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# Antioxidant Activity and Hepatoprotective Effect of an Aqueous Extract of *Alchornea cordifolia* Leaves

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## Abstract

*Alchornea cordifolia* is a medicinal plant, whose ethanolic and methanolic extracts have shown antioxidant activity which could confer hepatoprotective effect, knowing that liver cells are attacked by free radicals. The hepatoprotective effect of these extracts has been demonstrated in models of hepatotoxicity induced by paracetamol high doses in animals. However, anti-tubercular drugs at the usual dose present hepatotoxicity risk. Could *Alchornea cordifolia* help to limit hepatotoxicity induced by anti-tubercular drugs? This work aimed to evaluate the antioxidant activity and the hepatoprotective effect of an aqueous extract of *A. cordifolia* leaves (AEAC). The antioxidant activity of *A. cordifolia* leaves was studied *in vitro* by 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals scavenging assay and by the iron reduction ability. A phytochemical screening was carried out to identify the chemical groups that could be responsible for this activity. The hepatoprotective effect was demonstrated in a model of hepatotoxicity induced by isoniazid and rifampicin in rats. Two hours after induction of hepatotoxicity, the animals were orally administered the AEAC at 200 mg/kg, 400 mg/kg, 800 mg/kg for 10 consecutive days. A blood sample was taken on the 11$^{th}$ day for the evaluation of transaminases, markers of hepatic cytolysis. A totally of 96 rats were used in this study. AEAC showed dose-dependent antioxidant activity. Phytochemical screening revealed the presence of flavonoids, tannins and alkaloids. Administrated alone, aqueous extract of *A. cordifolia* leaves didn't modificate the transaminases, isoniazid and the isoniazid + rifampicin combination resulted in increasing transaminases (ALT and AST) by more than 48%. AEAC at 800 mg/kg reduced AST and ALT levels by more than 45%. AEAC at 200 mg/kg and 400 mg/kg decreased ALT more than 40%. Knowing that antioxidant activity protects liver, the AEAC may by its antioxidant activity, contribute to protect against the hepatotoxicity induced by an-

ti-tubercular drugs in the rat.

**Keywords**

Antioxidant, Hepatoprotective, Aqueous Extract, *Alchornea cordifolia*

## 1. Introduction

Herbal treatments are becoming increasingly important within populations. These plants are mostly endowed with antioxidant properties able to inhibit free radicals formation and to oppose macromolecules oxidation [1]. Free radicals have been implicated in a number of pathological processes, such as asthma, cancer, cardiovascular disease, cataracts, diabetes, inflammatory diseases, liver diseases and degenerative diseases [2]. Antioxidants can inhibit or retard the oxidation of an oxidizable substrate in a chain reaction [3]. The properties of these plants used in traditional medicine were attributed mainly to the presence of polyphenols [4].

Several works of our department have dealt with *Alchornea cordifolia* [5] [6]. *A. cordifolia* is a very popular herb in African traditional medicine for its many properties. Many studies have demonstrated its antioxidant properties [7] [8]. This antioxidant activity is manifested by free radical scavenging [9] [10], which can confer a hepatoprotective effect [7] [8] [11]. The liver is indeed the main organ involved in metabolism and detoxification for the excretion of various endogenous and exogenous substances. However, liver cells are prone to be attacked and necrotic by free radicals [12].

The antioxidant properties of a methanolic extract and an ethanolic extract of *A. cordifolia* have been studied [7] [8]. The hepatoprotective properties of these extracts have also been demonstrated against high-dose paracetamol-induced hepatotoxicity in an animal model [7] [11].

However, the antioxidant properties and the hepatoprotective effect of the aqueous extract, which is the most usual form in traditional human medicine, seem to have been little studied. In addition, the hepatoprotective effect of *A. cordifolia* has not been found in the literature against the hepatotoxicity of anti-tubercular drugs. Anti-tubercular drugs (isoniazid (INH) and rifampicin (RIF)) are responsible for many adverse effects in liver at the usual doses [13] [14]. These effects are cytolytic and result in an increase in serum transaminases [15] [16]. Aouam *et al.* (2007) [17] reported hepatic disorders in 10% to 20% of users in Tunisia with INH at therapeutic dose, when Blumberg *et al.* (2003) [13] reported disturbances of hepatic markers in 0.5% to 2% of patients in the USA. When the RIF was associated with the INH, this disturbance affected more patients [17], which was 2.5% to 6% of the patients according to Blumberg *et al.* (2003) [13].

This work aimed to evaluate the antioxidant properties and the hepatoprotective effect of an aqueous extract of *A. cordifolia* leaves in order to overcome the

hepatotoxicity of anti-tubercular drugs in rats.

## 2. Material and Method

**Plant material**

The plant material consisted of leaves of *Alchornea cordifolia* (Schum. and Thonn.) collected at Yakasse-Mé (in the city of Adzopé about 75 km from Abidjan, Ivory Coast). Voucher samples (AC 2016) are kept in the Pharmacology laboratory. The leaves were authenticated at the National Floristic Center of Abidjan, affiliated to Université Félix Houphouët Boigny (Abidjan). These leaves were air-dried in the laboratory at 18˚C.

**Extraction method**

Fine powder of dried leaves (100 g) was macerated for 24 h at room temperature in 1 L of distilled water. The resulting filtrate was dried in a MEMMERT oven at 60˚ for 72 h. The obtained dry extract (aqueous extract of *Alchornea cordifolia*: AEAC) was conserved at 4˚C; and aliquots of dry powder were used for pharmacological studies after being suspended in physiological saline.

**Animal material**

The animal material consisted of rats, *Rattus norvegicus*, Wistar strain weighing between 150 g and 220 g. 96 rats were used for 3 series of studies. They were obtained from the laboratory animals of the Pharmacology Laboratory of the Faculty of Pharmacy and Biological Sciences of Université Félix Houphouët Boigny Abidjan (Côte d'Ivoire). All animals were kept under controlled environmental conditions of 24˚C ± 1˚C with a cycle of 12 hours of light and 12 hours of darkness. Food and water and are given *Ad libitium*. Before the beginning of the experiment, they were subjected to fasting for 12 hours with free access to water.

**Chemicals materials used**

In this study, we used isotonic saline solution 0.9%, ether (Gifrer), distilled water, anti-tubercular drugs (INH (Lupine LTD), RIF (Remedica LTD), methanol, DPPH (1,1-diphenyl-2-picrylhydrazyl) (Sigma Aldrich, Germany), ascorbic acid (Sigma Aldrich, Germany) and silymarin (Sigma Aldrich, Germany).

Silymarin was used as a reference liver protector substance in this study. It is a mixture of three flavonoids (silychristin, silydianine and silybin) used as a hepatoprotective agent. It is extracted from the seeds and fruits of Silybum marianum (Parthasarathy *et al.*, 2007) [18].

**Chemical analysis**

Screening for different chemical groups was done using the method as described in the works of Békro *et al.* (1973) [19], Ronchetti and Russo (1971) [20] and Wagner (1983) [21].

**Antioxidant screening**

**Measurement of the reducing power of iron**

**Principle**

The reducing power of iron ($Fe^{3+}$) in preparations is determined according to the method described by Oyaizu M. (1986) [22] and Bougandoura and Nassima

(2013) [23]. The iron reduction method is based on the reduction of ferric iron to iron salt by the antioxidants which give the blue color in the reaction medium at 700 nm. The increase in the absorbance indicates an increase in the reduction of ferric iron and therefore of the reducing power of the extracts tested.

**Procedure**

One milliliter of the extract at different concentrations (0.007 to 2.5 mg/mL) is mixed with 2.5 ml of a 0.2 M phosphate buffer solution (pH 6.6) and 2.5 ml of a solution of potassium ferricyanide $K_3Fe(CN)_6$ at 1%. The whole was incubated in a water bath at 50˚C. for 30 min. Then, 2.5 mL of 10% trichloroacetic acid has been added to stop the reaction. The tubes are centrifuged at 3000 rpm for 10 min.

An aliquot (2.5 mL) of the supernatant was combined with 2.5 mL of distilled water and 0.5 mL of a 0.1% aqueous solution of $FeCl_3$ (Ferric Chloride); and incubated for 10 min at room temperature. The absorbance of the reaction medium was red at 700 nm against a similarly prepared white, replacing the extract with distilled water which makes it possible to calibrate the apparatus (UV-VIS spectrophotometer HACH made in USA).

The positive control was represented by a standard of an antioxidant, trolox (1.5 to 25 µg/mL) whose absorbance was measured under the same conditions as the samples. An increase in absorbance corresponds to an increase in the reducing power of the extracts tested.

**DPPH radical scavenging activity**

**Principle**

The method used was described by Parejo *et al.* (2000) [24] which is the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging. Indeed, the reduction of DPPH by a free radical sensor is accompanied by its passage from the violet color to the yellow color, measurable at 517 nm. A low absorbance reflects a strong inhibition of DPPH and therefore a strong antiradical activity.

**Procedure**

An aliquot of 0.5 ml of 0.1 mM DPPH in methanol was added to test tubes containing 2.5 ml of different concentrations (0.00625 - 0.1 mg / ml) of the methanol extract. The reaction mixture was mixed at room temperature and held for 20 minutes. The absorbance was read at 517 nm against a blank. The percentage inhibition of the DPPH radical was calculated according to the formula:

$$\text{DPPH inhibition}(\%) = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of the control}} \times 100$$

**Hepatoprotective activity**

**Principle**

The study involved inducing hepatotoxicity in laboratory rats by using antitubercular drugs in different combination [25] [26], and then evaluating the effect of different preparations on hepatic markers.

**Procedure**

**Effect of AEAC on hepatotoxicity markers**

Rats of both sexes were divided into 4 batches of 6 rats each and were treated

for 10 days as follows:

1) The rats in lot 1 (negative control) received saline solution by gavage;

2) The rats in lot 2 received AEAC at 200 mg/kg/day by gavage;

3) The rats in lot 3 received AEAC at 400 mg/kg/day by gavage;

4) The rats in lot 4received AEAC at 800 mg/kg/day by gavage.

**Hepatoprotective effect against INH-induced hepatotoxicity:**

Rats of both sexes were divided into 6 batches of 6 rats each and were treated for 10 days as follows:

1) The rats in lot 1 (negative control) received saline solution by gavage;

2) The rats in lot 2 received INH (100 mg/kg/day) by gavage;

3) The rats in lots 3, 4 and 5 received AEAC (200, 400 and 800 mg/kg) orally 2 hours after administration of INH (100 mg/kg/day);

4) The rats in lot 6 (positive control) received silymarin (100 mg/kg/day) orally, 2 h after administration of INH (100 mg/kg/day).

**Hepatoprotective effect against INH+RIF-induced hepatotoxicity:**

Rats of both sexes were divided into 6 batches of 6 rats each and were treated for 10 days as follows:

1) The rats in lot 1 (negative control) received saline solution by gavage;

2) The rats in lot 2 received INH (100 mg/kg/day) + RIF (100 mg/kg/day) by gavage;

3) The rats in lots 3, 4 and 5 received AEAC (200, 400 and 800 mg/kg/day) orally 2 hours after administration of INH (100 mg/kg/day) + RIF (100 mg/kg/day);

4) The rats in lot 6 (positive control) received silymarin (100 mg/kg/day) orally, 2 h after administration of INH (100 mg/kg/day) + RIF (100 mg/kg/day).

**Liver Biochemical Indices measured**

At the end of the 10 days of treatment, a blood sample was taken the $11^{th}$ day by cardiac puncture for the determination of markers of hepatotoxicity. They were alanine aminotransferase (ALT) and aspartate aminotransferase (AST) [27], using standard kits.

**Statistical analysis**

The results were expressed as mean ± SD. Statistical analysis used the Wilcoxon test. The difference between the mean values was considered significant if $p < 0.05$.

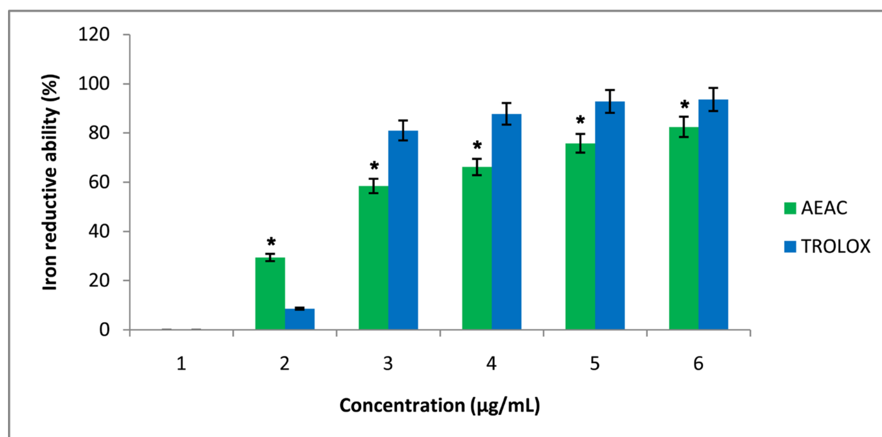
## 3. Results

**Extraction yield**

Extraction with distilled water (AEAC) gave 8.34 g of dry residue, a yield of 8.34%.

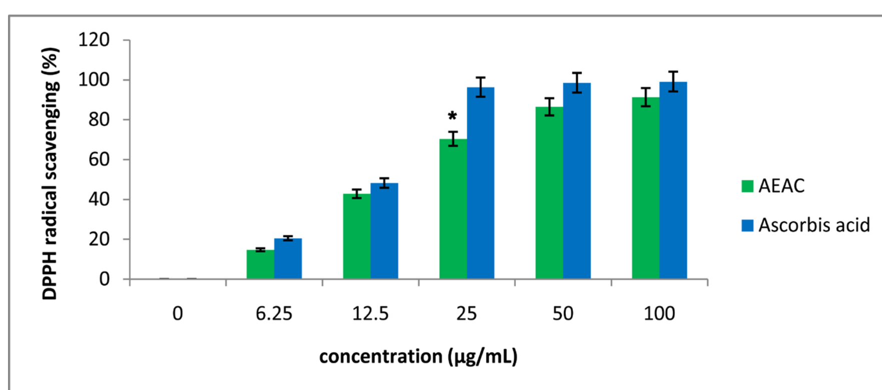
**Result on measurement of reducing power**

Figure 1 shows the results of iron reductive ability by AEAC and trolox. The AEAC has a reducing power which increases proportionally with the concentration. The same is true of the trolox. However, the AEAC activity is statistically inferior to that of the trolox.




*: $p < 0.05$ compared to trolox; AEAC: aqueous extract of *A. cordifolia.*

Figure 1. *In vitro* iron reductive ability of AEAC and trolox.




*: $p < 0.05$ compared to ascorbic acid; AEAC: aqueous extract of *A. cordifolia.*

Figure 2. *In vitro* anti-radical activity of AEAC and ascorbic acid.

**Measurement of anti-radical activity: DPPH**

The results of the anti-radical activity of AEAC and vitamin C are recorded in Figure 2. The AEAC shows an antiradical activity which increases with the concentration as well as the ascorbic acid. The anti-radical activity of the AEAC remains statistically comparable to that of the vitamin C at 100 μg/mL.

**Phytochemical characterization test**

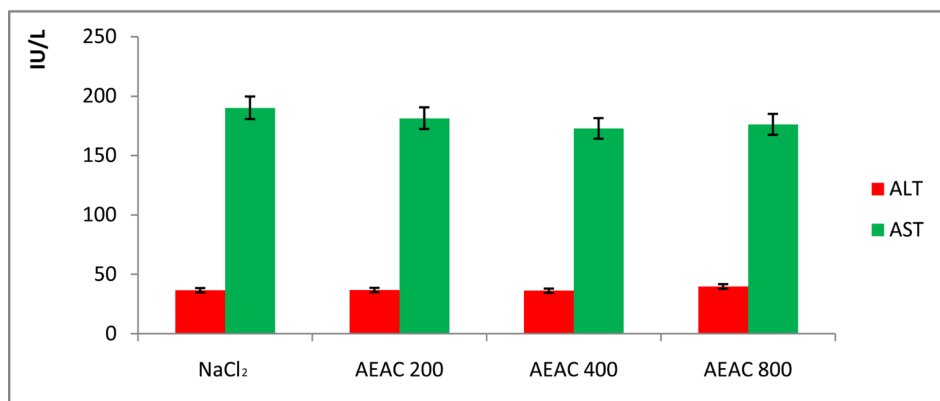
The characterization tests of the large phytochemical groups carried out on the AEAC revealed the presence of flavonoids, tannins and alkaloids, and an absence of saponosides.

**Results of the EAAC effect on transaminases**

The effect of AEAC alone was evaluated on serum transaminases and the various mean values are recorded in Figure 3. The different doses of AEAC alone did not increase the transaminase values compared to the $NaCl_2$ ($p > 0.05$).

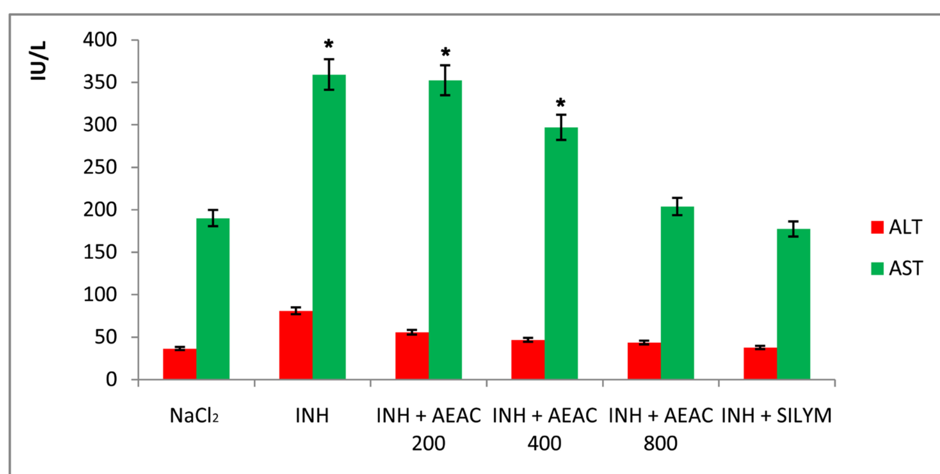
**Results on hepatoprotective effect against hepatotoxicity induced by isoniazid**

The mean of transaminases values are shown in Figure 4. Isoniazid resulted in elevated transaminases (ALT and AST) ($p = 0.028$) in rats receiving it compared




AEAC: aqueous extract of *A. cordifolia*.

**Figure 3.** Effect of AEAC on perturbations of serum transaminases.



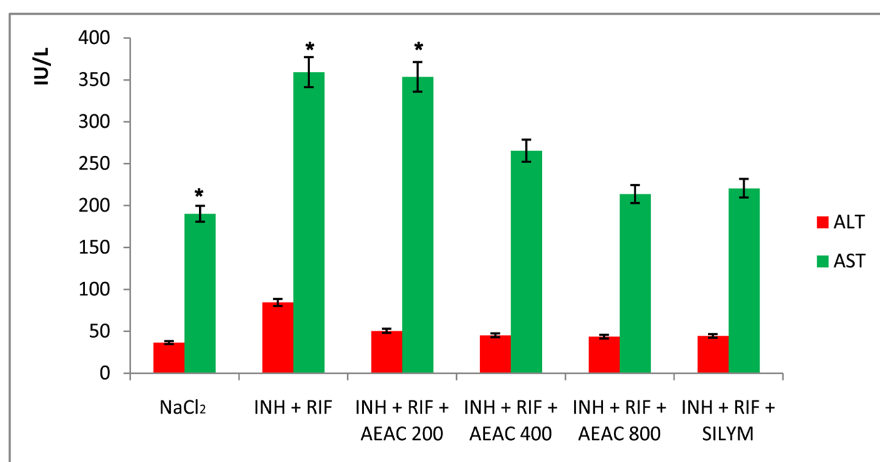

*: $p < 0.02$ compared to the control; INH: Isoniazid; AEAC: aqueous extract of *A. cordifolia*; SILYM: Silymarin.

**Figure 4.** Effect of AEAC on perturbations of serum transaminases induced by INH.

to rats given $NaCl_2$. Administration of AEAC to 800 mg/kg and silymarin to rats significantly reduced the different values of disturbed transaminases ($p < 0.05$). The AEAC at 200 mg/kg and 400 mg/kg reduced only ALT, with AST remaining high ($p = 0.143$). The comparison of the different doses of the AEAC between them showed a marked activity at 800 mg/kg ($p > 0.05$) on the AST. The AEAC at 800 mg/kg showed similar activity to silymarin ($p > 0.05$).

**Results on hepatoprotective effect against hepatotoxicity induced by isoniazid + rifampicin**

The mean of transaminases values for evaluation of liver function are shown in Figure 5. The combination of INH + RIF resulted in an elevation of ALT and AST ($p = 0.02$) in rats receiving it compared to rats given $NaCl_2$. The administration of AEAC at 400 mg/kg, 800 mg/kg and silymarin in rats resulted in a significant reduction in ALT and AST disturbed levels ($p < 0.05$), AEAC 200 mg/kg having been active only on the ALT. Comparison of AEAC to 400 mg/kg and 800 mg/kg, as well as to silymarin, showed no significant difference ($p < 0.05$).




*: $p < 0.02$ compared to the control; INH: Isoniazid; RIF: Rifampicin; AEAC: aqueous extract of *A. cordifolia;* SILYM: Silymarin.

**Figure 5.** Effects of AEAC on perturbations of serum transaminases induced by INH + RIF.

## 4. Discussion

This study aimed to evaluate the *in vitro* antioxidant activity of an aqueous extract of *A. cordifolia* leaves and its hepatoprotective effect *in vivo* in rats.

The antioxidant activity of the aqueous extract of *A. cordifolia* leaves was evaluated by iron reductive ability [28] and free radical scavenging [24].

The presence of reducing agents in an extract causes the reduction of ferric iron ($Fe^{3+}$) to ferrous iron ($Fe^{2+}$). Antioxidants are considered oxidant reducing agents and inactivators [29]. Other studies have also shown that the reducing power of a compound could serve as a significant indicator of its potential antioxidant activity [30] [31].

DPPH radical involves a hydrogen atom transfer process [32]. The antiradical activity results in a donation of electrons or protons reducing the hydrazyl radical form of DPPH in non-radical form hydrazine. All substances with free radical scavenging are known as antioxidants [33]. The aqueous extract of *A. cordifolia* would trap the DPPH radical and thus could have antioxidant activity.

There is a potential antioxidant activity of the aqueous extract of *A. cordifolia*. This antioxidant activity is statistically less than that of trolox and vitamin C. Osadebe *et al.* (2012) [8] showed comparable antioxidant activity of the methanolic extract of *A. cordifolia* to that of vitamin C in their studies. Other studies on an ethanolic extract of *A. cordifolia* showed better antioxidant activity than vitamin E [7].

Comparative study of the chemical constituents revealed the presence of alkaloids, saponins, tannins, and flavonoids both in the methanolic extract [8] and in the ethanol extract [7], while the aqueous extract contained alkaloids, tannins and flavonoids. It emerges almost a similarity of the major chemical groups likely to be responsible for antioxidant activity. Indeed, Manga *et al.* (2004) [34] found that flavonoids possess antioxidant activities. Various other studies have shown that the

antioxidant activity was mainly due to the presence of polyphenols [4]. Huong *et al.* (1998) [35] and Ince *et al.* (2014) [36] also showed that the saponosides were endowed with antioxidant properties. Based on this finding, a quantitative determination of the large chemical groups in these different extracts could justify the lower antioxidant activity of the aqueous extract. Since methanol and ethanol are more polar than water, they are able to extract more active ingredients from a plant, and thereby give better activity to the final extract.

This antioxidant activity of the aqueous extract of *A. cordifolia* could confer a protective effect of the liver which is subject to attack and necrosis by free radicals [12].

The study of the hepatoprotective effect of the aqueous extract of *A. cordifolia* was demonstrated using high-dose anti-tubercular drugs for 10 days in different combinations to induce hepatotoxicity in laboratory animals [25] [26]. They produce various categories of lesions in the liver, including centrilobular necrosis, hepatic cell degeneration [37]. These lesions resulted in an increase in serum transaminases [15] [16] as observed in hepatotoxic rats.

Isoniazid alone and the Isoniazid + Rifampicin combination resulted in a significant increase in ALT and AST levels. ALT and AST are two well-known diagnostic indicators of liver damage. In liver damage with hepatocellular lesions and parenchymal cell necrosis, these marker enzymes are released from damaged tissues and their levels increase in blood flow [38]. These are two hepatic enzymes linked to the subcellular functions of the mitochondria [39].

Administration of the aqueous extract of *A. cordifolia* at 200 mg/kg, 400 mg/kg and 800 mg/kg significantly decreased disturbed ALT values. The extract at 800 mg/kg decreased the AST. The extract at 800 mg/kg had an effect on ALT and AST which was superposable to that of silymarin, a hepatoprotectant used as a reference in this study [18]. The lower doses of the extract were active only on ALT, which is more specific to the liver. Nonetheless, the AST values were reduced, although not significant, at these doses ($p > 0.05$).

This ability of the aqueous extract of *A. cordifolia* to lower these values may suggest hepatoprotective activity of *A. cordifolia* leaves, especially at 800 mg/kg. This activity would be manifested by stabilization of the hepatic membrane and regeneration of the hepatocytes. The extract would also prevent the release of hepatic enzymes at the level of the blood stream by a reduction of the tissue lesions [40] [41].

This hepatoprotective activity of an ethanol extract and a methanolic extract of *A. cordifolia* leaves has already been demonstrated in rats against hepatotoxicity induced by paracetamol at high levels [7] [11] [42] and carbon tetrachloride [8]. The results of our work on the aqueous extract of *A. cordifolia* confirm this property of *A. cordifolia*. Moreover, at all the doses used, the aqueous extract of *A. cordifolia* was not hepatotoxic itself, in contrast to the methanolic extract which was hepatotoxic at doses greater than or equal to 800 mg/kg in of male rats [43]. More polar solvents, in addition to extracting more active ingredients for better efficiency, would also extract more toxic principles. The aqueous ex-

tract of *A. cordifolia* would therefore be safer to use.

## 5. Conclusion

The present study demonstrated that the aqueous extract of *A. cordifolia* leaves could have antioxidant activity and confer significant protection against the hepatotoxic effects of anti-tubercular drugs.

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