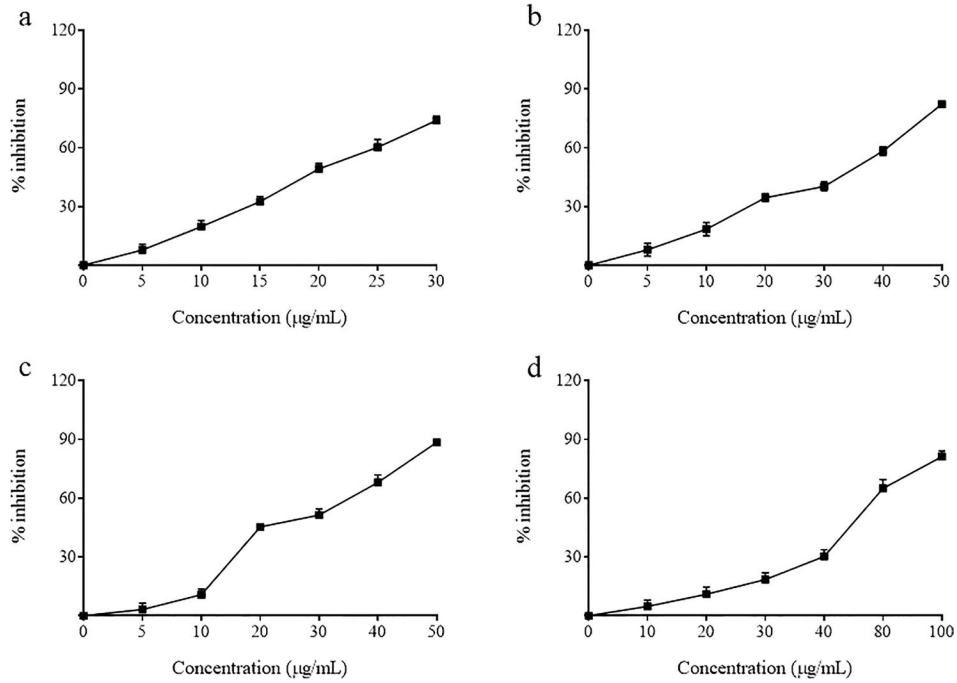

Figure 1. In vitro antioxidant activity of the methanolic extract of *Borassus flabellifer* haustorium in terms of DPPH (a), hydrogen peroxide (b), ABTS (c) radical scavenging and inhibition of AAPH induced oxidative hemolysis (d).

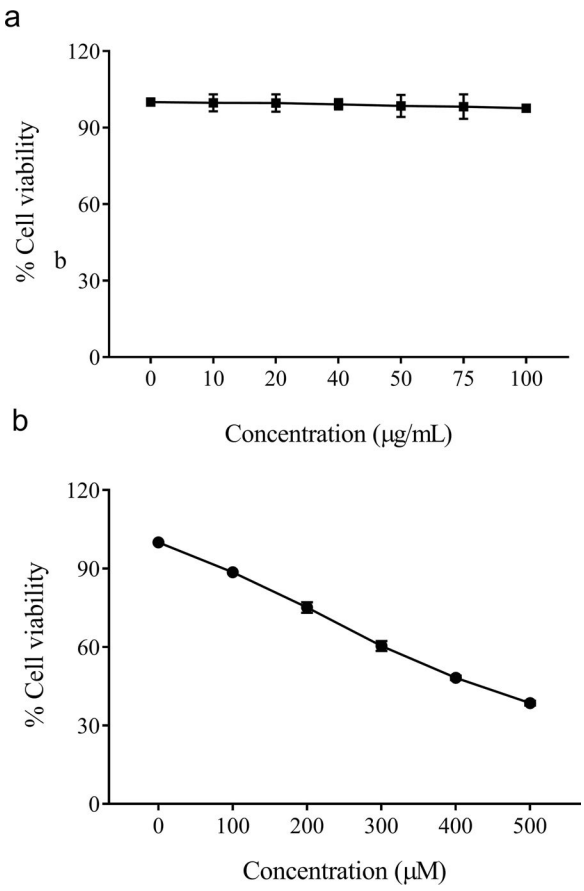

Figure 2. The cytotoxicity analysis of *Borassus flabellifer* haustorium methanolic extract (a) and 2, 2′- Azobis (2-amidinopropane) dihydrochloride (AAPH) (b) in normal murine intestinal epithelial cells (IEC-6) determined using MTT assay.

In cultured intestinal cells, the AAPH treatment (400 µM) induced significant cell death ($p < 0.001$) and reduced the viability to 47.5 ± 2.9%). On contrary, the pretreatment with 10, 25, and 50 µg/mL improved the overall cell viability of IEC-6 cells to 59.3 ± 1.6, 74.8 ± 2.7, and 87.3 ± 2.5% (Figure 3a). It has also been noted that when BHE is added together with buthionine sulfoximine (BSO), an inhibitor of glutathione biosynthesis, the protective effect of the BHE was significantly reduced.

Activities of cellular glutathione metabolizing enzymes and lipid peroxidation level

The protective effect of BHE was evident in terms of the cell viability; however, to provide further inputs in terms of the mechanism of action, the activities of the glutathione metabolizing enzymes has been evaluated (Table 2). When the cells were treated with different doses of BHE alone for 48 hours, there observed marginal increase in the activities of γ-glutamyl cysteine synthetase (γ-GCS), glutathione synthetase (GS), glutathione-s-transferase (GST), and glutathione reductase (GR); however, the activity of glutathione peroxidase (GPx) remained unaltered. The glutathione biosynthetic enzymes γ-GCS and GS were elevated significantly ($p < 0.05$) in their highest dose. Likewise, the activity of GR was also elevated in a similar manner in the 25 and 50 µg/mL treatments of BHE. Besides, as shown in Table 3, the intracellular glutathione levels were increased significantly ($p < 0.05$). On the contrary, the lipid peroxidation parameters were unaltered.

The treatment with AAPH induced significant reduction in the glutathione biosynthetic enzymes and glutathione reductase concomitantly increasing lipid peroxidation

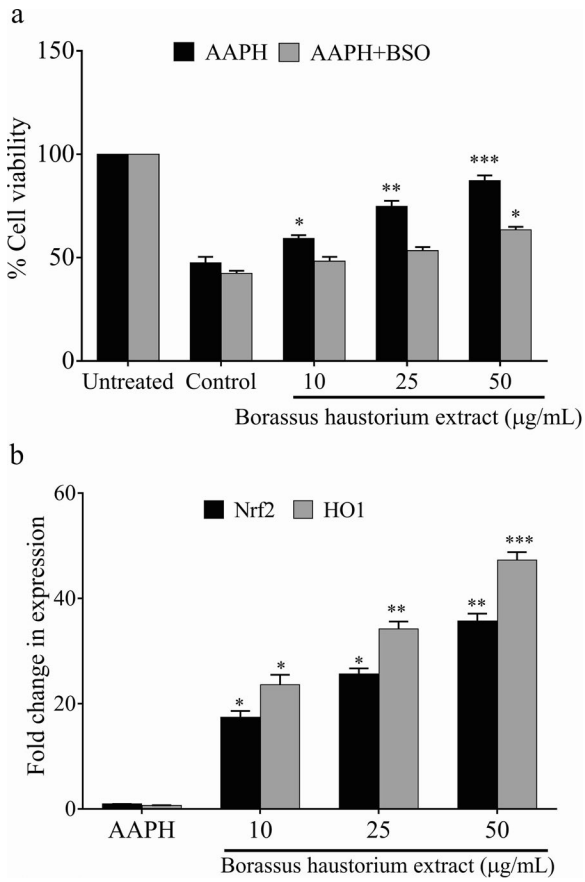

Figure 3. Pretreatment of *Borassus flabellifer* haustorium methanolic extract (BHE) protects against 2, 2′- Azobis (2-amidinopropane) dihydrochloride (AAPH, 400 µM) induced cell death in intestinal epithelial cells (IEC-6 cells) and co-treatment of buthionine sulfoximine reduced the protective efficacy of BHE (a). The fold change in the expression of antioxidant genes Nrf2 and haeme oxygenase-1 determined by qPCR in AAPH alone treated and the BHE- pretreated cells (b).

parameters ($p < 0.05$). On contrary, pretreatment with BHE prior to AAPH exposure reduced the AAPH-induced glutathione depletion by increasing the activity of glutathione metabolizing enzymes ($p < 0.05$). In addition, the activity of GPx, the GSH-dependent detoxification enzyme, which was increased during AAPH treatment, was restored back by BHE pretreatment (Table 2). Together with the increased antioxidant defense in BHE pretreatment, the lipid peroxidation markers, TBARS and CD, were significantly reduced dose-dependently (Table 3).

Expression of genes like Nrf2 and HO-1 genes

As shown in Figure 3b, the expression of both nuclear factor erythroid 2–related factor 2 (Nrf2) and haeme oxygenase −1 was significantly lower in AAPH alone treated cells ($p < 0.05$). However, pretreatment with different doses of BHE induced the expression of Nrf2 and HO-1 genes at a significantly higher level compared to the AAPH alone treated cells ($p < 0.01$).

Discussion

Borassus flabellifer is emerging as a functional food or nutraceuticals; the different parts, especially its haustorium have been reported to have health benefits such as antioxidant and anti-inflammatory properties.[7, 22] The present study evaluated the mechanistic basis of the antioxidant mediated cytoprotective effect of the methanolic extract of Borassus flabellifer in cells against a free radical generator, 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH). Under physiological temperature and aqueous conditions in a cell, AAPH undergo quickly decompose to generate a volume of nitrogenous and carbonyl moieties, which lead to the formation of highly reactive peroxyl (ROO*) radicals[23,24] and alkoxyl radicals.[23] The physiological equivalents of the alkoxyl radicals are formed as a result of lipid peroxidation, especially by the oxidation of polyunsaturated fatty acids.[8]

The results showed significant reduction in the cell viability upon treatment with AAPH. Further, there observed significantly elevated intracellular lipid peroxidation products in AAPH alone treated cells. The results are in line with the previous report that, the alkoxyl radicals do not penetrate the cell membrane, instead, they react with the membrane lipids and inducing lipid peroxidation and[25] subsequent DNA damages.[26] Corroborating with these, there observed a significant increase in the in glutathione peroxidase (GPx) activity of AAPH treated cells; possibly, the increased intracellular peroxides might have contributed to the increased GPx activity. Further, this increased GPx activity to cop-up with AAPH mediated oxidative stress might have depleted the cellular reduced glutathione pool as observed in these cells. Substantiating these observations, enzymatic activity of γGCS and GS, which are responsible for the de novo

Table 2. Changes in the glutathione metabolizing enzyme activities in the untreated IEC-6 cells (Normal), those exposed to different concentrations of *Borassus flabellifer* haustorium methanol extract (BHE) and BHE-pretreated cells further exposed to AAPH (alkoxyl radical). The enzymes analyzed include γ-glutamyl cysteine synthetase (γ-GCS), glutathione synthetase (GS), glutathione peroxidase (GPx), glutathione-s-transferase (GST), and glutathione reductase (GR).

Treatment	γ-GCS (U/mg protein)	GS (U/mg protein)	GPx (U/mg protein)	GST (U/mg protein)	GR (U/mg protein)
Normal	5.32 ± 0.19	8.05 ± 0.14	3.29 ± 0.20	9.45 ± 0.35	7.41 ± 0.15
BHE 10 µg/mL	5.25 ± 0.21	8.07 ± 0.25	3.18 ± 0.33	9.49 ± 0.27	7.38 ± 0.17
BHE 25 µg/mL	5.85 ± 0.18	8.63 ± 0.19*	3.26 ± 0.23	9.81 ± 0.25	8.17 ± 0.22*
BHE 50 µg/mL	6.01 ± 0.19*	8.92 ± 0.16*	3.34 ± 0.13	10.12 ± 0.33*	8.09 ± 0.11*
AAPH (400 µM)	2.11 ± 0.08	4.76 ± 0.32	5.13 ± 0.11	5.88 ± 0.29	4.11 ± 0.27
AAPH + BHE 10 µg/mL	3.45 ± 0.27**	5.12 ± 0.21	4.84 ± 0.17	6.42 ± 0.46*	5.22 ± 0.08*
AAPH + BHE 25 µg/mL	3.92 ± 0.13**	5.79 ± 0.14*	4.26 ± 0.29*	7.03 ± 0.31**	5.76 ± 0.21**
AAPH + BHE 50 µg/mL	4.19 ± 0.20***	6.32 ± 0.33**	3.76 ± 0.14**	7.67 ± 0.42***	6.15 ± 0.23***

The values are represented as mean ± SD of three independent experiments, each carried in triplicate. (*indicate significant difference $p < 0.05$; ** indicate significant difference $p < 0.01$and *** indicate significant difference $p < 0.001$).

Table 3. Changes in the reduced glutathione content as well as lipid peroxidation indicators (thiobarbituric acid reactive substance-TBARS and conjugated dienes) in normal IEC-6 cells, cell exposed to different concentrations of *Borassus flabellifer* haustorium methanol extract (BHE) along, and those pretreated with BHE and later exposed to AAPH (alkoxyl radical).

Treatment	GSH (µmoles/ mg protein)	TBARS (µmoles/ mg protein)	Conjugated dienes (µmoles/ mg protein)
Normal	6.12 ± 0.31	1.99 ± 0.12	29.25 ± 2.50
BHE 10 µg/mL	6.19 ± 0.23	2.06 ± 0.14	36.55 ± 4.13
BHE 25 µg/mL	6.30 ± 0.16	2.01 ± 0.21	34.91 ± 3.72
BHE 50 µg/mL	6.71 ± 0.18*	1.95 ± 0.16	40.11 ± 4.28
AAPH (400 µM)	2.35 ± 0.24	5.44 ± 0.23	134.30 ± 3.43
AAPH + BHE 10 µg/mL	3.11 ± 0.19*	4.87 ± 0.18*	113.42 ± 5.31*
AAPH + BHE 25 µg/mL	3.77 ± 0.22**	4.33 ± 0.17**	96.12 ± 5.05**
AAPH + BHE 50 µg/mL	4.27 ± 0.32**	3.88 ± 0.13***	80.62 ± 4.76***

The values are represented as mean ± SD of three independent experiments, each carried in triplicate. (*indicate significant difference $p < 0.05$; ** indicate significant difference $p < 0.01$and *** indicate significant difference $p < 0.001$).

biosynthesis of glutathione,[27] are also found to be inhibited by the AAPH derived alkoxyl radicals. To intensify the glutathione depletion, the activity of glutathione reductase (GR), which is involved in the regeneration of GSH from oxidized form of glutathione,[28] has been inhibited by the AAPH.

The pretreatment with BHE mitigated the AAPH-induced cell death mediated through the enhancement of glutathione biosynthetic enzymes γGCS and GS, subsequently restoring the cellular GSH pool. It has been also evident that treatment with buthionine sulfoximine, an inhibitor of glutathione biosynthesis reduces the efficacy of BHE against AAPH-derived free radicals. It confirms the role of glutathione system on the cytoprotective effect of BHE in intestinal epithelial cells. The supplementation of BHE alone showed an enhancement of glutathione system, which also supports the assumption that BHE promotes the glutathione biosynthesis in cells. Besides, there observed an increased expression of Nrf2/HO1 in the BHE treatment. Nrf2/HO1 is the key regulator of cellular antioxidant machinery and contributes to the glutathione mediated protection in cells . It is thus possible that the BHE antioxidant compounds enhance the glutathione metabolism via upregulation of Nrf2/HO1 signaling. Apart from the de novo metabolism of GSH, the increased activity of GR may have partially contributed to the increased cellular GSH levels. Pretreatment with BHE also brought down the activity of GPx and reduced intracellular cellular peroxidation products such as TBARs and conjugated dienes. It is possible that antioxidant components present in BHE as observed in the in vitro and ex vivo hemolysis models may have contributed to the scavenging of AAPH derived free radicals in the cellular conditions.

The protective effect of BHE against alkoxyl and peroxyl radical in intestinal epithelial cells may be applicable in the prevention of oxidative diseases of the tissue. Being the dietary sources of alkoxyl and peroxyl radicals, thermally oxidized edible oils intake often pauses concern to the health of intestinal epithelial cells;[9] it is also reported that these radicals increases the risk for ulcerative colitis and progression to colorectal cancers.[29] It is thus possible that the protective effect of BHE against alkoxyl radicals may be useful in preventing the intestinal inflammatory diseases and cancers in long-term. Therefore, the present study recommends further animal model studies on the protective effect of BHE against ulcerative colitis and colon cancer prevention to ascertain these facts.

Conclusion

In view of the results obtained, it is clear that the AAPH mediated alkoxyl radicals induce oxidative stress mediated cell death, where glutathione depletion plays significant roles. Treatment with polyphenol rich BHE improved the glutathione biosynthesis and regeneration as well as maintained the activities of glutathione-dependent detoxification systems. Overall, *Borassus flabellifer* haustorium improves cellular antioxidant defense by efficiently modulation glutathione metabolism; therefore, the haustorium of *Borassus flabellifer* may evolve as a functional food.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Availability of data and material

Available on genuine request by authorized persons.

Authors contributions

JTJ- Cytoprotective studies in cells exposed to AAPH, qPCR analysis and Manuscript draft preparation (along with AN); **AA** and **RR**- Phytochemical and nutritional analysis, In vitro antioxidant properties; **YOK**- Impact of BHE alone on the antioxidant status of IEC-6 cells; **HJK**- Study on the effect of BSO on the cytoprotective effect of BHE; **AN** designed the concept, analyzed data and prepared the manuscript. All authors approved the manuscript.

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