Allium Cepa Ameliorates Ethanol-Induced Gastric Injury in Rats Via Reduction in Gastric Neutrophils Infiltration

1*S.F. Ige, A. O. 1Oguntade and 2 T. Olatunji
1Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.
2Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Oyo state, Nigeria
[Corresponding author: Email: funkeige2006@yahoo.com; sfige@lautech.edu.ng; +2348060743616]

ABSTRACT
Neutrophil-derived oxygen free radicals have been implicated in the aetiology of gastric tissue damage. In this study, the ameliorative effects of Allium cepa on neutrophil infiltration, lipid peroxidation and antioxidant enzyme activities in the ethanol-induced gastric injury were examined. Twenty four adult male Wistar rats were used for this study and divided into four groups; Control, Allium cepa, Allium cepa + Ethanol and Ethanol. Allium cepa was administered (1mL/100g body weight) daily for twenty eight days while 0.2mL of 98% (v/v) ethanol per 23g Body Weight was used to induced gastric damage. Macroscopic measurement of ulcer area, histological examination and biochemical analyses (Malondialdehyde level, Myeloperoxidase (index of neutrophil accumulation), Superoxide dismutase (SOD) and Catalase (CAT) activities) were carried out in plasma and gastric tissue. Ethanol administration significantly (p<0.05) increased ulcer score and ulcer index, decreased percentage ulcer inhibition, increased MDA and MPO, decreased SOD and CAT activities. Histological findings show glandular destruction in the gastric mucosa and infiltration of inflammatory cells in ethanol only group. These effects were ameliorated with Allium cepa pre-treatment. The results obtained from this study demonstrate the ameliorative effect of Allium Cepa on ethanol-induced gastric injury by reduction in gastric neutrophils infiltration and increased antioxidant activities.

Keywords: Gastric, Allium Cepa, Neutrophil, Myeloperoxidase

INTRODUCTION
Gastric injury is one of major diseases affecting 5–10 % of people during their life time (Sumbul et al., 2011; Al - Asmari et al., 2016) owning to the fact that the stomach plays major role in the gastrointestinal functions (O’Connor and O’Moráin, 2014). It is opened to wide range of injurious substances such as gastric acid, Helicobacter pylori, non-steroidal anti-inflammatory drugs and excessive alcohol (Mowat et al., 1997) which affect the gastric tissue layers.

Reactive oxygen species (ROS) have been implicated in the aetiology of gastrointestinal pathologies, which include gastritis and gastric ulcers (Oliveira et al., 2003; Onasanwo et al., 2011). Characteristic features of gastric injury have been exemplified and it include decreased tissue blood flow, tissue death, neutrophil (2014; Alqasomi, 2015). The use of folk medicine (phytotherapy) has a great therapeutic efficacy and it is affordable.

infiltration, inflammatory mediator release and oxidative stress (Viana et al., 2013). Production of reactive oxygen species and lipid peroxidation have been known to correlate with tissue neutrophil infiltration which has a major association with the degree of tissue damage resulting from ethanol induced gastric tissue damage (Ozer et al., 2008). Excessive ethanol intake resulted in marked destruction of the gastric tissue (Guslandi, 1987), thus ethanol-induced gastric tissue damage in animal models is widely used to assess the protective ability of majority of drugs used in the treatment of gastric injuries (Hajrezaie et al., 2015; Al - Asmari et al., 2016). Meanwhile, much has been reported about the benefits of natural products of herbs in conferring gastro protective benefits owning majorly to their antioxidant properties (Wallace, 2013; Kangwan et al., 2016).

Allium cepa commonly known as “onion” is a member of Liliacea species and is one of the important vegetables consumed daily. Its...

available online at http://www.ajol.info/index.php/njbas/index
Nigerian Journal of Basic and Applied Science (December, 2016), 24(2): 01-08
DOI: http://dx.doi.org/10.4314/njbas.v24i2.1

ISSN 0794-5698

Full Length Research Article

NJBAS

Allium Cepa Ameliorates Ethanol-Induced Gastric Injury in Rats Via Reduction in Gastric Neutrophils Infiltration

1*S.F. Ige, A. O. 1Oguntade and 2 T. Olatunji
1Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.
2Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Oyo state, Nigeria
[Corresponding author: Email: funkeige2006@yahoo.com; sfige@lautech.edu.ng; +2348060743616]
antioxidant properties had been established and ascribed to its constituents such as flavonoids, anthocyanins and dihydroflavonols (Griffiths et al., 2002; Slimestal et al., 2007). *Allium cepa* is beneficial in the treatment of heart diseases, cancers and also diabetes (Formica and Regelson, 1995; Taj Eldin et al., 2010). Its aqueous suspension alleviates gastric tissue damage (Alqasomi, 2015). However, its role in generation of reactive oxygen species, lipid peroxidation and neutrophil infiltration in gastric tissue is yet to be elucidated. Therefore, this study was aimed at investigating the effects of *Allium cepa* on gastric neutrophils infiltration in ethanol-induced gastric injury since neutrophils have been implicated in the aetiology of gastric tissue damage.

MATERIALS AND METHODS
Experimental animals
Twenty four male Wister rats (with weight ranging from 150-200g) were used for this study. The animals were acclimatized for 2 weeks in animal Holdings of the Department of Physiology, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria. Animals were cared for according to United States National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH publication No 85-23) and allowed free access to animal feed and water. The animals were grouped into four and treated as follows; Group one served as control, *Allium cepa* group were treated with *Allium cepa* juice (1.0 ml/ 100g body weight) for 28 days. *Allium cepa* + ethanol group were also treated as *Allium cepa* group but also administered 0.2ml of 98% (v/v) ethanol per 23g body weight and then sacrificed 4 hours later on the 28th day. Ethanol group were administered 98% (v/v) ethanol (0.2ml/ 23g body weight) four hours before sacrifice on the 28th day of the experiment.

Preparation of *Allium cepa* juice.
*Allium cepa* juice was prepared as earlier reported (Ige and Akhigbe, 2013). Briefly, *Allium cepa* bulbs were purchased from local market in Ogbomosho, Oyo state, Nigeria. They (200g) were peeled and rinsed thoroughly with distilled water and air dried and then blended. The juice was then filtered using a sterile and tight sieve. The juice was prepared daily.

Induction of Gastric Injury
Induction of gastric injury was done by administration of (0.2ml / 23gBW of 98 % (v/v) ethanol) after the animals have been fasted for 36 hours according to Ige et al., (2012a).

Evaluation of Gastric tissue damage
Stomachs were opened along greater curvature, rinsed in normal saline and examined using magnifying lens (Hi-Tech Zone, Ningbo, Zhejiang, China). The degree of gastric damage was scored according to Praveen and Paradhasaradhi, (2013) as follows; normal coloured stomach = 0; red colouration = 0.5; spot ulcer = 1; haemorrhagic streak = 1.5; deep ulcers = 2; perforation = 3. The overall total scores divided by 10 was designated the ulcer index. The percentage ulcer inhibition was calculated as described by Anosike et al. (2013) using the formula percentage ulcer inhibition (%UI) = (1- Ul/Uc) x 100 was used. The gastric wall thickness was measured with the help of a vernier calliper (Mono-Block, Wuxi Numit, Jiefandanglu, Wuxi, Jiangsu. P.R.China).

Preparation of Samples for Biochemical analyses.
The animals were sacrificed and their blood was collected by cardiac puncture and centrifuged at 3,000 r.p.m high speed refrigerated centrifuge (Gulfex Medical and Scientific, England) the plasma collected was used for biochemical analyses which included superoxide dismutase (SOD), malondialdehyde (MDA), and catalase (CAT). Stomachs were harvested and divided into two. A part was homogenized in phosphate buffer (pH 7.0) and centrifuged at 4000 r.p.m for ten minutes and the supernatants used for biochemical analyses namely, superoxide dismutase (SOD), malondialdehyde (MDA), and catalase (CAT). The other part was homogenized in phosphate buffer (pH 6.0) for the determination of myeloperoxidase (MPO) activity according to Rao et al., (2003). Plasma and tissue SOD,
CAT and MDA were carried out as previously described (Ige et al., 2012b).

**Histological processing and examination**
Histological section was done according to the method of Ogihara and Okabe, (1993). Small portions of the stomach tissues were collected and fixed in 10% formalin for studying its histo-architecture. After fixing, it was cut into 5µm sections, and stained with haematoxylin and eosin stain.

**Statistical analysis**
Statistical analyses were performed with Graph Pad Prism 5 statistical package. All values were reported as mean ± S.E.M. Data were analysed by using a one – way analysis of variance (ANOVA) followed by unpaired Student’s t-test. Values of P < 0.05 were considered statistically significant.

**RESULTS**

**Effect of Allium cepa on Gastric tissue damage**
Administration of ethanol resulted in gastric injury with manifestation of ulcer and increase in thickness of the gastric wall; treatment with Allium cepa juice significantly ameliorated these effects of ethanol (Table 1).

**Effect of Allium cepa juice on Antioxidant status of plasma and Gastric tissue**
There was a significant increase in MDA level and significant decrease in SOD and catalase activities in the plasma and gastric tissue of animals given ethanol alone, pre-treatment with Allium cepa improving the lipid peroxidation status in the plasma and the gastric tissue by significantly increasing the SOD and catalase activities and decreasing the MDA level (Tables 2 and 3).

**Table 1: Effect of Allium cepa on Ulcer score, Ulcer index, percentage ulcer inhibition and stomach wall thickness**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ulcer score</th>
<th>Ulcer index</th>
<th>% Ulcer inhibition (%)</th>
<th>Gastric wall thickness(mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>—</td>
<td>1.67±0.11</td>
</tr>
<tr>
<td>Allium cepa</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>100</td>
<td>1.67±0.11</td>
</tr>
<tr>
<td>Allium cepa + Ethanol</td>
<td>0.50±0.13**</td>
<td>0.06±0.01**</td>
<td>62.5</td>
<td>3.17±0.11**</td>
</tr>
<tr>
<td>Ethanol</td>
<td>1.60±0.19*</td>
<td>0.16±0.02*</td>
<td>0</td>
<td>4.0±0.12*</td>
</tr>
</tbody>
</table>

* p<0.05 vs. ethanol *p<0.05 vs. Control.

**Table 2: Effect of Allium cepa juice on Plasma Antioxidant status**

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD(U/g)</th>
<th>CAT(U/mg)</th>
<th>MDA(µmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.64±0.04</td>
<td>0.39±0.04</td>
<td>0.31±0.02</td>
</tr>
<tr>
<td>Allium cepa</td>
<td>1.73±0.04*</td>
<td>0.49±0.04**</td>
<td>0.27±0.03*</td>
</tr>
<tr>
<td>Allium cepa + ethanol</td>
<td>1.64±0.06*</td>
<td>0.41±0.03*</td>
<td>0.32±0.02</td>
</tr>
<tr>
<td>Ethanol</td>
<td>1.31±0.06*</td>
<td>0.30±0.03*</td>
<td>0.34±0.03*</td>
</tr>
</tbody>
</table>

* p<0.05 vs. ethanol *p<0.05 vs. Control.

**Table 3: Effect of Allium cepa juice on Antioxidant status in Gastric tissue.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD(U/g)</th>
<th>CAT(U/mg)</th>
<th>MDA(µmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.59±0.07</td>
<td>0.36±0.04</td>
<td>0.38±0.05</td>
</tr>
<tr>
<td>Allium cepa</td>
<td>1.73±0.04*</td>
<td>0.43±0.04*</td>
<td>0.33±0.06</td>
</tr>
<tr>
<td>Allium cepa + ethanol</td>
<td>1.62±0.06*</td>
<td>0.34±0.04*</td>
<td>0.83±0.10**</td>
</tr>
<tr>
<td>Ethanol</td>
<td>1.35±0.06*</td>
<td>0.23±0.03*</td>
<td>1.19±0.01*</td>
</tr>
</tbody>
</table>

* p<0.05 vs. ethanol *p<0.05 vs. Control.
Effect of Allium Cepa on Myeloperoxidase activity of ethanol-induced gastric injury
Administration of ethanol significantly (p<0.05) increased the MPO activity when compared with the control, but there was no significant (p>0.05) difference in MPO activity in the Allium cepa group when compared with control. MPO activity was significantly (P<0.05) higher in Allium cepa + ethanol group when compared with the control. The MPO activity of the Allium cepa + ethanol group was significantly lower (p>0.05) compared to the ethanol group (Figure 1).

*Figure 1: Effect of Allium cepa Juice on Myeloperoxidase Activity in Ethanol-induced Gastric Injury in Rats*

Effect of Allium cepa juice on the histo-architecture of the gastric tissues
Histological examination show glandular destruction in the mucosa, fussed external muscle and muscularis mucosa, oedema and infiltration of inflammatory cell in ethanol group, while Allium cepa juice ameliorated the effect of ethanol, thus maintaining the cyto-architecture by decreasing the degree of inflammatory cells infiltration. (A) Control group rat with intact epithelial surface with normal mucosa, submucosa and muscularis layers. (B) Allium cepa group rat with no histological modifications in the tissue layers. (C) Allium cepa + ethanol group rat showing mild enlargement of the epithelia, mild glandular destruction and mild infiltration of inflammatory cells. (D) Ethanol group rats showing glandular destruction in the mucosa, fussed external muscle and muscularis mucosa, edema and infiltration of inflammatory cell. (black arrow, mucosa; yellow arrow, inflammatory cell).

DISCUSSION
This study seeks to elucidate the preventive effects of Allium cepa juice on ethanol – induced gastric injury in rats. This study finds that treatment with ethanol resulted in gastric injury and this is in agreement with previous studies (Szabo et al., 1991; Ige et al., 2012a). The gastric injury produced was characterized by ulcer, gastric neutrophil infiltration and decreased gastric antioxidant activities. This study also reveals that pre-treatment with Allium cepa significantly decreased gastric damage caused as a result of ethanol administration and increased the percentage ulcer inhibition. Not only that, it significantly decreased the MDA which is a marker for involvement of Allium cepa in the inhibition of gastric lipid peroxidation.
Gastric injury pathogenesis has been known to involve neutrophils (Wallace et al., 1996). Injury produced by neutrophils occurs as a result of their capacity to produce Reactive Oxygen Species (ROS) (Wallace et al., 1992). Study by Kozol et al., (1994) implicated superoxide in neutrophil-mediated gastric injury. This present study demonstrates that Allium cepa increased gastric antioxidant activities, which is important in the body’s defense system (Dhanprakash and Garima, 2007; Hwan et al., 2011), as earlier reported (Ige et al., 2011; Ige et al., 2012b). Likewise Superoxide dismutase (SOD), and catalase (CAT) are part of cellular enzymes that regulate reactive oxygen species such as superoxide, hydroxyl radicals and H$_2$O$_2$ (Buffinton and Doe, 1995; Furrie et al., 2004). This study finds that treatment with ethanol resulted in decrease gastric antioxidant activities and this is in agreement with previous studies (Liu et al., 2012; Arab et al., 2015). This reduction was ameliorated as a result of pre-treatment with Allium cepa, this is in consonance with the previous findings where Allium cepa has been shown to improve oxidative status (Ige et al., 2011, Ige et al., 2012b).

Studies have established the generation of free radicals and the resultant lipid peroxidation in ethanol gastric damage, with ethanol as the pathogenesis (Kvietsys et al., 1990; Salim, 1990). Ethanol has also been shown to induce vasoconstriction, followed by sharp arteriolar dilation, thus leading to reperfusion (oxygen-radicals generation due to ischemic reperfusion resulted in cellular destruction), (Glavin and Szabo, 1992). Inflammatory mediators which are linked to granulocyte and proteases are released, thus invoking cascade of activities leading to gastric damage (Tesyssen and Singer, 2003). This occurred due to the production of reactive oxygen species. From the present study, Allium cepa increased the gastric antioxidant activity which resulted in reduction in reactive oxygen species from the neutrophils, hence reducing the tissue damage.
Malondialdehyde level was examined in gastric tissue as marker of gastric lipid peroxidation in this study, so as to establish the level of tissue damage. MDA was significantly increased with ethanol treatment which confirmed the increase in the tissue damage, this was significantly reduce with Allium cepa pre-treatment. The significant decreases in MDA with Allium cepa pre-treatment confirm its role in ameliorate gastric damage.

Activation of neutrophil leads to generation of reactive metabolites of oxygen (Bilici et al., 2002) and increased myeloperoxidase activities which results in tissue damage (Granger, 1988). In this study, MPO was used to assess the degree of neutrophil infiltration (Xing et al., 2013; Al - Asmari et al., 2016). MPO activity was found to significantly decrease as suggested by our result consequent to pre-treatment with Allium cepa.

Histological examination of the gastric tissue also reveals glandular destruction in the gastric mucosa and inflammatory cells infiltration in ethanol group. This is in agreement with previous studies (Ige et al., 2012a; Al - Asmari et al., 2016), where administration of ethanol resulted in inflammatory cells infiltration and loss of glandular cells in the gastric mucosa. Pre-treatment with Allium cepa juice reduced these effects and this is in consonance with previous studies (Ige et al., 2011; Ige et al., 2012b), where Allium cepa has been shown to ameliorate tissue injury.

The MPO and histological results obtained from this study substantiated the fact that there was a significant decrease in the infiltration of neutrophil due to pre-treatment with Allium cepa. This study demonstrates the ameliorative effect of Allium Cepa in ethanol-induced gastric injury probably by reduction in gastric neutrophils infiltration and increased antioxidant activities.

REFERENCES


Ige et al.: Allium Cepa Ameliorates Ethanol-Induced Gastric Injury in Rats Via…………


