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Assessment of the health risk in rats of the pyrrolizidine alkaloids of *Vernonia amygdalina* Del. used by hepatic patients before their hospitalization at the University Hospital Center (UHC) of Cocody (Côte d'Ivoire)

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Abstract

Vernonia amygdalina is a medicinal plant used in Côte d'Ivoire for the treatment of various diseases, such as malaria and diabetes. This work evaluated the health risk related to the presence of pyrrolizidine alkaloids in this plant consumed by liver diseases patients before hospitalization in the department of Medicine and Hepato-gastroenterology at the University Hospital Center of Cocody. The biochemical parameters of the hepatic functions of wistar rats were accessed under repeated administration of the aqueous total extract (EAVA) and pyrrolizidine alkaloids (PAs) enriched extract (EEPAs) prepared from the leaves. Fourty rats were divided into eight groups of five rats each. The doses were 6.25; 12.5; 25 and 50 mg/Kg body weight (BW) for EAVA and 3; 6 and 9 mg/Kg BW for EEPAs during 14 days, twice per days. The results showed a significant increase in transaminase and specific liver protein concentrations, regardless of the type of extracts. High significant increase ($p < 0.05$) in ALT concentrations was observed with EEPAs. The values were respectively 195.825 IU/L at 3 mg/Kg BW, 215.354 IU/L at 9 mg/Kg BW and 234.748 IU/L at 6 mg/Kg BW. In the control group, a concentration of 84.304 IU/L was recorded. No significant difference between concentrations of ALT of rats treated with EAVA and control groups ($p > 0.05$). At 50 mg/Kg BW, the concentration was 88.548 IU/L, compared to control group (84.304 IU/L). These results showed a potential exposure of populations to toxic compounds, due to risk related to the consumption of this medicinal plant.

Keywords: *Vernonia amygdalina*; Pyrrolizidine alkaloids; Hepatotoxicity; Biochemical parameters

1. Introduction

Despite the huge advances in modern medicine, 80% of the population in developing countries still uses traditional medicine for their primary health care [1]. The use of medicinal plants is therefore gaining renewed interest, given the inadequacy of health services, rising cost of modern medicines and rapid population growth [2]. Medicinal plants play an important role in the control of diseases affecting Human and animal [3]. Empirical uses of medicinal plants expose people to poisoning that can sometimes be tragic [4]. Plants contain natural phytotoxins such as pyrrolizidine alkaloids (PAs) that are present in about 3% of all flowering plants [5; 6]. Ingestion of low doses of pyrrolizidine alkaloids and/or their N-oxides may be a source of intoxication. These molecules can contaminate food crops (cereals) and animal

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products such as eggs and milk [7]. Men may inadvertently be exposed to the consumption of these foods and contaminated remedies. Many episodes of PAs poisoning related to herbal medicines have been reported around the world and several thousand people in Uzbekistan, Afghanistan and India have died. In developing countries, characteristic liver diseases such as cirrhosis and primary tumors with high mortality occur due to occasional or continued consumption of medicinal plants [6]. *Vernonia amygdalina* Del. (Asteraceae) is a well-known plant in most countries of tropical Africa, including Côte d'Ivoire for its use in traditional medicine. The leaves, stem bark, roots and leafy twigs are used in drenching and bathing for the treatment of malaria, jaundice, tiredness and hypertension [8]. Also, in many other countries as, Democratic Republic of Congo [9], Nigeria [10], Zimbabwe and Tanzania [11], the leaves and roots are used in the treatment of digestive disorders, headaches, epilepsy. Diabetes, hemorrhoids, sexually transmitted diseases and parasitic diseases. The leaves of *V. amygdalina* are eaten as leafy vegetables in Côte d'Ivoire, Cameroon [12] and Benin [13; 14].

The objective of this study was to evaluate the effects of *V. amygdalina* on some specific biochemical parameters of liver injury in rats (*Rattus norvegicus*). This investigation focused on this species because it was consumed by patients prior to their admission to the Medicine and Hepato-gastroenterology department of the University Hospital Center (UHC) of Cocody in Côte d'Ivoire [15]. Also, the genera *Vernonia* contains pyrrolizidine alkaloids [16].

2. Material and methods

2.1. Collection of the leaves

Fresh leaves of *V. amygdalina* were collected in Abidjan from July 2016. A sample of this plant has been authenticated at the herbarium of the National Floristic Center at the University Felix Houphouët Boigny (Côte d'Ivoire) under voucher no 11694. The sample were dried during 2 weeks under air-conditioned room (18±2 °C) and then grounded in mortar to obtain powders.

2.2. Preparation of plant extracts

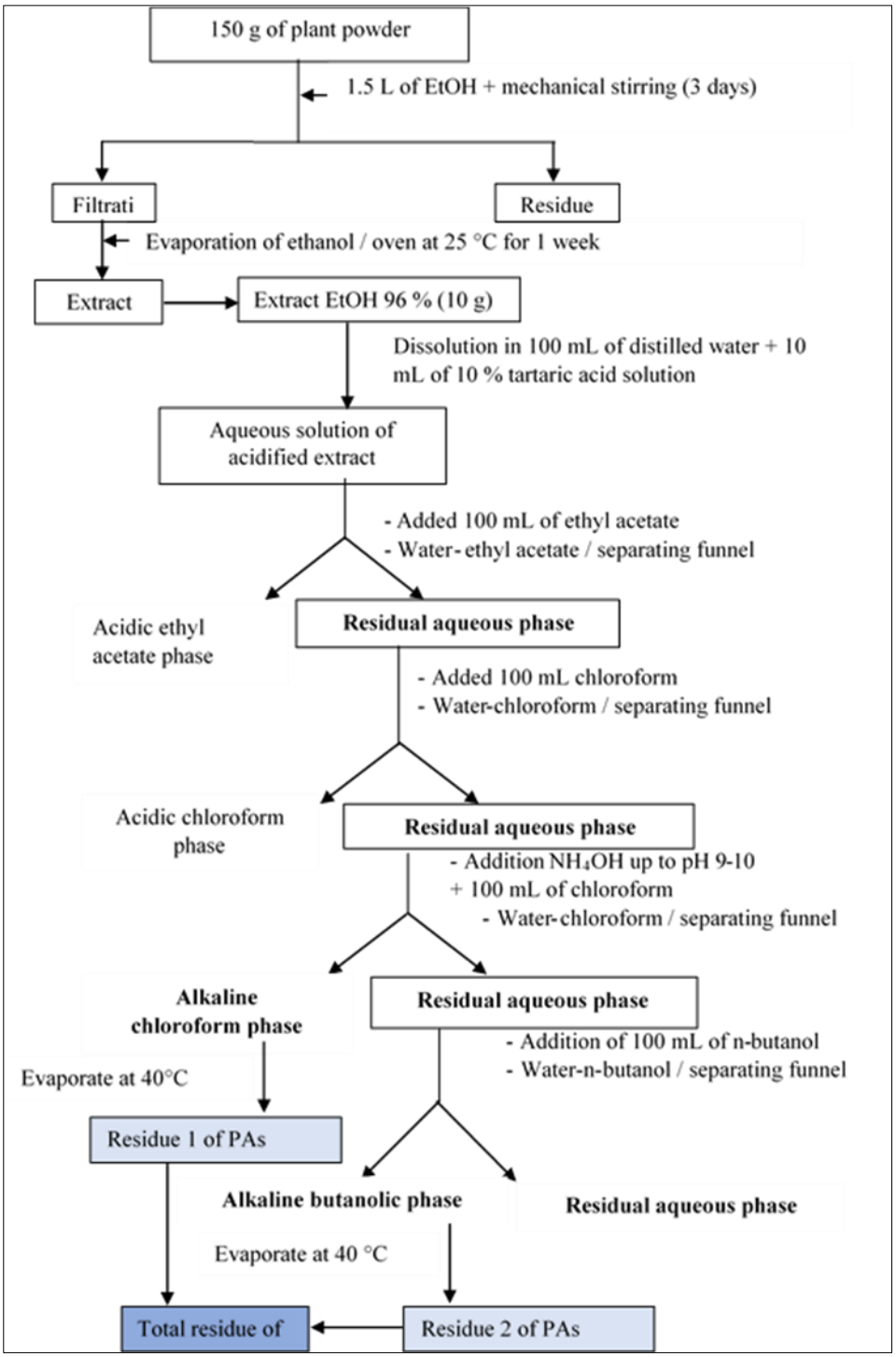
2.2.1. Preparation of aqueous extracts (AEVA)

The aqueous extract was a decoction prepared from 150 g of powder in 1.5 L of distilled water. After filtration on Whatman paper (no 1), the extract obtained were subsequently dried in desiccators (Mark Memmert, Germany) at 45 °C for one week.

2.2.2. Preparation of pyrrolizidine alkaloids enriched extracts (EEVA)

The liquid-liquid partition technique applied was inspired by the method used by Fabiana *et al*. [17]. For the preparation of the PAs-enriched extract (EEPAs), maceration was made from 150 g of powder in 1.5 L of 96% ethanol, under mechanical stirring for 3 days. The macerate was vacuum filtered on Whatman paper (no 1). The filtrate obtained was concentrated on a rotary evaporator at 40 °C and dried in desiccators (Mark Memmert, Germany) at 25 °C for one week. Ten (10) g of dried ethanolic extract were acidified with 10% tartaric acid and filtered again to obtain a waxy residue and an acidic solution which was dissolved in 100 mL of distilled water. After perfect solubilisation, this aqueous solution was transferred to a separating funnel in which 100 mL of ethyl acetate were added. The ampoule containing this mixture was stirred and left completely at the settling point. After perfect separation of the two phases (organic phase and aqueous phase), the ethyl acetate phase was removed. Subsequently, the residual aqueous phase was partitioned with chloroform in an acidified medium. The chloroform acid phase was removed while the residual aqueous phase was again partitioned with chloroform in an alkaline condition. The alkaline chloroform phase was evaporated to give the residue 1 of PAs.

The new aqueous phase was once again partitioned with butanol and an alkaline agent (NH_4OH, 0.5 M) was added to reach a pH 9-10. The butanol phase was recovered and evaporated to obtain the residue 2 of PAs. The EEVA was obtained by mixing residues 1 and 2 (Fig. 1)



PAs: Pyrrolizidine alkaloids, *NH_4OH*: Ammonium hydroxide, *EtOH*: Ethanol

Figure 1 Synoptic diagram of the liquid-liquid partition

2.3. Maintenance of animals

Experiments were performed on 40 male rats (*Rattus norvegicus*; strain Wistar) with weight between 180 and 280 g. They were marked for individual identification and then kept in plastic cages. The difference in weight of the animals in each group did not exceed ± 20% of the average weight. These animals were fed with commercial pellets and tap water *ad libitum*, at the animal facility of the Botany and Valorization of Plant Diversity Laboratory (NANGUI ABROGOUA University, Côte d'Ivoire), according to the principles of care and use of laboratory animals from the University's Ethics Committee. Research was conducted in accordance with the internationally accepted principles for laboratory animal use as found in the European Community Guidelines [18]. They were acclimatized in the cages for 7 days in the

laboratory and subjected daily to the room temperature of 25±2 °C and photoperiod of 12 hours of light and 12 hours of darkness. Before administration of the extracts, the animals of each batch were individually marked and weighed.

2.4. Preparation of doses administered to rats

The doses were prepared by solving the aqueous extract and PAs-enriched extracts in 10 mL physiological saline buffer solution (pH 7). Rats in the experimental groups were treated twice daily with 0; 6.25; 12.5; 25; 50 mg/Kg for the decoction extract and 3; 6; 9 mg/Kg for the PAs-enriched extract following the guidelines of the United States (US) National Toxicology Program [19].

2.5. Administration of different extracts

This study of toxicity was conducted following the guidelines of the US National Toxicology Program [19] and the principles of good laboratory practices of the European Community in relation to substances causing toxicity [20].

The different doses of the solutions were orally administered to rats every morning and evening for two weeks. The rats received a solution volume of 5 mL/Kg body weight (BW). Thus the doses administered to the rats were 0 (control group); 6.25 (group A); 12.5 (group B); 25 (group C) and 50 (group D) mg/Kg body weight for the aqueous extract and 3 (group E); 6 (group F) and 9 (group G) mg/Kg BW for the PAs-enriched extract [17]. The animals were observed for 30 minutes after the administration of the extracts.

2.6. Blood sampling

After 14 days of treatment, the animals were anesthetized with ether. According to the method described by Coles [21] the blood was collected using a capillary hematocrit tube through the retro-orbital sinus at the orbital vein. The blood was coagulated in the tubes without heparin for 2 hours at room temperature and then centrifuged at 850 rpm for 15 min at 4 °C. The resulting serum was aliquoted and stored at -20 °C for biochemical analyzes.

2.7. Biochemical assays

Transaminases such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) were determined using a semi-automatic biochemical analyzer (model RT-9200 Rayto, China). Total bilirubin (T Bil) and conjugate bilirubin (C Bil) were accessed as biomarkers.

2.8. Statistical analysis

Biochemical data were subjected to a one-way analysis of variance with Xlstat version 2017.1 software. This software was used to calculate the means and standard deviations of the analyzed parameters and the Dunnett tests was used to compare the averages when a significant variation was established by the Anova one way. The significance of the test is determined by comparing the probability $p < 0.0001$ associated with Fisher's F test statistic to the theoretical threshold $\alpha = 0.05$ [22]

3. Results

3.1. Behaviors of rats after treatment with aqueous extracts (AEVA) and pyrrolizidine alkaloids enriched extracts (EEPAs) of *V. amygdalina* Del.

The administration of AEVA and EEPAs from the low to high doses did not cause any change in behaviors of treated animals compared to the control group. No mortality was recorded during the 14 days of treatment. During the experiment, no cases of loss of alertness, breathing disorders, convulsions and coma were recorded. No alteration of locomotion activity or piloerection was observed during treatment. Fecal examination did not reveal any diarrhea during the experiment. The general behavior of the rats of the different batches remained unchanged; obviously all the animals were doing well.

3.2. Effect of AEVA and EEPAs of *V. amygdalina* Del. on rats body weight

The rats were weighed every two days during the experiments. The weight gain and the weight variation of the animals treated with both types of extracts were determined.

3.2.1. Effect of AEVA on weight variation in rats

For animals treated with AEVA, there was a general reduction in food consumption. This resulted in a loss of body weight of these rats compared to the control group (Fig. 2). However, rats treated with 6.25 mg/Kg BW and 12.5 mg/Kg BW of AEVA showed reduced food consumption throughout the experimental period. Animals in all other groups exposed to the 25 mg/Kg BW and 50 mg/Kg BW doses of AEVA also showed a reduction in body weight gain over the control group rats. Compared with the control group, weight loss was significant at the level of experimental groups ($p < 0.05$).

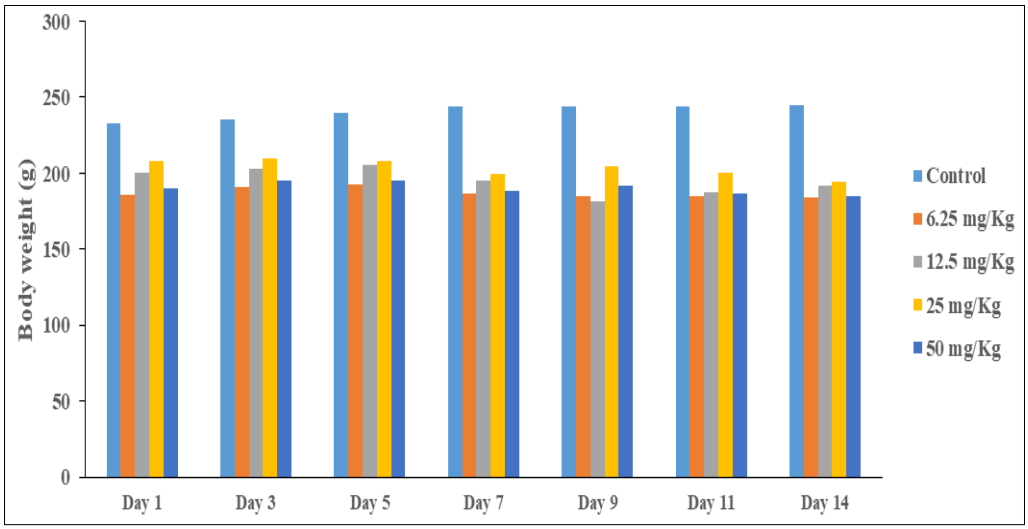


Figure 2 Body weight of rats treated with the aqueous extract of *V. amygdalina* Del.

3.2.2. Effect of EEPAs on the weight variation of rats

For animals treated with EEPAs, a significant gain in mass was noted 14 days post-treatment (Fig. 3). This observation was made in the rats treated at the respective doses of 3 mg/Kg BW weight and 6 mg/Kg BW compared to the control rats. However, a difference in body weight loss was observed between the animals treated with the dose 9 mg/Kg BW and those of the control group. Overall, weight gain was significant in the treated groups ($p > 0.05$) compared to the control.

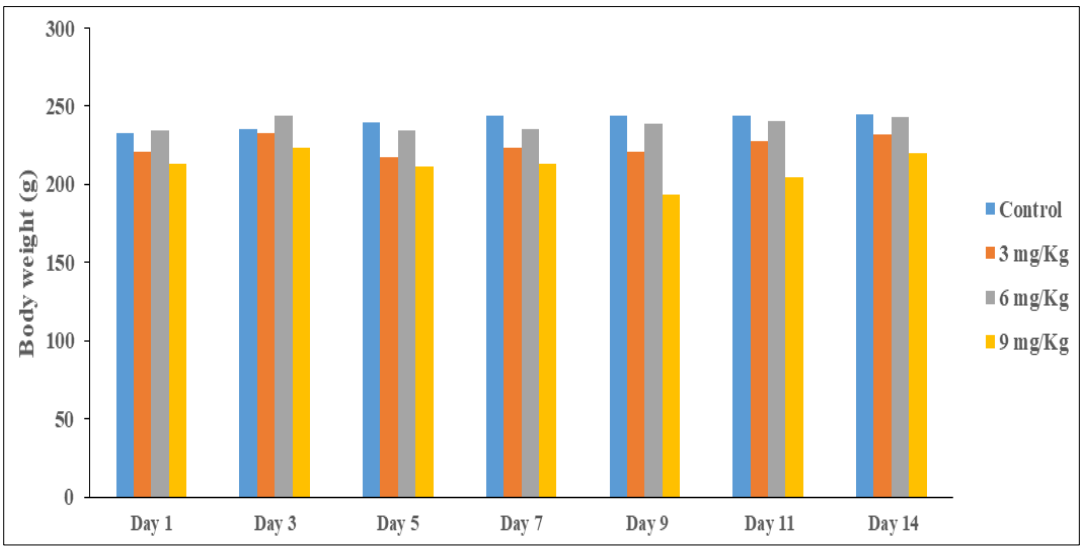


Figure 3 Body weight of rats treated with the pyrrolizidine alkaloids enriched extract of *V. amygdalina* Del.

3.3. Biochemical parameters of hepatic functions

3.3.1. Effects of extracts on alanine aminotransferase (ALT) and gamma-glutamyl transferase (GGT)

The results showed an increase in alanine aminotransferase (ALT) concentrations in groups of rats treated with EAVA (Table 1). There was no significant difference between concentrations of ALT of treated and control groups (p> 0.05). At the highest dose of 50 mg/Kg BW, the ALT concentration was 88.548 IU/L, compared to that of the control group which was 84.304 IU/L

Table 1 Effect of the aqueous extract of *V. amygdalina* on alanine aminotransferase (ALT)

Doses	Groups	Concentration ± SD (IU/L)
0 mg/Kg BW	Control	84.304 ± 13.350[a]
6.25 mg/Kg BW	A	78.831 ± 14.929[a]
12.5 mg/Kg BW	B	81.509 ± 14.929[a]
25 mg/Kg BW	C	88.448 ± 13.350[a]
50 mg/Kg BW	D	88.548 ± 13.350[a]
Statistical	dl	4
parameters	F	0.091
of ANOVA	p > F(model)	0.984

F Fisher statistic, *p* probability (ANOVA), *dl* degree of liberty, *ALT* alanine aminotransferase, *BW* body weight, *IU/L* international unit per liter, [a] p > 0.05 no significant difference/control treated with saline, Values are mean ± SD for 5 animals in each group

On the other hand, with EEPAs, an increase in ALT concentrations was observed. The concentration increased from 195.825 IU/L at 3 mg/Kg BW to 215.354 IU/L at 9 mg/Kg BW and 234.748 IU/L at 6 mg/Kg BW (Table 2). The difference was significant between the control group and the treated groups.

Table 2 Effect of pyrrolizidine alkaloids enriched extracts of *V. amygdalina* on alanine aminotransferase (ALT)

Doses	Groups	Concentration ± SD (IU/L)
0 mg/Kg BW	Control	84.304 ± 18.366[b]
3 mg/Kg BW	E	195.825 ± 18.366[a]
6 mg/Kg BW	F	234.748 ± 18.366[a]
9 mg/Kg BW	G	215.354 ± 18.366[a]
Statistical	dl	3
parameters	F	13.468
of ANOVA	p < F(Model)	0.000

F Fisher statistic, p probability (ANOVA), dl degree of liberty, ALT alanine aminotransferase, BW body weight, IU/L international unit per liter, a p < 0.05 significant difference/control treated with saline, Values are mean ± SD for 5 animals in each group

The dosage of GGT following administration of EAVA showed an increase of concentration to 5.906 IU/L (group A) and 5.766 IU/L (group B) respectively at 6.25 mg/Kg BW and 12.5 mg/Kg BW, compared to that of the control group which was 2.972 IU/L. At the highest dose (50 mg/Kg BW), there was an increase to 3.969 IU/L (Table 3). However, the difference between these values was not significant.

However, at doses of 3 and 6 mg/Kg of BW EEPAs caused an increase (p > 0.05) of 7.633 IU/L and 6.318 IU/L of GGT after 14 days of treatment, compared to the control group with 2.972 IU/L (Table 4). The difference between these values was significant.

Table 3 Effect of the aqueous extract of *V. amygdalina* on γ-glutamyl transferase (GGT)

Doses	Groups	Concentration ± SD (IU/L)
0 mg/Kg BW	Control	2.972 ± 1.461[a]
6.25 mg/Kg BW	A	5.906 ± 1.634[a]
12.5 mg/Kg BW	B	5.766 ± 1.634[a]
25 mg/Kg BW	C	3.997 ± 1.461[a]
50 mg/Kg BW	D	3.969 ± 1.461[a]
Statistical	dl	4
parameters	F	0.667
of ANOVA	p < F(Model)	0.623

F Fisher statistic, *p* probability (ANOVA), *dl* degree of liberty, *GGT* γ-glutamyl transferase, *BW* body weight, *IU/L* international unit per liter, [a] $p > 0.05$ no significant difference/control treated with saline, Values are mean ± SD for 5 animals in each group

Table 4 Effect of pyrrolizidine alkaloids enriched extracts of *V. amygdalina* on γ-glutamyl transferase (GGT)

Doses	Groups	Concentration ± SD (UI/L)
0 mg/Kg BW	Control	2.972 ± 1.595[b]
3 mg/Kg BW	E	7.633 ± 1.595[a]
6 mg/Kg BW	F	6.318 ± 1.595[a]
9 mg/Kg BW	G	4.122 ± 1.595[a]
Statistical	dl	3
parameters	F	1.741
of ANOVA	p < F(Model)	0.199

F Fisher statistic, *p* probability (ANOVA), *dl* degree of liberty, *GGT* γ-glutamyl transferase, *BW* body weight, *IU/L* international unit per liter, [a] $p < 0.05$ significant difference/control treated with saline, Values are mean ± SD for 5 animals in each group

Throughout the experiment, the ALT activity of the EAVA-treated groups remained significantly lower ($p > 0.05$) than that of the control group. This activity was dose-dependent. These results indicated the aqueous extract of *V. amygdalina* at the doses tested may cause damage to the liver. ALT activity was high in all groups treated with EEPAs, showing a deleterious effect on the liver of the animals. The significant increase ($p < 0.05$) in ALT activity showed that PAs are responsible for these alterations of the liver. An increase in the level of ALT in the blood reflects an acute or chronic hepatic pathology.

3.3.2. Effect of different extracts on aspartate aminotransferase (AST) and alkaline phosphatase (ALP)

The mean values (Table 5) of AST concentrations of the treated groups were high compared to those of the control group (AST = 258.847 IU/L). This increase in the AST values in EAVA-treated groups compared to the values of control group was not significant ($p > 0.05$).

With EEPAs, the mean values of 193.419 IU/L and 250.045 IU/L of AST (Table 6) of groups treated at 3 and 6 mg/Kg of BW, respectively, remained low compared to those of control group (AST = 258.847 IU/L). This decrease of the AST values in treated groups compared to control group was not significant ($p > 0.05$). At 9 mg/Kg BW, a concentration 280.452 IU/L was obtained against 258.847 IU/L for the control group.

Changes in serum levels of ALP in the presence of EAVA administered to rats indicated that the mean values of this marker for group A (6.25 mg/Kg of BW); group C (25 mg/Kg of BW) and group D (50 mg/Kg of BW) were below that of the control group 14 days post-treatment (Table 7). This decrease was not significant ($p > 0.05$).

With the EEPAs (Table 8), the average concentrations of ALP measured were 660.726 IU/L and 741.576 IU/L for group A (3 mg/Kg BW) and group G (9 mg/Kg BW). These values were high compared to that of the control (595.719 IU/L). However, these changes in ALP were not significant ($p > 0.05$).

Table 5 Effect of the aqueous extract of *V. amygdalina* on aspartate aminotransferase (AST)

Doses	Groups	Concentration ± SD (UI/L)
0 mg/Kg BW	Control	258.847 ± 32.003[a]
6.25 mg/Kg BW	A	273.701 ± 35.720[a]
12.5 mg/Kg BW	B	257.915 ± 35.780[a]
25 mg/Kg BW	C	268.768 ± 32.003[a]
50 mg/Kg BW	D	286.888 ± 32.003[a]
Statistical	dl	4
parameters	F	0.131
of ANOVA	*p* > F(Model)	0.969

F Fisher statistic, *p* probability (ANOVA), *dl* degree of liberty, *AST* aspartate aminotransferase, *BW* body weight, *IU/L* international unit per liter, [a] $p > 0.05$ no significant difference/control treated with saline, Values are mean ± SD for 5 animals in each group

Table 6 Effect of pyrrolizidine alkaloids enriched extracts of *V. amygdalina* on aspartate aminotransferase (AST)

Doses	Groups	Concentration ± SD (IU/L)
0 mg/Kg BW	Control	258.847 ± 35.223[a]
3 mg/Kg BW	E	193.419 ± 35.223[a]
6 mg/Kg BW	F	250.045 ± 35.223[a]
9 mg/Kg BW	G	280.452 ± 32.223[a]
Statistical	dl	3
parameters	F	1.110
of ANOVA	*p* > F(Model)	0.374

F Fisher statistic, *p* probability (ANOVA), *dl* degree of liberty, *AST* aspartate aminotransferase, *BW* body weight, *IU/L* international unit per liter, [a] $p > 0.05$ no significant difference/control treated with saline, Values are mean ± SD for 5 animals in each group

Table 7 Effect of the aqueous extract of *V. amygdalina* on alkaline phosphatase (ALP)

Doses	Groups	Concentration ± SD (IU/L)
0 mg/Kg BW	Control	595.710 ± 67.852[a]
6.25 mg/Kg BW	A	593.833 ± 75.861[a]
12.5 mg/Kg BW	B	669.903 ± 75.861[a]
25 mg/Kg BW	C	537.102 ± 67.852[a]
50 mg/Kg BW	D	570.652 ± 67.852[a]
Statistical	dl	4
parameters	F	0.452
of ANOVA	*p* > F(Model)	0.770

F Fisher statistic, *p* probability (ANOVA), *dl* degree of liberty, *ALP* alkaline phosphatase, *BW* body weight, *IU/L* international unit per liter, [a] $p > 0.05$ no significant difference/control treated with saline, Values are mean ± SD for 5 animals in each group

The results showed a slight increase in AST concentrations with both types of extracts. However, this slight increase does not exclude severe liver injury. The elevated level of ALP indicated a possible liver injury, especially cholestatic.

Table 8 Effect of pyrrolizidine alkaloids enriched extracts of *V. amygdalina* on alkaline phosphatase

Doses	Groups	Concentration ± SD (IU/L)
0 mg/Kg BW	Control	595.710 ± 105.247[a]
3 mg/Kg BW	E	660.726 ± 105.247[a]
6 mg/Kg BW	F	590.700 ± 105.247[a]
9 mg/Kg BW	G	741.576 ± 105.247[a]
Statistical	dl	3
parameters	F	0.449
of ANOVA	p > F(Model)	0.721

F Fisher statistic, *p* probability (ANOVA), *dl* degree of liberty, *ALP* alkaline phosphatase, *BW* body weight, *IU/L* international unit per liter, [a] $p > 0.05$ no significant difference/control treated with saline, Values are mean ± SD for 5 animals in each group

3.3.3. Effect of different extracts on total and conjugated bilirubin

The concentration of total bilirubin (T Bil) increased insignificantly ($p > 0.05$) during the treatment with both extracts. However, a decrease was observed at doses of 25 mg/Kg of BW (2.267 IU/L), 50 mg/Kg BW (1.787 IU/L) for EAVA (Table 9) and 6 mg/Kg of BW (1.328 IU/L), 9 mg/Kg BW (1.086 IU/L) for EEPAs. In the control group, a concentration of 2.316 IU/L was obtained (Table 10).

Table 9 Effect of the aqueous extract of *V. amygdalina* on total bilirubin (T Bil)

Doses	Groups	Concentration ± SD (UI/L)
0 mg/Kg BW	Control	2.316 ± 0.626[a]
6.25 mg/Kg BW	A	3.724 ± 0.700[a]
12.5 mg/Kg BW	B	2.511 ± 0.700[a]
25 mg/Kg BW	C	2.267 ± 0.626[a]
50 mg/Kg BW	D	1.787 ± 0.626[a]
Statistical	dl	4
parameters	F	1.143
of ANOVA	p < F(Model)	0.368

F Fisher statistic, *p* probability (ANOVA), *dl* degree of liberty, *T Bil* total bilirubin, *BW* body weight, *IU/L* international unit per liter, [a] $p > 0.05$ no significant difference/control treated with saline, Values are mean ± SD for 5 animals in each group

No significant effect ($p > 0.05$) of the extracts was observed on bilirubin conjugate levels of EAVA-treated groups compared to the control group (Table 11).

However, the conjugated bilirubin mean values of the EEPAs-treated groups were higher than that of the control rats during the two weeks of treatment. This increase was clear ($p < 0.05$) for group E and group G (Table 12). The difference between these values was not significant.

The increase in blood levels of total bilirubin reflects poor liver and gall bladder function, or increased destruction of red blood cells. Elevation of conjugated bilirubin suggests extrahepatic and intrahepatic cholestasis.

Table 10 Effect of pyrrolizidine alkaloids enriched extracts of *V. amygdalina* PAs on total bilirubin (T Bil)

Doses	Groups	Concentration ± SD (IU/L)
0 mg/Kg BW	Control	2.316 ± 0.385[a]
3 mg/Kg BW	E	2.418 ± 0.385[a]
6 mg/Kg BW	F	1.328 ± 0.385[a]
9 mg/Kg BW	G	1.086 ± 0.385[a]
Statistical	dl	3
parameters	F	3.112
of ANOVA	p < F(Model)	0.056

F Fisher statistic, *p* probability (ANOVA), *dl* degree of liberty, *T Bil* total bilirubin, *BW* body weight, *IU/L* international unit per liter, [a] $p > 0.05$ no significant difference/control treated with saline, Values are mean ± SD for 5 animals in each group

Table 11 Effect of the aqueous extract of *V. amygdalina* on conjugated bilirubin (C Bil)

Doses	Groups	Concentration ± SD (IU/L)
0 mg/Kg BW	Control	1.999 ± 0.686[a]
6.25 mg/Kg BW	A	2.521 ± 0.767[a]
12.5 mg/Kg BW	B	1.013 ± 0.767[a]
25 mg/Kg BW	C	2.015 ± 0.686[a]
50 mg/Kg BW	D	1.673 ± 0.686[a]
Statistical	dl	4
parameters	F	0.532
of ANOVA	p > F(Model)	0.714

F Fisher statistic, *p* probability (ANOVA), *dl* degree of liberty, *C Bil* = conjugated bilirubin, *BW* body weight, *IU/L* international unit per liter, [a] $p > 0.05$ no significant difference/control treated with saline, Values are mean ± SD for 5 animals in each group

Table 12 Effect of pyrrolizidine alkaloids enriched extracts of *V. amygdalina* on conjugated bilirubin (C Bil)

Doses	Groups	Concentration ± SD (IU/L)
0 mg/Kg BW	Control	1.999 ± 1.493[a]
3 mg/Kg BW	E	3.587 ± 1.493[a]
6 mg/Kg BW	F	1.148 ± 1.493[a]
9 mg/Kg BW	G	2.042 ± 1.493[a]
Statistical	dl	3
parameters	F	3.112
of ANOVA	p < F(Model)	0.056

F Fisher statistic, *p* probability (ANOVA), *dl* degree of liberty, *C Bil* conjugated bilirubin, *BW* body weight, *IU/L* international unit per liter, [a] $p > 0.05$ no significant difference/control treated with saline, Values are mean ± SD for 5 animals in each group

4. Discussion

PAs are mainly known for their hepatotoxic and potentially carcinogenic properties. PAs are distributed in plant families of Asteraceae, Boraginaceae, and Fabaceae. However, they became a matter of concern due to their toxicity associated with the high risk of intake within herbal preparations, phytopharmaceutical formulations, medicinal teas, or other plant-derived drug products [23].

V. amygdalina (Asteraceae) is a plant species used as a medicinal and food plant in Côte d'Ivoire as elsewhere in Africa [24; 25]. The objective of this study was to evaluate the probable hepatotoxic effects of the aqueous and pyrrolizidine alkaloids enriched extracts of this plant. Biological and serum biochemical parameters of liver injury were accessed using a rat model.

Following the administration of the extracts, a significant increase in weight of rats was observed as early as the first six days. At the end of the experiments, a slight decrease in body weight was recorded in the animals treated with the aqueous extract of *V. amygdalina* (EAVA). This weight loss may be due to a decrease in appetite due to the likely development of hepatitis. The effects considered in risk assessment are adverse effects including all changes in morphology, physiology, growth, development or life span of an organism resulting from a deterioration of functional capacity or the ability to compensate for additional stress or increased sensitivity. According to Lu [26], the presence of hepatomegaly can result from the stimulation of hepatic oxidase functions or from protein synthesis by the endoplasmic reticulum, thus inducing a decrease in body weight which can come from a decrease in food consumption induced by stress behavior noticed in treated animals.

In contrast, in rats treated with the enriched PAs extract (EEPAs), a weight gain was noted regardless of the dose administered. This body weight gain in this case may be related to a stimulation of the appetite increasing food consumption [27]. Mingatto *et al*. [28] also reported high weight gain in rats following the administration of monocrotaline, a pyrrolizidine alkaloid. These authors concluded that this hepatotoxic PAs is responsible for the increase in glycolysis and glycogenolysis, resulting in a large intake of food.

One of the signs of hepatic damage in the evaluation of liver function is the leakage of cellular enzymes into the plasma. Thus, following damage to the plasma membrane of hepatocytes, a variety of enzymes normally located in the cytosol released into the bloodstream. This hepatic cytolysis results in a blood release of transaminases. The increase of their rate in serum is a useful marker for assessing the extent and type of hepatocellular damage [29]. The values of biochemical parameters revealed that the two studied extracts possess hepatotoxic actions, the hepatotoxic activity of the aqueous extract at different doses was not significant between the treated groups. On the other hand, with the PAs enriched extract, pronounced hepatotoxic effects were noted. This hepatotoxicity is expressed as increase or decrease in transaminase and protein levels associated with liver injury. High significant increase in ALT concentrations was observed with EEPAs extracts. The values were respectively 195.825 IU/L at 3 mg/Kg BW, 215.354 IU/L at 9 mg/Kg BW and 234.748 IU/L at 6 mg/Kg BW. In the control group, a concentration of 84.304 IU/L was recorded. The results of Johanna *et al*. [30], showed the same trends. They observed with lasiocarpine at a dose of 3.3 mg/Kg BW a significant increase (p <0.05) in ALT to 160.5±19.4 IU/L compared to control (85.8 ±22.5 IU/L). For AST, the average values of the concentrations in treated groups remain slightly higher than that in the control group.

According to Lazare *et al*. [31], transaminases (ALT and AST) are enzymes with significant metabolic activity inside cells; the increase in their serum levels reflects a cellular injury, especially in the liver. This indication of liver injury is reinforced by the elevation of GGT concentrations obtained with EAVA. The values were 5.906 IU/L and 5.766 IU/L respectively at doses of 6.25 mg/Kg BW and 12.5 mg/Kg BW. After 14 days of treatment, there was a dose-dependent increase AST and ALT concentrations in serum for EAVA and EEPA extracts. Administration of EEPAs extract at doses of 3, 6 and 9 mg/Kg of BW, induced two-fold ALT concentrations. This significant increase in transaminase activity is explained by severe hepatocyte necrosis [32].

Gamma-glutamyl transferase (GGT) is a glycoprotein found in the liver and other organs such as pancreas, kidneys and spleen. In the liver, it is localized in the bile ducts and hepatocytes.

Elevation of GGT and transaminases is observed in hepatitis and cirrhosis, particularly inducible by alcohol and many drugs. The administration of the EEPAs extract at 3 and 6 mg/Kg BW resulted in a significant increase of 7.633 IU/L and 6.318 IU/L for GGT. According to Morgan, [33], GGT is an enzyme of hepatic origin, synthesis of which is induced by toxic compounds. The results obtained in the present study indicate the presence of toxic compounds in the extracts studied.

In addition, the increase of serum levels of bilirubin is associated with various liver functions, a small rise is an important indicator of liver damage. In animal laboratory, these levels may be a sign of obstruction of the bile ducts [34], as well as probable hyper hemolysis of liver cells [35].

5. Conclusion

The values of the biochemical parameters showed an increase in their serum concentrations, indicating a probable liver injury. These results show a potential exposure of populations to toxic compound, with a risk related to the consumption of *V. amygdalina* (Asteraceae) in Côte d'Ivoire. In overall, the results suggest that the causality of liver damage due to the use of medicinal plants may be taken into account in diagnostic. However, histological study need to be completed before confirming the safety use of this plant.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors have not declared any conflict of interests.

Statement of ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

References

[1] WHO. Promotion of the role of traditional medicine in the health system: strategy of the African region.AFR/RC50/9, 2002 ; p. 12-15. (www.who.int).

[2] Sofowora A. Medicinal plants and traditional African medicine. Edition Karthala, 1996 ; 378 p.

[3] Grurib-Fakim A. Medicinal plants: traditions of yesterday and drugs of tomorrow. Molecular Aspect of Medecine. 2006; 27(1):1-93. https://doi: 10.1016/j.mam.2005.07.008.

[4] Diaz HJ. Poisoning by Herbs and Plants: Rapid Toxidromic Classification and Diagnosis. Wilderness & environmental medicine. 2016; 27(1):136-52. https://doi: 10.1016/j.wem.2015.11.006.

[5] Bull LB, Dick AT and Culvenor CCJ. The pyrrolizidine alkaloids: their chemistry, pathogenicity and other biological properties. In:Amsterdam: North-Holland. 1968; 293p. https://lib.ugent.be/catalog/rug01:000059352.

[6] Roeder E. Medicinal plants in Europe containing pyrrolizidine alkaloids. Pharmazie. 1995 ; 50: 83-98.

[7] Cao Y, Colegate SM and Edgar JA. Safety assessment of food and herbal products containing hepatotoxic pyrrolizidine alkaloids: interlaboratory consistency and the im-portance of N-oxide determination. Phytochemical Analysis. 2008; 19(6):526-33. https://doi: 10.1002/pca.1079.19, pp 526-533.

[8] Yapi AB, Kassi NJ, Fofie NBY and Zirihi GN. Ethnobotanical study of medicinal Asteraceae sold on the markets of the autonomous district of Abidjan (Côte d'Ivoire). International Journal of Biological and Chimical Sciences. 2015; 9: 2633-47. http://dx.doi.org/10.4314/ijbcs.v9i6.10.

[9] Konda KM, Kabakura M, Mbembe B, Itufa Y, Mahuku K, Mafuta M, Mpoyi K, Ndemankeni I, Kadima K, Kelela B, Ngiuvu V, Bongombola M and Dumu L. Medicinal plants traditions from the province of ecuador - R.D. Congo Kinshasa. 2012 ; 420 p. ISBN 978-0-9554208-5-6.

[10] Okafor JC and Ham R. Identification, Utilization and Conservation of Medicinal plant in Southeastern Nigeria. Issues in Africa Biodiversity. 1999; 3: 1-7.

[11] Huffman MA, Koshimizu K and Ohigashi H. Ethnobotany and zoopharmacognosy of Vernonia amygdalina, a medicinal plant used by humans and chimpanzees, in CALIGARI P.D.S., HIND D.J.N. (eds.) Compositae : Biology & Utilization, Kew, The Royal Botanical Gardens. 1996; 2: 351-360.

[12] Kahane R, Temple L, Brat P and De-Bon H. Leafy vegetables from tropical countries : diversity, economic wealth and health value in a very fragile context. Angers Conference September 7-9, 2005 ; 1-9. https://agritrop.cirad.fr/529974/.

[13] Vodouhe SE, Tossou RC and Soumanou MM. Perception of consumers on the nutritional and sanitary quality of some local leafy vegetables produced in the coastal zone of South Benin. Bulletin of Agronomic Research of Benin. 2012 ; ISSN 1840-7099.

[14] Agbankpé AJ, Dougnon TV, Bankole HS, Yèhouénou B, Yedomonhan H, Legonou M and Dougnon TJ. Ethnobotanical study of therapeutic leafy vegetables used in the treatment of diarrhea in southern Benin (West Africa). International Journal of Biological and Chemical Sciences. 2014; 8:1784-95. https://doi:10.4314/ijbcs.v8i4.36.

[15] Kandé B, Yao K, Allah-Kouadio E and Koné MW. Enquête sur l'utilisation et l'effet des médicaments à base de plantes chez les patients hépatiques hospitalisés au service de médecine et d'hépatogastroentérologie du Centre Hospitalier Universitaire (CHU) de Cocody en Côte d'Ivoire. Journal of Applied Biosciences. 2018 ; 130: 13220-31.

[16] Kandé B and Koné MW. Analyse qualitative et quantitative des alcaloïdes pyrrolizidiniques chez quelques Asteraceae, Boraginaceae et Leguminosae utilisées en médecine traditionnelle en Côte d'Ivoire. European scientific journal. 2017 ; 13(12): 70-83.

[17] Fabiana E, Andreia OL, Fernando P, Mitsue H, Silvana LG, and Isis MH. "Haematological and immunological effects of repeated dose exposure of rats to integerrimine N-oxide from Senecio brasiliensis" Food and chemical toxicology. 2011 ; 49(9): 2313-2319.

[18] EU. Commission implementing decision of 14 november 2012 establishing a common format for the submission of the information pursuant to Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes (notifed under document Cf (2012) 8064) Text with EEA relevance. Special edition in Croatian: Chapter 15 Vol. 028, 2012. p. 163-180. https://data.europa.eu/eli/dec_impl/2012/707/oj.

[19] NTP. Technical report on toxicity studies of riddelliine (cas n°. 23246-96-0) administered by Gavage to F344/N Rats and B6C3F1 Mice. NTP toxicity report.1993; 27: p 105.

[20] OECD. Harmonized integrated hazard classification system for human health and Environmental effects of chemical substances as endorsed by the 28th joint meeting of the chemicals committee and the working party on chemicals in November 1998. Part2. p11.

[21] Coles H. Veterinary clinical pathology, 4th edn. W.B. saunders company, Philadelphia; 1986.

[22] Dagnelie P. Theories and statistical methods. Agricultural press Gembloux (2e éd.). Gembloux (Belgique); 1999.

[23] Letsyo E, Jerz G, Winterhalter P, Lindigkeit R and Beuerle T. Incidence of pyrrolizidine alkaloids in herbal medicines from German retail markets: Risk assessments and implications to consumers. Phytotherapy Research. 2017; 31(12): 1903-09. https://doi.org/10.1002/ptr.5935.

[24] Eleyinmi AF, Sporns P and Bressler DC. Nutritional composition of Gongronema latifolium and Vernonia amygdalina. Nutrition and Food Science. 2008; 38(2):99-109.

[25] Bhattacharjee B, Lakshminarasimhan P, Bhattacharjee A, Agrawala DK and Pathak MK (2013) Vernonia amygdalina Del. (Asteraceae) – An African medicinal plant introduced in India. Zoo's Print 28, pp 18-20.

[26] Lu FC. Acceptable Daily Intake: inception, evolution and application. Regulatory Toxicology and Pharmacology. 1988; 8(1) : 45-60. http://doi: 10.1016/0273-2300(88)90006-2.

[27] Gome MB, Kouakou K, Toure A and Traore F. Acute toxicity study and sub-chronic aqueous extract of Passiflora foetida L. (Passifloraceae) in rats and mice. International Journal of Biological and Chemical Sciences. 2011; 5: 1777-89. https://doi: 10.4314/IJBCS.V5I5.1.

[28] Mingatto FE, Maioli MA, Bracht A and Ishii-Iwamoto EL. Effects of monocrotaline on energy metabolism in the rat liver. Toxicology Letters. 2008; 10;182(1-3):115-20. http://doi: 10.1016/j.toxlet.2008.09.004.

[29] Kumar G, Sharmila B, Vanitha PP, Sundararajan M and Rajasekara PM. Hepatoprotective activity of Trianthema portulacastrum L. against paracetamol and thioacetamide intoxication in albino rats. Journal of Ethnopharmacology. 2004; 92(1):37-40. http://doi: 10.1016/j.jep.2003.12.009.

[30] Ebmeyer J, Rasinger JD, Hengstler JG, Schaudien D, Creutzenberg O, Lampen A and Hessel-Pras S. Hepatotoxic pyrrolizidine alkaloids induce DNA damage response in rat liver in a 28-day feeding study. Archives of Toxicology. 2020; 94(5): 1739-51. http://doi: 10.1007/s00204-020-02779-2.

[31] Lazare T, Jacques DY and Michel OA. Chronic alcoholization of rats (Rattus norvegicus) of wistar strain with a traditional brandy produced in Côte d'Ivoire (Koutoukou). Journal of Applied Biosciences. 2011; 41: 2772-79.

[32] Dobbs NA, Twelves CJ, Gregory W, Cruikshanka C, Richards MA and Rubens RD. Epirubicin in patients with liver dysfunction: development and evaluation of a novel dose modification scheme. European Journal of Cancer. 2003; 39(5):580-6. https://doi: 10.1016/s0959-8049(02)00669-x. pp 580-586.

[33] Morgan M. Oxford Textbook of Clinical Hepatology. Oxford University Press, 1999. p. 1186-1238.

[34] Hor SY, Ahmad M, Farsi E, Yam MF, Hashim MA, Lim CP, Sadikun A and Asmawi MZ. Safety assessment of methanol extract of red dragon fruit (Hylocereus polyrhizus): Acute and subchronic toxicity studies. Regulatory Toxicology and Pharmacology. 2012; 63(1):106-14. https://doi: 10.1016/j.yrtph.2012.03.006.63.

[35] Atsamo AD, Nguelefacka TB, Dattéb JY and Kamanyia A. Acute and subchronic oral toxicity assessment of the aqueous extract from the stem bark of Erythrina senegalensis DC (Fabaceae) in rodents. Journal of Ethnopharmacology. 2011; 134(3):697-702. https://doi: 10.1016/j.jep.2011.01.023.