Introduction

Excessive consumption of alcohol causes the liver to become fatty. Fatty liver is the accumulation of triglyceride (TG) and other fats in the liver cells. This condition makes the liver vulnerable to inflammation and varieties of disorders such as alcoholic hepatitis, fibrosis, cirrhosis, and even hepatocellular carcinoma and its associated complications. In recent years, there has been an escalation in alcohol abuse, and inevitably alcohol-related disorders are becoming an increasingly important cause of morbidity and mortality. [1]
Increased intake of alcohol damages mitochondria, endoplasmic reticulum, and other cellular structures, which contributes to the inhibition of fatty acid oxidation. The amount of fatty acid in the liver depends on the balance between the processes of delivery and removal\(^2\).

The liver is the principal site for alcohol metabolism. Alcohol in the liver causes a shift in metabolic pathways and could result in a buildup of fatty acids. Fatty acid overload in hepatocytes acts as both a substrate and an inducer of microsomal cytochrome P-450 (CYP) 2E1 and fatty acid oxidation systems that generate reactive oxygen species resulting in oxidative stress\(^3\). Increasing evidence indicates that multiple mechanisms contribute to the development of the alcoholic liver diseases (ALD), involving oxidative stress, inflammation, excess lipid synthesis, as well as complex interactions between alcohol metabolism, lipid metabolism and the immune system\(^4,5\).

Recently, researchers are working on therapeutic modalities to halt or reverse the pathogenesis and progression of ALD. Previously performed clinical and experimental studies have demonstrated that oils from different plant sources work as a scavenger of various reactive oxygen species, including superoxide anion and hydroxyl radicals. The possible mechanism(s) of their action may be due to free radical-scavenging potential caused by the presence of antioxidant component(s) in the oils\(^6-8\).

With the current resurgence of medicinal herbs in the world, some natural compounds isolated from plants, seeds or fruits have been used to treat alcoholic fatty liver. *Zingiber officinale* and *Curcuma longa* are of such plants having both medicinal and nutritive values, and popularly used as herbal remedy against a wide range of ailments, both in Nigeria and several other countries in the world\(^9\).

*C. longa* is a rhizomatous perennial herb that belongs to family Zingiberaceae. It is a tropical plant and is the source of the spice turmeric, which is derived from the dried, ground rhizome. It is extensively grown and used as dietary pigment and spices. *C. longa* possesses antioxidant, antitumor, antimicrobial, anti-inflammatory, wound healing, lipid-reducing, chemopreventive, immunomodulatory, and gastroprotective activities and all these are well documented\(^10-13\).

*Z. officinale*, commonly known as ginger, belongs to family Zingiberaceae. It is one of the most commonly used spices in Nigeria and around the world; it is an indispensable component of curry\(^13\). It has long been used to treat gastrointestinal disorders and its constituents have shown anti-inflammatory, chemopreventive, antidiabetic, antihypertoxic and antioxidant properties\(^14-17\).

Though many documented reports have implicated the rhizomes of *Z. officinale* and *C. longa* in folk medicine and herbal preparations for treatment of liver disorders, this informed our decision to extract the oils, evaluate antioxidant properties of the oils and study the effect of the oils in acute ethanol exposure in rats and we have compared our results with commercial multivitamin standard drug Livolin Forte\(^8\).

2 Materials and methods

2.1 Experimental animals

Thirty male albino rats procured from the animal house of the Biochemistry Department, College of Medicine, University of Ibadan, Nigeria, weighing between 120-200 g were randomly distributed into five groups of six animals each. The animals were acclimatized to standard laboratory conditions and were allowed free access to standard rat chow (Ladokun Feed, Ibadan, Nigeria) and water was given *ad libitum* for two weeks. Group I served as normal control and was given corn oil (200 mg/kg). Group II was a positive control given only 45% ethanol (4.8 g/kg). Group III received *Z. officinale* oil (200 mg/kg) and 45% ethanol (4.8 g/kg). Group IV received *C. longa* oil (200 mg/kg) and 45% ethanol (4.8 g/kg). Group V was given standard drug (Livolin Forte) (20 mg/kg) and 45% ethanol (4.8 g/kg). Rats were pretreated by oral gavage for 28 d with standard drug (Livolin Forte) and oils from *Z. officinale* and *C. longa* before they were exposed to 45% ethanol (4.8 g/kg) by oral gavage to induce acute fatty liver. Animals were sacrificed after 24 h of ethanol exposure. Animal experiments followed protocols established by National Institute of Health for the Care and Use of Laboratory Animals.

2.2 Plant collection and extraction

The rhizomes of *Z. officinale* and *C. longa*, were purchased from Ibole, Oja oba market, Ibadan, Nigeria. They were identified and authenticated in the Botany Department, University of Ibadan, Nigeria. One kilogram of each of the plant materials was air-dried at room temperature, grounded into powder and Soxhlet extracted using n-hexane for 72 h. Oils were stored in sample bottles and refrigerated until needed.

2.3 Ferric reducing antioxidant power activity

The ferric reducing antioxidant power (FRAP) assay of oils from *Z. officinale* and *C. longa* was done on a multiscan spectrum plate reader by using method developed by Benzie and Strain\(^18\). This assay uses antioxidants as reductants in a redox-linked colorimetric method which measures the reduced oxidant at low pH (pH = 3.6). Ferric tripyridyltriazine (Fe\(^{III}\)-TPTZ) complex is reduced to the ferrous form, forming an intense blue color with an absorption maximum at 593 nm. The change in absorbance is directly related to the reducing power of the electron-donating antioxidants present in the reaction mixture.

2.4 Oxygen radical absorbance capacity

The oxygen radical absorbance capacity (ORAC) of *Z.
C. longa and Z. officinale oils was determined using fluorescein
C₂₃H₂₁NO₄ as the fluorescent probe and 2,2'-azobis (2-methyl-
propionamidine) dihydrochloride (AAPH) as the peroxyl
determined by the method of Misra and Fridovich
centrifugation at 3 000×
for half an hour and the serum was obtained by blood
puncture allowed to coagulate at room temperature
C. longa, the animals were sacrificed 24 h after ethanol
drug (Livolin Forte) and oils from
Z. officinale
histological examination
2.6 Preparation of tissues for biochemical analyses and
histological examination
Following the daily treatment for 28 d with standard
drug (Livolin Forte) and oils from Z. officinale and
C. longa, the animals were sacrificed 24 h after ethanol
exposure. Blood samples were collected by retro-orbital
puncture allowed to coagulate at room temperature
for half an hour and the serum was obtained by blood
centrifugation at 3 000×g for 10 min and kept at 20 °C
until analyses were done. Liver samples were quickly
excised and washed in ice-cold 1.15% KCl solution, dried
using filter paper and weighed. They were then homogenized
in 4 volumes of 56 mmol/L Tris-HCl buffer (pH 7.4)
containing 1.15% KCl, and then centrifuged at 10 000×g
for 15 min.
The supernatant was collected and stored until needed
for assays. Small pieces of liver sections were fixed in
10% formal saline. Sections were cut and stained with
haematoxylin and eosin. The stained tissue sections
were observed under a light microscope for histological
assessment.
2.7 Statistical analysis
All values were expressed as the mean ± standard error
of mean of six animals. Data were analyzed using one-
way analysis of variance followed by the post-hoc LSD-
t test for analysis of biochemical data using SPSS (10.0)
statistical software. P < 0.05 was considered statistically
significant.

3 Results

3.1 FRAP activity and ORAC
Prior to the commencement of the animal experiment,
FRAP and ORAC activities of the oils from Z. officinale
and C. longa were determined. FRAP values obtained from
the result showed that Z. officinale oil ((976.91±1.32) µmol
AA/g) possessed the highest reducing power while C. longa
oil possessed the lowest reducing power ((113.10±5.67) µmol
AA/g). The ORAC value of the oils determined also
showed that Z. officinale oil ((2 649.00±0.91) µmol
TE/g) possessed a higher ORAC value than C. longa oil
((587.00±0.78) µmol TE/g).

3.2 Effects of Z. officinale and C. longa oils on the
marker of LPO in the liver, and serum TC and TG
levels
The effects of Z. officinale and C. longa oils on the marker
of LPO in the liver are presented in Table 1. Malondialdehyde
(MDA) level of ethanol-treated rats (Group II) was significantly
(P<0.05) higher than those of the normal control
rats (Group I). The increase observed in the MDA level of
the ethanol-treated rats was significantly (P<0.05) reduced
as a result of pretreatment with the standard drug (Livolin
Forte) and oils from Z. officinale and C. longa. In like manner,
significant (P<0.05) increases noted in serum TC and TG
levels of ethanol-treated rats (Group II) when compared
with Group I, were significantly (P<0.05) reduced as a
result of pretreatment with the standard drug (Livolin
Forte) and oils from Z. officinale and C. longa.

3.3 Effects of Z. officinale and C. longa oils on hepatic
GSH level, and SOD and GST activities
GSH level, and SOD and GST activities in liver homogenate
were significantly (P<0.05) decreased in ethanol-treated
rats (Group II) compared with the normal control (Group
I). Moreover, in the pretreated groups (Groups III, IV and V),
GSH level, and SOD and GST activities in liver
homogenate were significantly (P<0.05) lower than that of
the ethanol-treated rats (Group II). Group IV rats
showed significantly (P<0.05) higher GSH level, and SOD
and GST activities than corresponding GSH level, and SOD
and GST activities in Groups III and V rats (Table 2).

3.4 Effects of Z. officinale and C. longa oils on liver
marker enzymes
The effects of Z. officinale and C. longa oils on liver
marker enzymes are presented in Table 3. Exposure to
ethanol caused abnormal liver function in ethanol-treated
rats (Group II). The levels of serum hepatospecific enzymes
such as ALP, ALT and AST were significantly (P<0.05)
increased when compared with the normal control (Group I). It was observed that the elevated levels of ALP, ALT and AST were significantly ($P<0.05$) lowered in the pretreated groups (Groups III, IV and V) when compared with the ethanol-treated rats (Group II).

### 3.5 Histological analysis of liver sections

The results of the histological assessment of the liver section are presented in Figure 1. Group I showed normal liver architecture, with no visible lesions seen. There were marked portal congestion and cellular infiltration by mononuclear cells in Group II. Group III was characterized by periportal hydropic degeneration of hepatocytes with cellular infiltration by mononuclear cells. Mild portal congestion and mononuclear cellular infiltration with mild hydropic degeneration were observed in Group IV while in Group V, there were mild portal congestion, mild hydropic degeneration and few foci of cellular infiltration by mononuclear cells.

### 4 Discussion

Many pathways have been suggested as playing a key role in how alcohol induces oxidative stress. Toxic substances generated during the metabolism of alcohol in the liver may contribute greatly to the development of ALD. Importantly, oxidation of alcohol through the CYP 2E1 generates superoxide anion radical and hydrogen peroxide. These free radicals are capable of damaging many cellular components such as DNA, protein, and lipid$^{[24-27]}$.

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>MDA in liver tissue (nmol/g)</th>
<th>Serum TC (mg/dL)</th>
<th>Serum TG (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (corn oil 200 mg/kg)</td>
<td>6</td>
<td>2.89±0.08</td>
<td>3.59±0.09</td>
<td>4.13±0.05</td>
</tr>
<tr>
<td>Group II (45% ethanol)</td>
<td>6</td>
<td>3.36±0.12$^*$</td>
<td>4.82±0.06</td>
<td>9.35±0.02$^*$</td>
</tr>
<tr>
<td>Group III (Z. officinale oil 200 mg/kg + 45% ethanol)</td>
<td>6</td>
<td>2.39±0.10$^{△△}$</td>
<td>3.94±0.07$^{△△}$</td>
<td>3.88±0.04$^{△△}$</td>
</tr>
<tr>
<td>Group IV (C. longa oil 200 mg/kg + 45% ethanol)</td>
<td>6</td>
<td>2.03±0.06$^{△△}$</td>
<td>3.51±0.16$^{△△}$</td>
<td>4.03±0.42$^{△△}$</td>
</tr>
<tr>
<td>Group V (Livolin Forte 200 mg/kg + 45% ethanol)</td>
<td>6</td>
<td>2.33±0.02$^{△△}$</td>
<td>4.09±0.02$^{△△}$</td>
<td>4.86±0.12$^{△△}$</td>
</tr>
</tbody>
</table>

$^*$P < 0.05, vs Group I; $^{△}$P < 0.05, vs Group II.

### Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>GSH (µg/mL)</th>
<th>SOD (U/mg protein)</th>
<th>GST (µmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (corn oil 200 mg/kg)</td>
<td>6</td>
<td>6.91±0.14</td>
<td>8.55±0.36</td>
<td>0.56±0.03</td>
</tr>
<tr>
<td>Group II (45% ethanol)</td>
<td>6</td>
<td>5.95±0.41$^*$</td>
<td>7.45±0.36$^*$</td>
<td>0.27±0.05$^*$</td>
</tr>
<tr>
<td>Group III (Z. officinale oil 200 mg/kg + 45% ethanol)</td>
<td>6</td>
<td>6.75±0.23$^{△△}$</td>
<td>8.61±0.38$^{△△}$</td>
<td>0.49±0.03$^{△△}$</td>
</tr>
<tr>
<td>Group IV (C. longa oil 200 mg/kg + 45% ethanol)</td>
<td>6</td>
<td>6.92±0.29$^{△△}$</td>
<td>8.79±0.21$^{△△}$</td>
<td>0.81±0.04$^{△△}$</td>
</tr>
<tr>
<td>Group V (Livolin Forte 200 mg/kg + 45% ethanol)</td>
<td>6</td>
<td>6.39±1.10$^{△△}$</td>
<td>8.09±0.16$^{△△}$</td>
<td>0.35±0.03$^{△△}$</td>
</tr>
</tbody>
</table>

$^*$P < 0.05, vs Group I; $^{△}$P < 0.05, vs Group II; $^{△△}$P<0.05, vs Group IV.

GSH: glutathione; SOD: superoxide dismutase; GST: glutathione-S-transferase.

### Table 3

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>ALP (U/L)</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (corn oil 200 mg/kg)</td>
<td>6</td>
<td>30.03±0.70</td>
<td>37.87±0.23</td>
<td>4.35±0.17</td>
</tr>
<tr>
<td>Group II (45% ethanol)</td>
<td>6</td>
<td>50.00±3.26$^*$</td>
<td>65.80±1.71$^*$</td>
<td>7.10±0.55$^*$</td>
</tr>
<tr>
<td>Group III (Z. officinale oil 200 mg/kg + 45% ethanol)</td>
<td>6</td>
<td>35.14±0.87$^{△△}$</td>
<td>49.47±0.41$^{△△}$</td>
<td>4.94±0.51$^{△△}$</td>
</tr>
<tr>
<td>Group IV (C. longa oil 200 mg/kg + 45% ethanol)</td>
<td>6</td>
<td>31.16±1.87$^{△△}$</td>
<td>45.80±1.74$^{△△}$</td>
<td>4.64±0.77$^{△△}$</td>
</tr>
<tr>
<td>Group V (Livolin Forte 200 mg/kg + 45% ethanol)</td>
<td>6</td>
<td>44.01±1.24$^{△△}$</td>
<td>47.60±2.20$^{△△}$</td>
<td>5.90±0.60$^{△△}$</td>
</tr>
</tbody>
</table>

$^*$P < 0.05, vs Group I; $^{△}$P < 0.05, vs Group II.

ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase.
In recent years, researchers have shown that a way of preventing or delaying the pathogenesis of free radical-mediated cellular injuries is to augment the oxidative defense capacity of the cell through intake of antioxidants. Sequel to this, much attention has been focused on the health beneficial role of naturally occurring antioxidants in biological systems. Phytochemicals derived from plants are being considered to play an important role as nutraceuticals and are being utilized for treatment and prevention of metabolic diseases related to oxidative stress, even though their modes of action may still not be fully elucidated.

The results of the present study indicate that ethanol treatment caused significant morphological abnormalities in the liver, as were demonstrated by the appearance and observed changes in the histoarchitecture of the liver sections. This was confirmed by the noticeable elevation in the serum transaminases and ALP activities in Group II rats following administration of single dose of 45% ethanol (4.8 g/kg) compared to the control rats (Group I). The elevated levels of these biochemical parameters are a direct reflection of alterations in the hepatic structural integrity, possibly, a necrosis of hepatocytes that results in the leakage of transaminases and the elevation of serum ALP. The significantly decreased serum transaminases and ALP activities in the groups pretreated with standard drug (Livolin Forte) and oils from *Z. officinale* and *C. longa* prior to ethanol administration demonstrated their hepatoprotective ability.

We noticed that ethanol administration resulted in a significant elevation in the hepatic MDA level. Oxidative injury induced by ethanol can be monitored in animal models by measuring the extent of LPO. Ethanol administration results in excessive generation of free radicals such as hydroxethyl radical, superoxide radical, hydroxyl radical, peroxyl radical and hydrogen peroxide. All these radicals formed from the ethanol-mediated process have a great potential to react rapidly with lipids which in turn leads to LPO. It is generally accepted that the enhanced LPO is one of the toxic manifestations of ethanol ingestion. However, the significant decrease in the MDA level of the treated groups, indicated the anti-fatty liver effects of oils from *Z. officinale* and *C. longa*.

Disturbance observed in lipid metabolism in ALD is often important in determination of the disease status. The increase in serum concentration of TG may be attributed to inhibition of cholesterol catabolism or mobilizations of fatty acids from adipose tissues by lipolysis while the increase in serum TG level may be due to increased biosynthesis or dismissed clearance from the blood. Previous works reported that chronic ethanol ingestion results in hypercholesterolemia and hypertriglyceridemia and increased concentration of lipids in liver. The increased reduced form of nicotinamide adenine dinucleotid/nicotinamide adenine dinucleotide ratio during alcohol intake enhances the concentration of glycerophosphate, which favors hepatic TG accumulation by trapping fatty acids. However, these were significantly reduced in the serum of pretreated rats.

The reduction observed in hepatic antioxidant (GSH, SOD and GST) status of ethanol-treated rats compared with the control is related to oxidative stress and elevation of LPO that resulted in the leakage of hepatic enzymes to serum in the ethanol-treated rats. The endogenous GSH, synthesized mainly in the liver, plays an important role in

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**Figure 1** Representative histological section of liver in acute toxicity (Light microscopy, ×400; haematoxylin and eosin staining)

A: Group I, no visible lesions seen. B: Group II, there is marked portal congestion and cellular infiltration by mononuclear cells. C: Group III, there are periporal hydropic degeneration of hepatocytes with cellular infiltration by mononuclear cells. D: Group IV, there are mild portal congestion and mononuclear cellular infiltration, and mild hydropic degeneration. E: Group V, there are mild portal congestion, mild hydropic degeneration and few foci of cellular infiltration by mononuclear cells.
the system of cell defence. It is involved in detoxification of many xenobiotics through conjugation of toxic metabolites in the second phase of biotransformation. The substrate of SOD is the superoxide radical anion (O$_2^-$) which is generated by the transfer of one electron to molecular oxygen. This is responsible both for the direct damage of biological macromolecules and for generating other reactive oxygen species. SOD keeps the concentration of superoxide radicals at low levels and therefore plays an important role in the defense against oxidative stress. Taken together, the pretreated groups generally show a level of protection and restoration in hepatic antioxidant (GSH, SOD and GST) status. Excessive generation of free radicals, caused by ethanol metabolism, can be suppressed by enzymatic and molecular antioxidants such as catalase, glutathione peroxidase, SOD, GST, and GSH$^{[37-40]}$.

ORAC and FRAP are two of the most popular total antioxidant capacity assays. Based on the results obtained from this study, significant correlations exist between ORAC and FRAP values obtained which may be responsible for the ameliorative and antioxidant activities observed in other biochemical parameters estimated.

5 Conclusions

The present study demonstrates that pretreatment of rats for 28 d with oils from *Z. officinale* and *C. longa* (200 mg/kg) exhibits a significant hepatoprotective effect in acute ethanol-induced fatty liver. This was ascertained by a comparative analysis of the results obtained in rats pretreated with oils from *Z. officinale* and *C. longa* and Livolin Forte®. Histopathological findings further confirmed the protective effect of the oils, with *Z. officinale* oil identified to have better effects than *C. longa* oil.

6 Acknowledgements

We are grateful to the Antioxidant Research Laboratory, Cape Peninsula University, Cape Town, Nigeria for the FRAP and ORAC analysis of the oils.

7 Competing interests

The authors declare that they have no competing interests.

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