**Allablanckia floribunda** hypotensive activity on ethanol induced hypertension in rats

Danielle Claude Bilanda, Paul Désiré Djomeni Dzeufiet, Orelion Mtopi Bopda, Pierre Kamtchouing, Théophile Dimo

**ABSTRACT**

**Background:** Chronic alcohol intake is related to hypertension. In the present work, we investigated the effect of *Allablanckia floribunda* Oliver (Clusiaceae) aqueous extract in alcohol-induced hypertensive rats and on related oxidative stress damages. **Methods:** Alcohol-induced hypertensive rats (AHR) was obtained by oral administration of ethanol (3 g/kg/day during 8 weeks). Blood pressure and heart rate were evaluated using the direct cannulation method. The effects of the extract on lipid profile as well as kidney and liver functions were studied. Free radical scavenging and antioxidant properties of the extract were evaluated by colorimetric method. The effects of *A. floribunda* were evaluated after 4 weeks of treatment with alcohol. **Results:** At the doses of 200 and 400 mg/kg/day, *A. floribunda* significantly decreased the mean blood pressure of AHR by 14.06 and 23.25% respectively. Administration of the plant extract lead to the reduction of total cholesterol by 41.50% and 43.06%, HDL-cholesterol by 22.16 and 30.15% and artherogenic index by 69.78 and 74.43%, respectively at the doses of 200 and 400 mg/kg, as compared to untreated hypertensive rats. *A. floribunda* (200 and 400 mg/kg) decrease bilirubine (12.98 and 16.88%), urea (23.32% and 32.26 %), ALT (10.73 and 27.97%) and AST (29.80 and 42.22%) of treated AHR, respectively. The plant extract also reduced superoxide dismutase (SOD), malondialdehyde (MDA) and catalase and increased the reduced glutathione (GSH) concentration in aorta, heart, kidney and liver of AHR. **Conclusion:** These results suggest that the aqueous extract of *A. floribunda* possesses antioxidant and hypotensive activity in alcohol-induced hypertension.

**Keywords:** *A. floribunda*, Alcohol, Hypertension, Oxidative stress.

**INTRODUCTION**

Chronic ethanol consumption is associated with hypertension [1] and oxidative stress [2]. Alcohol abuse is now recognized as an important determinant of an elevated blood pressure. An initial interest in the relationship between alcohol and hypertension dates back to 1915 when Lian [3], a French physician, described his findings amongst wine drinking French men [3]. We now know that the relationship between hypertension and chronic intake of alcohol appears to be independent of sex, race, type of alcoholic beverage, education, smoking, and salt intake [4-5]. The relationship between alcohol intake and blood pressure is probably even stronger than the relationship between salt and blood pressure [4]. A positive association between heavy alcohol consumption and elevated blood pressure has been found in several large observational studies. Ethanol consumption is able to induce oxidative stress in the liver and in extra hepatic tissues, due to an imbalance between the pro-oxidant and the antioxidant systems in favour of the former [6]. Chronic ethanol consumption is associated with oxidative stress, mainly by an increase in lipid peroxidation (LPO) [7-8].

Bioactive phytochemical molecules have long been recognized as essential in maintaining healthy body systems. Epidemiological studies have shown that dietary intake of polyphenol-rich foods is inversely associated with the incidence of cardiovascular diseases [9-10]. A large number of plant species which are important sources of traditional medicine are widely being used to treat hypertension and/or oxidative stress. *Allablanckia floribunda* Oliver (Clusiaceae) is a medicinal plant, widely used in Africa to manage hypertension and many other ailments. Its hypotensive effects on sugar-induced hypertension have already been demonstrated [11]. In order to know if the hypotensive activity of the plant can also be applied to another model of hypertension, the present study was designed to investigate the hypotensive and antioxidant activities of *A. floribunda* on alcohol-induced hypertensive rats.
MATERIALS AND METHODS

Plant material and extraction

The stem barks of *A. floribunda* were collected in February at Nkolossan, in the centre region of Cameroon. The plant was authenticated at the National Herbarium by comparison with the existing voucher specimen Nº 1380/HNC collected by R. Letouzey. The bark was dried at room temperature and reduced to a powder (1 kg). The material was added to six liters of hot water (80 ºC) and allowed to macerate during 48 hours. The solution obtained after filtration was lyophilized and gave 154 g (15.4% yield) of a brown powder.

Animals

Male Wistar rats, 4-6 weeks old, weighing between 120-130 g were used. They were raised in the animal house of the Faculty of Sciences, University of Yaounde I, Cameroon. Animals were exposed to daily 12 hours light – dark cycle, maintained in a room temperature (25º ±3 C) with free access to a standard animal diet and tap water. Prior authorization for the use of laboratory animals in this study was obtained from the Cameroon National Ethics Committee (Reg. Nº. FWA-IRD 0001954). To determine the antihypertensive and antioxidant activity of *A. floribunda*, the alcohol-induced hypertensive rat (AHR) model was used.

Drugs and chemicals

All the drugs and biochemicals used in this experiment were purchased from Sigma Chemical Company (St. Louis, MO, USA). The chemicals were of analytical grade.

Procedure

The Alcohol Hypertensive Rats (AHR) model was obtained from normal rats by giving a 35 degree ethanol (3 g/kg, 1 ml/100 g body weight) orally once a day, while normotensive group was receiving the same volume of distilled water, during 8 weeks [12]. After hypertension induction, rats were divided into five groups of five rats each and treated daily for 4 weeks. Throughout the experiment, body weight was taken. Control normotensive rats received distilled water (group 1). Control AHR received ethanol and distilled water (group 2). The test groups (3-5) made up only with AHR, received simultaneously, once a day, ethanol and *A. floribunda* aqueous extract (200 and 400 mg/kg) or nifedipine (10 mg/kg). All the treatments were giving orally. At the end of the respective treatments, arterial blood pressure and heart rate of all rats were recorded as described by Bilanda et al. [12]. Briefly, the rats were anesthetized using an intraperitoneal injection of urethane (1.5 g/kg). The trachea of each rat was exposed and cannulated to facilitate spontaneous respiration. Hemodynamic parameters were assessed from the right carotid artery via an arterial cannula connected to a pressure transducer coupled with a hemodynamic recorder (Biopac Student Lab., MP35) and a computer. The rats were then sacrificed and the blood was collected. Serum was separated for the determination of serum total cholesterol, HDL-cholesterol, triglycerides, urea and bilirubin levels using commercial diagnostic kits (Fortress, UK). Atherogenic index was calculated following Wakayashi and Kobabas’ formula [13]; Atherogenic index= Total-cholesterol-HDL-cholesterol / Total- cholesterol. The activities of alanine and aspartate aminotransaminases were also determined using the method of Reitman and Frankel [14]. After blood collection and the heart, aorta, liver and kidneys were dissected out. Those organs were weighed and homogenized in Mc Even solution for aorta and heart, or in Tris-HCl 50 mM buffer solution for liver and kidneys to make a 20% homogenate. Each homogenate was centrifuged at 10000 g for 30 minutes and stored at -20 ºC. Tissue protein concentration was assayed according to Gornall et al. [15] using the Biuret reagent and bovine serum albumin as a standard. Catalase was determined according to Sinha [16], whereas reduced glutathione (GSH) and superoxide dismutase (SOD) were determined using the method of Ellman [17] and Misra and Fridovich [18], respectively. Malondialdehyde (MDA), the end-product of lipid peroxidation was determined using the procedure of Wilbur et al. [19].

Statistical analysis

Results are expressed as the mean ± SEM. The difference between treatment groups was compared using one-way analysis of variance (ANOVA) followed by the Dunnett’s post hoc test, and a value of P < 0.05 was considered statistically significant.

RESULTS

Effect of *A. floribunda* on body weight

The effect of *A. floribunda* on the evolution of the body weight of alcohol hypertensive rats is represented in Figure 1. The body weight of all experimental rats increased normally from the beginning of the extract treatment until the second week. From the third week, we observed a significant (P< 0.05) decrease in the alcohol-treated group. Body weight in all the AHR groups was lower at the end of the treatment period as compared to normotensive rats. However, the decrease in body weight of the extract-treated animals was less marked and significantly (P< 0.05) low compared with AHR untreated group. Those differences were 11.20, 13.54 and 11.90 g, respectively, for the groups treated with the plant extract (200 mg/kg and 400 mg/kg) and nifedipine (10 mg/kg).

![Figure 1: Effect of *A. floribunda* aqueous extract on body weight of experimental animals.](image-url)

Each point represents the mean ± S.E.M.; n=5; *P< 0.05, ***P< 0.001, significantly different compared to normal rats. $^*$P< 0.05, significantly different compared to alcohol hypertensive rats. Al, alcohol, Ea, aqueous extract.)
**A. floribunda** activity on blood pressure and heart rate

Figure 2 summarizes the effect of *A. floribunda* aqueous extract on blood pressure and heart rate of the experimental animals. Administration of alcohol resulted in an increase in blood pressure of the rats. The increase in blood pressure was 55.26, 33.42, 18.67 and 17.98% higher, respectively, on AHR untreated group and *A. floribunda*-treated rats with 200 and 400 mg/kg or the nifedipine-treated group as compared to normal rats. The blood pressure of treated animals was significantly lower as compared to the untreated rats. The reduction in blood pressure produced by the plant extract was 14.06 and 23.25%, respectively, at the doses of 200 and 400 mg/kg as compared to AHR untreated rats. The reduction obtained with nifedipine was 24.01% after 4 weeks of treatment. No change on heart rate was observed in all groups after 4 weeks of treatment.

![Figure 2: Effect of *A. floribunda* on mean arterial blood pressure and heart rate of alcohol-induced hypertension in rats.](image)

Each bar represents a mean ± S.E.M.; n=5; ***P< 0.001, significantly different compared to normal rats. ³P< 0.05, ²²²P< 0.001, significantly different compared to alcohol hypertensive rats. Al, alcohol; Ea, aqueous extract.

**A. floribunda** activity on blood biochemical parameters

Table 1 shows the effect of *A. floribunda* aqueous extract on the lipid profile of experimental animals. The treatment (4 weeks) with the plant extract or nifedipine decreased the blood concentrations of total cholesterol, HDL-cholesterol and artherogenic index. However, there was no significant change in the concentration of triglyceride in the groups treated with *A. floribunda* while a decreased was observed with nifedipine treatment. *A. floribunda* aqueous extract decreased total-cholesterol (41.50% and 43.06%), HDL-cholesterol (22.16 and 30.15%) and artherogenic index (69.78 and 74.43%) at the doses of 200 and 400 mg/kg, respectively.

<table>
<thead>
<tr>
<th>Table 1: Effect of <em>A. floribunda</em> on lipid profile of alcohol hypertensive rats</th>
</tr>
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<tbody>
<tr>
<td><strong>Water</strong></td>
</tr>
<tr>
<td>Total Cholesterol (mg/dl)</td>
</tr>
<tr>
<td>HDL-Cholesterol (mg/dl)</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
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<td>Arterogenic index</td>
</tr>
</tbody>
</table>

Values are expressed as means ± S.E.M.; n=5; ³P< 0.05, ²²²P< 0.001, significantly different compared to normal rats. ³³³P< 0.01, ²²²²²P< 0.001, significantly different compared to alcohol hypertensive rats.

The results in table 2 showed that *A. floribunda* reduced dose-dependently the activity of ALT (10.73 and 27.97%) and AST (29.80 and 42.22%) at the doses of 200 and 400 mg/kg respectively, as compared to AHR untreated group. The plant extract also dropped significantly (P <0.001) the concentration of bilirubin by 12.98 and 16.88%, respectively, at the doses of 200 and 400 mg/kg as compared to the AHR untreated group. *A. floribunda* also had a benefic effect on urea levels reducing them significantly by 23.32% and 32.26 % at the doses of 200 and 400 mg/kg, respectively, as compared with AHR untreated group. Nifedipine reduced the activity of ALT (27.35%) and AST (43.85%), the concentration of bilirubin (20.77%) and of urea (35.63%) as compared with the AHR untreated group.
Table 2: Effect of *A. floribunda* on liver and kidney function parameters

<table>
<thead>
<tr>
<th></th>
<th>Water</th>
<th>Alcohol</th>
<th>Al + Ea200mg/kg</th>
<th>Al + Ea400mg/kg</th>
<th>Al + nif10mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bilirubine</strong></td>
<td>1.20±0.03</td>
<td>1.54±0.05</td>
<td>1.34±0.03</td>
<td>1.28±0.03</td>
<td>1.22±0.02</td>
</tr>
<tr>
<td><strong>Urea (mg/dl)</strong></td>
<td>46.66±1.72</td>
<td>76.36±1.67</td>
<td>58.55±2.48</td>
<td>51.72±2.23</td>
<td>49.15±2.43</td>
</tr>
<tr>
<td><strong>ALT (UI)</strong></td>
<td>181.56±5.81</td>
<td>273.89±4.60</td>
<td>244.49±3.43</td>
<td>197.28±3.18</td>
<td>198.97±3.42</td>
</tr>
<tr>
<td><strong>AST (UI)</strong></td>
<td>80.00±5.65</td>
<td>173.92±3.35</td>
<td>122.08±4.46</td>
<td>104.84±4.72</td>
<td>97.64±4.69</td>
</tr>
</tbody>
</table>

Values are expressed as means ± S.E.M.; n=5; *P< 0.05, **P< 0.01, ***P< 0.001, significantly different compared to normal rats. $P< 0.01, $$$P< 0.001, significantly different compared to alcohol hypertensive rats.

*A. floribunda* activity on oxidative stress marker variables

The effect of *A. floribunda* on oxidative stress marker parameters is summarized in Figure 3. The oxidative stress parameters evaluated in this study was SOD (A), catalase (B), reduced glutathione (C), and MDA (D) on the aorta, heart, liver and kidneys. In all organs, we observed a significant increase in SOD, catalase and MDA concentration in alcohol-treated animals. *A. floribunda* and nifedipine significantly reduced these parameters as compared with the alcohol untreated group. The level of glutathione in aorta, liver and kidneys was significantly (P< 0.01) increased by *A. floribunda* extract and nifedipine as compared with the alcohol untreated rats.

DISCUSSION

In the present study, the chronic administration of alcohol in rats resulted in a significant increase of mean arterial blood pressure. Similar results were previously observed by [12], and Husain et al. [20] who reported that administration of ethanol significantly elevates the systolic and mean blood pressure. Several mechanisms of chronic ethanol consumption action on cardiovascular diseases have been fully described elsewhere [24-25]. *A. floribunda*, used in the present study significantly and dose-dependently reduced the blood pressure of AHR rats with no effect on heart rate. The hypotensive effect of *A. floribunda* has already been reported in sucrose-induced hypertension [11]. Our results confirmed that activity and reveal that it was not only linked to the model of hypertension, since the same extract was used. Weight can play an important role in the reduction of blood pressure [23]. In our study, the body weight of *A. floribunda*-treated rats was similar to the normal control while untreated AHR showed a significant (P<0.001) reduction in body weight as compared to normal rats. This suggests that in our case, body weight does not appear to be a major factor in the reduction of the blood pressure. The same can also be said with the reduction of body volume. In fact, *A. floribunda*...
doi not exhibit a diuretic effect on normal and hypertensive rats (Data not shown).

To investigate the mechanism by which A. floribunda exerts its hypotensive effect, we evaluated its action on lipid profile and oxidative stress markers of AHR. It is well established that chronic consumption of alcohol or fructose can lead to dyslipidemia [24-25] which can enhance vascular resistance and leads to the increase in blood pressure. In the present study, the chronic administration of alcohol to rats, as expected, resulted in a significant increase in serum total cholesterol, triglyceride levels and atherogenic index values. Those parameters were significantly reduced after treatment with the aqueous extract of A. floribunda, confirming the improvement of lipid profile by the plant extract [11, 12, 26]. Our results are consistent with those of Aikawa et al. [27] who reported that lipid lowering can improve vascular functions and some indices of oxidative stress. This finding suggests that lipid lowering is one of the ways by which A. floribunda exerts its hypotensive effect [11-12].

Ethanol is believed to generate oxygen radicals, inhibit GSH synthesis and deplete GSH levels in tissues, increase MDA levels and generally impair the antioxidative defense system [28]. In the present study, we observed in untreated AHR a significant increase in SOD, MDA and catalase levels while glutathione was significantly decreased. Reactive oxygen species (ROS) production is a naturally occurring process and a variety of enzymatic and nonenzymatic mechanisms have evolved to protect cells against ROS [29]. Enzymes involved in the elimination of ROS include SOD, catalase and glutathione peroxidase. Alcohol has been shown to deplete GSH levels, particularly in the mitochondria, which normally are characterized by high levels of GSH needed to eliminate the ROS generated during activity of the respiratory chain. The increase in the GSH level on A. floribunda treated rats suggests that it has direct free-radical scavenging property and also strengthens the enzymatic defense system. These results confirm the findings of many authors [11, 30] who have shown that the plant is rich in polyphenolic compounds and has a strong antioxidant activity.

The plant extract might stimulate GSH synthesis and inhibit the depletion of GSH due to alcohol in tissues [31]. Treatment with A. floribunda extract attenuates the increase of MDA in the aorta, heart, liver and kidney from the AHR, confirming the antioxidant activity of the plant. El-Sokkary et al. [31] obtained the similar reductions of alcohol toxicity following antioxidant (melatonin) administration. Furthermore, the flavonoids of our extract may be involved in vasorelaxation of rat aortic rings which is NO and endothelium-dependent; flavonoids can also attenuate the development of hypertension and reduce high blood pressure [32]. In addition, flavonoids are potent antioxidant compounds that possess free radical-scavenging abilities, and their antiradical property is directed toward OH and O2-, which are highly reactive oxygen species implicated in the initiation of lipid peroxidation [33-34]. Thus, the presence of flavonoids and phenols in the aqueous extract of A. floribunda may partially explain their effect on increasing antioxidant enzyme activity. Since this plant extract effectively improved lipid profile and tissues oxidation, it might be more appropriate to prevent or alleviate lipid profile and tissue oxidation responsible for the development and progression of oxidation-associated diseases such as atherosclerosis and cardiovascular diseases. Ethanol administration can disturb the delicate balance between the pro- and anti-oxidant systems of the organism, leading to oxidative stress [35-36]. Increased generation of ROS/free radicals is able to cause auto-oxidation of the hepatic cells, kidney and other organs resulting in marked hepatic lesions [35]. Our results revealed a significant increase in serum ALT and AST activities in untreated alcohol-induced hypertensive rats as compared with normal rats.

The obvious sign of hepatic injury is the leakage of cellular enzymes into plasma [37]. In the present study, the increased activities of serum enzymes (AST and ALT) imply the increased permeability and damage of hepatocytes [38]. Because the enzyme ALT is located in the cytoplasm and the soluble enzyme AST is located mainly in organelles such as mitochondria [39], increased levels of AST and ALT suggested damage of both hepatic cellular and mitochondrial membranes in alcohol-treated rats. On the other hand, serum bilirubin levels can be used as a predictor of liver damage [40]. Elevated serum bilirubin levels in untreated AHR may confirm the adverse effects of alcohol in the liver. The kidney is one of the important target organs in hypertension. Increase level in urea has a positive correlation with oxidative stress index and kidney damage [41] associated with hypertension. In the present study, the level of urea was significantly increased in untreated hypertensive rats as compared to normal animals. These results are similar to those of Maho et al. [42] and suggest the toxicity of alcohol on the kidney. When A. floribunda or nifedipine was administered to the rats, levels of ALT, AST and bilirubin in serum were in the same range as the normal control. The same decrease was observed in urea levels. These results confirm that A. floribunda possesses the ability to repair and to prevent, at least in part, the adverse effects of ethanol both on liver and kidneys [11-12]. These results are similar to those of Kumar et al. [39] who worked with the leaf extract of Cissia auriculata in rats.

These results confirm that the aqueous extract of A. floribunda has beneficial effects on alcohol-induced hypertensive rats by decreasing mean arterial blood pressure and improving the oxidative balance. The decrease in blood pressure may be related to lipid lowering and antioxidative effects. The antioxidative effect is reflected in the improvement and the prevention of damages induced by alcohol consumption. The present study also indicates that A. floribunda treatment can suppress the alcohol-induced inflammatory processes in the kidney and liver. Further studies need to be carried out to determine the mechanism of action and to isolate the active principle(s) of A. floribunda.

Competing interests

The authors declare that they have no competing interests

REFERENCES
