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

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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 this acrylamide was initially reported to be a probable carcinogen (Lyon, 1994), therefore, its presence in human foods invites a serious attention. From time, acrylamide has wide application in many industries and research laboratories. In line with this, human tend to experience some form of exposure. This exposure to acrylamide 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 Igisu, 2007; Sumizawa and Igisu, 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 (Oyewole and Kalejaiye, 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 ant- 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 (Rodríguez-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 (H_2O_2) 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 72hours first with fine musele 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 -20ºC 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 pelletized 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+E_{100}) - 17.5 mg/kg b.wt AA and 100 mg/kg b.wt ELEBa (as reported by Bamidele *et al*., 2010) Group IV (AA+E_{250})- 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 anesthetic influence of petroleum ether 24hours after the last treatment. Serum was obtained by centrifuging the blood at 3000xg for 10mins. Kidney and Liver tissues were expunged, weighed and then homogenized in 50 mmol/l Tris–HCl buffer (pH 7.4) and then spun at 10000×g for 15 min with table top centrifuge to obtain post mitochondrial fraction for Supernatants were kept frozen at -20^{o}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 viathe 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) formed during lipid peroxidation by using the procedure described by Rice- Evans *et al*., (1986) Ohkawa *et al*., (1979).

Histopathological Analysis

Tissues of the liver and kidney were expunged 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 paraffined tissue sections were collected, and then 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 ethanol 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 1 Analysis of active principles in *Basella alba* (ELEBa) by GC-MS

S/N	Retention time	Compound name	Molecular formula	Molecular weight	Peak area (%)
1	24.789	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	5.03
2	25.978	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	32.94
3	26.272	Cis-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282	19.51
4	29.606	Cis-vaccenic acid	$C_{18}H_{34}O_2$	282	16.29
5	9.953	Oleic acid	$C_{18}H_{34}O_2$	282	9.95
6	30.029	Octadecanoic acid	$C_{18}H_{36}O_2$	284	8.58

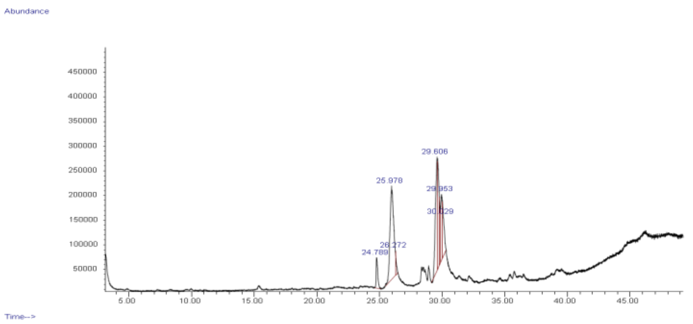

Figure 1 Chromatogram of GC/MS for *Basella alba* (ELEBa)Ethanol Leaf Extract

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

Parameter	Control	AA	AA +E_{100}	AA +E_{250}
AST	22.34±0.78	33.57±1.59[a]	23.17±0.16 [b]	19.67±0.28 [b]
ALT	13.07±0.96	26.42±1.44 [a]	15.74±0.76 [b]	11.98±0.76 [b]
GGT	2.35±1.72	5.75 ±1.23 [a]	2.99.0.92 [b]	3.01±0.11 [b]
ALP	23.46±9.40	37.26±7.01[a]	28.06±8.53 [b]	24.14±7.23
UREA	6.12±0.07	9.67±0.17 [a]	8.64±0.64 [a]	6.97 ±0.17 [b]
CREATININE	1.05±0.03	1.09 ±0.07	1.12 ± 0.38	0.92±0.18

Legend: Control: = distilled water, AA: = acrylamide, AA+E_{100}: +acrylamide and 20mg/kg bodyweight of ELEBa, AA+E_{250}: + 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.

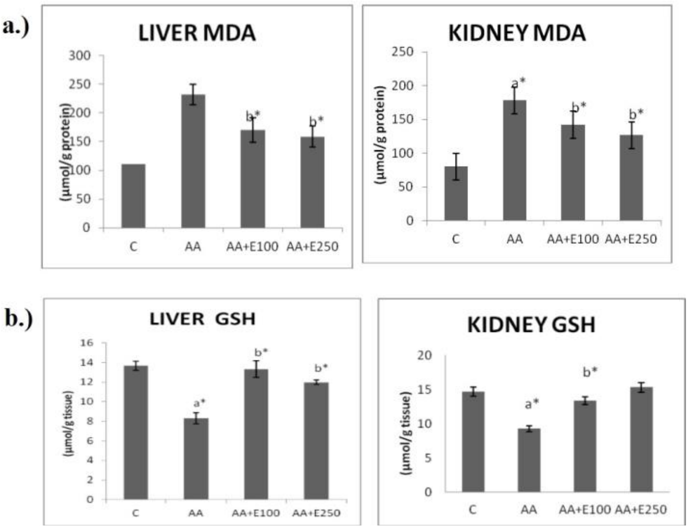

Figure 2 (a) Results of *Basella alba* (ELEBa) ethanol leaf extract on level of MDA in liver and kidney tissues of models exposed to acrylamide. MDA: malondialdehyde, (b) Results of *Basella alba* (ELEBa) ethanol leaf extract on level of GSH in liver and kidney tissues of models exposed to acrylamide. C: control animals given distilled water, AA: = acrylamide, AA+E_{100}: +acrylamide and 20mg/kg bodyweight of ELEBa, AA+E_{250}: + acrylamide and 250 mg/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.

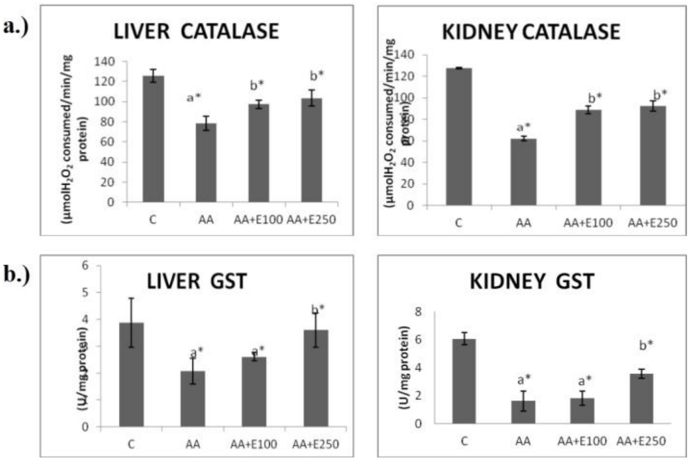

Figure 3 (a) Results of *Basella alba* (ELEBa) ethanol leaf extract on activities of catalase in liver and kidney tissues of models exposed to acrylamide. (b) Results of *Basella alba* (ELEBa) ethanol leaf extract on activities of GST in liver and kidney tissues of models exposed to acrylamide. GST: Glutathione S Transferase, C: control animals given distilled water, AA: = acrylamide, AA+E_{100}: +acrylamide and 20mg/kg bodyweight of ELEBa, AA+E_{250}: + acrylamide and 250mg/kg bodyweight of ELEBa . Data are expressed using mean ± standard deviation (SD); n =5. a and b mean data are significant at (P < 0.05) as likened to control and acrylamide respectively.

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.

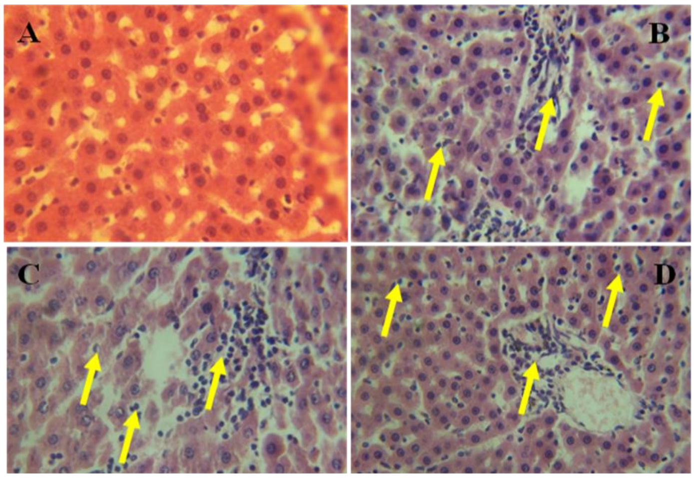

Figure 4 The photomicrograph results of *Basella alba* (ELEBa) ethanol leaf extract on the liver of models exposed to acrylamide (17.5mg/kg.bw) (Mag x400). (A) Control (distilled water): showing no visible lesion, (B) Acrylamide (17.5mg/kg.bwt): showing moderate mononuclear infiltration of periportal cells (arrows). (C) Acrylamide (17.5mg/kg.bwt) and ELEBa (100mg/kg bwt) showing very mild mononuclear infiltration of periportal cells (arrows). (D) Acrylamide (17.5mg/kg.bwt) and ELEBa (250mg/kg bwt): showing very mild mononuclear infiltration of periportal cells (arrow). H&E stain at 400x Magnification.

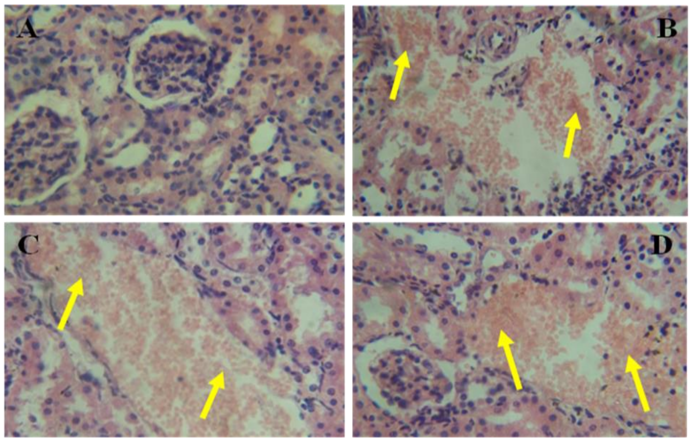

Figure 5 The photomicrograph results of *Basella alba* (ELEBa) ethanol leaf extract on the kidney of models exposed to acrylamide (Mag x400). (A) Control (distilled water): showing no visible lesion, (B) Acrylamide (17.5mg/kg.bwt): showing marked renal cortical congestion and interstitial cellular infiltration (arrow). (C) Acrylamide (17.5mg/kg.bwt) and ELEBa (100mg/kg bwt): showing mild renal congestion (arrow), (D) Acrylamide (17.5mg/kg.bwt) and ELEBa (250mg/kg bwt): showing very mild renal congestion (arrow). H&E stain at 400x Magnification.

DISCUSSION

Production of oxidative stress is counted to be part of the consequence of exposure to acrylamide in experimental animals. Reports abound on the elevated level of free radicals with significant decreased level of antioxidants as a result of this exposure **(Venkataswamy *et al.*, 2013)** Several reports also have indicated the modulation of acrylamide- induced toxicities by antioxidants which buttress the contribution of oxidative stress in its induced toxicities **(Adewale *et al.*, 2015).** The various health benefits offered by plants are tremendous, and these have been in a way linked to the several phytochemicals which they possess since secondary metabolites and other constituents in plants have been reported to be responsible for their medicinal properties **(Varadarajan *et al.*, 2008; Manubolu *et al.*, 2014; Goodla *et al.*, 2019).** Initial information about the protective effect of *Basella alba* against oxidative stress in rat exists **(Bamidele *et al.*, 2015)**, however, the anti oxidative effect of *Basella alba* against acrylamide-induced oxidative stress has not been reported hitherto. In the current study, the potential ameliorative effect of ELEBa was assessed in acrylamide induced toxicities. Both the liver and the kidney perform key roles in detoxification of body harmful substances, converting them to less harmful substances or breaking them down before they get excreted out of the body. Several drug effects on these tissues emphasize their importance in the metabolism of exogenous substances. Acrylamide could be metabolized into a highly potent intermediate called glycidamide **(Calleman *et al.*, 1990; Sumner *et al.*, 1992)**, which can undergo further conjugation reaction during phase two metabolism to less toxic more water- soluble metabolites that are excreted from the body in bile via the liver or in urine via the kidney **(Airman *et al.*, 2003).** Both liver and kidney function bio markers have been reported to be significantly 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 with ELEBa 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 polyunsaturated fatty acids that constitute 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; Hasanin, 2017),** this is due in part to its ability to induce oxidative stress. In the current study, treatment with acrylamide significantly increased liver and kidney MDA concentrations signifying the induction of lipid per oxidation as a consequence 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 soluble non-enzymatic antioxidant which is highly abundant in all cell compartments. It plays a critical role in the metabolism of exogenous substances, it also precisely mops-up 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 animals exposed to acrylamide, this may not be disconnected from acrylamide's ability to generate oxidative stress. Acrylamide is reported to belong a large chemical class called type-2 alkenes **(LoPachin *et al*., 2007a)** , it is highly electrophilic due the possession of an alpha double bond that is conjugated and therefore taking part in nucleophilic process together with active nitrogen functional groups these include: 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 suggested to be related to some pathophysiological conditions **(Krolow, 2014),** therefore, decrease activity of catalase after acrylamide treatment may 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 adjustment in activity of catalase.

The lethal result of acrylamide treatment in metabolism was demonstrated by observed weakness in rats followed by sores (not shown) on some of them during the treatment period and co-treatment with ELEBa at the tested doses modulated these observations. This toxicity of acrylamide may not be unrelated to the activity of its active metabolite, glycidamide which is a product from epoxidation reaction of acrylamide **(Calleman *et al*., 1990; Sumner *et al*., 1992)** and is capable of forming adducts with essential cellular macromolecules and as a result inducing oxidative damage .

GC/MS characterization of ELEBa clearly revealed six peaks corresponding to six unsaturated fatty acids. These compounds in ELEBa have been reported for their abilities to maintain growth and reduce the risk of diseases (**Tapiero *et al*., 2002)** and hence, suggested to be part of the explanation for the use of *ELEBa as* protective plant against risk of diseases.

Basella alba is a very important plant with high nutritional capacity. It is considered an excellence basis of vitamin C, vitamin A, magnesium, folic acid and calcium, **(Duke and Ayenshu, 1985; Palada and Crossman, 1999).** It is established to likewise possess many primary metabolites, ash, fibre, calcium, some vitamin B complex **(Grubben *and* Denton, 2004).** Reports have also revealed its *in vitro* antioxidant capacity such free radical and metal ion mopping activities, with ability to inhibit peroxidation **(Reshmi *et al*., 2012b; Anusuya *et al*., 2012)** which make it a good candidate for medicinal purposes. Many compounds including *Basella* saponinins A-D **(Toshiyuki, *et al*., 2001),** Beta-carotene- (Greuter and Raus, 2006), Bioflavonoid (Rutin)- **(Khare, 2007**) ,Gomphrenin I-III (**Glassgen *et al*., 1993; Lin *et al*., 2010)**, have similarly been extracted from various portions of *Basella alba* and many of these have been reported for their antioxidant activities. This study have also been able to report newly isolated fatty acids in the ELEBa. The antioxidative effects exhibited by ELEBa against acrylamide –induced oxidative stress may not be unrelated to the anti oxidative effects of some or combination of the isolated active compounds. Further studies are however suggested at the molecular level to evaluate antioxidative results of ELEBa on oxidative stress generated by drugs.

CONCLUSION

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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