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Protective Effects of Aqueous and Hydroethanolic Extracts of *Secamone afzelii* (Asclepiadaceae) Leaves on Liver Transaminases, Serum Vitamin D and Zinc Levels against Carbon Tetrachloride (CCl_4)-Induced Hepatotoxicity in Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author KDS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors GG and DBN managed the analyses of the study and provided technical support. Author BA managed the literature searches. Author YHF supervised the work. All authors read and approved the final manuscript.

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ABSTRACT

Purpose: This study was carried out to evaluate effect of aqueous and hydroethanolic extracts of Secamone afzelii leaves on Liver transaminases, serum vitamin D and Zinc levels in hepatotocixity induced by carbon tetrachloride (CCl_4) in rats.
Methods: The experiment was performed on 7 groups of 6 rats by the method of Mekky and collaborators. Rats were pre-treated with aqueous, hydroethanolic extracts Secamone afzelii (100 and 200 mg/kg) and Silymarin (SIL) an hepatoprotective reference prior to CCl_4. Hepatotoxicity was induced by the intraperitoneal injection of Carbon tetrachloride (CCl_4) in rats. Hepatotoxicity implied a significant rise of Liver transaminases (ALT and AST) by hepatocyte alteration rate. The

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parameters evaluated in the study were alanine aminotransferase (ALT), aspartate aminotransferase (AST), vitamin D and Zinc in serum. Vitamin D and Zinc levels in serum were respectively determined by HPLC analysis and Atomic absorption method.

Results: Carbon tetrachloride injection to rats every 2 days showed a significant rise of Liver transaminases (ALT and AST) and a significant lowering of vitamin D and zinc levels in serum compared to normal. However, pre-treatments with aqueous and hydroethanolic extracts of Secamone afzelii caused a significant decrease of Liver transaminases (ALT and AST) and restored vitamin D and zinc levels in serum of animals ($P < 0.001$ and $P < 0.01$) compared to rats treated with CCl_4 only (Negative control). Moreover, hydroethanolic extract (200 mg/kg) and Silymarin both reduced very well carbon tetrachloride effects by protecting Liver.

Conclusion: This study reveals that Secamone afzelii leaves extracts (aqueous and hydroethanolic) possess protective properties of the Liver. It also shows a significant association between low serum vitamin D and zinc levels and hepatotoxicity. The most active extract is Hydroethanolic extract at the dose of 200 mg/kg which can be used for preventives purposes.

Keywords: Hepatoprotective; Secamone afzelii; carbon tetrachloride; vitamin D; zinc; rat.

1. INTRODUCTION

Liver is the principal and metabolic organ playing major functions including metabolism, detoxification, excretion, synthesis of protein. It involved in the metabolism of vital molecules and with almost all the biochemical pathways [1,2]. Liver disorders are one of the world problems with high morbidity and high mortality. So far not yet any therapy has successfully prevented the progression of hepatic disease, even though newly developed drugs have been used to treat chronic liver disorders, these drugs have often side effects [3]. Liver is known to be the major organ involved in the detoxification of xenobiotics, and is thus the main target of tissue injury produced by these chemicals and their metabolites [4]. Liver is also responsible for regulating homeostasis in the body and is involved with almost all the biochemical pathways related to growth, nutrient supply, maintenance of immunity and reproduction [5].

Carbon Tetrachloride (CCl_4) is a hepatotoxin used extensively for inducing liver injury in various experimental models to elucidate the mechanisms underlying hepatotoxicity [6]. Liver damage involved imbalance nutrients metabolism as vitamin D and zinc in Liver damage [7,8]. These elements exhibit various important physiological and biological properties in *in-vivo* and *in-vitro* [9].

Plants are commonly used to treat or prevent many disease and are benefit for the health. Nowadays, traditional medicine is one of the multiple option in prevention or treatment of hepatic disorders because of their antioxidant and anti-inflammatory properties [10,11].

Secamone afzelii is a tropical plant belonging to the family of Asclepiadaceae. It has been found in many countries in Africa. It is used in traditional medicine for stomach problems, colic, dysentery and also for kidney problems [12]. It has antioxidant, anti-inflammatory and antimicrobial properties [13,14].

The present study was conducted to evaluate effect of aqueous and hydroethanolic extracts of *Secamone afzelii* on Liver transaminases serum zinc and vitamin D in hepatotoxicity induced by Carbon Tetrachloride (CCl4) in albino rats.

2. MATERIALS AND METHODS

2.1 Plant Material

The fresh leaves of *Secamone afzelii* were collected in Abobo (Abidjan) in 2017. The plant species was later identified and authenticated in the National Floristic Center (CNF) under herbarium number 13866. These leaves were collected, washed and dried at room temperature under the shade for two weeks and pulverized using the crushing assistance (IKAMAG RCT®). The powder of leaves served to prepare different extracts.

2.2 Extract Preparation

2.2.1 Aqueous extract

Fifty grams (50 g) of plant powder were added to one Liter (1L) of distilled water in a liquidizer and blend 3 times during 3 minutes. After blending, the mixture obtained was first spun in a clean square fabric, and then filtered fifth in successively with cotton wool. The filtrate was evaporated at 55°C for 48 hours [15].

2.2.2 Hydroethanolic extract

Fifty grams 50 g of powder of Secamone afzelii leaves were macerated for 24 hours in 1 Liter of ethanol-water mixture 70% (70:30, v/v). The obtained macerate was then filtered twice on white cotton and once on Whatman filter paper N°3. The filtrate was evaporated and dried at temperature of 40°C using a rotary evaporator type BUCHI 161 Water Bath [16].

2.3 Animal Material

Wistar albino rats weighing between (110 - 160) g were used for the study. The rats were provided and kept in the laboratory animal house of High Normal School of Abidjan, Côte d'ivoire. They had free access to food and water and were maintained under standard laboratory conditions which included 12-hour light-dark cycle and temperature of 25-30°C. The animals were housed in hygienic plastic cages during the experimental period.

2.4 Methods

2.4.1 Animals treatment

Animals were divided into 7 lots of 6 rats each (n= 6). The treatments are carried out every day at the same hour for 6 days. Before starting treatment blood sampling was done in rats of each group.

The evaluation of the hepatoprotective activity of Secamone afzelii aqueous and hydroethanolic extracts was conducted using the method described by [17]. The animals were divided according to weight in seven groups each of six rats:

Group I (Normal): Normal control treated daily with distilled water and olive oil.

Group II (CCl_4): Negative control treated daily with distilled water and CCl_4 (2 ml/kg in olive oil v/v).

Group III (SIL): Positive control treated daily with Silymarin (100 mg/kg) and CCl_4 (2 ml/kg in olive oil v/v).

Group IV (EA 100): Rats treated daily with aqueous extract of Secamone afzelii (100 mg/kg) and CCl_4 (2 ml/kg in olive oil v/v).

Group V (EA 200): Rats treated daily with aqueous extract of Secamone afzelii (200 mg/kg) and CCl_4 (2 ml/kg in olive oil v/v).

Group VI (EE 100): Rats treated daily with hydroethanolic extract of Secamone afzelii (100 mg/kg) and CCl_4 (2 ml/kg in olive oil v/v).

Group VII (EE 200): Rats treated daily with hydroethanolic extract of Secamone afzelii (200 mg/kg) and CCl_4 (2 ml/kg in olive oil v/v).

The test extracts of Secamone afzelii and the control groups were given by oral route every day for 6 days; 1 hour prior to the olive oil and the Tetrachloride of carbon (CCl_4) intraperitoneal injection every 2 days in the different groups.

2.5 Collection and Storage of Blood

After blood sampling on Day 7^{th} animals were sacrificed under ether anaesthesia. The blood of each animal was collected (tail vein) in a tube without anticoagulant after experiment. The blood was centrifuged at 3000 rpm for 10 minutes (Centrifuge B4i) to separate serum. Serum was kept at -20°C until the analysis in eppendorf tubes for the determination of certain biochemical parameters.

2.6 Biochemical Assays

The serum samples were used to analyse the biochemical parameters such as: transaminase (ALT and AST) using an automatic analyzer (Cobas C311, Hitachi Rock), serum Zinc analysed by atomic absorption method [18] and serum Vitamin D analysed by HPLC (High Performance Liquid Chromatography) [19].

2.7 Statistical Analysis

Graph pad 5.01 was used for statistical analyses. Data were expressed as mean ± SEM (Standard Error Mean). Mean values of the different lots were analysed by using one-way ANOVA followed by Dunnett test and $p<0.05$ was considered as statistically significant.

3. RESULTS

3.1 Before Treatment

Blood sampling was done in rats of each group and biochemical parameters in serum (ALT, AST, vitamin D and Zinc) were evaluated. Parameters were statistically equal when compared each group to others groups.

3.2 After Treatment

Rats treated only with Carbon Tetrachloride (CCl_4) produced a significant increase on Liver

transaminases activities: ALT (105.2 ± 8.75 IU/L) and AST (302 ± 13 IU/L) compared to normal rats ALT (66.5 ± 2.28 IU/L) and AST (235 ± 7.7 IU/L).

But treatment with aqueous and hydroethanolic extracts of Secamone afzelii at doses 100 mg/kg and 200 mg/kg and Silymarin (positive control) significantly reduced ($P < 0.001$) respectively ALT activity compared to negative control (105.2 ± 8.75 IU/L). However, the aqueous and hydroethanolic extracts respectively at dose 200 mg/kg (71.33 ± 3.52 IU/L; 73 ± 2.64 IU/L) and Silymarin (67.67 ± 2.18 IU/L) had statistically the same effect against CCl_4 on ALT activity in rats (Fig. 1).

Aqueous and hydroethanolic extracts of Secamone afzelii (100 and 200 mg/kg) and Silymarin (positive control) significantly decreased respectively AST activity ($P < 0.05$; $P < 0.01$ and $P < 0.001$) compared to negative control (302 ± 13 IU/L). Therefore, the hydroethanolic extract at the dose of 200 mg/kg (238 ± 6.7 IU/L) and Silymarin (236 ± 5.5 IU/L) had statistically the same effect against CCl_4 on AST activity in rats (Fig. 2).

Intraperitoneal injection of Carbon Tetrachloride in rats showed significant decrease ($P<0.001$) of serum vitamin D (4.70 ± 0,47 ng/mL) and serum zinc (0.34 ± 0.025 mg/dL) levels compared to normal rats (7.5 ± 0.24 ng/mL; 0.58 ± 0.007 mg/dL). But, pretreatment with Silymarin, aqueous and hydroethanolic extracts significantly increased ($P < 0.05$; $P < 0.01$; $P < 0.001$) serum vitamin D and serum zinc levels compared to negative control (Table 1).

4. DISCUSSION

Medicinal plants are commonly used in treating or preventing specific diseases and they are considered to play a beneficial role in health care [20,21]. The present study was carried out to evaluate the protective effects of the aqueous and hydroethanolic extracts of *Secamone afzelii* leaves on carbon tetrachloride-induced hepatotoxicity in rats and to establish the scientific evidences for the usage of Secamone afzelii plant in hepatic conditions.

Liver responsible of detoxification in the organism is exposed to these toxics effects during its functioning. During the metabolism of carbon tetrachloride (CCl_4), liver can be its target. Carbon tetrachloride (CCl_4) is biotransformed in liver by the cytochrome P-450 system. Its catabolism involved to free radical production (CCl3- and CCl3OO-) responsible of toxic effect of solvent. Its metabolites lead intracellular nucleophile structure and initiate cellular membranes polyunsaturated fatty acids leading to centrolubular hepatocyte cytolyse or necrosis [22,23,24].

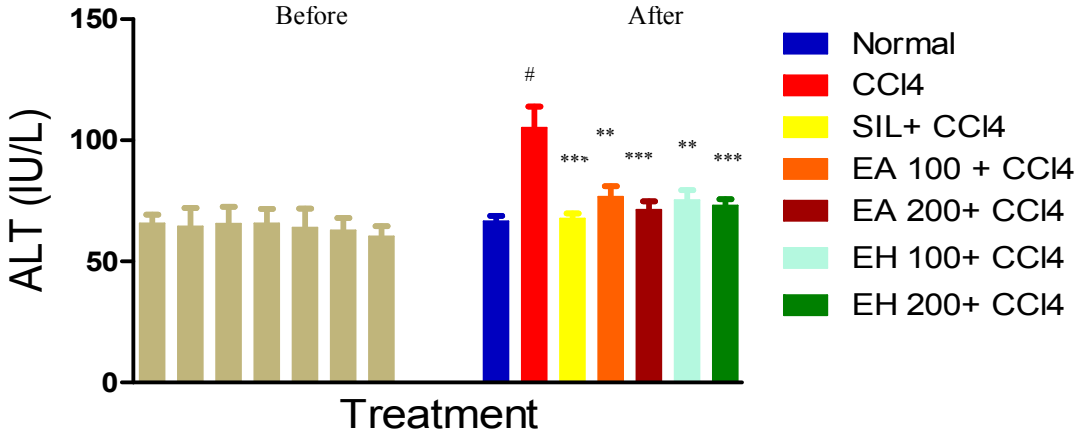

Fig. 1. Effect of *Secamone afzelii* extracts (aqueous and hydroethanolic) and silymarin on ALT activity in CCl_4-treated rats compared to negative control group

*Normal group: Distilled water; CCl_4: Carbon Tetrachloride; SIL: Silymarin; EA 100: aqueous extract (100 mg/kg); EA 200: aqueous extract (200 mg/kg); EH 100: hydroethanolic extract (100 mg/kg); EH 200: hydroethanolic extract (200 mg/kg); Before = before treatment; After = after treatment; #: Significant difference compared to normal group; *:P<0.05, **: P<0.01 and ***: P<0.001 Significant difference compared to negative group (CCl_4)*

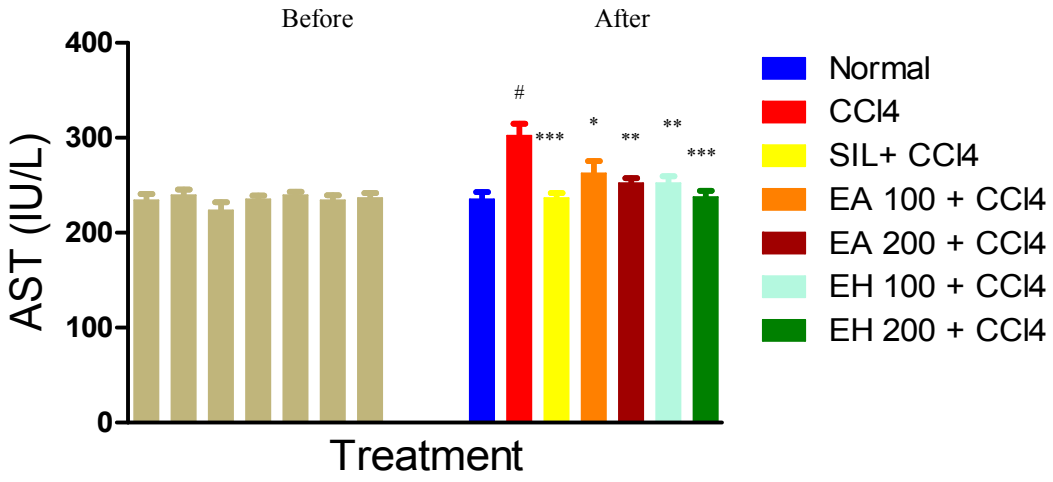

Fig. 2. Effect of *Secamone afzelii* extracts (aqueous and hydroethanolic) and silymarin on ALT activity in CCl_4-treated rats compared to negative control group

Normal group: Distilled water; CCl_4: Carbon Tetrachloride; SIL: Silymarin; EA 100: aqueous extract (100 mg/kg); EA 200: aqueous extract (200 mg/kg); EH 100: hydroethanolic extract (100 mg/kg); EH 200: hydroethanolic extract (200 mg/kg); Before = before treatment; After = after treatment; #: Significant difference compared to normal group; *:P<0.05, **: P<0.01 and ***: P<0.001 Significant difference compared to negative group (CCl_4)

Table 1. Variation of serum vitamin D and zinc levels before and after treatment with *Secamone afzelii* extracts (aqueous and hydroethanolic) and carbon tetrachloride (CCl_4)

	Serum			
	Vitamin D (ng/mL)		Zinc (mg/dL)	
	Before	After	Before	After
Normal	7.1±0,39	7.5 ± 0.24	0.567± 0.51	0.58 ± 0.007
CCl_4	7.47±0,26	4.70 ± 0.47$^{\#\#\#}$	0.571± 0.51	0.34 ± 0.025$^{\#\#\#}$
EA 100+CCl_4	7.76±0,47	5.90 ± 0.31*	0.559± 0.13	0.42 ± 0.012*
EA 200+ CCl_4	7.52±0,63	6.30 ± 0.32**	0.541± 0.35	0.46 ± 0.020**
EE 100+ CCl_4	7.29±0,44	6.00 ± 0.14*	0.553± 0.22	0.43 ± 0.021*
EE 200+ CCl_4	7.66± 0,28	6.90 ± 0.13^{b***}	0.562± 0.40	0.49 ± 0.013***

Normal group: Distilled water; CCl_4: Carbon Tetrachloride; SIL: Silymarin; EA 100: aqueous extract (100 mg/kg); EA 200: aqueous extract (200 mg/kg); EH 100: hydroethanolic extract (100 mg/kg); EH 200: hydroethanolic extract (200 mg/kg); Before = before treatment; After = after treatment; #: Significant difference compared to normal group; :P<0.05, **: P<0.01 and ***: P<0.001 Significant difference compared to negative group (CCl_4)

The hepatoprotective potential of remade to reduce harmful effect or protect normal hepatic physiologic mechanisms disturbed by toxin is an indicator of protection [25]. In the case of Silymarin that neutralize free radical sensitive to damage cells exposed to toxins. It stimulates proteins synthesis in liver leading to an increase of news cells production to displace damaged cells [26]. However, many natural agents with antioxidant activity had been proposed to prevent hepatotoxicity induced by different toxins [27,28,29]. Serum transaminases (ALT and AST) are the sensible indicators of hepatic affections [30].

Hepatoprotectrive effect of aqueous and hydroethanolic extracts of Secamone afzelii has been evaluate by variations of transaminases (ALT and AST). Intraperitoneal administration of Tetrachloride of Carbon (CCl4) significantly increased transaminase activities in rats indicating hepatic cells damage [31,32]. Ours results showed that Silymarin, aqueous and hydroethanolic (100 and 200 mg/kg) administration in rats reduced hepatotoxicity by decreasing transaminases (ALT and AST) activities in serum compared to rats treated with CCl_4. Ours results are similar to those obtained in others studies in which plants extracts

significantly reduced hepatotoxicity effects induced by CCl_4 [33,34,35]. Transaminase decreasing serum activities indicate extracts and Silymarin protection by maintaining functional and integrity of hepatocytes cells membranes. It must be explained by the presence of secondary metabolites like Flavonoids.

Minerals and vitamins are commonly used to treat or prevent many diseases. Therefore, serum vitamin D and zinc levels could be an indicators of hepatic dysfunctions. Injection of CCl_4 in rats significantly reduced serum vitamin D and zinc levels in comparison to normal group. This reduction is the result of hepatic lesions induced by CCl_4 [36,24]. Liver products protein like vitamin D binding protein that transport vitamin D in plasma [37]. Then liver storage and activate vitamin D. Decreasing of serum vitamin D level could be due to a endogen vitamin D binding protein production in liver which decrease in presence of cirrhosis, a diminution vitamin D hepatic hydroxylation in 25(OH)D and an elevation of 25(OH)D catabolic suppression [7].

Yet administration of Silymarin, aqueous and hydroethanolic (100 et 200 mg/kg) indicated significant increase of vitamin D level in serum compared to negative group (CCl_4). This result is supported by Seamans & Cashman [38] who shown significant increase of serum vitamin D level after supplementation with vitamin D_2 and/or vitamin D_3. Elevation of serum vitamin D (25(OH)D) level is due to the decreasing utilisation of 25(OH) D as substrate, to the delayed metabolism clearance of 25(OH)D in liver and to the inhibition of 25-hydroxylase by 1,25(OH)2D as result of negative feedback [39].

Zinc is necessary for the normal functioning of liver and vice versa, liver play a central role in zinc homeostasis [40]. Injection of CCl_4 in rats significantly reduced serum Zinc levels in comparison to normal group. This result is similar to those of Nkechi and coll [41] who observed significant decrease of zinc level in serum in rats treated with CCl_4 compared to normal group. It could be due to imbalance of metabolism product by zinc status diminution in zinc-dependant enzymes which is necessary for multiple metabolic process [42]. It could be also due to inflammation and hormonal stress [43,44,45].

But, administration of Silymarin, aqueous and hydroethanolic (100 et 200 mg/kg) showed significant increase of zinc level in serum compared to negative group (CCl_4). Our result is corroborating by the study of Sahel and coll [46] in which administration of *Majoram* leaves extract increase serum zinc level. It is also due to the reinforcement of zinc-dependant enzymes as glutathione peroxidase [47]. According to Baltaci and coll [48] the large absorption of zinc decreased copper and iron absorption. It could lead to high level of zinc in serum.

5. CONCLUSION

The present study indicated that the aqueous and hydroethanolic extracts of Secamone afzelii leaves restored Liver transaminases activities. The aqueous and hydroethanolic extracts at the dose of 200 mg/kg body weight possessed profound heptoprotective properties. It also revealed that low serum vitamin D and Zinc levels are associated with hepatic toxicity. Hydroethanolic extract had an hepatoprotective properties near to those of Silymarin.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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