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Hepatic tolerance study of hydro-alcoholic extract of *Terminalia mantaly* H. Perrier (Combretaceae) in rats

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Abstract

Objective: The effect of the hydro-alcoholic extract of *Terminalia mantaly* H. Perrier (Combretaceae) was evaluated on liver tissue and biochemical markers in Rats were analyzed. **Methods:** The animals were divided into four groups of 10 animals each (5 males and 5 females). Group 1 received 1 mL/100g body weight of distilled water and served as control. Groups 2, 3 and 4 received extract doses of 150, 300 and 600 mg/kg body weight, respectively. Blood sampling was carried out to evaluate various biochemical parameters. **Results:** Biochemical analyzes showed significant reductions in ALT, AST, total and conjugated bilirubin, glucose, total protein, TG, total cholesterol in the treated rats depending on changes in time. On the other hand, this study showed a significant elevation of serum ALP in treated rats with doses of 300 and 600 mg / kg compared to controls and a significant increase of GGT in treated rats with doses of 150 and 600 mg / kg during the third and fourth week. Histopathological study revealed no significant damage in rats liver tissues in groups treated with the hydro alcoholic extract of *Terminalia mantaly* when compared with the control groups. **Conclusion:** These results revealed that the hydro-alcoholic extract of *Terminalia mantaly*, when used in the dose range evaluated in this study, may be well tolerated by the liver.

Keywords: *Terminalia mantaly*, hepatic tolerance, Biochemical parameters

Introduction

Medicinal plants is recognized to have a very important role on the health of men [1]. WHO [2] has identified more than 22 000 medicinal plants used by traditional medicine. These medicinal plants continue to be, subject to different research work that give preference generally to ethnobotanical studies, pharmacological and phytochemical [3]. *Terminalia mantaly* H. Perrier is a plant of the family of *Combretaceae* [4] used in traditional medicine practice in Madagascar where its stem bark and leaves are used for the treatment of dysentery [5], mouth candidiasis and postpartum care [6].

In Côte d'Ivoire [7], the leaves are used in the treatment of malaria [7]. *Terminalia mantaly* has several pharmacological properties. In vitro studies have shown that the hydro-alcoholic extract has the best antifungal and antibacterial activities than the aqueous extract of bark of *Terminalia mantaly* [8, 9]. Phytochemical investigations on the plant led to the isolation of saponins, flavonoids, terpenes, steroids, phenol and alkaloids [9]. From these studies it was shown that the extracts possess anti-infective properties [9].

To guarantee the safety use of *Terminalia mantaly* by populations, early study of the hematologic parameters assessment revealed that the hydro alcoholic extract of *Terminalia mantaly* could be well tolerated by blood cells mice and rat model [10]. But up now, there are no available scientific data concerning the influence of this extract on vital organs such as liver.

Liver is a target organ and the primary site of detoxification. Being a major site of intense metabolism and, therefore, prone to various disorders as a consequence of exposure to toxins of extrinsic and intrinsic nature, the liver plays an important role in metabolism to maintain energy level and structural stability of the body [11]. It is also a site of biotransformation where toxic compounds are transformed to less harmful substances [11].

This study was, therefore, conducted to assess the effect of the hydro-alcoholic extract of *T. mantaly* on some biochemical markers and tissue of the liver.

Materials and methods

Plant material

The material used was the bark of *Terminalia mantaly* H. Perrier harvested in the region of Azaguié (southern area of Abidjan) in the month of February 2014.

Pieces of bark from the trunk of *Terminalia mantaly* H. Perrier were harvested, cut and dried in the shade. After drying, the pieces of this plant were finely ground using an electric grinder IKAMAG-RCT@type. The powder obtained is brown. The extracts were prepared according to the method described by Zihiri and Kra^[12]. For the preparation of ethanolic extracts 70%, 100 g of plant powder were extracted in blender (the process is repeated 3 times) with one liter of distilled water or a mixture of ethanol - water (729 ml of ethanol 96% et 271 ml of distilled water). After crushing, the mixture obtained was first spun in a clean square fabric, and then filtered successively twice with cotton wool and once with Whatman 3mm paper. The filtrate was concentrated using a rotary evaporator at 70 °C. The concentrate was evaporated at 50 °C in an oven for 48 hours. The extract obtained is the hydro-alcoholic extracts 70%.

Experimental Animals

Animals were selected as per the Organization of Economic Co-Operation and Development (OECD) guidelines no. 423^[13]. Healthy young and nulliparous, non-pregnant Wistar rats weighing from 100-120 mg of 8-10 weeks old obtained from the animal house of Pharmaceutical science, Abidjan (Côte d'Ivoire) were selected. The animals are randomly selected, marked to permit individual identification, and kept in plastic cages with wood chips renewed every two days for 5 days prior to dosing to allow for acclimatization of the laboratory conditions (room temperature 25°C (± 3°C), moisture 35 to 60%, light and dark period 12/12 hours, bedding cleaned and sterilized). All animals had a regular supply of clean drinking water and food.

Experimental design

Repeated oral dose of toxicity study was carried out according to OECD Guideline 407^[14]. The animals were divided into four groups of 10 animals each (5 males and 5 females). Group 1 received 1 ml/100g body weight of distilled water and served as control. Groups 2, 3 and 4 received extract doses of 150, 300 and 600 mg/kg body weight, respectively. Mortality, body weights, food and water consumption as well as observation for general toxicity signs of the animals were evaluated daily for 28 days.

Blood sample and organ collection

At the end of each week, the animals were anesthetized with diethyl ether. The blood was drawn through cardiac puncture and collected in sterile tubes without anticoagulant. Plasma was obtained in one set by centrifuging the blood at 3000 revolutions/min for 10 min and stored at -20°C in Eppendorff bottles until required for enzymatic activities and concentration of biochemical metabolites assays. The organs (livers) were collected and fixed with 10% buffered formalin for further analysis^[15].

Assay of hepatic parameters in rat serum

Hepatic parameters of the serum were measured with an automatic analyzer, Hitachi 902 (Roche), using commercial kits and certified controls (Spinreact S.A., Ctra Santa Coloma, Spain) based on the manufacturer's instructions, as summarized in Tables 1 and 2.

Table 1: Operating parameters for the quantitative determination of enzymes

Parameter	Enzyme kinetic method	Wavelength (nm)
ALT	Disappearance of NADH	340
AST	Disappearance of NADH	340
ALP	Rate of p-nitrophenol formation	405
GGT	Rate of NAMB	405
LDH	Disappearance of NADH	340

ALT=alanine aminotransferase; AST=aspartate aminotransferase; ALP=alkaline phosphatase; GGT=gamma-glutamyltransferase; LDH=lactate dehydrogenase; NAMB=2-nitro-5-aminobenzoic acid formation; NADH=nicotinamide adenine dinucleotide; NADPH=nicotinamide adenine dinucleotide phosphate.

Table 2: Operating parameters for the quantitative determination of metabolites

Parameter	Colorimetric method	Wavelength (nm)
Total Cholesterol	Cholesterol esterase, oxidase and peroxidase	500
HDL Cholesterol	Cholesterol esterase, oxydase and peroxidase	500
Triglycerides	Lipase, glycerol kinase, oxydase and peroxidase	500
Glucose	Glucoseoxydase and peroxidase	500
Total bilirubin	Sulfanilic acid, Hydrochloric acid and dimethylsulfoxide	555
Conjugated bilirubin	Sulfanilic acid, Hydrochloric acid	555

Preparation of tissue sections and histopathology

The liver tissues were cut into transverse blocks. An automatic processor (RH-12EP Sakura, Fine Technical Co.Ltd, Tokyo, Japan) was used for further processing blocks. About 12 hours were required for deshydration (96% alcohol for one hour x four changes, and 100% alcohol for one hour x one change).

Clearing in three changes of toluène for one hour each. Tissues were impregnated in two changes of paraffin wax with a melting point of 50° C for period of 2 hours. Embedding of tissue was done in paraffin using L-shaped metallic moulds. These blocks were put in the refrigerator for a period of 4-6 hours. Each block was cut on a rotary microtome (MicromGmbH, Waldorf, Germany). About 5 micro meter thick tissue section were obtained and placed in the water bath with temperature of 50°C below the melting point of paraffin wax. Cut ribbons of tissues were placed on the albumenized glass slide. The sample slides were subsequently stained with haematoxylin-eosin (HE) and examined under a light microscope; photomicrographs of the samples were recorded^[16].

Statistical Analysis

The results are presented as mean ± standard deviation (SD). Analysis of variance (ANOVA) with repeated measures was employed to compare the results according to the administered doses and times of treatment. Analysis of variance was considered significant when the level of probability (p) was < 0.05; if p < 0.01, this difference is considered as very significant; if highly significant P < 0.001.

Results

Biochemical results

Effect of *Terminalia mantaly* on hepatic enzyme activities of rats

Effect of *Terminalia mantaly* on the level of ALT

The ALT activity did not change significantly (p> 0.05) in treated animals compared to control animals. Over time, the ALT activity decreased significantly in animals controls from 37.33± 4.5 (S1) to 29.50±2.5 (S2) corresponding to -20.97%; in treated animals group II from 38 ± 10.39 (S1) to 30.6 ± 6.36 (S3) to 39.67 corresponding to -19.47 % (p <0.01); Lot III from 36.67 ± 10.79 (S1) to 30 ± 2.83 (S4) corresponding to -18.18 % (P <0.05).

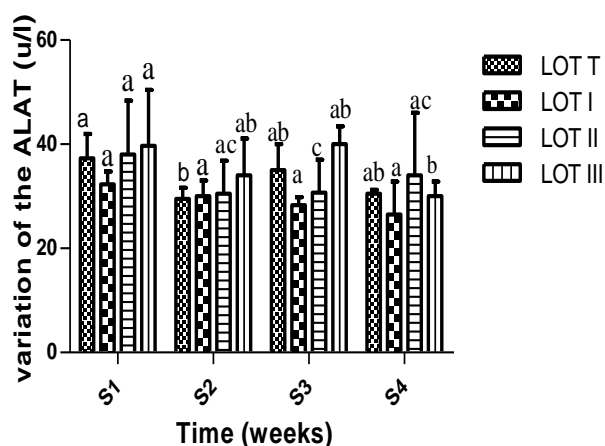


Figure 1: variation of the ALAT activity (IU/L) versus time

Each bar represents the mean \pm STDEV, n = 10 with t = control; Lot I = 150mg / kg; Lot II = 300mg / kg; Lot III = 600mg / kg body weight of the animal; the letters indicate significant differences for each group according to the time (a>b>c).

Effect of Terminalia mantaly on the level of the ASAT

The AST activity did not vary significantly (P > 0.05) in the treated animals compared to control animals during the four weeks.

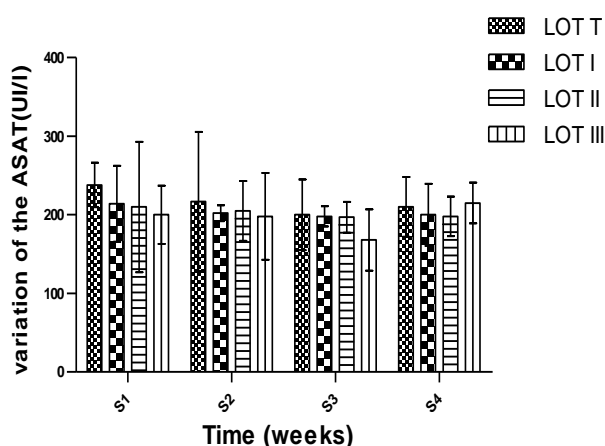


Figure 2: variation of the ASAT activity (IU/L) versus time

Each bar represents the mean \pm STDEV, n = 10 with t = control; Lot I = 150mg / kg; Lot II = 300mg / kg; Lot III = 600mg / kg body weight of the animal.

Effect of Terminalia mantaly on the level of PAL

The PAL activity increased significantly in treated animals compared to control animals. In group II the PAL activity varied from 310 \pm 16.6 to 398 \pm 62 (S1) corresponding to 28.38 % (p < 0.001) of increase; in group III, the PAL activity varied from 310 \pm 16.6 to 384 69 (S1) corresponding to 23.87 % and from 330 \pm 8 to 400.5 \pm 6.36 (S2) corresponding to 21.67 % (p < 0.01).

Over time, the PAL activity decreased significantly in animals treated group I from 300 \pm 5 (S1) to 364 \pm 45 (S4) corresponding to -21.33 % (p < 0.01); in group II from 398 \pm 62 (S1) to 331 \pm 51 (S4) corresponding to -16.83 % and from 380.5 \pm 5 (S2) to 325.7 \pm 45 (S3) to -14.28% (p < 0.05); group III from 400.5 \pm 6.4 (S2) to 333 \pm 74 (S3) corresponding to -16.85 % and from 400.5 \pm 6.4 (S2) to 337.5 \pm 38 (S4) corresponding to -15.73 % (p < 0.05).

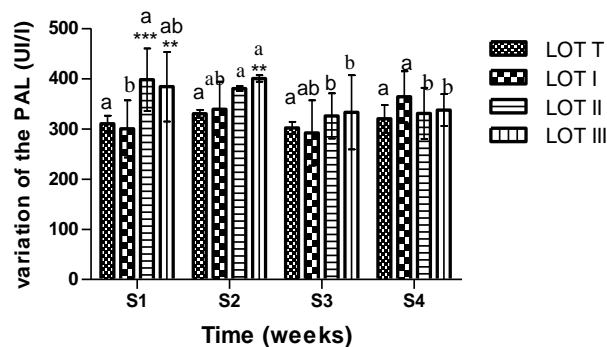


Figure 3: variation of the PAL activity (IU/L) versus time

Each bar represents the mean \pm STDEV, n = 10 with Lot T =control; Lot I = 150mg / kg; Lot II = 300mg / kg; Lot III = 600mg / kg body weight of the animal. the letters indicate significant differences for each group according to the time (a>b>c); the asterisk indicate significant differences in the groups treated animals compared to controls in each week (** P < 0.01; *** P < 0.001)

Effect of Terminalia mantaly on the level of the GGT

The GGT activity did not vary significantly in treated animals compared to control.

Over time, the activity of GGT was significantly increased in animals treated group I and varied from 4.67 \pm 2.89 (S3) to 7 \pm 2.83 (S4) corresponding to 49.86 % (p < 0.05). In group III, the activity of GGT is enhanced from 5.67 \pm 1.5 (S1) to 8 \pm 2.1 (S4) corresponding to 41 % of increasing (p < 0.05) and 5 \pm 2 (S3) to 8 \pm 2.1 (S4) corresponding to 60% of increasing (p < 0.01).

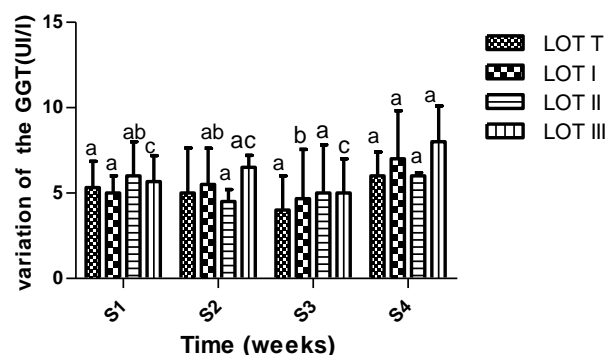


Figure 4: Variation of the GGT activity (IU/L) versus time

Each bar represents the mean \pm STDEV, n = 10 with Lot T=control; Lot I = 150mg / kg; Lot II = 300mg / kg; Lot III = 600mg / kg body weight of the animal; the letters indicate significant differences for each group according to the time (a>b>c).

Effect of Terminalia mantaly on the level of the LDH

The LDH activity did not change significantly with in treated animals compared to control animals (p > 0.05).

Over time, the activity of LDH levels varied significantly in the treated animals: in the group I, the activity of LDH increased from 820 \pm 173 (S1) to 930 \pm 62.2 (S2) corresponding to 13.41 % (p < 0.01) and then, decreased from 930 \pm 62.2 (S2) to 810 \pm 66.7 (S3) corresponding to -12.90 % (p < 0.01) and 930 \pm 62.2 (S2) to 790 \pm 14.14 (S4) corresponding to -15.05 % (p < 0.001); it decreased in the group II, from 902 \pm 28.9 (S2) to 740 \pm 83.73 (S3) corresponding to -19.96% p < 0.01 and 28.9 \pm 902 (S2) 760 \pm 32 (S4) of -15.74 % (p < 0.001) and in the group III, from 940 \pm 17.7 (S2) to 786 \pm 46 (S3)

corresponding to -12.86 % ($p < 0.01$) and from 940 ± 17.7 (S2) to 795 ± 86.9 (S4) corresponding to -15.42 % ($p < 0.001$).

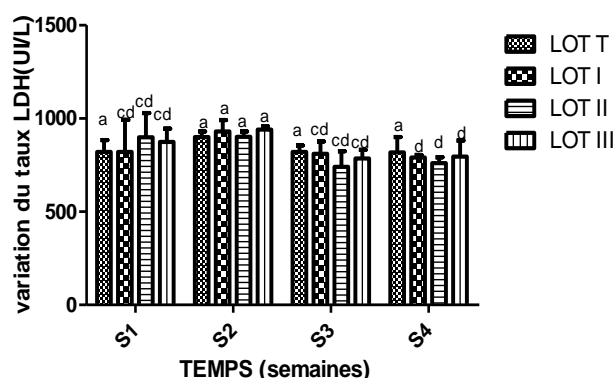


Figure 5: Variation of the LDH activity (IU/L) versus time

Each bar represents the mean \pm STDEV, $n = 10$ with Lot T = control; Lot I = 150mg / kg; Lot II = 300mg / kg; Lot III = 600mg / kg body weight of the animal; the letters indicate significant differences for each group according to the time (a>b>c).

Effect of Terminalia mantaly on hepatic metabolism

Effect of Terminalia mantaly on the level of the triglycerides

The Triglyceride rate decreased significantly in treated animals compared to control. In group II the Triglyceride rate varied from 1.15 ± 0.05 to 0.82 ± 0.05 (S4) corresponding to -28.69 % ($p < 0.05$); in group III ($p < 0.05$), it decreased from 1.15 ± 0.05 to 0.79 ± 0.02 (S4) corresponding to -31.30%. Over time, the triglycerides rate did not change significantly in treated animals ($p > 0.05$).

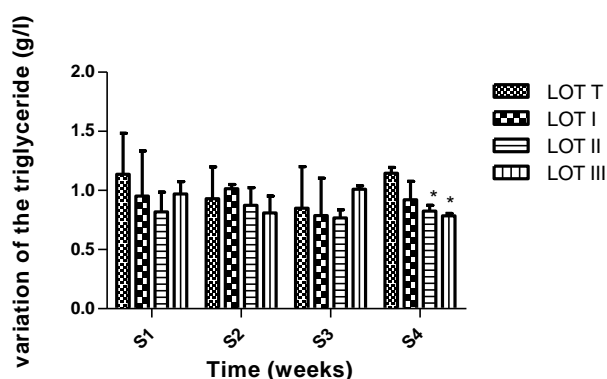


Figure 6: variation of the rate triglycerides (g/l) versus time

Each bar represents the mean \pm STDEV, $n = 10$ with Lot = control; Lot I = 150mg / kg; Lot II = 300mg / kg; Lot III = 600mg / kg body weight of the animal; the asterisk indicate significant differences in the groups treated animals compared to controls in each week * $p < 0.05$

Effect of Terminalia mantaly on the level of the bilirubin

The total bilirubin rate varied significantly in treated animals compared to control.

The total bilirubin concentration decreased, in group II, from 14 ± 3 to 11 ± 4 (S1) corresponding to -21.42 % ($p < 0.05$) and 14.5 ± 2.1 to 11 ± 2.8 (S2) corresponding to 24.13 % ($p < 0.01$); in group III ($p < 0.01$) from 14 ± 3 to 10 ± 4 (S1) corresponding to -28.57 % and from 14.5 ± 2.1 to 10.5 ± 0.71 (S2) corresponding to -27.58 %.

Over time, the total bilirubin rate did not change significantly in animals treated ($p > 0.05$).

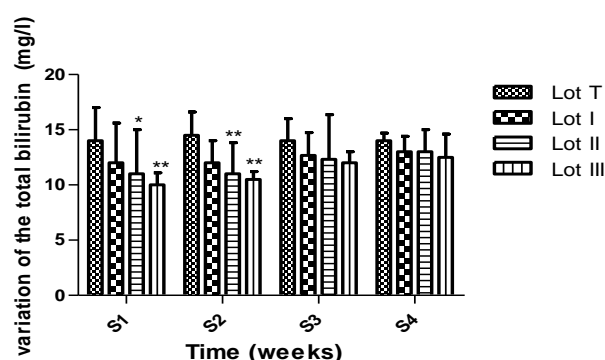


Figure 7: Variation of the rate total bilirubin versus time

Each bar represents the mean \pm STDEV, $n = 10$ with Lot T= control; Lot I = 150mg / kg; Lot II = 300mg / kg; Lot III = 600mg / kg body weight of the animal; the asterisk indicate significant differences in the groups treated animals compared to controls in each week (* $P < 0.05$; ** $P < 0.01$).

Effect of Terminalia mantaly on the level of the conjugated bilirubin

The Conjugated bilirubin rate did not change significantly ($p > 0.05$) in treated animals compared to control, excepted in animals treated with doses 600mg/kg where it decreased significantly from 6.5 ± 3.53 to 4 ± 0.71 (S4) corresponding to -38.46 % ($p < 0.01$).

Over time, the conjugated bilirubin levels did not change significantly in the treated animals ($p > 0.05$).

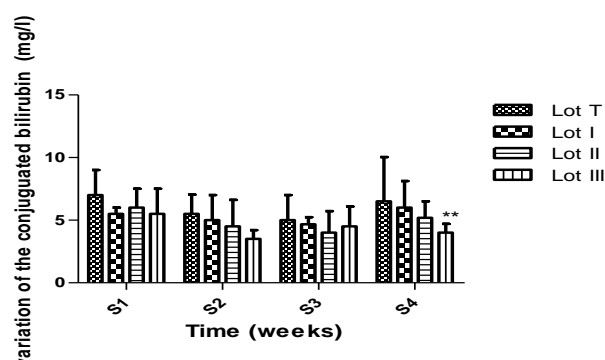


Figure 8: Variation of the levels conjugated bilirubin versus time

Each bar represents the mean \pm STDEV, $n = 10$ with Lot T=control; Lot I = 150mg / kg; Lot II = 300mg / kg; Lot III = 600mg / kg body weight of the animal; the asterisk indicate significant differences in the groups treated animals compared to controls in each week (** $P < 0.01$; *** $P < 0.001$).

Effect of Terminalia mantaly on the level of the total cholesterol

The total cholesterol rate did not change significantly in animals treated compared to control.

Over time, the cholesterol rate decreased significantly in animals treated in group I from 0.84 ± 0.11 (S2) to 0.65 ± 0.12 (S4) corresponding to -22.61% ($p < 0.01$); in group III from 0.79 ± 0.15 (S1) to 0.63 ± 0.05 (S4) corresponding to -20.25 % ($p < 0.05$); from 0.85 ± 0.02 (S2) to 0.63 ± 0.05 (S4) corresponding to -25.88 % ($p < 0.001$); 0.79 ± 0.16 (S3) to 0.63 ± 0.05 (S4) corresponding to -20.25 % ($p < 0.05$).

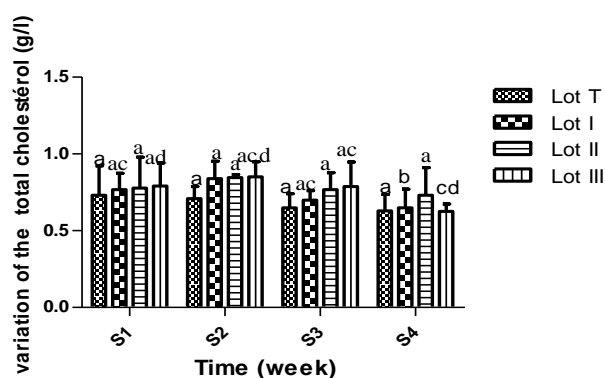


Figure 9: variation of the rate total cholesterol versus time

Each bar represents the mean \pm STDEV, n = 10 with Lot T= control; Lot I = 150mg / kg; Lot II = 300mg / kg; Lot III = 600mg / kg body weight of the animal; the letters indicate significant differences for each group according to the time (a>b>c).

Effect of *Terminalia mantaly* on the level of the HDL cholesterol

The HDL cholesterol rate increased significantly in treated animals compared to control. In group II the HDL cholesterol value varied from 0.19 ± 0.01 to 0.24 ± 0.02 (S4) corresponding to 26.31 % (p <0.05) of increasing; in group III (p <0.001), it increased from 0.19 ± 0.01 to 0.25 ± 0.01 (S4) corresponding to 31.51%.

Over time, the HDL cholesterol rate increased significantly in animals treated in group II from 0.18 ± 0.01 (S2) to 0.24 ± 0.02 (S4) corresponding to 33.3% and from 0.21 ± 0.05 (S3) to 0.24 ± 0.02 (S4) corresponding to 12.5 % (p <0.01); in group III, from 0.19 ± 0.01 (S2) to 0.25 ± 0.02 (S4) corresponding to 24% (p <0.05).

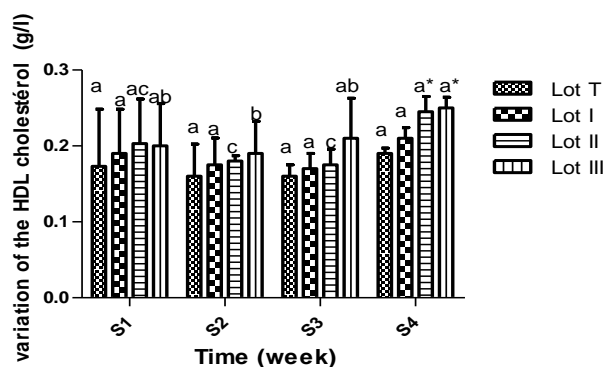


Figure 10: Variation of the rate HDL cholesterol versus time

Each bar represents the mean \pm STDEV, n = 10 with Lot T= control; Lot I = 150mg / kg; Lot II = 300mg / kg; Lot III = 600mg / kg body weight of the animal; the letters indicate significant differences for each group according to the time (a>b>c); the asterisk indicate significant differences in the groups treated animals compared to controls in each week (* P <0.05).

Effect of *Terminalia mantaly* on the level of the glucose

The glucose rate decreased significantly (P< 0.05) in treated animals compared to control. In group II the glucose value varied from 1.27 ± 0.34 to 1.03 ± 0.05 (S1) (p <0.05) corresponding to -18.90% of decreasing; in group III, it varied from 1.41 ± 0.37 to 1.39 ± 0.25 (S3) corresponding to -1.41 % of decreasing (p <0.05).

Over time, the glucose rate was significantly decreases in controls animals from 1.41 ± 0.36 (S2) to 1.14 ± 0.21 (S4) corresponding to -13.58% and from 1.43 ± 0.17 (S3) to 1.14 ± 0.21 (S4) corresponding to -13.98%; treated animals of group II from 1.34 ± 0.3 (S2) to $1.06 \pm$

0.08 (S4) of -20.89 % and 1.30 ± 0.1 (S3) to 1.06 ± 0.08 (S4) corresponding to -18.46 % (p <0.05).

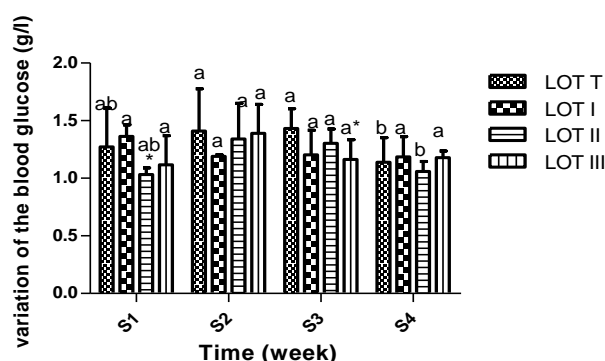


Figure 11: Variation of the rate of blood glucose versus time

Each bar represents the mean \pm STDEV, n = 10 with Lot T= control; Lot I = 150mg / kg; Lot II = 300mg / kg; Lot III = 600mg / kg body weight of the animal. the letters indicate significant differences for each group according to the time (a>b>c); the asterisk indicate significant differences in the groups treated animals compared to controls in each week (* P <0.05).

Effect of *Terminalia mantaly* on the level of the total protein

The total protein rate decreased significantly in treated animals compared to control. In group I, the total protein value varied (p<0.05) from 59 ± 3.6 to 53 ± 2.08 (S1) corresponding to -10.16% and from 66 ± 6 to 59 ± 9.07 (S3) corresponding to -10.60% of decreasing. In group II, it varied from 59 ± 3.6 to 50 ± 9.07 (S1) corresponding to -15.25% (p<0.001) and in group III from 66 ± 6 to 59 ± 1 (S3) corresponding to -10.60% of decreasing (p<0.05).

Over time, the total protein levels did not change significantly in the treated animals (p > 0.05).

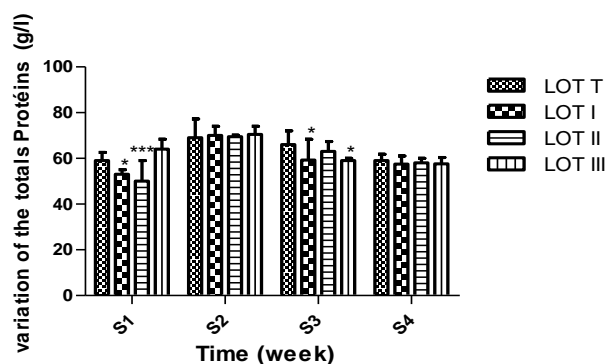


Figure 12: Variation of the rate of total protein versus time

Each bar represents the mean \pm STDEV, n = 10 with Lot T= control; Lot I = 150mg / kg; Lot II = 300mg / kg; Lot III = 600mg / kg body weight of the animal; the asterisk indicate significant differences in the groups treated animals compared to controls in each week (* P <0.05; *** P <0.001)

Histopathological result

No significant damage was observed in rats liver tissues in groups treated with the hydro alcoholic extract of *Terminalia mantaly* when compared with the control groups (Figures 13 to 16).

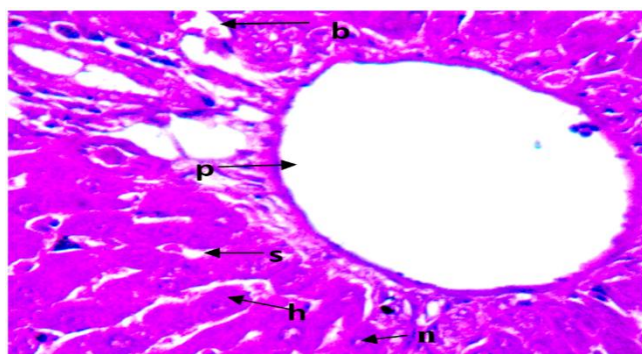


Figure 13: Photomicrograph of the liver tissues of controls rats (H&E x400), b=bilarcanaliculi;

p= portal vein; s = sinusoid capillary; h= hepatocyte; n= nucleus.

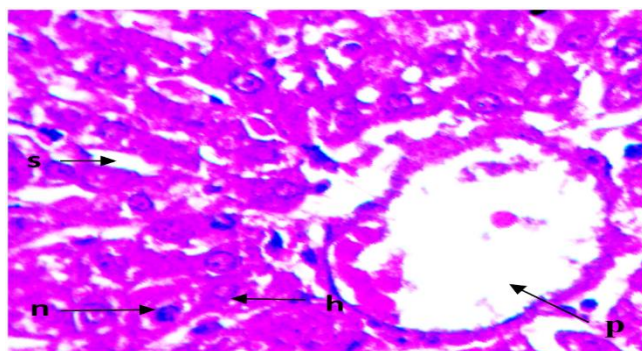


Figure 14: Photomicrograph of the liver tissues of rats treated with 150 mg/kg body weight of *Terminalia mantaly* (H&E x400)

p= portal vein; s= sinusoid capillary; h= hepatocyte; n= nucleus.

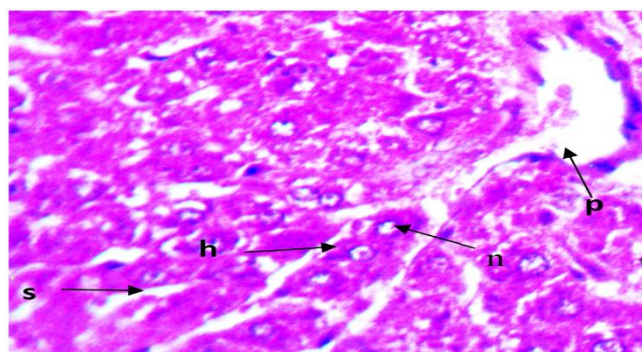


Figure 15: Photomicrograph of the liver tissues of rats treated with 300 mg/kg body weight (H&E x400) of *Terminalia mantaly*. p= portal vein ;s=sinusoid capillary; h= hepatocyte; n= nucleus.

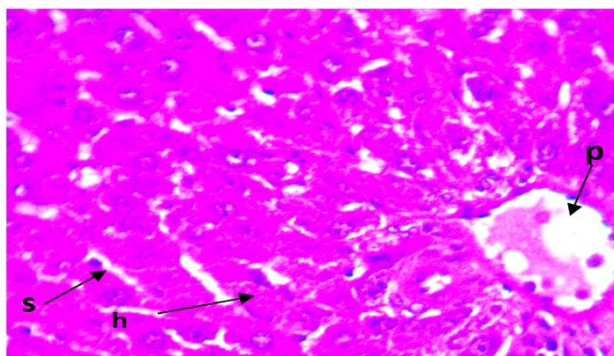


Figure 16: Photomicrograph of the liver tissues of rats treated with 600 mg/kg body weight. (H&E x400) of *Terminalia mantaly*.

p= portal vein; s=sinusoid capillary; h= hepatocyte; n= nucleus.

Discussion

Most toxic compounds, including secondary metabolites of medicinal plants, accumulate in the liver where they are detoxified [16]. Therefore, a study of hepatic tissue integrity and metabolism may be useful in the evaluation of toxic effects of medicinal plants on the liver. The ALT, AST, LDH, GGT and PAL are key enzymes used in order to assess the hepatic tissue integrity [17].

In general, the AST and ALAT are enzymes of mitochondrial and cytoplasmic origin. Thus, any cell necrosis or destruction of the hepatic parenchyma or an increase in the membrane permeability of the hepatocytes may lead to the disposal of such enzymes into the bloodstream and therefore an increase in their activity in serum [18, 19]. On the other hand, the PAL and GGT are enzymes present in particular in the liver, biliary tract, kidney, bone and placenta, where they are highly concentrated [20].

Their increase in serum generally indicates liver failure, cholestasis, and the presence of an obstruction of the intrahepatic bile ducts, biliary cirrhosis or primary disruption of liver architecture [21]. Another indicator appears to be involved in the assessment of liver tissues integrity, bilirubin, a breakdown product of hemoglobin. The hyperbilirubinemia may be a sign of liver tissue damage, obstruction of the bile ducts or hemolytic anemia [22, 23].

Subacute treatment for four weeks by the hydro-alcoholic extract of *Terminalia mantaly* did not induce significant variation of transaminases activities (AST and ALAT) in treated animals compared to control animals. The opposite was reported by Lamchouriet al. [24], their chronic treatment (12 weeks) with the alkaloids extract of *Peganum harmala* increased significantly the levels of these enzymes in treated rats. This can be attributed to the difference the duration of treatment and the composition of the extracts used.

In our study, the activity of ALAT decreased in treated animals versus time with doses of 150, 300 and 600 mg / kg.

The reduction in ALAT's activities by the extract suggest that the extract was not toxic for the integrity of the liver but possibly hepatoprotective [25, 26].

Furthermore, Ramasami et al. [27] obtained a decrease in transaminases activities. These authors have demonstrated that the ethanol extract of fruit of *Passiflora foetida* had a hepatoprotective effect. In fact, this plant is rich in flavonoids such as apigenin and naringenin [28], molecules known as hepatoprotective [29]. The effect of this plant on the activity of liver enzymes was therefore due to the presence of flavonoids [30]. Moreover previous studies have shown that alcoholic extract of *Terminalia mantaly* is rich in flavonoids [9].

According to Xavier [31], liver plays a role in the metabolism and regulation of glucose, lipids and proteins and any damage to the liver would lead to change the levels of these metabolites.

In our study, a significant decrease in the concentration of glucose, total protein, TG and cholesterol was observed in treated animals compared to control animals during the period of test. These parameters give information about the state of carbohydrate, protein and lipid metabolism in the liver [32]. Identical results on cholesterol have been obtained by Mosaddegh et al. [33] and by Sharmila et al. [34] respectively with the aqueous extracts of *Paliurus spina christi* and *Trichosanthes dioica* in rats. Others authors have proven the hypoglycemic effect of the seeds of *Peganum harmala* [35], this extract reduced the rate of serum glucose and lipid in rats.

There was also a significant decrease in the rate of total and conjugated bilirubin in treated animals versus time with the dose 600 mg / kg. This result would not influence the hepatic metabolism and biliary excretion.

Overall, the absence of any change or decrease in these parameters (AST, ALT, total and conjugated bilirubin glucose, TG, cholesterol) indicated that the hydro-alcoholic extract of *Terminalia mantaly* have not effect on the cell membrane permeability and the lipid and carbohydrate metabolism in the liver, and therefore, on hepatocytes [19, 32]. The same observation was made with *Morinda morindoides* by Tra-Bi *et al.* [36].

However, this study showed a significant elevation in the serum activity of the PAL in rats treated with doses of 300 and 600 mg / kg compared to controls. Also that the activity of the GGT does it significantly increased in treated rats of 150 and 600 mg / kg during the third and fourth week.

This leads to the suggestion that this plant would be able to cause direct adverse effects on the liver parenchyma and specifically on roads or bile ducts in animals [21, 22].

Moreover, the increases observed in this study are less than the double of their values obtained in control rats and also the reference values [37]. These increases are, therefore, classified as moderate increases [38] and would therefore not due to pathological aggression liver tissue.

Histopathological studies revealed undamaged structures of the liver (hepatocyte, sinusoid). These results showed that the hydro alcoholic extract of *Terminalia mantaly* would not disrupt the main functions and the integrity of the liver. These results are consistent with previous studies where it has been reported that the extract of *Morinda morindoides* was well tolerated by the liver [36, 39].

Conclusion

After prolonged administration of different doses, our results showed disturbances in the studied biochemical parameters. Such differences are minor and transient, not due to disturbances of functions and the integrity of the liver tissue. This leads us to say that the hydro alcoholic extract of *Terminalia mantaly* would be well tolerated by rat model and it could exert hepatoprotective action. Therefore, it can be concluded that the extract is non-toxic and can be safely used orally.

It would, however, be necessary to carry out further studies, including cardiovascular, and renal tolerance studies in order to obtain a fuller picture of the safety profile of the extract.

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