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Hepatic tolerance of an ethyl acetate extract of *Holarrhena floribunda* (G. Don) Durand and Schinz leaves in *Wistar* Rats

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ABSTRACT

Purpose: This study aims to evaluate the hepatic biochemical effects associated with the use of ethyl acetate extract of *Holarrhena floribunda* leaves as a traditional medicine. ***Methods***: The rats were randomly divided into four groups of 10 (male control, female control, male test and female test) and combined with four groups of 5 (male control satellite, female control satellite, male test satellite and female test satellite) were force-fed for 90 days. The control and control satellite control rats received distilled water at a rate of 2 mL / 100g PC, and treated and treated satellite treated rats received the 1000 mg / kg PC dose of the acetate extract of ethyl leaves of *Holarrhena floribunda*. The blood collected each week during the first month, then at the end of each month as of the 2nd month allowed to assay liver serum markers such as ALT, ASAT, PAL, LDH, total bilirubin, direct bilirubin, albumin, glucose, HDL-cholesterol, LDL-cholesterol, total cholesterol and triglycerides. ***Results***: The animals were well supported gavage of the extract since no sign of pathological manifestation was observed on them. The biochemical analysis indicated on the one hand a significant decrease in the level of total cholesterol, glucose (in both sexes), LDL cholesterol (in the female rats), and on the other hand an increase in the HDL cholesterol level (in both sexes), the rate of ASAT activities (in female rats) and PAL (in rats). On the other hand serum levels of total bilirubin, direct bilirubin, albumin, triglycerides, activities of ALT and LDH did not significantly vary. Microscopic observations of the liver tissue sections of the rats in the test lot did not show any lesions. ***Conclusion***: Administration of the ethyl acetate extract of *Holarrhena floribunda* leaves to the rats for 90 days did not interfere with liver function or cause liver tissue damage.

Keywords: *Holarrhena floribunda*, biochemical parameters, liver tolerance, histopathology, wistar rats.

INTRODUCTION

The use of complementary traditional medicine, which includes herbal medicines for the treatment of various diseases, has developed rapidly in both developed and developing countries because of their accessibility and effectiveness. According to the World Health Organization (WHO), more than 80% of the world's population uses traditional medicine to cope with their health problems [1]. It is with this in mind that WHO has recommended the use of herbal medicines whose quality assurance, safety, appropriate use and efficacy are guaranteed [2]. *Holarrhena. floribunda* is one of the medicinal plants of the flora of Ivory Coast used for the treatment of several diseases such as dysentery, diarrhea, colic, sterility and especially diabetes. This tree, 10 to 25 m tall, with a white latex abundant in all parts, has opposite, single whole and cuneiform leaves [3]. It has been subject of many previous studies. Some researchers showed its in vitro inhibitory effect of the aqueous extract of *H. floribunda* on the growth of non-pathogenic amoebae of the species Amoeba proteus [4]. Others demonstrated the estrogenic effects in the ovariectomized rat and the antioxidant potential of the methanolic extract of *H. floribunda* leaves [5, 6]. Phytochemical investigations on the plant led to isolation of flavonoids, phytohormones, and alkaloids [7, 8]. More recently, our research team has shown the hypoglycemic activity of the ethyl acetate extract with 1000 mg/kg b.w. as a therapeutic dose [9]. To guarantee the use of this plant which offers a promising therapeutic perspective, an earlier study by [10] showed that ethyl acetate extract from *Holarrhena floribunda* leaves at a dose of 1000 mg / kg body weight did not have significant effects on heart integrity in wistar rats. So far, there are no studies on the effect of this hypoglycemic extract on liver structure. Given the key role in the metabolism to maintain the energy level and structural stability of the body on the one hand and secondly in the detoxification of xenobiotics and thus the high risk of exposure of this organ to toxic entering in the body such a study concerning the tolerance of this extract could be conducted.

MATERIALS AND METHODS

Plant material

The plant material consists of the leaves of *Holarrhena floribunda*. They were harvested in the department of Agboville (Côte d'Ivoire) from July to September 2013. The plant has been identified at the national floristic center of Felix Houphouët-Boigny University (Côte d'Ivoire) was a voucher specimen was deposited (Holarrhena floribunda (G. DON) DURAND AND SCHINZ (Apocynaceae) n° 13240).

Animal material

Animals were selected as per the Organization of Economic Co-operation and Development (OECD) guidelines no. 408 [11]. Healthy young and nulliparous, non-pregnant Wistar rats weighing from 90-127 mg of 6-8 weeks old. The animals were bred in the animal house of the Center for Ecological Research (CRE) of the University of Nangui Abrogoua (Côte d'Ivoire). The animals are randomly selected, marked to permit individual identification, and kept in plastic cages with wood chips renewed every three days

Methods

Preparation of ethanol extract

Holarrhena floribunda leaves were dried out of the sun at room temperature of 27 ± 2 ° C. The dried leaves were pulverized using a mill (RETSCH S M ® 100). The ethanol extract of H. floribunda is obtained considering the traditionally method used by traditional healers to treat diabetes. Hundred grams of the previously obtained powder were dissolved in two liters of ethanol 80 %. The solution was homogenized with a magnetic stirrer (STURART SB 162) for 48 hours and filtered on cotton wool and on whatmann n° 1 paper. The residue was re-extracted with ethanol 80 % twice for 6 hours. The solution is also filtered on cotton wool and whatmann n° 1 paper. The filtrates were put together and concentrated using a rotary evaporator (Buchi R110/NKE 6540/2) at reduced pressure at 45 ° C and then dried in an oven (Retch type SM 100, Haan, Germany) at 45° C for 48 hours. This extraction obtained a yield of 13.01%.

Preparation of the ethyl acetate extract

With a separation final, Ten (10) g of the ethanol extract was dissolved into 100 mL of distilled water. 100 mL of hexane was added to the preparation to obtain 200 mL of a solution of water-hexane in the ratio 1:1. After shaking and then decanting the solution, the aqueous layer was collected. The mixture was further successively partitioned (1:1, v/v) by dichloromethane and ethyl acetate. Solvents were evaporated using a rotary evaporator (Buchi Rotavapour) at 45 ° C and extracts were dried using an oven. The powder obtained was the ethyl acetate extract from the leaves of *Holarrhena floribunda*. The yield of the ethyl acetate fraction from the 10 g of ethanol extract was 15.23 %.

Subchronic Toxicity Test

Subchronic toxicity tests were conducted in accordance with OECD Guideline No. 408 [11].

The rats were divided into four groups of 10 (male control, female control, male and female treated) and in four groups of 5 (male control, female control, male-treated and female-treated satellite) were force-fed for 90 days. Rats in Control and Control Satellite were administered by gavage with distilled water daily at 2 mL / 100 g b.w. Those of Treated and Treated Satellite groups received orally and daily doses of 1000 mg / kg b.w. of EAHf, the dose which induces the hypoglycaemic effect in hyperglycemic rats according to Gnangoran *et al*., [9].

Collection of blood samples

Rats were fasted for 24 hours and then anesthetized with ether; 1 ml / 100 g of blood was taken from the retro-orbital sinus. Blood samples were taken a day before the administration of either the extract or distilled water to rats and every week during the first month and every month from the second month until the end of the Experiments. The whole collected blood in dry tubes was centrifuged immediately in a JOUAN BR4i centrifuge (Buckinghamshire, England) at 3000 rpm for 5 min to obtain a serum for the determination of biochemical parameters.

Determination of serum hepatic markers

Hepatic enzymes activities, serum albumin, bilirubin (Total and Direct) and lipids levels were determined using a Cobas C311® HITACHI biochemistry automaton (Roche Diagnostics, France). The experiments were conducted using commercial kits (Roche Diagnostics, France) based on the manufacturer's instructions, as summarized in Serum glycemic level were determined by glucose meter (Tables 1). The LDL cholesterol level has been calculated according to Friedwald's formula [12].

$$LDL\text{-}c = TC - HDL\text{-}c - TG/2{,}17 \text{ (mmol/L)}$$

Table 1: Operating parameters for the quantitative determination of serum hepatic markers

Hepatic biochemical markers	Methods	Waves length (nm)
albumin	turbidimetric	570
Total bilirubin	Colorimetric (formation of azobilirubine)	546
Direct bilirubin	Colorimetric (formation of azobilirubine)	546
Glucose	Blood glucose meter (glucose dehydrogenase)	
Cholestérol total	Colorimetric (formation of phenolic chromogen)	505
Cholestérol HDL	Colorimetric in homogeneous phase	600

Triglycerides	Colorimetric (formation of phenolic chromogen)	505
Alanine aminotransferase (ALT)	Absorption kinetics (Disappearance of NADH	340
Aspartate aminotransferase (AST)	Absorption kinetics (Disappearance of NADH	340
Lactate dehydrogenase (LDH)	Absorption kinetics (Disappearance of NADH)	340
Alkaline phosphatase (ALP)	Colorimetric (Rate of p-nitrophenol formation)	450

Hepatic tissue sampling and analysis of histological sections

At the end of the third month, the rats of the main test and those of the satellite batches at the end of the fourth month were euthanized by overdose with ether. Their livers were removed and attached to 10% formalin. On the cores removed from the formalin, longitudinal sections of 5 mm of tissue pieces were taken using scalpel blade and then deposited in cassettes. These cassettes were introduced into an automaton (Technicon® Tissue Tek) where the tissue pieces were respectively subjected to fixing baths with 10% formaldehyde, dehydration baths with 70% and 90% ethanol, Lightening with toluene and impregnation baths with paraffin using microtome (Microme® GmbH (walldorf, Germany), cuts of 3 µm thickness were made. The sections obtained were mounted on slides and then stained with hematoxylin-eosin. Blade readings were made using a binocular optical microscope (Motic®); and the photos were taken using an electronic photography device (Am Scope® FMA050, 8.0 PIXEL) adapted to the objective of the microscope.

Statistical Analysis

The results are expressed as averages followed by the standard error on the mean (M ± ESM). The repeated variance analysis (ANOVA) was used to compare the administered dose (Control/ Test) effects and the treatment time effects. The tests were supplemented by the Bonferroni post-Hoc test. The differences were considered significant for a probability level $p < 0.05$. All these analyzes were carried out using the GraphPad prism Version 5.0 software

RESULTS

Biochemical study

Effects of ethyl acetate extract of leaves of *Holarrhena floribunda* on serum levels some metabolites of rat liver

The ethyl acetate extract of *Holarrhena floribunda* leaves did not cause significant effects on the serum albumin, total bilirubin and total bilirubin levels of the rats in the treated group compared to the control rats in both sexes gender.In regards to blood glucose, significant decreases ($p < 0.05$) of (-12.53%) to J7, and (-11.55%) to J60 in male rats and (-12.97 %) at day 7, of, (-17.62%) at day 28 and (-17.26%) at day 60 in female rats were observed (Table 2)

Table 2: Effects of ethyl acetate extract of leaves of *Holarrhena floribunda* on serum levels some metabolites of rat liver

Parameters	Groups	Day0	Day7	Day14	Day21	Day28	Day60	Day90	Day120
					Males				
Albumin	Control	31,750 ± 0,69	34,440 ± 1,20	32,380 ± 1,35	34,140 ± 1,00	35,240 ± 0,71	34,960 ± 1,18	36,880 ± 0,96	32,400 ± 1,39
	Treated	33,270 ± 0,86	35,030 ± 1,52	36,100 ± 0,96	36,870 ± 1,62	39,290 ± 1,28	34,180 ± 0,91	36,070 ± 0,88	36,180 ± 1,15
Direct bilirubin	Control	0,303 ± 0,03	0,290 ± 0,02	0,297 ± 0,02	0,323 ± 0,02	0,292 ± 0,03	0,311 ± 0,02	0,301 ± 0,03	0,302 ± 0,03
	Treated	0,308 ± 0,03	0,283 ± 0,02	0,278 ± 0,03	0,281 ± 0,03	0,273 ± 0,03	0,290 ± 0,03	0,263 ± 0,03	0,261 ± 0,03
Total bilirubin	Control	0,8678 ± 0,04	1,134 ± 0,09	0,853 ± 0,06	1,136 ± 0,09	0,955 ± 0,11	0,974 ± 0,11	0,956 ± 0,11	0,802 ± 0,05
	Treated	0,9078± 0,02	0,955 ± 0,11	0,980 ± 0,10	0,955 ± 0,11	1,183 ± 0,18	1,141 ± 0,18	1,184 ± 0,18	0,970 ± 0,10
Glucose	Control	107,400 ± 3,40	111,700 ± 3,47	113,300 ± 5,37	106,300 ± 5,17	106,100 ± 5,11	112,500 ± 2,64	119,400 ±5,96	115,800 ± 5,05
	Treated	110,100 ± 4,42	97,700 ± 1,51 (*)	107,100 ± 3,70	96,900 ± 3,72	107,100 ± 2,19	99,500 ± 5,07 (*)	111,800 ± 3,54	108,600 ± 3,59
					Females				
Albumin	Control	34,680 ± 0,69	35,410 ± 1,11	35,960 ± 1,11	37,790 ± 1,83	37,200 ± 1,02	36,880 ± 1,05	33,210 ± 0,80	36,710 ± 1,00
	Treated	33,620 ± 0,71	36,730 ± 1,25	35,880 ± 1,59	36,800 ± 0,96	35,770 ± 1,40	38,500 ± 1,17	34,220 ± 0,61	33,850 ± 1,68
Direct bilirubin	Control	0,246 ± 0,03	0,263 ± 0,02	0,259 ± 0,03	0,259 ± 0,01	0,257 ± 0,03	0,254 ± 0,02	0,271 ± 0,03	0,276 ± 0,03
	Treated	0,267 ± 0,02	0,233 ± 0,03	0,248 ± 0,03	0,248 ± 0,03	0,276 ± 0,02	0,244 ± 0,03	0,227 ± 0,02	0,302 ± 0,03
Total bilirubin	Control	1,227 ± 0,11	1,094 ± 0,15	1,137 ± 0,15	1,1093 ± 0,14	1,145 ± 0,12	1,146 ± 0,11	1,232 ± 0,14	1,0951 ± 0,12
	Treated	1,258 ± 0,14	1,213 ± 0,13	1,301 ± 0,14	1,145 ± 0,12	1,280 ± 0,12	1,183 ± 0,10	1,326 ± 0,13	1,178 ± 0,16
Glucose	Control	114,200 ± 5,74	114,100 ± 4,92	117,800 ± 4,10	113,900 ± 3,50	121,400 ± 4,61	114,700 ± 3,61	105,900 ± 3,58	114,200 ± 2,76
	Treated	111,700 ± 4,92	99,300 ± 2,03 (*)	105,100 ± 3,12	102,300 ± 3,15	100,000 ± 2,91 (**)	94,900 ± 1,76 (**)	102,500 ± 4,12	112,400 ± 2,74

Values are means ± SEM. Comparisons are made between the control and the corresponding treated group according to gender.The asterisk indicate significant differences (*) = P< 0.05; (**) = p< 0.01; (***) = p< 0.001

Effect of ethyl acetate extract of leaves of *Holarrhena floribunda* on the serum lipids levels of rats.

Serum triglyceride levels in treated rats in both sexes compared to control rats were not significantly changed. The extract significantly decreased the serum total cholesterol level by (-16.56%) on day 14 in male rats and (-8.99%) in female rats compared to their counterparts in control groups. At the level of cholesterol. LDL, a significant reduction ($p < 0.05$) of (-21.76%) compared to the rats of the control group was observed in the female rats on day 14. The extract also significantly increased (+ 12.98%) and (+ 10.63%) at day 28 the HDL cholesterol level respectively in the rats and rats treated with respect to their counterparts in the batches. controls (Table 3).

Table 3: Effect of ethyl acetate extract of leaves of *Holarrhena floribunda* on the serum lipids levels of rats

Parameters	Groups	Day0	Day7	Day14	Day21	Day28	Day60	Day90	Day120
					Mâles				
HDL-c	Control	0,540 ± 0,01	0,565 ± 0,02	0,563 ± 0,01	0,494± 0,02	0,523 ± 0,03	0,524 ± 0,01	0,547 ± 0,01	0,542 ± 0,01
	Treated	0,513 ± 0,02	0,523 ± 0,01	0,521 ± 0,00	0,542 ± 0,02	0,592 ± 0,01 (*)	0,542 ± 0,01	0,553 ± 0,01	0,523 ± 0,01
TC	Control	0,986 ± 0,02	1,038 ± 0,04	1,123 ± 0,01	0,977 ± 0,03	0,950 ± 0,02	0,923 ± 0,02	0,986 ± 0,02	0,970 ± 0,04
	Treated	0,943 ± 0,03	0,916 ± 0,03	0,936 ± 0,03 (**)	0,958 ± 0,04	0,974 ± 0,02	1,041 ± 0,02	1,076 ± 0,02	1,020 ± 0,05
LDL-c	Control	0,173 ± 0,03	0,155 ± 0,02	0,185 ± 0,03	0,195 ± 0,03	0,164 ± 0,02	0,136 ± 0,02	0,143 ± 0,02	0,167 ± 0,04
	Treated	0,166 ± 0,03	0,138 ± 0,03	0,159 ± 0,03	0,145 ± 0,03	0,129 ± 0,02	0,160 ± 0,02	0,183 ± 0,02	0,195 ± 0,03
Triglycérides	Control	0,590 ± 0,05	0,688 ± 0,05	0,812 ± 0,07	0,622 ± 0,08	0,568 ± 0,02	0,567 ± 0,01	0,588 ± 0,00	0,566 ± 0,05
	Treated	0,571 ± 0,06	0,551 ± 0,07	0,554 ± 0,00	0,586 ± 0,02	0,549 ± 0,06	0,731 ± 0,08	0,708 ± 0,10	0,651 ± 0,14
					Females				
HDL-c	Control	0,552 ± 0,01	0,541 ± 0,00	0,559 ± 0,01	0,562 ± 0,01	0,554 ± 0,01	0,543 ± 0,00	0,551 ± 0,00	0,567 ± 0,01
	Treated	0,533 ± 0,00	0,563 ± 0,01	0,577 ± 0,01	0,557 ± 0,01	0,614 ± 0,01 (*)	0,562 ± 0,01	0,553 ± 0,01	0,566 ± 0,01
TC	Control	0,996 ± 0,02	1,020 ± 0,03	1,089 ± 0,01	1,026 ± 0,01	1,017 ± 0,03	0,985 ± 0,02	1,0166 ± 0,02	1,0214 ± 0,03
	Treated	1,003 ± 0,02	1,026 ± 0,02	0,992 ± 0,01 (*)	1,027 ± 0,02	1,030 ± 0,02	1,021 ± 0,02	1,076 ± 0,02	1,046 ± 0,01
LDL-c	Control	0,128 ± 0,02	0,136 ± 0,02	0,169 ± 0,01	0,125 ± 0,02	0,129 ± 0,02	0,132 ± 0,02	0,147 ± 0,02	0,135 ± 0,03
	Treated	0,137 ± 0,01	0,115 ± 0,02	0,132 ± 0,00 (*)	0,129 ± 0,01	0,107 ± 0,02	0,126 ± 0,02	0,138 ± 0,02	0,130 ± 0,01
Triglycérides	Control	0,683 ± 0,01	0,682 ± 0,01	0,693 ± 0,01	0,690 ± 0,01	0,678 ± 0,01	0,670 ± 0,01	0,688 ± 0,01	0,692 ± 0,01
	Treated	0,675 ± 0,01	0,710 ± 0,01	0,710 ± 0,01	0,695 ± 0,01	0,755 ± 0,01	0,718 ± 0,00	0,702 ± 0,01	0,701± 0,02

Values are means ± SEM. Comparisons are made between the control and the corresponding treated group according to gender. The asterisk indicate significant differences (*) = P< 0.05; (**) = p< 0.01; (***) = p< 0.001

Effect of ethyl acetate extract of leaves of *Holarrhena floribunda* on serum liver activities some enzymes of rat liver

In this study, serum ALT and LDH activities in rats treated in both sexes were not significantly different from controls in rats. The serum activity of AST and ALP respectively indicate a significant decrease in serum AST activity in treated rats and an increase in those of ALP in treated male rats (Table 4)

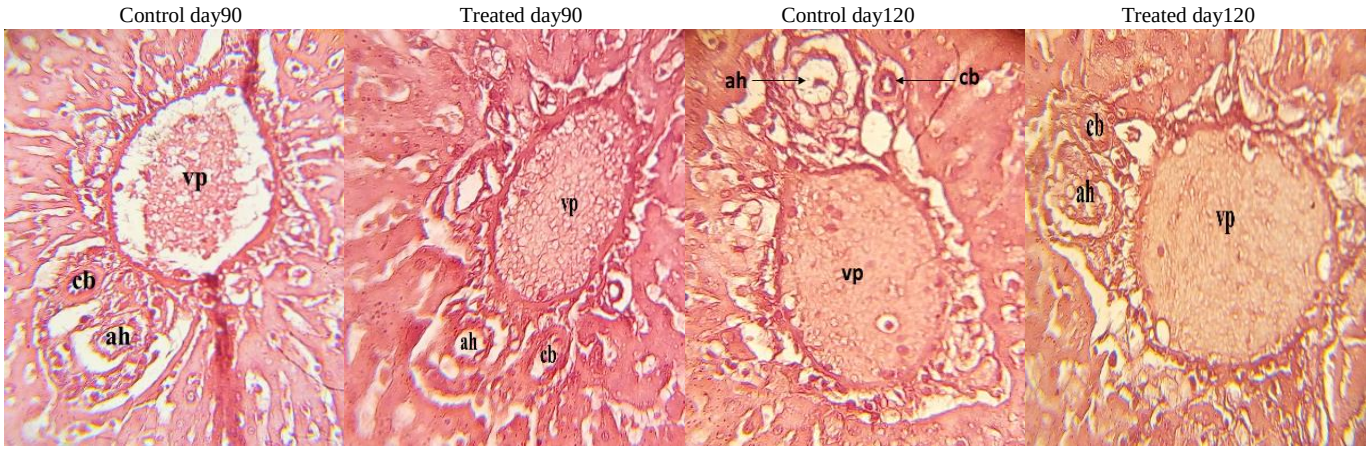
Table 4: Effect of ethyl acetate extract of leaves of *Holarrhena floribunda* on serum liver activities some enzymes of rat liver

Parameters	Groups	Day0	Day7	Day14	Day21	Day28	Day60	Day90	Day120
					Mâles				
ALT	Control	27,936 ± 3,10	29,300 ± 2,69	36,500 ± 5,48	36,100 ± 1,16	29,800 ± 3,08	26,900 ± 3,71	27,200 ± 1,97	26,900 ± 3,67
	Treated	30,236 ± 2,70	31,660 ± 2,80	29,100 ± 2,23	34,771 ± 3,25	31,471 ± 3,01	24,660 ± 3,26	28,200 ± 2,20	23,799 ± 3,39
AST	Control	43,900 ± 4,44	46,300 ± 3,10	53,200 ± 3,53	51,300 ± 6,54	43,400 ± 2,09	47,800 ± 5,87	56,300 ± 4,27	44,800 ± 4,94
	Treated	45,800 ± 5,07	42,200 ± 6,04	56,200 ± 3,55	49,600 ± 4,62	59,300 ± 3,16	43,100 ± 4,98	48,400 ± 4,19	55,300 ± 4,13
LDH	Control	469,000 ± 26,27	596,900 ± 44,56	596,700 ± 50,28	549,900 ± 37,29	628,100 ± 43,58	542,400 ± 36,21	562,700 ± 26,57	546,700 ± 46,11
	Treated	494,100 ± 32,47	649,500 ± 46,41	644,300 ± 45,70	584,400 ± 36,27	683,200 ± 46,45	681,400 ± 47,21	597,100 ± 58,07	538,300 ± 31,26
PAL	Control	428,600 ± 15,25	406,300 ± 20,94	434,700 ± 12,16	388,700 ± 21,26	407,200 ± 13,90	337,500 ± 14,88	293,100 ± 18,05	226,300 ± 14,36
	Treated	436,900 ± 10,27	423,900 ± 13,66	406,300 ± 20,94	454,700 ± 8,45 (*)	434,700 ± 12,16	369,100 ± 6,90	305,100 ± 18,15	208,700 ± 15,76
					Femelles				
ALT	Control	18,58 ± 2,84	24,900 ± 2,29	25,100 ± 2,46	20,100 ± 3,02	26,000 ± 1,67	23,100 ± 3,47	20,300 ± 3,84	27,498 ± 1,02
	Treated	23,116 ± 2,85	23,740 ± 3,06	20,800 ± 2,65	22,965 ± 2,61	25,510 ± 3,07	22,740 ± 3,72	25,100 ± 2,45	26,825 ± 2,13
AST	Control	39,000 ± 4,41	40,100 ± 1,82	40,500 ± 2,66	39,700 ± 4,05	39,600 ± 1,55	40,300 ± 3,91	40,600 ± 2,29	39,800 ± 3,06

	Treated	39,900 ± 2,21	42,100 ± 2,90	41,900 ± 3,99	41,800 ± 2,18	50,700 ± 1,42 (*)	41,600 ± 2,71	41,800 ± 1,32	41,400 ± 1,97
LDH	Control	440,200 ± 49,72	450,200 ± 19,08	466,300 ± 29,48	473,500 ± 29,92	413,000 ± 32,73	524,100 ± 47,98	488,500 ± 45,54	540,300 ± 48,66
	Treated	397,300 ± 23,76	561,500 ± 39,76	552,800 ± 41,02	490,500 ± 23,26	547,000 ± 42,57	529,400 ± 44,43	585,800 ± 32,87	455,400 ± 50,58
PAL	Control	388,800 ± 13,96	404,900 ± 7,95	363,200 ± 14,94	397,400± 26,61	343,600 ± 19,46	257,000 ± 12,06	218,200 ± 4,02	209,900± 9,86
	Treated	383,900 ± 13,44	386,000 ± 14,01	404,900 ± 7,95	363,200 ± 14,94	347,200 ± 15,66	261,800 ± 12,69	214,200 ± 2,76	252,500 ± 19,30

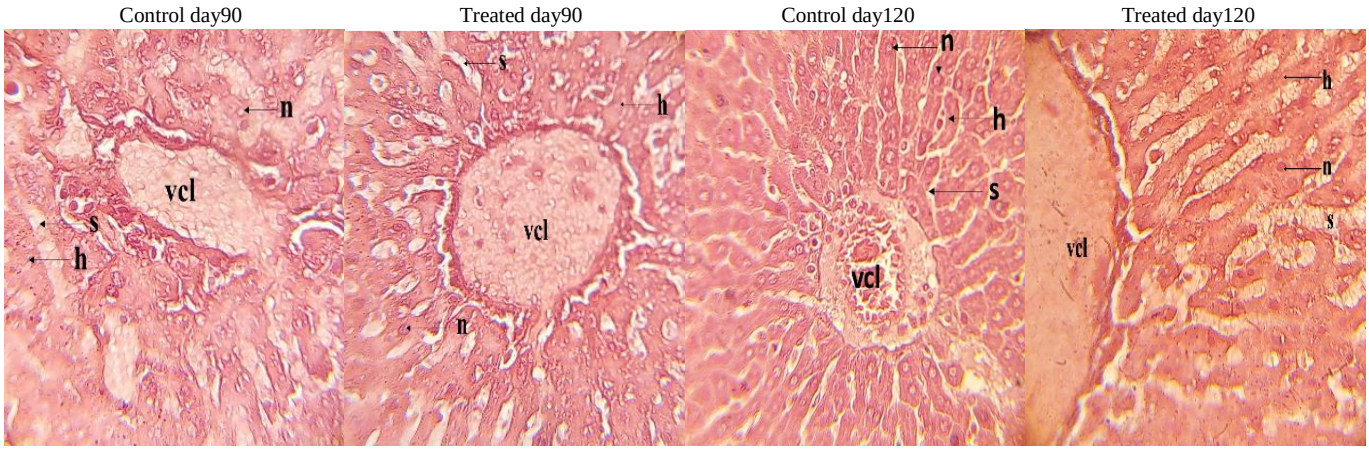
Values are means ± SEM. Comparisons are made between the control and the corresponding treated group according to gender. The asterisk indicate significant differences (*) = P< 0.05; (**) = p< 0.01; (***) = p< 0.001

Histological study of rat liver



hematoxylin-eosin stain; Magnification:x400
The images show a representative example of the batches of animals after 90 and 120 days of study
***vp** = portal vein; **ah** = hepatic artery; **cb** = biliary canal*

Figure 1: Photomicrograph of the Hepatic parenchyma of rats showing portal space at different dates



hematoxylin-eosin stain; Magnification:x400
The images show a representative example of the batches of animals after 90 and 120 days of study
Vcl= centrilobular vein; **n** = nucleus; **h** = hepatocyte; **s** = sinusoids

Figure 2: Photomicrograph of Hepatic parenchyma of rats showing the centrilobular vein at different dates

Figures present photomicrograph of portal space (**fig 1**) and centrilobular vein **(fig 2)** of liver of rat in control and treated group at different dates. Compared to the control group, the sections of the rats in the test group show normal structures. The integrity of the liver tissue seems to be preserved. The liver cells, the blood vessels and the bile duct are not damaged, there is an absence of white vacuoles

DISCUSSION

The liver is the main organ that is able to convert drugs into forms that can be easily removed from the body. A wide range of adverse effects of many drugs on liver function and integrity has been documented. These reactions range from mild transient changes to complete liver destruction. In this work, the impact on the liver of the ethyl acetate extract of *Holarrhena floribunda* used in the treatment of diabetes was evaluated by assaying some markers of liver integrity and functional.

In terms of liver integrity, ASAT, ALT, PAL and LDH are serum markers of the liver. The increase in serum activity of these enzymes is evidence of alterations or lesions in liver cells [13]. In this study, serum ALT and LDH activities in rats treated in both sexes were not significantly different from controls in rats. This assumes that the extract of *Holarrhena floribunda* did not cause membrane lysis of the liver cells that could allow the escape of these enzymes into the blood. Assay results for ASAT and PAL respectively indicate a significant decrease in ASAT serum activity in treated rats and an increase in

PAL in treated male rats. The reduction in serum activity of ASAT obtained in this study is identical to that observed by [14] and [15] respectively with the aqueous extracts of the leaves of *Artemisia afra* and *Passiflora foetida* Linn. in the rats. It could reflect a hepatoprotective effect of *Holarrhena floribunda* extract in these rats. On the other hand, the increase of the serum activities of the PAL is similar to that obtained by Kamo *et al*. (2015) [16] in the study of hepatic biotolerance of the aqueous-alcoholic extract of Terminalia mantaly H. Perrier in rats. It could be explained either by an obstruction of the intrahepatic bile ducts (inducing a cholestasis), or by a disorganization of the hepatic architecture [17]. In this case, the action of *Holarrhena floribunda* extract could result in a possible toxic effect on the hepatobiliary pathway. However, the average value of serum activities of the PAL observed in treated male rats is less than twice the value of activities obtained in the control rats and also lower than the reference values published by [18]. In addition, the microscopic study of the tissue sections of the liver revealed that the portal and centrolobular spaces observed in the present study kept their normal anatomical structure in treated groups as in the control groups. In fact, the portal spaces each contain a branch of the portal vein, the hepatic artery and an unobstructed bile duct. No degeneration in centrolobular spaces, nor steatosis or necrosis was observed as suggested by [19]. As a result, the increase in ALP activities observed in this study, described as moderate increases [20], can not be attributed to liver injury.

Functionally, serum albumin, bilirubin (total and direct), and triglyceride levels in treated rats in both sexes compared to control rats were not significantly modified. The extract, however, significantly decreased blood glucose, serum total cholesterol, and LDL cholesterol, and increased HDL cholesterol levels in both sexes compared to rats in the corresponding control groups. The reduction in blood glucose observed could be due to either the secretion of insulin, the potentiation of the action of insulin on peripheral organs, or the inhibition of glucose uptake at the level of the insulin. intestine with *Holarrhena floribunda* extract. These observations support the results of [21] and [9] who respectively showed the inhibition of alpha amylase activity and a significant decrease in blood glucose with ethanolic and acetic extracts of leaves of *Holarrhena floribunda*. As for the decrease in serum levels of total cholesterol, and LDL-c and the increase in HDL-c levels obtained in this study, they could suggest an implication of *Holarrhena floribunda* extract in the transformation of cholesterol into acids. bile ducts, LDL reduction and hepatic synthesis of HDL lipoproteins, as reported by [22]. Since in clinical practice, effective and intensive lowering of CT, TG and LDL levels and increased cholesterol levels are important in order to reduce and prevent [23] coronary heart disease, the effects of the extract of *Holarrhena floribunda* on the serum lipids obtained in this study testify to the protective role of this extract in the occurrence of cardiosclerotic diseases.

CONCLUSSION

After oral administration for 90 days at a dose of 1000 mg / kg body weight, the ethyl acetate extract of the leaves of *Holarrhena floribunda* did not result in functional disturbances or hepatic damage in rats. However, biochemical and histological studies of other organs would be necessary in order to provide additional information to those already obtained in this study.

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