ETHANOL EXTRACT OF Basella alba Linn MODULATES ACRYLAMIDE-INDUCED OXIDATIVE STRESS IN WISTAR RATS

Omowumi O. Adewale1,2, Ekundayo S. Samuel1, Michael A. Gbadegesin1, Solomon E. Owumi1 and Oyeronke A. Odunola∗

Address(es): Oyeronke Odunola,
1 Cancer Research and Molecular Biology Unit, Department of Biochemistry, College of Medicine, University of Ibadan, Ibadan, Nigeria.
2 Department of Biochemistry, Faculty of Basic and Applied Sciences, Osun State University, Osogbo, Nigeria.

*Corresponding author: ronudmol@yahoo.com

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ABSTRACT
Acrylamide (AA), a common toxicant in processed foods is associated with cancer development via induction of oxidative stress. Therefore, the need for a potent antioxidant substance or compound that could ameliorate this toxic effect. Basella alba has been reported to have medicinal properties, and in this study, the anti-oxidative potentials of ethanol leaf extract of Basella alba (ELEBa) were assessed against oxidative stress induced by acrylamide in male Wistar rats (120-150g). Twenty (20) animals were grouped into four, Group 1: 1 ml/kg body weight (bwt.) distilled water (control), Group 2: 17.5 mg/kg bwt AA, Group 3: 17.5 mg/kg bwt AA+100 mg/kg bwt ELEBa, Group 4: 17.5 mg/kg bwt AA + 250 mg/kg bwt ELEBa. Treatment of animals was done orally and once daily for 14 days before sacrifice. The liver and kidney tissues were processed for the analyses of antioxidant activities. Serum was analyzed for hepatic and renal function bio markers in the treated animals. The plant’s bio active constituents were characterized by GC-MS. Acrylamide caused a significant (p < 0.05) ameliorated these values. GC/MS analysis revealed the presence of pentadecanoic acid, n-hexadecanoic acid, cis-13-octadecenoic acid, cis-vaccenic acid, oleic acid and octadecanoic acid. Our findings suggest that, ELEBa is a potential chemopreventive agent against acrylamide-induced oxidative stress in wistar rats.

Keywords: Acrylamide, oxidative stress, Basella alba, antioxidants

INTRODUCTION
Close to two decades now, acrylamide was reported among food carcinogens as a result of its discovery in some high-temperature processed carbohydrate-rich foods (Tareke et al., 2002; Houston, 2013; Mastovska and Lehotay, 2006). This discovery had led to a major concern for scientific researchers, food policymakers and food surveillance institutions, as well as to the public (Dirk, 2009), because acrylamide is a highly electrophilic molecule can occur in the body through different routes creates its bioavailability in the body system, got metabolized and converted to excretable macapturic acid metabolites which are excreted out of the body (Botcher et al., 2006; Dybing et al., 2005). However, accumulation of acrylamide being a highly electrophilic molecule can occur in the body when it binds to important macro molecules in the cells such as DNA and proteins especially the hemoglobin (Fuhr et al., 2006) thereby eliciting it toxicities. The major mechanism of toxicity by acrylamide was reported to be via oxidative stress (Sumizawa and Igsu, 2007; Sumizawa and Igsu, 2009; Alturfan, 2012; Lakshmi, 2012), a potent anti-oxidative agent is therefore proposed to be capable of mediating acrylamide-induced toxicities. One of the plants that have gained researchers’ interest is Basella alba (Ba). It is one of the neglected underutilized species yet with several medicinal potentials (Fluorite, 2006). There have been reports on the anti-inflammatory (Krishna, 2012), antimicrobial (Oyeswole and Kalejaye, 2012) and anti-oxidative properties (Nirmala et al., 2009) of B.alba. With several reports (Haskell et al., 2004; Bamidele et al., 2010; Nantia et al., 2011; Rhoda et al., 2012) on the pharmacological activities of B.alba, many data are still expected, especially from its activities against toxic anti-induced biological changes in order to further elucidate its beneficial properties. Based on this background, and most particularly because exposure to AA is considered a lifetime affair in humans (Rodriguez-Ramiro et al., 2011). Our objective is to investigate the anti-oxidative activities of Basella alba leaf ethanol extract in acrylamide-intoxicated rats.

MATERIALS AND METHODS
Chemicals
Acrylamide (79-06-1) was gotten from British Doghouses (Poole, Dorset, UK). 5’,5’-dithiobis-2-nitrobenzene (DTNB), Glutathione (GSH), Biuret, 2-thiobarbituric acid (TBA), hydrogen peroxide (H2O2), and 1-chloro-2, 4-dinitrobenzene (CDNB) and were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were of analytical grade.

Collection of fresh leaves of Basella alba
The fresh leaves of B.alba were purchased from a market in Oke Baale, Osogbo and were identified in, Botany Department, Faculty of Science, University of Ibadan, Nigeria and were was given voucher number (UIH-22501).

Plant extract preparation
Fresh leaves of B. alba were dried under shade and then pulverized to powder using blender. 500g of the plant powder was stirred in 2000ml of 50% ethanol and kept for 72 hours with regular manual agitation. The mixture was decanted after 72 hours first with fine museline in muslin cloth and later with filter paper. The ethanol leaf extract of Basella alba (ELEBa) passed through concentration process using rotary evaporator; the concentrate was freeze dried and then stored at -20°C for this experiment. % yield for ethanol leaf extract of B.alba (ELEBa) = 8.43%.

Analysis of ELEBa by GC-MS
The analysis of ELEBa was achieved by GC-MS with model 7890A. Various constituents were detected by the detector at different retention times, the detected constituents were sent as signals to the chart recorder, and these signals were then recorded as peaks on the chromatography. (Zarshenas et al., 2014)
Experimental design

A total number of twenty (20) male Twister rats (120-150g) were gotten from College of Health Sciences Animal House in Osun State University, Osogbo and housed at the Central animal house of the University. The animals were given pelleted feed (Vita Feeds, Mokola, Ibadan, Nigeria) and water ad libitum and were permitted to adapt to the environment for one week. They were kept under natural photo period of about 12 h light/12h dark throughout the experimental phase. The animals were nurtured in agreement with NIH Guide for the care and use of laboratory animals.

Group I (C) – 1ml/kg body weight distilled water.
Group II (AA) - 17.5 mg/kg b.wt of Acrylamide (1/10th of LD50 reported by Fullerton and Barnes, 1966; McCollister et al., 1964). Group III (AA+ELEBa) - 17.5 mg/kg b.wt AA and 100 mg/kg b.wt ELEBa (as reported by Bamidele et al., 2010) Group IV (AA+ELEBa) – 17.5 mg/kg b.wt of AA and 250 mg/kg b.wt ELEBa (as reported by Bamidele et al., 2015)

Treatment was oral and was done once a day for 14 days. Animals were bled retro-orbitally (blood was collected into plain bottles for clotting) and then sacrifice was achieved by cervical dislocation under the anaesthetic influence of ether 2-hours after the last treatment. Serum was obtained by centrifuging the blood at 3000xg for 10mins. Kidney and Liver tissues were excised, weighed and then homogenized in 50 mmol/l Tris–HCl buffer (pH 7.4) and then spun at 10000g for 15 min with table top centrifuge to obtain post mitochondrial fraction for Supernatants were kept frozen at -20°C until needed.

Evaluation of hepatic and renal function biomarkers

The hepatic function biomarkers: aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were evaluated via the principle reported by Reitman and Frankel (1957), gamma -glutamyl transferase (GGT) and alkaline phosphatase (ALP) activities were evaluated via the principles reported by Englehardt et al., (1970) and Szasz (1969) respectively. The renal function bio markers: Creatinine and blood urea nitrogen concentrations were evaluated via the principles described by Henry et al., (1974); Weatherburn, (1967) and Maaroufi et al., (1996) respectively. Protein contents of the post mitochondrial fractions from kidney and liver tissues were evaluated via the method of Biuret as described by Gornal et al., (1949).

Determination of antioxidant status

Activity of catalase enzyme was evaluated via the principle explained by Sinha (1972). Reduced glutathione (GSH) levels in the samples was estimated using the principle explained by Butler et al., (1963). The activity of Glutathione-S-transferase was evaluated through the principle described by Habig et al., (1974).

Level of Lipid peroxidation was evaluated by quantifying the thiobarbituric acid reactive substances (TBARS) formation during lipid peroxidation by using the procedure described by Rice- Evans et al., (1986) Olikawa et al., (1979).

Histopathological Analysis

Tissues of the liver and kidney were excised separately from the experimental animals following sacrifice and then fixed in 10% formalin solution, to be used for tissue sections and subsequent examination of histopathology. These tissues were then immersed in paraffin. Through a rotary microtome, five micrometer thick paraffinized tissue sections were collected, a series of these sections stained with Hematoxylin and Eosin (H&E). These specimens were studied and snapped underneath a light microscope

Statistical analysis

The experimental data were presented using mean ± standard deviation. One-way analysis of variance (ANOVA) was utilized to analyze the differences between the groups and aided by means of Statistical Package for Social Sciences (SPSS) software, SPSS Inc., Chicago, Standard version 10.0.1. Tukey’s test was used as the post hoc test. P-value of < 0.05 was taken as the level of statistical significance for mean differences.

RESULTS

GCMS analysis of ethanolic leaf extract of Basella alba (ELEBa)

The Subjection of ELEBa to analysis by GC-MS showed six peaks corresponding to Pentadecanoic acid, Cis-13-Octadecenoic acid, n-Hexadecanoic acid, Cis-vaccenic acid, Oleic acid and Octadecanoic acid (Table 1 and figure 1)

<table>
<thead>
<tr>
<th>S/N</th>
<th>Retention time</th>
<th>Compound name</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
<th>Peak area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24.789</td>
<td>Pentadecanoic acid</td>
<td>C_{15}H_{28}O_{2}</td>
<td>242</td>
<td>5.03</td>
</tr>
<tr>
<td>2</td>
<td>25.978</td>
<td>n-Hexadecanoic acid</td>
<td>C_{16}H_{30}O_{2}</td>
<td>256</td>
<td>32.94</td>
</tr>
<tr>
<td>3</td>
<td>26.272</td>
<td>Cis-13-Octadecenoic acid</td>
<td>C_{17}H_{32}O_{2}</td>
<td>282</td>
<td>19.51</td>
</tr>
<tr>
<td>4</td>
<td>29.606</td>
<td>Cis-vaccenic acid</td>
<td>C_{18}H_{30}O_{2}</td>
<td>282</td>
<td>16.29</td>
</tr>
<tr>
<td>5</td>
<td>9.953</td>
<td>Oleic acid</td>
<td>C_{18}H_{34}O_{2}</td>
<td>282</td>
<td>9.95</td>
</tr>
<tr>
<td>6</td>
<td>30.029</td>
<td>Octadecanoic acid</td>
<td>C_{20}H_{40}O_{2}</td>
<td>284</td>
<td>8.58</td>
</tr>
</tbody>
</table>

Figure 1 Chromatogram of GC/MS for Basella alba (ELEBa)Ethanol Leaf Extract

Effects of ELEBa on the hepatic and renal function biomarkers in the serum of rats treated with acrylamide

Exposure of experimental rats to acrylamide led to elevation in AST, ALT, GGT and ALP activities and urea concentration in the serum significantly meanwhile no alteration that was significant was observed in serum creatinine concentration when compared with the control, this is an indication of renal and hepatic damage in the rats exposed to acrylamide at the tested dose. Treatment with ELEBa at 100 and 250mg/kg body weight significantly reduced these changes in hepatic and renal biomarkers (Table 2), to show the ability of ELEBa in hepatic and renal damage.

Table 2 Effects of ELEBa on the serum hepatic and renal markers in rats exposed to acrylamide

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>AA</th>
<th>AA+ELEBa</th>
<th>AA +ELEBa</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>22.34±0.78</td>
<td>33.57±1.59</td>
<td>23.17±0.16</td>
<td>19.67±0.28</td>
</tr>
<tr>
<td>ALT</td>
<td>13.07±0.96</td>
<td>26.42±1.44</td>
<td>15.74±0.76</td>
<td>11.98±0.76</td>
</tr>
<tr>
<td>GGT</td>
<td>2.35±1.72</td>
<td>5.75±1.23</td>
<td>2.99±0.92</td>
<td>3.01±0.11</td>
</tr>
<tr>
<td>ALP</td>
<td>23.46±5.40</td>
<td>37.26±7.01</td>
<td>28.06±8.53</td>
<td>24.14±7.23</td>
</tr>
<tr>
<td>UREA</td>
<td>6.12±0.07</td>
<td>9.67±0.17</td>
<td>8.64±0.64</td>
<td>6.97±0.17</td>
</tr>
<tr>
<td>CREATININE</td>
<td>1.05±0.03</td>
<td>1.09±0.07</td>
<td>1.12±0.38</td>
<td>0.92±0.18</td>
</tr>
</tbody>
</table>

Legend: Control; = distilled water, AA: = acrylamide, AA+ELEBa = acrylamide and 20mg/kg bodyweight of ELEBa, AA+ELEBa = acrylamide and 250mg/kg bodyweight of ELEBa. Data are expressed using mean ± standard deviation (s.d.); n = 5, a and b mean data are significant at (P < 0.05) as likened to control and acrylamide respectively.

Effects of ELEBa on malondialdehyde (MDA) and reduced GSH concentration in liver and kidney tissues of rats treated with acrylamide

Exposure of experimental rats to acrylamide led to elevation in MDA concentration and decreased GSH concentration significantly in both liver and kidney at the tested dose (figures 2a and 2b). This indicates significant generation of lipid peroxidation and oxidative stress by acrylamide. Simultaneous treatment with ELEBa and acrylamide led to significant depression MDA with elevation of GSH concentrations both in the tissues of liver and kidney (figures 2a and 2b), this is an indication of antioxidative ability of ELEBa.

Effects of ELEBa on the activities of some antioxidant enzymes in liver and kidney tissues of rats treated with acrylamide

Catalase and Glutathione- S- transferase activities decreased significantly following acrylamide treatment in rat liver and kidney tissues when likened to control rats showing the antioxidant depleting activities of acrylamide in tissues. Simultaneous treatment with ELEBa and acrylamide resulted in moderation in these enzymes activities at p < 0.05 level of significance (figure 3a and 3b), a revelation of the capacity of ELEBa to significantly restore depleted antioxidant.
Results of ELEBa on tissues of liver and kidney histology in rats treated with acrylamide

Evaluation of liver and kidney histology revealed that acrylamide caused perturbation in both tissues while administration of ELEBa at the two showed mild pathological alteration (Fig. 4 and 5) revealing ameliorative ability of acrylamide.
Increased after exposure to acrylamide (Toker, 2016; Alwan et al., 2016), this was also observed in the present study. Some acrylamide molecules can form adduct with some vital macro molecules or generate free radicals in the cells, and since these organs have been directly involved in the metabolism, they become highly susceptible to the generated toxicities. Co-administration of ELEBa with ELABs at the tested doses significantly moderated these changes as also confirmed by the photomicrographs of liver and kidney. Cell damage and membrane destruction are regarded as the consequences of lipid per oxidation which is a process elicited via the action of reactive oxygen species (ROS) on highly oxidizable polysaturated fatty acids of the integral part of biological membranes structures. Elevated lipid peroxidation as manifested via increased level of Malondialdehyde (MDA) has been a regular feature of acrylamide exposure (Pan, 2015; Hasinan, 2017), this is due in part to its ability to induce oxidative stress. In the present study, treatment with acrylamide significantly increased liver and kidney MDA contents, which is a measure of lipid peroxidation, it may be an indication of the antioxidant defense mechanisms of breakdown. Treatment with ELEBa at the tested doses upturned these observations leading to a substantial decrease in both organs MDA levels, indicating its modulative impact on oxidative damage created by acrylamide. Glutathione (GSH) is the major soluable non-enzymatic antioxidant which is highly abundant in all cell compartments. It plays a critical role in the metabolism of exogenous substances, it also play an important ROS including lipid peroxides (Livingstone and Davis, 2007). The mopping up of ROS by GSH is done by donating electron (being electron- rich) to peroxide to reduce it to non-toxic metabolites, thereby, preserving macromolecules including lipids from being oxidized. In the present study, reduction in GSH level is observed in the categories of test groups (ELEBa-alone) this may not be disconnected from acrylamide’s ability to generate oxidative stress. Acrylamide is reported to belong to a large chemical class called type-2 alkene (LoPachin et al., 2007a), it is highly electrophilic due to the possession of an alpha double bond that is conjugated and therefore taking part in nucleophilic process together with active nitrogen from the thiol group on glutathione (Friedman, 2003). The reaction of acrylamide with GSH which results in the formation of glutathione S-conjugates which occurs in the metabolism of acrylamide into mercapturic acid or other more water- soluble metabolites with subsequent excretion through urine (Boettcher, 2006). Therefore, acrylamide is detoxified and excreted from the body by conjugation with GSH, hence, the reduction of GSH concentration in this study is possibly, due to depletion of glutathione reserves in order to detoxify acrylamide. This results agrees with earlier reports of (Raju et al., 2013; Batoryna et al., 2017) who reported that there was a resultant lowered level of GSH in various tissues following acrylamide exposure. The observed elevated GSH level in organs of rats exposed to acrylamide and ELEBa may be an indication of ROS-scavenging ability of ELEBa or its ability to increase GSH synthesis. Catalase is one of the enzymatic antioxidants. It performs a critical function in decomposing hydrogen peroxide to water and oxygen thereby reducing the deleterious effect of the so called ree radicals. Decreased catalase activity has been reported to be related to its induced toxicity on the antioxidant system. This study is in agreement with Venkataswamy et al. that accounted for a significantly lower catalase activity following acrylamide exposure (Venkataswamy et al., 2013). Treatment of acrylamide exposed rats with ELEBa at the tested doses resulted in a significant activation of catalase activity. The lethal result of acrylamide treatment in metabolism was demonstrated by observed weakness in rats followed by sores (not show). The observed elevated GSH level in organs of rats exposed to acrylamide and ELEBa may be an indication of ROS-scavenging ability of ELEBa or its ability to increase GSH synthesis.

The study presents results that suggest the antioxidative efficiency of ELEBa in oxidative damage created by acrylamide both in liver and the kidney. Data are however required from future research on the molecular mechanism of anti oxidative efficacy of ELEBa against acrylamide-induced oxidative stress.

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