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Full Length Research Paper

Alchornea cordifolia extract protects wistar albino rats against acetaminophen-induced liver damage

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At the therapeutic doses, aetaminophen (*N*-acety1-*p*-aminophenol, paracetamol, AAP) is considered a safe drug, intake of toxic dosage could lead to liver disease. The hepatoxicity of AAP is mainly as a result of oxidative stress mediated by the metabolites of APP. The present work seeks to evaluate phytochemical constituents, antioxidative properties and hepatoprotective activities leaf extract of *Alchornea cordifolia* on acetaminophen-induced hepatoxicityin rats. Phytochemical analyses gave positive results for saponins, tannins and flavonoids. The antioxidative properties revealed total phenolic content of 0.22 mg/ml and reducing power 0.062 mg/ml as compared to vitamin E with a reducing power of 0.042 mg/ml. Oral administration of a single acetaminophen dose (2 g/kg) caused oxidative liver damage as determined by alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activities, cholesterol and bilirubin levels in the liver and the serum. The administration of 200-500 mg/kg *A. cordifolia* leaf extract for 2 weeks produced a significant dose-dependent curative/preventive effect on acetaminophen-nduced liver toxicity as reflected by the above biochemical markers. The protective effect compared favorably with putative antioxidant agents such as curcumin and Vitamin E. The results so far suggest that the hepatoprotective activity of this plant against acetaminophen-nduced liver damage is connected to its antioxidative properties.

Key words: *Alchornea cordifolia,* acetaminophen, aminotransferase, hepatoprotective agents.

INTRODUCTION

Medicinal and poisonous plants have always played an important role in African societies. However, among the more than 250, 000 species of higher plants, only about 10% have been chemically investigated (Nahrstedt, 1996). The folklore knowledge of medicinal plants has significantly contributed in discovering many important drugs of the modern system of medicine. Most developing countries are endowed with vast resources of medicinal plants. In fact, modern pharmaceuticals still contain at least 25% drugs derived from plants.

Medicinal plants have various effects on living systems. Some are sedatives, analgesics, antipyretics, cardioprotectives, antibacterials, antivirals and antiprotozoals. However, this study focuses on remedies for liver damage; there is an alarming increase in the incidence of alcohol and drug related liver damage.

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Acetaminophen (*N*-acety1-*p*-aminophenol, paracetamol, AAP) is a clinically important over-the-counter drug commonly used for its analgesic and antipyretic properties. At the therapeutic doses, AAP is considered a safe drug. However, it can cause hepatic necrosis, nephrotoxicity, extra hepatic lesions, and even death in humans and experimental animals when taken in overdoses (Ray et al., 1996; Webster et al., 1996). Lipid peroxidation resulting from oxidative stress contributes to the initiation and progress of liver damage (Albano et al., 1985;Kyle et al., 1987) and the data trom the studies of Michael et al., 1999 suggested that APP hepatotoxicity is mediated by an initial metabolic oxidation, covalent binding and subsequent activation of macrophages to form reactive oxygen and nitrogen species.

Conversely, a number of plants have been found to offer some hepatoprotection. These include *Trichilia roka* (Germano et al., 2004), *Hemidesmus indicus* (Prabakan et al., 2004), *Cassia fistula* leaf extract (Bhakta et al., 2004), legumes (Wu et al., 2004) and *Acanthus ilicifolius* (Babu et al., 2004).

Alchornea cordifolia (Thonn and schumach) Mull. Arg. (Euphorbiaceae) is an erect, sometimes scrambling, bushy perennial shrub or small tree, up to 4 m high reproducing from seeds. The stem is woody, greyish, much branched and bushy when young. The leaves are simple and alternate, broadly ovate, 10-28 cm long and 6.5-16.5 cm broad. The leaf blade is heart shaped at the base, a culminate at the apex, entire to sub-entire at the margin with long petioles. The leaf is mostly smooth to the touch but often has a few glands at the base. The inflorescence consists of auxiliary panicles, the flowers are greenish white. The male flowers are long spikes 8-36 cm long while the females are simple and have short stalks. The fruit is 3-chambered capsules with red seeds.

There are many convergence in its traditional use throughout tropical Africa as topical anti-inflammatory, chancre, yaws (Neuwinger, 2000), wounds and ulcers (Bouquet and Debray, 1974; Neuwinger, 2000), toothache (Delaude et al., 1971), gum inflammation (Neuwinger, 2000) and conjunctivitis (Neuwinger, 2000). Despite the popular uses of this plant in traditional medicine, there is dearth of information on its hepatoprotective potentials. In continued search for hepatoprotective medicinal plants in our Laboratory, this work aims to evaluate antioxidant and hepatoprotective activities of *A. cordifolia*.

MATERIAL AND METHODS

Sample preparation and extraction

The leaves of *A. cordifolia* were collected from a piece of land at Federal University of Technology, Akure, Nigeria. The sample was authenticated by Mr. Aduloju (Chief Technologist) in the Department of Crop Science and Pest Management, Federal University of Technology, Akure, Nigeria. Voucher samples (AC 2005) are kept in the Biochemistry laboratory. The leaf samples were air-dried at room temperature and blended to a mesh size of 1 mm. The blended samples (500 g) were soaked in 2 litres of 98% ethanol for 48 h, filtered and concentrated to dryness using rotary evaporator. The ethanolic extract was kept in the refrigerator for analyses.

Phytochemical screening

The ethanolic extract was screened for the presence of some secondary metabolites such as saponins, tannins, anthraquinones, alkaloids, flavonoids and cardiac glycosides. This was done according to the methods described by Sofowora (1993).

Total phenol

The concentrations of phenolic compounds in the ethanolic extract of the leaf of *A. cordifolia*, expressed as tannin equivalents, were measured using a modified method of Singleton et al. (1999). Ethanolic extract (0.2 g) was dissolved in 10 ml of acetone for 10 min in ice-cold water. To 0.5 ml of the solution, 0.5 ml of distilled water, 0.5 ml of folin's reagent (1: 1) and 2.5ml of 20% sodium carbonate were added. The reaction mixtures were kept in the dark for 40 min, after which the absorbance was read at 750 nm. Phenol contents were extrapolated from standard tannin calibration curve.

Reducing property

The ability of *A. cordifolia* leaf extracts to reduce ferric chloride was measured according to a modified method of Pullido et al. (2000). The reducing property was expressed as tocopherol (Vitamin E) equivalent. The ethanol extracts (5 g) was dissolved in 10 ml of water and filtered. To 2.5 ml of the filtrate, 2.5 ml of phosphate buffer (pH 6.6) and 2.5 ml of potassium ferrocyanide were added. The mixtures were incubated at a temperature 40°C. 10% trichloroacetic acid was added. The resulting mixtures were centrifuged for 10 min. The supernatant (5 ml) was mixed with 5 ml of distilled water and 1 lof 0.1% ferric chloride. The absorbance of the standard and the sample were read at 700 nm against reagent blank.

Free radical scavenging

The scavenging activity of the Vitamin E and *A. cordifolia* ethanolic leaf extract on DPPH radicals were determined according to the method of Chu chang and Hsu (2000). An aliquot of 0.5 ml of 0.1mM DPPH radical in methanol was added to test tubes containing 1 ml of different concentrations (5–50 mg/ml) of the ethanolic extract. The reaction mixture was mixed at room temperature and kept for 20 min. The absorbance was read at 520 nm against distilled water.

Hepatoprotective activities

Adult albino rats (male) of *Wistar strain* weighing 150-200 g were obtained from Federal University of Technology (Animal Production and Health Department), Akure, Nigeria. They were made to acclimatize to laboratory conditions for 10 days and were fed a commercial pelleted (Guinea feeds) rat chow and water *ad libitum*. The bioassay was conducted in accordance with the internationally accepted principles (European Community guidelines EEC Directives of 1986, 86/609/eec; US guideline, NIII publication #85-23, revised in 1985) for laboratory animal care. The animals were grouped into nine of five animals each as follows:

Group I: Control i.e. uninduced
Group II: Intoxicated control induced with 2 g/kg acetaminophen (paracetamol)
Group III: Induced with 2 g/kg paracetamol and treated with 100 mg/kg vitamin E standard.
Group IV: Induced with 2 g/kg paracetamol and treated with 100 mg/kg curcumin standard.
Group V: Treated with 300 mg/kg plant extract only.
Group VI: Induced with 2 g/kg paracetamol and treated with 200 mg/kg plant extract.
Group VII: Induced with 2 g/kg paracetamol and treated with 300 mg/kg plant extract Group VIII: Induced with 2 g/kg paracetamol and treated with 400 mg/kg plant extract Group IX: Induced with 2 g/kg paracetamol and treated with 500 mg/kg plant extract

Hepatotoxin (acetaminophen, 2 g/kg) was administered to all the groups except control followed by the plant extract of varying dosages (200-500 mg/kg) to all the groups except the intoxicated control (acetaminophen only) and those that were given the standards; vitamin E and curcumin (100 mg/kg) for a period of 14 days.

He animals were sacrificed 24 h after the last administration by cervical decapitation. The livers were removed and divided into two. One part was homogenized in ice-cold isotonic sucrose while the remaining part of the liver was used for histopathological studies. The blood obtained was centrifuged to give a clear serum. Both the serum and the liver homogenate were used for the estimation of the liver function parameters.

Biochemical studies on liver and serum

Serum and livers were collected for the analyses of the following: bilirubin (Doumas et al., 1973), cholesterol (Zoppi and Fellin, 1978), aspartate amino transferase and alanine amino transferase (Reltman and Frankel, 1957) and alkaline phosphatase (Babson et al., 1966).

Histopathological studies of livers

The sectioning method described by Lamb (1991) was used for the histological examination. A thin section of the organ (thickness being 6 microns) was used. This method has the advantage of preserving the relations of cells and tissues to one another. Small pieces of organs 3-5 mm thick were fixed in 10% (w/v) formosaline solution for 24 h. They were washed in running water for 24 h. They were then dehydrated by passing through ascending grades of alcohol: 50, 70, 90 and 100%, for 2-3 days following which they were cleared in benzene to remove alcohol till the tissue became more or less transparent. They were later passed through three cups containing molten paraffin at 58°C and finally embedded in a cubical block of paraffin made by the L moulds.

From the embedded samples, sections of 6 microns thick were cut using the microtone and fixed on a slide by Mayer's albumen. The sections were stained with haematoxylin to cover the section and kept for 6 min. Excess stain was removed with tap water. Eosin was added to cover the stem for 2 min. Excess Eosin was poured away and removed with tap water. This was covered with a cover glass to avoid air bubbles, and viewed with the use of both low power and high power microscope

Statistical analysis

All analyses were performed in triplicates. The data were recorded as means ± standard deviation and analyzed by SPSS (version 11 for windows SPSS Inc.). One-way analysis of variance (ANOVA) and Duncan test were carried out to test any significant differences between their means. Differences between means at 5% significant level (P – value <0.05) level were considered.

RESULTS

Phytochemical screening

The phytochemical screening revealed the presence of alkaloids, saponins, tannins, flavonoids and glycosides with steroidal rings (Table 1).

Antioxidant properties

In Table 2, the concentration of the total phenol present in the plant was 0.22 mg/ml as tannin equivalents, reducing property of 0.0625 mg/ml and that of Vitamin E was 0.04 mg/ml. The scavenging activity of the extract as measured by the inhibition of 1,1–diphenyl–2-Picrylhydrazyl (DPPH) radical (Figure 1) was related to the concentration of the extract added. 50 mg/ml of the extract showed 92% inhibition and 5 mg/ml of vitamin E showed 90.2% inhibition of DPPH radical.

Hepatoprotective activity

In Tables 3 and 4, the serum activities of aspartate amino transferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) of the animals treated with hepatotoxin plus the leaf-extract was significantly lowered than the group treated with hepatotoxin alone. However, there were significant increases in the activities of these enzymes in the serum of the animals treated with heaptotxin alone as compared with the control. This trend was opposite in the liver homogenate. Increase in the serum enzyme activity signifies damages to the liver membrane. The serum AST activity of animals treated with curcumin, vitamin E and 400 mg/kg of plant extract were not significantly different form each other, whereas the serum AST activity of the group fed with plant extracts only (183±3.74 IU/g) was significantly lower than that of the control

Table 1. Phytochemical constituents of *Alchornea cordifolia* leaf-extract.

Constituents	Results
Alkaloids	+
Saponins	+
Tannin	+
Phlobatannins	-
Anthraquinones	-
Flavonoids	+
+, Positive; -, Negative	

Table 2. Antioxidant indices of *Alchornea cordifolia* leaf extract

Indices	Level (mg/ml)
Total phenol	0.22
Reducing property	
Alchornea cordifolia	0.0625
Vitamin E	0.042

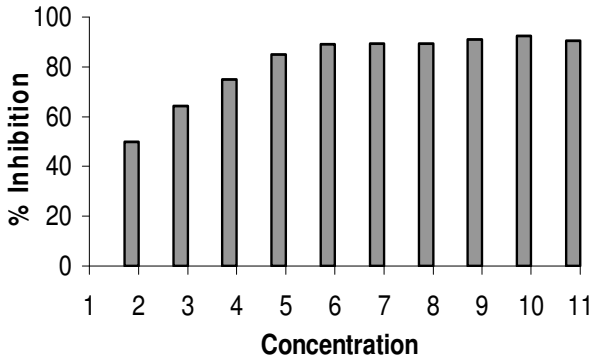

Figure 1. Free radical scavenging (DPPH) ability of extract of *Alchornea cordifolia* as compares with that of vitamin E. 1 –10 are various concentrations of the plant extract ranging from 5 to 50 mg/ml while 11 is the concentration of vitamin E (5 mg/ml).

Table 3. Effects of *Alchornea cordifolia* leaf extracts on some liver biochemical indices of rats given 2 g/kg acetaminophen.

Treatment	AST IU/g	ALT IU/g	ALP IU/g	Cholesterol IU/g	Total bilirubin IU/g	Direct bilirubin IU/g	GST µM/Abs/ g/L	Total protein min ($x 10^{-1}$)
Control	192.2^{d}±2.48	158.4^{abc}±3.28	50.29^{d}±2.93	335^{h}±3.74	2.36^{a}±0.26	1.72^{a}±0.84	0.33^{e}±0.08	1.74^{a}±0.18
Toxicant	86.0^{a}±2.48	63.4^{a}±3.44	46.01^{cd}±0.00	$229^{b\pm}$3.65	3.04^{cd}±0.59	2.64^{b}±0.69	0.25^{b}±0.03	3.96^{cd}±0.19
Tox. + Vit. E	85.3^{a}±3.89	157^{bc}±5.09	79.6^{d}±17.34	293.4^{f}±3.4	2.22^{a}±0.19	1.78^{a}±0.16	0.64^{e}±0.05	1.94^{ab}±0.29
Tox. + curcumin	190.6^{d}±5.27	157.5^{bc}±5.21	46.01^{cd}±0.00	284^{e}±3.81	2.58^{a}±0.19	2.44^{b}±0.3	0.50^{d}±0.04	1.86^{ab}±0.29
300 mg/kg PE only	176^{c}±3.39	183^{f}±3.74	18.64^{a}±0.24	294.2^{f}±2.95	2.78^{bc}±0.22	2.34b±0.32	0.14a±0.01	2.24^{b}±0.41
Tox. + 200 mg/kg PE	189.6^{d}±6.06	153.8^{b}±3.89	53.05^{d}±3.96	224.6^{a}±2.61	4.66^{e}±0.18	3.51^{c}±0.32	0.28^{bc}±0.06	4.24^{d}±0.09
Tox. + 300 mg/kg PE	189.8^{d}±6.01	164.4^{d}±3.05	39.8^{c}±1.16	234.8^{c}±4.17	5.73^{f}±0.41	3.96^{c}±0.21	0.46^{d}±0.08	2.20^{b}±0.45
Tox. + 400 mg/kg PE	116.4^{b}±6.22	162^{bc}±6.48	19.83^{ab}±1.62	276.0^{d}±3.81	3.24^{d}±0.15	1.70^{a}±0.16	0.81^{f}±0.06	3.93^{c}±0.31
Tox. + 500 mg/kg PE	115.6^{b}±2.99	171.42^{e}±8.73	27.36^{b}±1.97	304.8^{g}±4.27	2.34^{a}±0.15	1.66^{a}±0.11	0.89^{g}±0.04	3.70^{c}±0.32

PE: Plant extract.
Values with same superscript down the column are not statistically significantly different from one another.

Table 4. Effects of *Alchornea cordifolia* leaf extracts on some serum biochemical indices of rats given 2 g/kg acetaminophen.

Treatment	AST IU/g	ALT IU/g	ALP IU/g	Cholesterol IU/g	Total bilirubin IU/g	Direct bilirubin IU/g	GST µM/min/ ml
Control	36.20^{c}±1.79	32.40^{d}±1.82	44.47^{c}±2.11	227.2d±2.17	1.36^{a}±28	0.80^{ab}±28	0.8^{a}±0.02
Toxicant	596.00^{d}±16.73	98.40^{f}±4.21	98.80^{e}±7.42	595.60f±41.43	5.04^{f}±0.36	4.34^{g}±0.47	0.40^{b}±0.04
Tox. + Vit. E	36.80^{c}±2.28	28.60^{c}±1.67	27.75^{b}±2.17	190.4e±15.64	1.48^{a}±0.13	0.70^{a}±0.16	0.29^{a}±0.02
Tox. + curcumin	18.10^{ab}±1.14	13.10^{a}±2.13	46.01^{c}±0.00	16.42b±16.16	1.83^{b}±0.67	1.36^{cd}±0.23	0.31^{a}±0.02
300 mg/kg PE only	14.60^{a}±2.61	24.00^{b}±2.23	20.30^{a}±5.20	105.60a±3.64	2.36^{c}±0.39	1.50^{de}±0.25	0.17^{a}±0.0
Tox. + 200 mg/kg PE	37.80^{c}±2.28	46.66^{e}±2.89	46.02^{c}±0.00	249.00e±6.40	2.78^{d}±0.23	1.74^{e}±0.11	0.81^{a}±0.02
Tox. + 300 mg/kg PE	34.40^{c}±3.78	25.80^{bc}±3.03	46.01^{c}±0.01	256.40e±6.27	3.38^{e}±0.25	2.58^{f}±0.26	72.2^{a}±4.15
Tox. + 400 mg/kg PE	24.22^{b}±2.80	27.20^{bc}±2.77	45.61^{c}±0.88	197.60c±8.76	2.23^{c}±0.21	0.98^{a}±0.14	0.36^{a}±0.04
Tox. + 500 mg/kg PE	16.80^{ab}±2.17	$11.^{20a}$±1.79	91.80^{d}±0.45	179.20c±4.60	1.66^{ab}±0.09	1.10^{b}±0.12	0.23^{a}±0.04

PE: Plant extract.
Values with same superscript down the column are not statistically significantly different from one another.

Table 5. Observed features of histopathological studies of the liver of albino rats treated with *Alchornea cordifolia* compared with standard drugs.

Groups	Observation
Control	Normal
Hepatotoxin (2 g/kg)	Cellular in filtration, necrosis, disorientation of the parenchyma tissue of the liver, vacuolar formation in the parenchyma.
Toxin +Vit. E (100 mg/kg)	Normal orientation.
Toxin+curcumin (100 mg/kg)	Normal
Toxin+Plant extract (200 mg/kg)	Normal except for some level of cellular in filtration and vacuolar formation
Toxin+Plant extract (300 mg/kg)	Normal
Toxin+Plant extract (400 mg/kg)	Normal
Toxin+Plant extract (500 mg/kg)	Normal

(158.4 ± 3.28 IU/g). The serum activities of ALP of the treated animals were significantly different from that of the control except group fed with 200 mg/kg, which was not significantly different. Generally, the activities of these enzymes in the plant-treated groups compared favorably with the curcumin and vitamin E groups. Total bilirubin contents were reduced down the group i.e. in a dose dependent manner compared to the hepatotxin group alone. Cholesterol was also significantly reduced in the serum of the plant-extract treatment. In Table 5, the rats of the group fed with acetaminophen only showed some cellular infiltration and necrosis (Table 5). The group fed with 200 mg/kg showed reduced healing of the liver damage while other groups were able to heal the liver damage by restoring them back to normal.

DISCUSSION

Perchellet et al. (1996) found out that foliage tannins have potent antioxidants of anti-inflammatory activities, so also flavonoids have been said to possess antioxidant and anti-inflammatory activities (Manga, 2000). Okuda et al. (1983) reported that tannins and related compounds might prevent the destructive effects of lipid peroxide in liver cells by lowering the levels of lipid peroxide in liver cells. Okuda et al. (1983) elucidated that tannins in medicinal plants are effective against liver injury by inhibiting the formation of lipid peroxide, owing to this reducing effect in co-existing substances or by preventing their oxidation. The protective effects of the extract could be due to the presence of one or the other of the active principles in the plant (Benzouzi et al., 2002). The presence of these secondary metabolites might be responsible for the pharmacological activities of this plant extract.

The antioxidative effectiveness in natural resources was reported to be mostly due to phenolic compounds (Hagase and kato, 1984). Gutfinger (1981) discovered that phenolic compounds play an important role in inhibiting autoxidation of oils; Yen and Duh (1993) reportted that peanut hulls containing many phenolic compounds are closely related to the antioxidative activities. Apparently, the contents of phenolic compounds seem to relate to the antioxidant activity. Yen and Duh (1993) reported that phenolic content (0.1671 mg/ml) of hulls displayed strong antioxidative activity. The amount of total phenolic compounds in this plant extract (0.22 mg/ml) was greater than 0.1671 mg/ml. Yen and Duh (1993) reported that the reducing power of methanolic extracts of peanut hulls containing high levels of polyphenol significantly correlated to the extent of antioxidative activity. It appears that antioxidative activity might have much correlation with the reducing effect.

The reducing power of *A. cordiolia* leaf extracts shown in (Table 2) was greater than that of vitamin E, a reducing agent as well as a reductone (Schimada et al., 1992). Therefore, the leaf extract tested was an electron donor and could react with free radicals to convert them to more stable products to terminate radical chain reactions. Gordon (1990) reported that the antioxidative effect exponentially increased as a function of the development of the reducing power, indicating that the antioxidative properties are concomitant with the development of the reducing power. Therefore, the marked antioxidative activity of the leaf extract might be connected with their reducing power. The scavenging activity of the extract as measured by the inhibition of 1,1–Diphenyl–2-Picrylhydrazyl (DPPH) radical (Figure 1) was related to the concentration of the extract added. 50 mg/ml of the extract showed 92% inhibition and 5 mg/ml of vitamin E showed 90.2% inhibition of DPPH radical. DPPH has been used as a free radical to evaluate antioxidative activity of some natural sources (Shimada et al., 1992; Yen and Duh, 1995). These results indicated that the extract exhibited a potent scavenging effect on free radical and would have effective activities as hydrogen donor and as primary antioxidants by reacting with the lipid radical. Free radicals play an important role in autoxidation of unsaturated lipids in foodstuffs (Kaur the Perkins, 1991). For example, oxidation of muscle cholesterol may be initiated by free radicals generated during the oxidation of polyunsaturated fatty acids. Also, antioxidants are believed to interrupt the free radical chain of oxidation and to contribute hydrogen from the phenolic hydroxyl groups themselves, thereby forming stable free radical, which do not initiate or propagate further oxidat-

tion of lipids (Sherwin, 1978).

The serum activities of aspartate amino transferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) of the animals treated with hepatotxin plus the leaf-extract were significantly lowered than the group treated with hepatotoxin alone. Increase in the serum enzyme activity signifies damages to the liver membrane. In affected liver, both the levels of ALT, ALP increased (Strove, 1989). These results suggest that the plant extract may possess hepatoprotective activity especially at close range of (200-300 mg/kg).

The serum AST activity of animals treated with curcumin, vitamin E and 400 mg/kg of plant extract were not significantly different form each other. This shows that the plant extract is as effective as the standards whereas the serum AST activity of the group fed with plant extract only (183±3.74 IU/g) was significantly lower than that of the control (158.4±3.28 IU/g). The serum activities of ALP of the treated animals were significantly different from that of the control except group fed with 200 mg/kg, which was not significantly different.

Liver enzymes related to sub-cellular functions such as plasma membrane (ALP), mitochondrion (AST, ALT) were affected (Dwivedi et al., 1993), not only that the oxidative damage due to either obstruction of the bile ducts or leakage of the cells may be prevented, the plant extract was able to offer some protection to the liver by reducing the serum activities of ALT and AST compared to the control. The ability of the extract of this plant to protect acetaminophen induced liver damage coupled with its antioxidative potentials suggests that this plant extract might be suitable for the treatment of acetaminophen toxicity.

It has been shown from the results so far that *A. cordifolia* may confer significant protection against heaptotoxic, necrotic and peroxidative actions of acetaminophen and might also be able to prevent liver accumulation and inhibit formation of peroxides at a dosage between 300-500 mg/kg (Seyer et al., 1982).

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