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Hepatoprotective Effect of Aqueous Extract of *Lippia multiflora* Leaves against Ethanol-induced Toxicity in Wistar Rats

Rita Bouagnon[1], Dodehe Yeo[1], Konan Kouassi[1], Kadja Beugre[1], Joseph A. Djaman[1,2] and Jean David Nguessan[1*]

[1]Laboratory of Pharmacodymamy Biochemistry, Felix Houphouet Boigny University of Cocody, Abidjan, 22 BP 582 Abidjan 22, Côte d'Ivoire.
[2]Department of Clinical and Fundamental Biochemistry, Pasteur Institute of Côte d'Ivoire, 01 BP 490 Abidjan 01, Côte d'Ivoire.

Authors' contributions

This work was carried out in collaboration between all authors. Author RB carried out the hepatoprotective tests and drafted the manuscript. Author DY helped for the interpretation of the histopathological results. Author KK performed the histopathological analysis. Author KB made the statistical analysis. Author JAD participated in the design of the study and helped to draft the manuscript. Author JDN conceived the study, participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: Usually called Tea of Gambia, *Lippia multiflora* is traditionally used for its sedative, relaxing, febrifuge, anti-flu-like, antispasmodic, hypotensive, anti-inflammatory, anti-catarrhal, mucolytic, anti-infective and hepatoprotective properties. The present study was carried out to evaluate the hepatoprotective effect of aqueous extract of *Lippia multiflora* leaves against ethanol induced toxicity in rat livers.
Study Design: Thirty Wistar albino rats (100-162 g) were divided into six groups of five animals.

**Corresponding author: E-mail: nguessanjd@yahoo.fr;*

Group 1 served as control and received only distilled water. Group 2 received only 15%ethanol (3 mL/100 g body weight/day). Group 3 served as standard group and received silymarin (70 mg/kg b.w.). Groups 4, 5 and 6 served as extract treatment groups and received respectively 100, 300 and 900 mg/kg of *L. multiflora* aqueous extract. 15% ethanol (3 mL/100 g b.w./day) was administered 1h after treatment in groups 3, 4, 5 and 6.
Place and Duration of Study: The study was carried out in Laboratory of Biochemical Pharmacodynamy, Felix Houphouet-Boigny University of Cocody-Abidjan (Côte d'Ivoire) between September and November 2014.
Methodology: All treatment protocols followed 28 days. Animals received daily doses and were observed for psychomotor changes and other signs of toxicity including death throughout the period of study. At days 7, 14, 21 and 28 of experimental period, blood samples were collected from retro-orbital venous plexus in non-heparinized tubes and the serum levels of liver marker enzymes, biochemical metabolites and hematological parameters (numbers of red blood cells, white blood cells, platelet count and mean cell volume)were monitored.
Results: *L. multiflora* extract (300 and 900 mg/kg b.w.) provides significant protection ($P<0.05$) against ethanol induced toxicity in rat livers showed by reduction of enzymatic parameters activities (ALT, AST and GGT). Histopathological study shows a normal hepatic architecture in *L. multiflora* extract (300 and 900 mg/kg b.w.) group compared to 15% ethanol group. Additionally, hematological analyses revealan increase of red blood cells and platelet count. A decrease of white blood cells and mean cell volume values in groups treated with 300 and 900 mg/kg of *L. multiflora* compared to 15% ethanol group is also observed.
Conclusion: Our results prove that *L. multiflora* extract has protective effects against ethanol-induced toxicity.

Keywords: Lippia multiflora; ethanol; hepatoprotective effect; histopathology.

1. INTRODUCTION

Lippia multiflora Moldenke also known as *Lippia adoensis* Hochst is an herbaceous plant of the genus *Lippia.* It belongs to the family *Verbanaceae*, which is composed of 41 genera with approximately 220 species of herbs, shrubs and small trees [1-3]. *L. multiflora* is a stout woody, perennial and aromatic shrub mainly distributed throughout tropical Africa, South and Central American countries [4]. The distribution range of *L. multiflora* has its major concentrations in Guinea Savannah, Forest Savannah and Transitional and Coastal Savannah zones. This plant is commonly known as Lippia tea and commercially known as "Gambian Tea Bush", "Bush Tea" and "Healer Herb" [5]. *L. multiflora* has been used in many traditional and herbal medicines to treat bronchial inflammation, malaria fever, conjunctivitis, gastro-intestinal disturbances, enteritis, coughs and colds [4], and possesses hypotensive, fatigue relieving, and diuretic properties [6]. Some rural dwellers cook the herbs and use it to relieve stress and enhance sleep [7]. The tea is also used traditionally against, venereal diseases and as a laxative [5]. One of the characteristics of *Lippia multiflora* aqueous extract is its wealth in polyphenols, flavonoids and tannins. Some researchers on green teas and black teas indicate that phenolic compounds, exactly flavonoids have antioxidant properties [8,9]. Also, literary review reveals that *Lippia multiflora* aqueous extract would have hepatoprotective properties unconfirmed scientifically [10].

People in West and central African communities use savannah tea like drinker for its aroma. The leaves of savannah tea are generally consumed in the form of hot drink. Through its values and commercialization in Côte d'Ivoire [11], the use of *L. multiflora* is drunk from rural and urban areas.

Herbal medicinal products play an important role in the management of liver diseases for the lack of satisfactory liver protective drugs in allopathic medical practices. Searching for hepatoprotective drugs with high efficacy and safety is of great need. In regard of the popular consumption of *L. multiflora* as a tea, the present work aimed to study the effects of the aqueous extract of this plant against ethanol induced liver damages in rats. For this purpose, some liver biochemical markers, hematological parameters and histopathological examination were investigated.

2. MATERIALS AND METHODS

2.1. Extraction Methodology

Fresh leaves of *L. multiflora* were washed and air-dried in shade for one week. The dried samples were later powdered using a mechanical grinder to obtain a coarse powder. One hundred (100) g of powdered leaves was boiled in 1 L of distilled water for 15 min. The aqueous solution was then filtered through Whatman filter paper (3 mm) and dried with a vacuum evaporator below 40°C. The resultant extract constitutes the aqueous extract of *L. multiflora* [12].

2.2 Administration of the Treatment

Thirty wistar rats were randomly distributed into six groups of five animals each. Group I served as the control and received only distilled water. Group 2 received only 15% ethanol (3 mL/100 g/day p.o.). Group 3 served as standard group and received silymarin (70 mg/kg p.o.). Groups 4, 5 and 6 served as extract treatment groups and received 100,300 and 900 mg/kg of *L. multiflora* aqueous extract. 15% ethanol (3 mL/100 g/day p.o.) was administered 1h after treatment in groups 3, 4, 5 and 6. Animals received daily doses for 28 days and were observed daily for psychomotor changes and other signs of toxicity including death throughout the period of study.

All the experimental procedures were approved by the Ethical Committee of Health Sciences, Felix Houphouet-Boigny University of Abidjan. These guidelines were in accordance with the European Council Legislation 87/607/EEC for the protection of experimental animals.

Body weights of rats in all groups were measured at the beginning, the day 7, day 14, day 21 and the end (day 28) of the experimentation. In addition, the weight gains were calculated using theses equations:

Weight gain (g) at day 7 = weight at day 7 - initial weight (day 0)
Weight gain (g) at day 14 = weight at day 14 - initial weight (day 7)
Weight gain (g) at day 21 = weight at day 21 - initial weight (day 14)
Weight gain (g) at day 28 = weight at day 28 - initial weight (day 21)

While the body weight changes (%) were Calculated using the following equation:

Body weight change (%) = ((final b.w. - initial b.w.) / initial b.w.) x100.

2.3 Biochemical Estimation

At the days 7, 14, 21 and 28 of experimental period, blood samples were collected from retro-orbital venous plexus in non-heparinized tubes, centrifuged at 4000 rpm for 10 min. Serum was collected and stored at 4°C prior immediate determination of enzymatic parameters (ALT, AST, GGT) and substrates (CRP and TG). All of these parameters were measured using Chemistry Analyzer (SFRI BSA-300).

2.4 Hematological Studies

At the end of the study (day 28), blood samples were collected in ethylene diamine tetra-acetic acid (EDTA) coated bottles. Samples were then analyzed for the assessment of the number of red blood cells (RBCs), white blood cells (WBCs) and platelets, and mean cell volume (MCV), according to standard methods using a Blood Counter (Urit Coulter).

2.5 Histopathological Examination

Rats were sacrificed and liver was separated, washed in ringer's solutionand stored during one month in 10% formalin before histological analysis.For microscopic examination, sections were taken from liver tissues, dehydrated in gradual ethanol, cleared in toluene and infiltrated in paraffin using automatic tissue processor. After routine processing, paraffin sections of each tissue were cut into 4 µm thickness, stained with haematoxylin and eosin, and observed under light microscope [13].

2.6 Statistical Analysis

All the data were expressed as mean± standard error of means (S.E.M).Statistical analyses were performed by one way analysis of variance (ANOVA) and differences between means were determined by Turkey's Multiple Comparison test using Graph Pad Prism 5.0 program. A value of $P< 0.05$ was considered significant.

3. RESULTS

3.1 Body Weight and Liver Weight Study

The results of this study are shown in Tables 1, 2 and 3. Compared to control, all groups did not shown significant difference in rats body weight changes (P< 0.05), and in both absolute liver weight and relative liver weight. However, at the day 14, ethanol group has shown an increase in rat body weight (6.16%±4.359^{b}) compared to control group (1.01%±3.742^{a}) and in weight liver (6.910±1.002^{b}) compared to control animals (3.96±0.1867^{a}).

Table 1. Effect of treatment on weight variation of ethanol induced hepatotoxicity in rats

Groups	Weight (g)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Control	117±5.385^{a}	118.6±4.523^{a}	118.6±4.915^{a}	124±4.103^{a}	126±6.110^{a}
15% ethanol	148.6±2.804^{a}	146±3.559^{d}	141.7±4.410^{bcd}	136.7±2.404^{ab}	135.3±1.453^{ab}
15% ethanol + Sylimarin 70 mg/kg	140±5.553^{a}	141.8±4.055^{bd}	142.8±5.093^{cd}	143±4.889^{ab}	143.2±7.490^{ab}
15% ethanol + *L. multiflora* 100 mg/kg	132.3±2.417^{a}	132.3±3.180^{ad}	130.7±2.849^{ad}	128.7±3.333^{ab}	127±3.512^{a}
15% ethanol + *L. multiflora 3*00 mg/kg	128.8±9.595^{a}	129.3±8.667^{ad}	131±2.933^{ad}	138.8±8.430^{ab}	139±13.08^{ab}
15% ethanol + *L. multiflora 9*00 mg/kg	140.8±5.963^{a}	143.3±6.149^{cd}	147.2±4.954^{d}	149.6±4.895^{b}	154.6±3.326^{b}

The values of weight are expressed as Mean ± S.E.M for five rats (n=5). In the same column values, the same letters are not significantly different (P<0.05)

Table 2. Effect of treatment on weight gain

Groups	Body weight change (%)			
	Day 7 (week 1)	Day 14 (week 2)	Day 21(week 3)	Day 28 (week 4)
Control	1.7±0.8367^{a}	1.01±3.742^{a}	1.26±0.2887^{a}	4.667±1.856^{a}
15% ethanol	3.03±1.443^{a}	6.16±4.359^{b}	3.53±2.517^{a}	2.000±0.5774^{a}
15% ethanol + Sylimarin 70 mg/kg	4.57±3.326^{a}	1.27±0.9165^{ab}	0.98±0.4000^{a}	0.2000±0.2000^{a}
15% ethanol + *L. multiflora*100 mg/kg	3.02±3.000^{a}	1.007±0.333^{ab}	1.78±0.3334^{a}	1.000±0.5774^{a}
15% ethanol + *L. multiflora 3*00 mg/kg	4.66±2.082^{a}	5.93±1.856^{ab}	3.05±2.646^{a}	6.33±1.764^{a}
15% ethanol + *L. multiflora 9*00 mg/kg	1.98±0.9595^{a}	1.35±1.023^{ab}	1.63±0.5099^{a}	4.393±1.965^{a}

The values of body weight change are expressed as Mean ± S.E.M for five rats (n=5). In the same column values, the same letters are not significantly different (P< 0.05)

Table 3. Effect of treatmenton liver weight variation of ethanol induced hepatotoxicity in rats

Groups	Liver	
	Weight	Relative weight (%)
Control	3.96±0.1867^{a}	3.100±0.2858^{a}
15% ethanol	6.910±1.002^{b}	5.120±0.7802^{a}
15% ethanol + Silymarin 70 mg/kg	5.428±0.4374^{ab}	3.810±0.3316^{a}
15% ethanol + *L. multiflora* 100 mg/kg	6.313±0.2567^{ab}	4.980±0.2919^{a}
15% ethanol + *L. multiflora 3*00 mg/kg	5.545±0.7642^{ab}	4.537±0.8952^{a}
15% ethanol + *L. multiflora 9*00 mg/kg	5.5±0.7151^{ab}	3.508±0.3978^{a}

The values of liver weight are expressed as Mean ± S.E.M for five rats (n=5). In the same column values, the same letters are not significantly different (P< 0.05)

3.2 Effects of Treatment on Enzymatic Parameters

Table 4 shows serum ALT activities in rats treated at days 7, 14, 21, 28. The levels of ALT are significantly (P<0.05) increased in ethanol group compared to the control. There is a significant (P<0.05) decrease of ALT levels in *L. multiflora* extract (100, 300, 900 mg/kg) and silymarin groups compared to ethanol group.

Table 5 shows that there is a significant increase of serum AST levels in ethanol intoxicated animals compared to the control group. Treatments with *L. multiflora* extract (100, 300, 900 mg/kg) and silymarin significantly (P<0.05) decrease serum AST levels when compared to ethanol group during the experimental period, except the day 7.

Concerning GGT activity, results show a significant (P < 0.05) increase of serum GGT levels in ethanol intoxicated animals compared to the control group. Treatments with *L. multiflora* extract (100, 300, 900 mg/kg) and silymarin significantly (P<0.05) decrease serum GGT levels when compared to ethanol group (Table 6).

3.3 Effects of Treatment on Biochemical Substrates

In Table 7, results show that there is no significant difference between serum TG levels of all the animal groups.

In Table 8, treatment of rats with *L. Multiflora* extract (at the doses of 300 and 900 mg/kg b.w.) and silymarin (70 mg/kg b.w.) reveals no significant changes in serum levels of CRP compared to control animals. However, there is a significant difference (P < 0.05) of serum CRP levels between all the animal groups and the ethanol one.

3.4 Hematological Study

Results show a significant (P< 0.05) increase of RBCs and platelets counts in *L. multiflora* extract (900 mg/kg) and silymarin-treated rats compared to15% ethanol group. However, a significant (P< 0.05) reduction in WBCs and MCV values was recorded (Table 9).

Table 4. Alanine aminotransferase activities in treated rats

Groups	Alt (ui/l)			
	Day 7	Day 14	Day 21	Day 28
Control	59.40±1.030^{a}	60.60±1.077^{a}	61.60±1.691^{a}	61.60±1.288^{a}
15% ethanol	124.5±1.708^{f}	127±0.9129^{f}	131.3±0.75^{f}	144±1.155^{f}
15% ethanol + Silymarin 70 mg/kg	96±1.414^{bc}	79±1.871^{b}	76.67±0.8819^{b}	75±1.732^{b}
15% ethanol + *L. multiflora* 100 mg/kg	117±1.472^{def}	104.3±3.301^{de}	101.5±2.327^{e}	100±1.202^{e}
15% ethanol +*L. multiflora* 300 mg/kg	122±1.414^{ef}	104.8±1.315^{e}	93.75±1.250^{de}	90±2.082^{cd}
15% ethanol + *L. multiflora* 900 mg/kg	96.75±6.263^{c}	94.75±6.290^{cde}	93±6.042^{cde}	90±2.646^{d}

The values of ALT levels are expressed as Mean ± S.E.M. for five rats (n=5). In the same column values, the same letters are not significantly different (P < 0.05)

Table 5. Aspartate aminotransferase activities in treated rats

Groups	Ast (ui/l)			
	Day 7	Day 14	Day 21	Day 28
Control	143±3.072^{a}	143.6±2.909^{a}	146±2.72^{a}	145.8±3.262^{a}
15% ethanol	251.8±3.816^{cd}	297±6.868^{f}	350.3±19.76^{f}	381±6.658^{f}
15% ethanol +Silymarin 70 mg/kg	228±0.7071^{b}	217.4±7.414^{b}	185±1.581^{bc}	176.5±0.6455^{b}
15% ethanol + *L. multiflora*100 mg/kg	305.7±1.764^{f}	291.3±1.667^{ef}	248.7±2.667^{e}	237.7±3.180^{e}
15% ethanol + *L. multiflora* 300 mg/kg	296.3±4.404^{ef}	265.5±3.969^{de}	236.5±2.630^{de}	210±3.180^{d}
15% ethanol + *L. multiflora* 900 mg/kg	262.8±3.184^{d}	246±4.037^{cd}	187.6±2.839^{c}	207.2±5.190^{cd}

The values of AST levels are expressed as Mean ± S.E.M. for five rats (n=5). In the same column values, the same letters are not significantly different (P < 0.05)

Table 6. Gamma-glutamyl transferase activities in treated rats

Groups	Ggt (ui/l)			
	Day 7	Day 14	Day 21	Day 28
Control	258.40±1.503[a]	258.60±1.030[a]	260±1.703[a]	263.80±2.083[a]
15% ethanol	368±2.16[f]	276±1.934[f]	284±2.677[f]	313.5±7.467[f]
15% ethanol + Silymarin 70 mg/kg	277.8±1.744[b]	295.8±1.934[b]	310±5.666[b]	317.4±5.297[b]
15% ethanol + *L.multiflora* 100 mg/kg	349.7±3.80[e]	362.7±4.055[ef]	377±1.155[ef]	388.3±4.10[e]
15% ethanol + *L. multiflora* 300 mg/kg	335±1.652[d]	344±2.273[d]	360.3±2.780[de]	375.5±2.102[de]
15% ethanol + *L. multiflora* 900 mg/kg	303.6±4.490[c]	318.6±4.632[c]	343.6±2.731[c]	354±1.393[c]

The values of GGT levels are expressed as Mean ± S.E.M for five rats (n=5). In the same column values, the same letters are not significantly different (P < 0.05)

Table 7. Triglycerides levels in treated rats

Groups	Tg (g/l)			
	Day 7	Day 14	Day 21	Day 28
Control	0 .854±0.09315[a]	0.9240±0.05591[a]	0.8950±0.1024[a]	0.98±0.09[a]
15% ethanol	0.82±0.05132[a]	0.143±0.1419[a]	0.9±0.15[a]	1.230±0.14[a]
15% ethanol + Silymarin 70 mg/kg	0.752±0.08387[a]	0.7840±0.09511[a]	0.8060±0.09584[a]	1.135±0.1384[a]
15% ethanol + *L. multiflora* 100 mg/kg	0.82±0.04[a]	1.355±0.055[ab]	1.06±0.01[a]	0.6±0.04[a]
15% ethanol + *L. multiflora* 300 mg/kg	0.7675±0.08910[a]	0.9325±0.1574[ab]	0.8933±0.08876[a]	1.030±0.2702[a]
15% ethanol + *L. multiflora* 900 mg/kg	0.9720±0.2409[a]	1.004±0.07737[b]	0.7825±0.6060[a]	0.81±1.223[a]

The values of TG levels are expressed as Mean ± S.E.M. for five rats (n=5). In the same column values, the same letters are not significantly different (P < 0.05)

Table 8. C Reactive protein levels in treated rats

Groups	Crp (ui/l)	
	Day 21	Day 28
Control	0[a]	0[a]
15% ethanol	10.50±2.021[b]	12.25±1.750[c]
15% ethanol + Silymarin 70 mg/kg	0[a]	0[a]
15% ethanol +*L. multiflora* 100 mg/kg	8.75±1.750[b]	7±0[b]
15% ethanol +*L. multiflora* 300 mg/kg	0[a]	0[a]
15% ethanol + *L. multiflora* 900 mg/kg	0[a]	0[a]

The values of CRP levels are expressed as Mean ± S.E.M. for five rats (n=5). In the same column values, the same letters are not significantly different (P < 0.05)

Table 9. Hematological indices of rats at day28

Groups	Hematological parameters			
	Rbc	Wbc	Platelet	Mcv
Control	6 .733±0.04807[f]	6.4±0.6658[a]	891±36.04[e]	72.43±0.7219[bcd]
15% ethanol	3.917±0.2489[a]	15.97±0.5783[d]	303±26.31[a]	77.15±6.658[e]
15% ethanol +Silymarin 70 mg/kg	6.460±0.1222[ef]	6.333±0.2333[a]	776,7±33.41[de]	67.45±0.3617[a]
15% ethanol + *L. multiflora* 100 mg/kg	5.147±0.03180[bc]	12.4±0.3786[c]	450.3±35.69[a]	75.75±1.65[de]
15% ethanol + *L. multiflora* 300 mg/kg	5.303±0.1027[cd]	9.067±0.2166[b]	622.3±25.89[bc]	73.15±0.85[cde]
15% ethanol + *L. multiflora* 900 mg/kg	5.827±0.4667[d]	7.225±0.08539[a]	726±27.62[dc]	69.05±0.05[abc]

The values of hematological parameter are expressed as Mean ± S.E.M. for five rats (n=5). In the same column values, the same letters are not significantly different (P < 0.05)
RBC=Red Cell Count, WBC=White Blood Cell, MCV= Mean Cell Volume

3.5 Histopathological Study

Histopathological examinations of the liver sections are shown in Fig. 1. No visible lesions are observed in control group (Fig. 1A) while there are portal fibroplasias in the 15% ethanol one (Fig. 1B). Normal hepatic parenchymal architecture with mild dilatation and congestion of the central vein and blood sinusoids is observed in silymarin (70 mg/kg b.w.) treated rats (Fig. 1 C). Diffuse hydropic degeneration and cellular infiltration by mononuclear cells are observed in liver section of *L. Multiflora* extract 100 mg/kg treated rats (Fig.1 D). In *L. multiflora* extract 300 mg/kg (Fig.1E) and 900 mg/kg (Fig. 1F), liver sections show almost normal cells with less dilated congested portal venules.

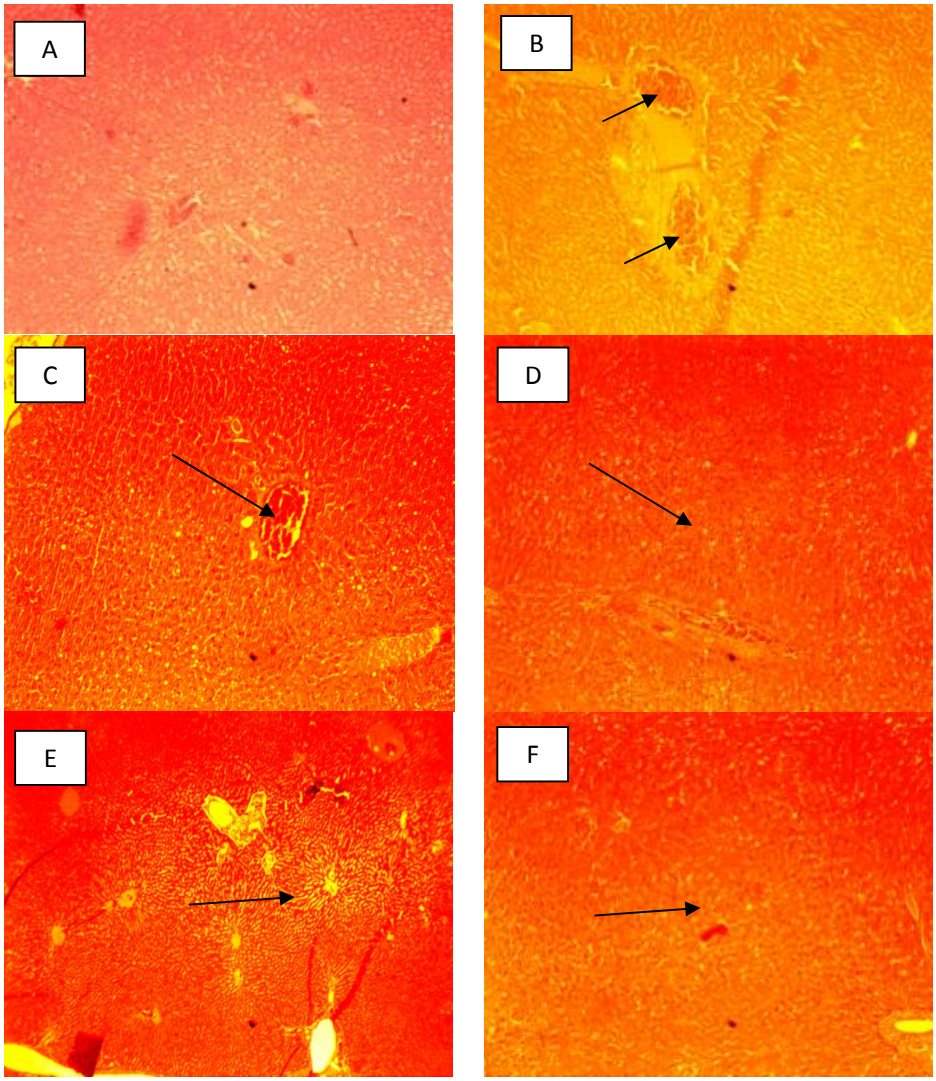

Fig. 1. Representative of histological assessment of rat liver sections after 28 days of treatments (A) Control (no visible lesions observed), (B) 15% ethanol (portal fibroplasias observed), (C) ethanol + Silymarin 70 mg/kg (Normal hepatic parenchymal architecture with mild dilatation and congestion of the central vein and blood sinusoids), (D) 15% ethanol + *L. multiflora* extract 100 mg/kg (hydropic degeneration and cellular infiltration), (E) ethanol + *L. multiflora* extract 300 mg/kg (normal cells with a few dilated congested portal venules), (F) ethanol + *L. multiflora* 900 mg/kg (x100) (normal cells with less dilated congested portal venules)

4. DISCUSSION

Consumption of alcohol affects the liver and other organs and could contribute to the development of alcohol liver disease [14]. The elevation observed in serum levels of ALT and AST is an indication of the degree of the liver damages caused by ethanol [15,16]. The increase of ALT, AST and GGT in ethanol group reflects cellular lesions, in particular at the hepatic level and certain cardiac cells [17,18]. Clinically, measurements of serum ALT, AST and GGT are widely used as markers in evaluating the degree of liver injury. ALT is the more specific measure of alcohol-induced liver injury because it is found predominantly in the liver, whereas AST is found in several organs, including the liver, heart, muscle, kidney, and brain [19]. The reduction in the levels of transaminases (ALT, AST) and the enhancement of GGT by *L. Multiflora* extract at doses of 300 and 900 mg/kg b.w. (at day 7, 14, 21 and 28), during liver damages induced by ethanol, suggests that the extract is not toxic or damaging for the integrity of the liver but possibly hepatoprotective.

Worthy of note that ethanol administration led to a significant increase in the level of serum GGT, which was markedly reduced by the dose of silymarin and *L. multiflora* extract (300 or 900 mg/kg, b.w.). Moreover, in the present study, the effects of silymarin and leaves of *L. multiflora* extract (300 or 900 mg/kg b.w.) on blood parameters were demonstrated by the significant reduction in WBC and MCV compared to ethanol group while RBC and platelet counts are improved. MCV and GGT are important in the biological screening of the alcoholization [20]. The effects of *L. multiflora* on GGT and MCV values point out the potent protective effects of this plant for the liver.

Wang et al. [21] has suggested that *L. multiflora* extract is one of the most widely consumed beverages in the world and more attention is paid to its health benefits effects notably in the prevention of cancer, cardiovascular diseases and also liver injuries, particularly those generated by ethanol consumption.

The triglycerides (TG) did not vary significantly during the study. However, a significant increase of CRP was noticed in ethanol group indicating the inflammatory process initiated by ethanol. The results also show that the inflammation is lowered by *L. multiflora* extract (300 and 900 mg/kg b.w.) as well as silymarin.

As we mentioned before, we evaluated body weight gain, absolute and relative liver weights ratio of rats. In general, obtained data showed no significant difference in the body weight gain and in both absolute and relative liver weight of all rats groups, compared group. Except the day 14, ethanol group showed an increase in both body weight gain and liver weight, compared to normal group. The results clearly showed that ethanol treatment induced significant hepatic histopathological injuries. The increase ($P< 0.05$) in liver weight of the alcohol-treated rats can be due to the accumulation of fats and water causing hepatocytic hypertrophy [22,23].

In the present study, we used an animal model to reveal the protective role of *L. multiflora* extract against ethanol-induced hepatic toxicity. The protection of liver by leaves of *L. multiflora* extract was detected by diagnostic indicators of liver damage (AST, GGT and ALT levels), and by histopathological analyses. These health benefit properties of *Lippia multipflora* are probably based on its main phytochemical compounds such as phenylated glycosides put it evidence by Arthur et al. [24].

5. CONCLUSION

Lippia multiflora was used in traditional middle to ill a lot of pathology. The results showed that leaves of *L. multiflora* aqueous extract could ameliorate hepatic damage caused by ethanol exposure in rat models.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that all manipulations of animals were approved by the Ethical Committee of Health Sciences, Felix Houphouet-Boigny University of Abidjan. Moreover, all procedures used in animal experimentation complied with the European Council Legislation 87/607/EEC for the protection of experimental animals. All efforts were made to minimize the number of animals used and their suffering, and the study met the ethical standards of Chronobiology International.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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