Aqueous Extract of Vernonia amygdalina Protects Against Alcohol-Induced Hepatotoxicity in Wistar Rats

Babatunde OGUNLADE 1, Godson G AKUNNA 1, Oluwaseun O FATOTA 2, Omolara J AYENI 2, Adebiyi A ADEGOKE 2, Sunday A ADELAKUN 2

ABSTRACT [ENGLISH/ANGLAIS]
Adverse health effects have been recognized to be caused by Alcohol has been shown to cause adverse health effect worldwide; and liver is one of the organs mostly affected. The effect of Vernonia amygdalina against alcohol-induced hepatic damage in rats was investigated in the present study. Rats were divided into three groups; the alcohol-alone group was given 5 ml/kg body weight of 56% ethanol (v/v) daily per oral for 35 days. The alcohol plus V. amygdalina group were similarly given alcohol, but had V. amygdalina 300 mg kg⁻¹ b.wt, daily p.o post-treatment for another 35 days. Another group of rats were given distilled water (the vehicle) 300 mg kg⁻¹ b.wt, daily p.o, for 35 days to serve as the control. The gross anatomical parameters of the liver and liver histology were assessed. Liver oxidative stress was evaluated by liver Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH) and malondialdehyde (MDA) assays. In addition, the activities of the biomarker enzymes of the liver (alanine transaminase, aspartate transaminase and alkaline phosphatase were assessed. An assessment of the histological profiles of the liver showed a derangement of the liver cytoarchitecture following alcohol abuse and a marked improvement was observed after V. amygdalina administration. Similarly, V. amygdalina improved the reduction of antioxidant parameters (SOD, CAT, GPx and GSH) and the increased MDA caused by alcohol ingestion. It was concluded that V. amygdalina may offer protection against free radical mediated oxidative stress of rats with alcohol-induced hepatotoxicity.

Keywords: Alcohol, liver, oxidative stress, antioxidants, Vernonia amygdalina

RÉSUMÉ [FRANÇAIS/FRENCH]
Effets néfastes sur la santé ont été reconnus pour être causés par l’alcool que l’alcool a été montré pour causer des effets néfastes sur la santé dans le monde entier et le foie est un des organes les plus touchés. Le Vernonia amygdalina effet contre les dommages hépatiques induit par l’alcool chez le rat a été étudiée dans la présente étude. Les rats ont été divisés en trois groupes: le groupe de l’alcool seul a donné 5 poids corporel ml / kg d’éthanol 56% (v / v) par jour et por voie orale pendant 35 days. the alcohol plus V. amygdalina groupe ont même donné de l’alcool, mais il a V. amygdalina 300 mg kg⁻¹ b.wt, tous les jours post-traitemt pendant encore 35 jours. Un autre groupe de rats ont reçu de l’eau distillée (le véhicule) 300 mg kg⁻¹ b.wt, tous les jours po, pendant 35 jours pour servir de contrôle. Les paramètres anatomiques brut de l’histologie hépatique et le foie ont été évalués. Le stress oxydatif hépatique a été évalué par la superoxide dismutase foie (SOD), la catalase (CAT), glutathione peroxidase (GPx), le glutathion réduit (GSH) et de malondialdéhyde (MDA) des essais. En outre, les activités des enzymes marques du foie (alanine aminotransférase, aspartate transaminase et de phosphatase alcaline ont été analysées. Une évaluation des profil histologiques du foie a montré un dérangement de la cytoarchitecture du foie suite à l’abus d’alcool et une évaluation a été observée après V. amygdalina administration. De même, V. amygdalina améliore la réduction des paramètres antioxydants (SOD, CAT, GPx et GSH) et l’augmentation de MDA causée par l’ingestion d’alcool. Il a été conclu que la protection contre V. amygdalina may offer protection against free radical mediated oxidative stress of rats with alcohol-induced hepatotoxicity.

Mots-clés: L’alcool, le foie, le stress oxydatif, antioxydants, Vernonia amygdalina

INTRODUCTION
Alcohol liver disease (ALD) remains a major treat, complication and causes of increase in mortality and morbidity rate (reaching about 6.1%) in heavy alcohol drinkers [1]. ALD poses a major health and economic concern and its treatment forms has remained a great challenge for researchers. ALD is a complex chronic disease process which typically progresses through the stages of alcoholic steatosis, alcoholic hepatitis and alcoholic cirrhosis to end-stage liver disease [2-3]. Chronic alcohol consumption has been reported to induce oxidative stress via cytochrome P450 2E1 [4]. Numerous studies to elucidate the pathogenesis of ALD have been done [5-9] and this is majorly because
approximately 80% of ingested alcohol is metabolized in
the liver by a process that produces numerous ROS (S).
In 2004, Lieber [10] reported that alcohol metabolism
could also lead to production of aldehydes which contain
a potent pro-inflammatory and profibrotic properties.
Damage to cell membranes and organelles has been
linked to lipid peroxidation through oxidative stress
leading to the release of reactive aldehydes [11].
Since indigenous plants have been the traditional source
of raw materials for the manufacture of medicines,
alternative practitioners thus routinely recommend
natural antioxidant supplement for ALD.
Considerable interest have developed in plants like
*Vernonia amygdalina*, Del (Compositae), which is a well-
known herbal medicine widely used as food with large
variety of beneficial effects. *V. amygdalina* is a well-
recognised African medicinal plant whose tree is about 1-
3 m in height [12-14]. It is a medium sized shrub whose
green leaf is about 6mm diameter and commonly called
“bitter leaf” and “ewuro” among Yoruba people in
southwest Nigeria. It has been reported to contain
“bitter leaf” and “ewuro” among Yoruba people in
southwest Nigeria. It has been reported to contain
antioxidants (luteolin, luteolin 7-O- _glucuronoside and
luteolin 7-O- _glucoside flavonoid) isolated from the
leaves [15]. Several reports on the antitumor [16-22],
antihelmintics [23-25], anti diabetic [26], hypolipidemic
[27-29], antimalarial [30-32], antiviral [33-34] and laxating
[35] activities of *V. amygdalina* has been documented.

The present investigation was therefore designed to
explore the hepatoprotective potential of aqueous extract
of *V. amygdalina* leaves on alcohol induced liver damage
in rats.

**MATERIALS AND METHODS**

*Vernonia amygdalina* and the Aqueous Extraction
Procedure

The leaves of *Vernonia amygdalina* used in this research
were collected from a grown plants located at a village
around Oke-Ogun in Oyo state, Nigeria on February 1,
2012. They were transported to the laboratory of the
Anatomy department of Lagos State University College of
Medicine, Ikeja, Lagos state, Nigeria and were
authenticated by a staff in the herbarium of the
Department of Botany, Lagos State University, Lagos
state, Nigeria. The leaves were thoroughly washed in
sterile water and the water was then drained from the
leaves.

Animals

Twenty four (24) adult male wistar rats were obtained
from a breeding stock maintained in the animal house of
the Lagos state university college of Medicine, Ikeja. The
animals were housed in well ventilated wire wooden
cages in the animal facility of the department of
Anatomy, Lagos state University College of Medicine,
Ikeja. The rats were maintained under standard natural
photoperiodic condition of twelve hours of light
alternating with twelve hours of darkness (i.e. L:D;12h:12h
photoperiod) at room temperature (25-26°C) and relative
humidity of 65±5%. They were allowed unrestricted
access to water and rat chow; and allowed to acclimatized
over a period of 20 days before the commencement of the experiment. The weights of the
animals were measuredat procurement, during
acclimatization, at commencement of the experiments
and whenthe experiment was completed using an
electronic analytical and precision balance (BA210S,
d=0.0001g) (Satorius GA, Goettingen, Germany).

Experimental procedures involving the animals and their
care were conducted in conformity with international
national and institutional guidelines for the care of
laboratory animals in biomedical research and use of
laboratory animals in bio-medical research promulgated
by the Canadian council of animal care [36].

**Acute Oral Toxicity Study of Vernonia amygdalina Extract**

The acute oral toxicity study for *V. amygdalina* extract was
conducted using the Organization for Economic
Cooperation and Development (OECD) [37] Guidance
Document on Humane End points that should reduce the
overall suffering of animals used in this type of toxicity
test. The test used was the limit dose test of the up and
down procedure.

Briefly, 5 animals were weighed and individually
identified. The first animal was given the test dose – *V.
amygdalina* extract 2000 mg per kg body weight. The
second and third animals were concurrently dosed and
the fourth and fifth animals sequentially dosed.

The results were evaluated as follows (S = Survival, X =
death). The animals were observed individually at least
once during the first 30 minutes after dosing, periodically
during the first 24 hours (with special attention given
during the first 4 hours), and daily thereafter for a total
period of 14 days. All observations were systematically
recorded with individual records maintained for each
animal.

**Animal Grouping and Experimental Design**

Twenty four adult male wistar rats weighing 190-230g
were used for this research work. The rats were
randomly divided into three groups (A-C) of eight rats each such that the weight difference between and within groups did not exceed ±20% of average weight of the sample population.

Rats in group A which served as control were given 10ml/kg/day of distilled water for 35 days.

Rats in group B (ethanol group) were given ethanol diluted with normal saline (56%; v/v) administered orally through an orogastric cannula into the stomach via the esophagus at a dose of 5ml/kg body weight, three times a day, for 35 consecutive days.

The appropriate quantity of alcohol was given. The administrations were done once daily.

Rats in group C (ethanol + V. amygdalina group) rats were fed V. amygdalina (300 mg/kg body weight/day) in drinking water administered for 35 consecutive days, and ethanol was given simultaneously as described for the ethanol group.

Animal Sacrifice and Sample Extraction

Twelve hours after the administration of the last ethanol dose, the rats were at the time of sacrifice first weighed and then cervical dislocation was carried out. Blood samples were collected and centrifuged at 1,500 g/min at 4 °C for 10 min to obtain serum. The abdominal cavity of each rat was opened up through a midline abdominal incision to expose the liver. The liver was excised and weighed; the liver was weighed with an electronic analytical and precision balance. The liver of each animal was fixed in 10% formol-saline for histological examination. (BA 210S, d=0.0001- Sartoriusen GA, Goettingen, Germany).

Histological Procedures and Analysis

This was done as described by Ogunlade et al [38]. Briefly, the organs were cut on slabs about 0.5cm thick and fixed in 10% formol saline for a day after which they were transferred to 70% alcohol for dehydration. The tissues were passed through 90% alcohol and chloroform for different durations before they were transferred into two changes of molten paraffin wax for 20min each in an oven at 57°C. Serial sections of 5µm thick were obtained from a solid block of tissue and were stained with haematoxylin and eosin stains, after which they were passed through a mixture of equal concentration of xylene and alcohol. Following clearance in xylene, the tissues were oven- dried. Photomicrographs were taken with a JVC colour video digital camera (JVC, China) mounted on an Olympus light microscope (Olympus UK Ltd, Essex, UK) to demonstrate the hepatocyte.

Determination of Serum ALT, AST and ALP Assay

The degree of liver damage was evaluated by ALT, AST and ALP in serum using a commercially available kit. Detailed procedures for the above measurements were performed according to the kit manufacturer’s instructions.

Determination of Oxidative Stress parameters

SOD activity in liver was determined according to the method described by Marklund and Marklund [39] and GSH-Px activity was determined by GSH-Px assay kit. Detailed procedures for the above measurements were performed according to the kits’ protocol. CAT was assayed by the method described by Ferro and Chagas [40]. The non-enzymic GSH was analyzed by the method of Moron, Dipierre, and Mannervik.

Determination of liver MDA contents

Lipid peroxidation was evaluated on the base of MDA level and MDA in liver was determined using the method described by Jain et al., [41].

Statistical Analysis

All data were expressed as mean ± S.D. Differences between groups were analyzed using one-way analysis of variance (ANOVA). A value of \( p < 0.05 \) was considered to be statistically significant.

RESULTS

Acute oral Toxicity Studies

There were no deaths of rats dosed 3000 mg/kg body weight of the plants extract both within the short and long outcome of the limit dose test of Up and Down method (Table 1). The LD50 was calculated to be greater than 3000 mg/kg body weight/orally.

Mean Body Weights (g), Liver Weights (g) and Liver Volumes (ml), in Control and Experimental Rats

As showed in table 2, ethanol induced rats lost weight as compared to the control group while ethanol + V. amygdalina rats reversed the lost weight to nearly that of the control. Mean liver weight in the ethanol group decreased by 17% compared to that of control group while ethanol + V. amygdalina group decreased by 8% revealing the restoring potential of V. amygdalina extract to the weight lost due to ethanol administration. The relative liver weight (liver weight/body weight)x100) was ~3% in the ethanol group compared to the control and ethanol + V. amygdalina that were ~4%.
Table 1: This table shows results of acute toxicity test for V. amygdalina extract (up and down procedure) in rats.

<table>
<thead>
<tr>
<th>Test serial number</th>
<th>Animal Identity</th>
<th>Dose of V. amygdalina (mg/kg)</th>
<th>Short term result (48hrs)</th>
<th>Long term result (14 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>REP</td>
<td>2000</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>2</td>
<td>LEP</td>
<td>2000</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>3</td>
<td>TC</td>
<td>2000</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>4</td>
<td>RLT</td>
<td>2000</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>5</td>
<td>LLT</td>
<td>2000</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>6</td>
<td>I</td>
<td>2000</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

S = Survival; REP = Right ear pierced; LEP = Left ear pierced; TC = Tail cut; RLT= Right leg tagged; LLT=Left Leg tagged, I = Intact rat

Table 2: This table shows mean body weight (g), liver weight (g) in control and experimental rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Body weight (g)</td>
<td>215.5±4.0</td>
<td>216.5±2.7</td>
<td>215.0±3.5</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>235.5±4.2</td>
<td>205.3±2.5</td>
<td>213.5±3.3</td>
</tr>
<tr>
<td>Body weight diff. (g)</td>
<td>20.0</td>
<td>11.2*</td>
<td>1.5**</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>9.0±0.2</td>
<td>7.5±0.2*</td>
<td>8.3±0.2*</td>
</tr>
<tr>
<td>Liver wt./body wt. ratio</td>
<td>0.04</td>
<td>0.03</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for n=8; *p < 0.05, **p < 0.001 significantly dissimilar from control

AST and ALT levels

As showed in table 3, the serum levels of AST and ALT were significantly increased in the ethanol group compared to the control group, but the serum AST and ALT activities in the ethanol + V. amygdalina group were significantly lower than in the ethanol group.

Table 3: This table shows AST and ALT levels in control and experiment rats.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>AST (U/l)</th>
<th>ALT (U/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (CONTROL)</td>
<td>25.0±2.2</td>
<td>23.5±1.0</td>
</tr>
<tr>
<td>B (ETHANOL)</td>
<td>102.2±1.2**</td>
<td>120.6±2.1**</td>
</tr>
<tr>
<td>C (V.amygdalina+ ETHANOL)</td>
<td>35.5±1.6*</td>
<td>40.2±3.0*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for n=8; *p < 0.05, **p < 0.001 significantly dissimilar from control

Antioxidant Levels (CAT, SOD, GSH, GPx) and MDA Levels

As showed in table 3, the MDA levels in the ethanol group increased compared with the control group but decreases in ethanol + V. amygdalina group compared to the ethanol group. The anti-oxidant levels (CAT, SOD, GSH and GPx) decreased significantly in ethanol group (**P<0.001) compared to the control group but the CAT, SOD, GSH and GPx levels in ethanol + V. amygdalina group decreases (*P<0.05) compared to the control group.

Table 4: This table shows effects of V. amygdalina on antioxidant levels and lipid peroxidation levels in rat liver.

<table>
<thead>
<tr>
<th></th>
<th>MDA (nmol/mg protein)</th>
<th>CAT (U/mg protein)</th>
<th>SOD (U/mg protein)</th>
<th>GSH (nmol/mg protein)</th>
<th>GPx (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>6.9±0.6</td>
<td>17.5±3.6</td>
<td>11.5±4.0</td>
<td>7.2±0.7</td>
<td>4.0±8.0</td>
</tr>
<tr>
<td>Group B</td>
<td>9.3±1.1**</td>
<td>10.2±3.0**</td>
<td>5.2±2.5**</td>
<td>5.0±0.6**</td>
<td>1.2±4.8**</td>
</tr>
<tr>
<td>Group C</td>
<td>7.2±0.7*</td>
<td>15.2±3.3*</td>
<td>11.2±3.9*</td>
<td>6.7±0.5*</td>
<td>3.4±6.8*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for n=8; *p < 0.05, **p < 0.001 significantly dissimilar from control
Administration of ethanol significantly (P<0.001) increased the serum intracellular enzymes such as alanine aminotransferase (AST) and aspartate aminotransferase (ALT) compared to the control group while administration of *V. amygdalina* decrease significantly (P<0.001) these enzyme levels. The reversal of elevated serum intracellular enzyme levels by *V. amygdalina* extract after ethanol administration may be attributed to the stabilizing ability of the cell membrane preventing enzymes leakages. In addition, it was reported that the reversal of increased levels of transaminases to nearly normal predicts the restoration of hepatocytes and regeneration of hepatic parenchyma [42].

In addition, the antioxidant enzymes and lipid peroxidation levels can be used to predict the severity of ethanol induced liver damage. Antioxidants enzymes such as SOD, CAT, GSH and GPx dependently act in the metabolic pathways that involve free radicals. Therefore, SOD, CAT, GSH and GPx levels decrease in liver suggest the toxic effects of ethanol on liver functions but the administration of *V. amygdalina* can counter the efficacy of ethanol on liver cells thereby blocking the decrease antioxidants levels. Since it was proved that the significance of GSH in the detoxification of chemically reactive metabolite in drug induced toxicity after decrease in GSH [43-45] then we can deduce that increased oxidation and decrease synthesis of GSH causes decrease in GSH levels. Therefore increase in antioxidant enzyme activities levels (SOD, CAT, GSH and GPx) after *V. amygdalina* extracts administration might contribute to the ameliorating effects of oxidative stress.

MDA is a known biomarker of lipid peroxidation and oxidative stress, the increase in MDA level signifies the toxic effects of ethanol on liver [45] but the counteractions of *V. amygdalina* in reducing MDA level suggest the potential attributes of *V. amygdalina* in the restoration of damaged liver tissues after ethanol administration. Therefore, the antioxidant potential of *V. amygdalina* improves the liver functions by promoting antioxidant enzyme activities, thus can be recommended as a therapeutic agent for heavy alcohol drinkers in alcohol related liver damage.

Furthermore, histological evaluation can be used to shows the severity and toxicity of alcohol induced liver damage. It was observed in the control group that the normal hepatic cytoarchitecture was evident with visible terminal hepatic lobules consisting of terminal hepatic venules, hepatocytes with intervening sinusoidal spaces radially accentuated. Alcohol induced group shows hepatic nodular fibrosis, moderate portal fibrosis with

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**Figure 1:** This figure shows photomicrograph of group A (Control) rat liver stained with H&E X 400

**Figure 2:** This figure shows photomicrograph of group B (Alcohol treated Groups) rat liver stained with H&E X 400

**Figure 3:** This figure shows photomicrograph of Group C (*V. amygdalina* Alcohol group) rat liver stained with H&E X 400

**DISCUSSION**

Administration of ethanol significantly (P<0.001) increased the serum intracellular enzymes such as alanine aminotransferase (AST) and aspartate aminotransferase (ALT) compared to the control group while administration of *V. amygdalina* decrease significantly (P<0.001) these enzyme levels. The reversal of elevated serum intracellular enzyme levels by *V. amygdalina* extract after ethanol administration may be attributed to the stabilizing ability of the cell membrane preventing enzymes leakages. In addition, it was reported that the reversal of increased levels of transaminases to nearly normal predicts the restoration of hepatocytes and regeneration of hepatic parenchyma [42].

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Vernonia amygdalina Protects Against Hepatotoxicity in Wistar Rats

portamento and porto-central extensions. There are observable improvements in the microscopic appearance of the liver after *V. amygdalina* administration showing restoration in the hepatocyte, mild congestion of the cytoplasm, absence of centrilobular necrosis with nearly visible central vein. Since *V. amygdalina* has antioxidant components that can alter the damage done by alcohol consumption, heavy drinkers can really on this naturally available plant as supplement after drinking. Therefore we can deduce from our findings that *V. amygdalina* tentatively mitigates the effects of alcohol on the liver of rats.

**REFERENCES**


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Nil.

CONFLICT OF INTEREST
No conflict of interest was declared by authors.

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