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Hepatic tolerance study of aqueous extract of *Mitracarpus scaber* (Rubiaceae) in rabbits

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This study aimed to evaluate the hepatic safety of *Mitracarpus scaber* (Rubiaceae) in rabbit. This plant is traditionally used for its antibacterial and antifungal properties. It is also used to treat skin diseases and many ailments in Côte d'Ivoire and around West Africa. Six groups of 6 rabbits each, were injected intraperitoneally, twice a week four six weeks, with increasing doses ranging from 12.5 to 200 mg/kg body weight of aqueous extract of *Mitracarpus scaber* (encoded Misca). Blood sampling was carried out to investigate changes in different serum biochemical markers: alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase ($_{\gamma}$GT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), glucose, total proteins, total cholesterol, and triglycerides. The statistical analysis showed significant decrease of triglycerides concentration and in serum activities of ALP, LDH ($P<0.05$). But there is no significant change in serum activity of ALT, AST, $_{\gamma}$GT as well as serum concentrations of glucose, total proteins and total cholesterol ($P>0.05$). In conclusion, the aqueous extract of Misca at dose of 100 mg/kg body weight for 4 weeks induces no hepatic dysfunction and is therefore well tolerated by the liver.

Keyword: *Mitracarpus scaber*, Hepatic tolerance, Serum biochemical markers

1. Introduction

Since the dawn of time man has always used medicinal plants to cure various ailments. This practice has survived the centuries. And it tends to intensify today in Côte d'Ivoire and in Africa as well as elsewhere in the world, despite the rise of modern medicine. However, the therapeutic use of plant is not always without danger to the user populations. The traditional use of plants may cause many therapeutic accidents [1]. Among the causes of these accidents we can mention ignorance of doses of extracts administered empirically as well as their biochemical, pharmacological and toxicological properties [2, 3]. Therefore it becomes imperative to make a contribution in the direction of the value of these medicinal plants by studying the toxicity of the extracts administered and their impact at some vital organs such as the liver. This is the case of *Mitracarpus scaber* (Rubiaceae), a plant traditionally used in Côte d'Ivoire and elsewhere in Africa to treat sores, ringworm and various ailments.

The antibacterial and antifungal activities of *Mitracarpus scaber* (encoded Misca) has been indicated by several studies [4, 5, 6, 7, 8]. It has a marked activity on 12 germs among which we can quote: *Cryptococcus, Aspergillus, Trichophyton, Candida, Staphylococcus, E. coli*, which are opportunistic pathogens of AIDS. Minimum Fungicidal Concentration (MFC) of Misca is 0.20 mg/mL while IC_{50} is 0.10 mg/mL [9, 10, 11, 12]. In addition to dermatitis, the cardiodepressive and hypotensive effects has been studied [13].

Given the excellent results of pharmacological tests and the wide use of this plant, a rationalization of its use is required in view of its use in cardiovascular therapeutics. The liver is a metabolic crossroads with a rich equipment enzyme that plays a very important role in the metabolism of several substances. The high hepatic blood flow predisposes liver to toxic drug injury. That is why it is important to investigate biochemical and toxicological activity of the extracts of *Mitracarpus scaber* (Rubiaceae), on this key organ.

The present study aims to evaluate the hepatic safety of the aqueous extract of Misca following changes of some serum specific biochemical markers in rabbits: enzymes (ALT, AST, $_{\gamma}$GT, LDH, ALP) and metabolites (glucose, totals proteins, total cholesterol, triglycerides).

Variations in these enzymes activities and metabolites in serum can thus assess the impact of this extract on hepatic function [14, 15, 16].

2. Material and methods

2.1 Plant material

The leaves of *Mitracarpus scaber* (Rubiaceae) were collected in Abobo-Adjame Campus University (Abidjan) and in some peripheral areas of Abidjan (Côte d'Ivoire). The plant was authenticated by Professor Ake Assi Laurent of Department of Botany, University of Cocody-Abidjan and a voucher specimen (N° 13612) of the plant was deposited in the herbarium of the National Floristic Center of University of Cocody-Abidjan.

2.2 Animals

Rabbits, *Oryctolagus cuniculus* (36) of 8-10 weeks old, weighing 1.17± 0,22 kg and bred at the Department of Biosciences, University of Cocody, Ivory Coast, were used for the experiments. They come from a rabbit cattle farm in Bingerville (Abidjan). The animals were kept in standard cages with good ventilation, free access to food and water. Experimental procedures and protocols used in this study were approved by the Ethical Committee of Health Sciences of University of Cocody-Abidjan. These guidelines were in accordance with the European Council Legislation 87/607/EEC for the protection of experimental animals [17].

2.3 Preparation of aqueous extract of *Mitracarpus scaber* (Rubiaceae)

Plant harvested were air dried at room temperature (28±1 °C) for one month. The dried leaves were ground into fine powder. 100 g of powder were soaked in two liters of distilled water for 48 hours on a magnetic agitator (Ikamag RCT). The extract was filtered twice through cotton wool, and then through Whatman filter paper (3 MM). The filtrate was evaporated to dryness in a rotary evaporator (Buchi) at 60° C. After drying, we get a greenish powder used to prepare the aqueous extract of Misca.

2.4 Experimental protocol

After randomization into 6 groups of 6 rabbits, and before initiation of experiments, the rabbits were acclimatized for a period of 14 days under standard environmental conditions of temperature, relative humidity, and 12 h dark/light cycle.

Animals had free access to food and water *ad libitum*.

Animals in each group were separated according to their sex in cages. Among these 6 groups, five experimental groups have received doses ranging from 12.5 to 200 mg/kg of bw (which is the Maximum Tolerated Dose (MTD) of the aqueous extract) in a geometric progression of ratio 2 [18, 19]. Twice a week for six weeks, the animals received intraperitoneally 0.2 mL of an injection according to their group. Each rabbit of batch 1 (control) received only 0.2 mL of physiological solution of 0.09% Nacl (B. Braun) used to administrate extracts. Rabbits of batch 2 to batch

6 received respectively 12.5; 25; 50; 100 and 200 mg/kg of bw.

Blood samples were collected in the morning (from 8 to 11 am) via the marginal ear vein of the animals, once a week using sampling needles. Blood sampling was carried out once a week in the one week preceding the first application of treatment (w0), during the five weeks of treatment (w1, w2, w3, w4, w5 and w6). These blood samples were collected in sterile tubes without anticoagulant. They were centrifuged at 3000 rpm for 10 min using a liquidizer Jouan.

2.5 Assay of hepatic parameters in rabbit serum

The principles of the determination of each parameter are described according to the manufacturer's instructions reagents. Hepatic parameters of the serum were measured with an automatic analyzer, Liasis based on the manufacturer's instructions as summarized in Table 1 and 2.

Table 1: Operating parameters for the quantitative determination of enzymes

Parameters	Enzyme kinetic method	Wavelength (nm)
ALT	Disappearance of NADH	340
AST	Disappearance of NADH	340
ALP	Rate of p-nitrophenol formation	405
$_{\gamma}$GT	Rate of NAMB	450
LDH	Disappearance of NADPH	340

ALT= alanine aminotransferase; AST= aspartate aminotransferase; ALP= alkaline phosphatase, $_{\gamma}$GT= 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

Parameters	Colorimetric method	Wavelength (nm)
Total cholesterol		500
Triglycerides	Lipase, glycerol kinase, oxidase and peroxidase	500
Glucose	Glucose oxidase and peroxidase	500
Total Protein	Copper salts and alkaline medium	550

2.6 Statistical Analysis

The data were processed using the software Graph Pad Prism 5.0 (Microsoft, USA).

The analysis of variance (ANOVA) was performed according to the multiple comparison test of Tukey for the comparison of mean values of biochemical markers of different groups but also to relative baseline in each group. Data are presented means ± standard error of mean (S.E.M) for the number of animals in each group (n = 6). The difference is said to be significant if ($P< 0.05$) and not significant if ($P>0.05$).

3. Results

The results of changes in serum activity of enzymes (ALT, AST, $_{\gamma}$GT, LDH, ALP) and metabolites (glucose, totals proteins, total cholesterol, triglycerides) are expressed in tables (3, 4, 5, 6,7,8,9, 10 and 11) are averages of six assays performed in each group.

Table 3: Effect of Misca on the serum activities (IU/L) of ALT over time on rabbits.

Serum activities of ALT (IU/L)						
Doses (mg/kg/w)	**0**	**12,5**	**25**	**50**	**100**	**200**
S_0	21.5±7	33.7±7	43.3±10.4	43.3±7.5	30.7±10	28.5±3.77
S_1	23.7±3.5	33±12	30±5	23,7±1,5	42±12.6	28.3±10.4
S_2	23.3±4.16	33±6.24	27.5±3.7	31.8±9.3	30.8±7.15	29.7±10.8
S_3	22±2.64	32.7±3	33.6±5.5	26.7±6.5	29.5±4.5	46.7±17
S_4	32±5.57	36.3±8	39.3±3	32.7±3.2	37±7.94	32±6.2
S_5	30±5.57	25.3±5,7	33.3±1.5	27.7±5	30.8±3.8	23.2±5.4
S_6	27.7±6	28±1,73	35.3±10	24,7±0.6	31.7±7.6	22.8±6.6
Lots	Lot_1	Lot_2	Lot_3	Lot_4	Lot_5	Lot_6

Values are expressed as mean ± S.E.M (n=6); P>0.05 compared to control and S_0 level
S_0: Week preceding the first application of treatment / S1 to S6: Weeks of treatment

The serum activity of ALT was 21.5±7 IU / L in the untreated lot (lot1). This value varied over time between 22±2.64 IU / L (minimum S_3) and 32±5.57 IU / L (maximum S_4), representing a change of 2.32 % (S_3) to 48.84 % (S_4) of the initial serum rate activity of ALT (table 3). In group 2 (12.5 mg / kg), serum activity of ALT was 33.7±7 IU / L before treatment. Over the past six weeks, this rate varied of 25.3±5.7 IU/L (minimum S_5) to 36.3±8 IU/L (maximum S_4). These values correspond to variations of -24.8 % (S_5) to 7.92 % (S_4).

Percentage changes registered in groups 3, 4, 5 and 6 are respectively -36.5% (S_2) to -9.23% (S_4); -45% (S_1) to -24% (S_4); -3.8% (S_3) to 36.9% (S_1) and -19.9 % (S_6) to 63.74% (S_3) (table 3).

The statistical analysis shows no significant change in serum activity of ALT with different doses (P>0.05).

Table 4: Effect of Misca on the serum activities (IU/L) of AST over time on rabbits.

Serum activities of AST (IU/L)						
Doses (mg/kg /w)	**0**	**12,5**	**25**	**50**	**100**	**200**
S_0	24±3	30.8±1.3	31.7±1.6	36.2±2.8	37.3±4.3	34.7±2.6
S_1	24.7±2.6	26.7±2.3	28.7±2.4	32±3	28.8±1.7	35±1.7
S_2	27.8±2.61	28.6±1.3	28.4±2.4	36.8±4.9	30.8±4.9	40±2.9
S_3	36.8±3.7	36.7±3.5	35.7±5.6	26.3±4.1	37.3±10.3	38.3±3.3
S_4	33.7±4.5	35.3±7.4	33±2.3	30.3±3.9	35±2	41.7±3.3
S_5	29.8±3.4	32.3±5	30.5±2.5	30±4	34.5±1.9	42±6.2
S_6	31.3±3	35.3±2	28.5±0.8	37±1.5	38.3±3.3	42.3±1.4
Lots	Lot_1	Lot_2	Lot_3	Lot_4	Lot_5	Lot_6

Values are expressed as mean ± S.E.M (n=6); P>0.05 compared to control and S_0 level
S_0: Week preceding the first application of treatment/ S1 to S6: Weeks of treatment

The serum activity of AST was 24±3 IU / L in the untreated lot (lot1). This value varied over time between 24.7±2.6 IU / L (minimum S_1) and 36.8±3.7 IU / L (maximum S_3), representing a change of 3.05 % (S_1) to 53.2 % (S_3) of the initial serum rate activity of AST (table 4). In group 2 (12.5 mg / kg), serum activity of GOT was 30.8±1.3 IU / L before treatment. Over the past six weeks, the rate varied of 26.7±2.3 IU/L (minimum S_1) to 36.7±3.5 IU/L (maximum S_3). These values correspond to variations of -13.3 % (S_1) to 18.92 % (S_3).

Percentage changes registered in groups 3, 4, 5 and 6 are respectively -10.5% (S_2) to 12.5% (S_3); -27.32% (S_3) to 2.11% (S_6); -22.63% (S_1) to 2.86% (S_6) and 0.86 % (S_1) to 23.92% (S_6) (table 4). The statistical analysis shows no significant change in serum activity of AST with different doses (P>0.05).

Table 5: Effect of Misca on the serum activities (IU/L) of $_{\gamma}$GT over time on rabbits.

Serum activities of $_{\gamma}$GT (IU/L)						
Doses (mg/kg /w)	**0**	**12,5**	**25**	**50**	**100**	**200**
S_0	23±1.3	26±2.3	28±2	30±4.3	28±3.1	29±4
S_1	24±2.7	23.7±4.5	27±5.1	29±1.9	26±3	30±2
S_2	23±3.1	27±0.17	26±6	30±5	30.8±4.4	27±5.1
S_3	23±0.57	25±3.6	31±3.2	25±7	27±1.54	28±3.4
S_4	25±2.4	29±6.1	28±4.1	27±4.2	27±5	30±3
S_5	24±3.1	31±3	28±5.3	29±5.3	28±6.5	29±2.2
S_6	24±1.8	29±2.05	27±4	25±3.2	27±7	30±5.5
Lots	Lot_1	Lot_2	Lot_3	Lot_4	Lot_5	Lot_6

Values are expressed as mean ± S.E.M (n=6); P>0.05 compared to control and S_0 level
S_0: Week preceding the first application of treatment/ S1 to S6: Weeks of treatment

The serum activity of $_{\gamma}$GT was 23±1.3 IU / L in the untreated lot (lot1). This value varied over time between 23±3.1 IU / L (minimum S_2) and 25±2.4 IU / L (maximum S_4), representing a change of 0 % (S_2) to 8.69% (S_4) of the initial serum rate activity of $_{\gamma}$GT (table 5). In lot 2 (12.5 mg / kg), serum activity of $_{\gamma}$GT was 26±2.3 IU / L before treatment. Over the past six weeks, the rate changed of 23.7±4.5 IU/L (minimum S_1) to 31±3 (maximum S_5). These values correspond to variations of -8.84% (S_1) to 19.23% (S_5).

Percentage changes registered in groups 3, 4, 5 and 6 are respectively -7.14% (S_2) to 10.71% (S_3); -16.66% (S_3) to 6.66% (S_6); -18.75% (S_1) to 21.87% (S_3) and -12.90 % (S_2) to -3.22% ($S_{1, 2, 3}$) (table 5). The statistical analysis shows no significant change in serum activity of $_{\gamma}$GT with different doses (P>0.05).

Table 6: Effect of Misca on the serum activities (IU/L) of LDH over time on rabbits.

Serum activities of LDH (IU/L)						
Doses (mg/kg/w)	**0**	**12,5**	**25**	**50**	**100**	**200**
S_0	853±74.7	853±74.7	990±63.5	1075±118	1060±176	1002±57.8
S_1	1027±112	1027±112	1065±30	1041±138	948±40	963.3±32
S_2	787±63.2	863±31.8	980±160	905±155	1130±101	940±66.5
S_3	1060±70.3	1060±70.3	890±49	1021±92	1097±38	939.3±37.7
S_4	1026±89.3	1026±89.3	1028±36	1040±31	1068±87	800±57.74
S_5	941±109	941±109	973±371	858±264	1095±64	550±28.87*
S_6	920±51.3	920±51.3	1080±60	1037±265	1028±88	533.3±33*
Lots	Lot_1	Lot_2	Lot_3	Lot_4	Lot_5	Lot_6

*Values are expressed as mean ± S.E.M (n=6); ***P<0.05** compared to control and S_0 level*
S_0: Week preceding the first application of treatment / S_1 to S_6: Weeks of treatment

The serum activity of LDH was 853±74.7 IU / L in the untreated lot (lot1). This value varied over time between 787± 63.2 IU / L (minimum S_2) and 1060 ±70. 3 IU / L (maximum S_3), representing a change of -7.73 % (S_2) to 24.26% (S_3) of the initial serum rate activity of LDH (table 6). In lot 2 (12.5 mg / kg), serum activity of LDH was 853±74.7 IU / L before treatment. Over the past six weeks, the rate changed of 863 ± 31.8 IU/L (minimum S_2) to 1060 ± 70.3 (maximum S_3). These values correspond to variations of 1.17% (S_2) to 24.26% (S_3).

Percentage changes registered in groups 3, 4, 5 and 6 are respectively-10.1% (S_3) to 9.09% (S_6); -20.17% (S_2) to -3.16% (S_5); -10.51% (S_5) to 6.64% (S_1) and -46.75 % (S_2) to 3.83% (S_6) (table 6).

Statistical analysis of the results indicate a significant change in serum activity of LDH ($P < 0.05$), especially with the dose of 200 mg / kg bw (lot 6) in the fifth and sixth week.

Table 7: Effect of Misca on the serum activities (IU/L) of ALP over time on rabbits

Serum activities of ALP (IU/L)						
Doses(mg/kg/w)	**0**	**12,5**	**25**	**50**	**100**	**200**
S_0	400±132.3	342±63.2	270±135	375±129.2	407±95.6	341±82.14
S_1	313±36,86	377±74.4	447±68.29	257±72.4	505±140.6	297±93.54
S_2	327±90.6	365±108.7	397±119.2	306±98.54	328±82.93	225±52.73
S_3	302±150	371±145.5	209±8.5	413±50.3	430±85.29	391±122.23
S_4	292±114.7	343±82.5	254±11	254±11	530±100.6	403±86.33
S_5	411±86.64	295±175	223±73.82	323±117.8	509±80.1	277±53.82*
S_6	438±78.47	287±68.25	318±96	315±77.58	427±99.62	270±98.31*
Lots	Lot_1	Lot_2	Lot_3	Lot_4	Lot_5	Lot_6

Values are expressed as mean ± S.E.M (n=6); ****P<0.05*** *compared to control and S_0 level*
S_0: Week preceding the first application of treatment / S_1 to S_6: Weeks of treatment

Serum activity of ALP was 400±132.29 IU / L in the untreated lot (lot1). This value varied over time between 292±114.71 IU / L (minimum S_4) and 438±78.47 IU / L (maximum S_6), representing a change of -27.08 % (S_4) to 9.58 % (S_6) of the initial serum rate activity of ALP (table 7). In group 2 (12.5 mg / kg/bw), serum activity of ALP was 342±63.22 IU / L before treatment. Over the past six weeks, this rate varied of 287 ±68.25 IU/L (minimum S_6) to 377±74.44 IU/L (maximum S_1). These values correspond to variations of -16.1 % (S_6) to 10.4 % (S_1).

Percentage changes registered in groups 3, 4, 5 and 6 are respectively -22.9% (S_3) to 65.4% (S_1); -32.3% (S_4) to 10.1% (S_3); -19.4% (S_2) to 30.1% (S_4) and -33.9 % (S_2) to 18.4% (S_4) (table 7).

Statistical analysis of the results indicate a significant change in serum activity of ALP ($P < 0.05$), especially with the dose of 200 mg / kg bw (lot 6) in the fifth and sixth week.

Table 8: Effect of MISCA on serum total cholesterol (g/L) levels over time.

Serum levels of total cholesterol (g/L)						
Doses (mg/kg/w)	**0**	**12,5**	**25**	**50**	**100**	**200**
S_0	0.59±0.08	0.75±0.47	0.85±0.08	0.89±0.53	0.93±0.42	1.13±0.25
S_1	1.03±0.22	0.63±0.42	0.68±0.1	0.49±0.05	0.72±0.32	0.91±0.53
S_2	0.88±0.06	0.48±0.06	1.15±0.09	0.78±0.2	0.73±0.41	0.6±0.27
S_3	1.08±0.37	0.64±0.08	1.45±0.05	0.58±0.13	0.41±0.1	0.52±0.1
S_4	0.93±0.49	0.48±0.03	1.03±0.22	0.66±0.1	0.54±0.27	0.65±0.31
S_5	0.95±0.04	0.56±0.15	0.88±0.54	0.72±0.1	1.2±0.17	1.07±0.51
S_6	0.93±0.12	1.05±0.54	0.82±0.17	0.87±0.29	0.9±0.43	0.7±0.44
Lots	Lot_1	Lot_2	Lot_3	Lot_4	Lot_5	Lot_6

Values are expressed as mean ± S.E.M (n=6); P>0.05 compared to control and S_0 level
S_0: Week preceding the first application of treatment/ S1 to S6: Weeks of treatment

The level of total cholesterol in serum (S_0) was 0.59±0.08 g /L in the untreated group (group 1). This value which varied over time from 0.88±0.06 g /L (minimum S_2) to 1.08±0.37 g /L (maximum S_3), represents a variation of 50% (S_2) to 84.66% (S_3) of the initial serum total cholesterol (table 8).
In lot 2 (12.5 mg / kg), serum total cholesterol was 0.75±0.47 g /L before treatment. Over the past six weeks, the rate changed of 0.48 ± 0.06 g / L (minimum S_2) to 1.05±0.54 g / L (maximum S_6). These values correspond to variations of -36.3% (S_2) to 39.38% (S_6). Percentage changes recorded in batches 3, 4, 5 and 6 were respectively -20% (S_1) to 70.59% (S_3); -44.8% (S_1) to -2.99% (S_6); -55.7% (S_3) to 28.57% (S_5) and - 54.4% (S_3) to -5.88% (S_5) of the initial serum total cholesterol (table 8).
The statistical analysis shows any change in total cholesterol serum with different doses (P > 0.05).

Table 9: Effect of MISCA on serum triglycerides (g/L) levels over time.

Serum levels of triglycerides (g/L)						
Doses (mg/kg)	**0**	**12,5**	**25**	**50**	**100**	**200**
S_0	0.75±0.25	1.75±0.21	1.8±0.3	1.3±0.64	0.95±0.13	0.85±0.3
S_1	0.73±0.07	1.73±0.21	1.89±0.16	1.43±0.42	1.09±0.33	0.75±0.45
S_2	0.7±0.1	1.78±0.65	1.35±1.01	1.23±0.27	0.9±0.28	0.72±0.19
S_3	0.65±0.06	1.43±0.45	1.36±0.02	1.3±0.35	1.21±1.02	0.64±0.06
S_4	0.75±0.1	1.45±0.1	1.17±0.35	0.91±0.14	0.75±0.09	0.72±0.21
S_5	1.05±0.13	1.15±0.4	1.17±0.37	1.1±0.32	**0.77±0.39***	**0.58±0.09***
S_6	0.9±0.13	1.13±0.36	1.78±0.78	1.56±0.41	**0.85±0.18***	**0.68±0.11***
Lots	Lot_1	Lot_2	Lot_3	Lot_4	Lot_5	Lot_6

*Values are expressed as mean ± S.E.M (n=6); ***P<0.05** compared to control and S_0 level*
S_0: Week preceding the first application of treatment / S_1 to S_6: Weeks of treatment

The initial rate of triglycerides was 0.75 ± 0.25 g/L in the untreated lot (lot1). This value varied over time between 0.65 ± 0.06 g/l (minimum S3) and 1.05 ± 0.13 g/l (maximum S5), representing a change of -13.33% (S3) to 40% (S5) -7.26 % (S_3) to 10.65% (S_6) of the initial serum rate of triglycerides (table 9). In lot 2 (12.5 mg / kg), serum level of TG was 1.75 ± 0.21 g/ L before treatment. Over the past six weeks, the concentration changed of 1.13 ± 0.36 g/L (minimum S6) to 1.78 ± 0.65 g/L (maximum S2). These values correspond to variations of -35.2% (S6) to 1.9% (S2).
Percentage changes registered in groups 3, 4, 5 and 6 are respectively -35% (S4) to 5% (S1) ; -30.3% (S4) to 20 % (S6) ; -21.1% (S4) to 27.02 % (S3) et -31.8% (S5) à -11.8% (S1) (table 9).
Statistical analysis of the results indicate a significant decrease in serum rate of TG (P <0.05), especially with doses of 100 and 200 mg / kg bw (lot 5, lot 6) in the fifth and sixth week.

Table 10: Effect of MISCA on serum glucose (g/L) levels over time.

Serum levels of glucose (g/L)						
Doses (mg/kg)	**0**	**12,5**	**25**	**50**	**100**	**200**
b	0.93±0.17	0.98±0.22	1.12±0.33	0.9±0.09	1.2±0.2	1.02±0.08
S_1	0.7±0.2	0.87±0.23	1.06±0.16	0.85±0.15	0.81±0.16	0.75±0.09
S_2	0.85±0.25	0.82±0.3	0.82±0.14	0.9±0.26	0.98±0.27	0.92±0.25
S_3	0.77±0.25	0.54±0.28	0.98±0.2	0.96±0.26	1.07±0.38	1±0.27
S_4	0.62±0.06	0.82±0.33	1.02±0.17	0.73±0.15	1±0.1	0.93±0.35
S_5	0.96±0.07	0.97±0.15	1.15±0.18	0.73±0.27	0.87±0.11	0.94±0.5
S_6	0.98±0.11	0.88±0.33	0.93±0.41	1.12±0.1	0.81±0.18	1.17±0.17
Lots	Lot_1	Lot_2	Lot_3	Lot_4	Lot_5	Lot_6

Values are expressed as mean ± S.E.M (n=6); P>0.05 compared to control and S_0 level
S_0: Week preceding the first application of treatment/ S1 to S6: Weeks of treatment

The level of glucose in serum (S_0) was 0.93 ± 0.17 g /L in the untreated group (group 1). This value which varied over time from 0.62 ± 0.06 g /L (minimum S_4) to 0.98 ± 0.11 g /L (maximum S_6), represents a variation of 50% (S_2) to 84.66% (S_3) of the initial serum glucose (table10). In lot 2 (12.5 mg / kg), serum glucose was 0.98 ± 0.22 g /L before treatment. Over the past six weeks, the rate changed of 0.54 ± 0.28 g / L (minimum S_3) to 0.97±0.15 g / L (maximum S_5). These values correspond to variations of -45.1% (S_3) to -1.69% (S_5).

Percentage changes recorded in batches 3, 4, 5 and 6 were respectively 26.9% (S_2) to 2.98% (S_5); -18.5% (S_4, S_5) to 24.07% (S_6); -3.8% (S_6) to -11.1% (S_3) to -26.2% (S_1) to 14.75% (S_6). The statistical analysis shows any change in concentration of serum glucose with different doses (P > 0.05).

Table 11: Effect of MISCA on serum total proteins (g/L) levels over time.

Serum levels of total proteins (g/L)						
Doses (mg/kg)	**0**	**12,5**	**25**	**50**	**100**	**200**
S_0	26.7±7.64	32.2±11.9	28.2±7.59	27.3±6.81	34.5±18.2	30±13.22
S_1	25.2±4.48	28.3±5.77	35±7	36.7±2.89	38.3±5.77	27±3.6
S_2	28.5±10.2	23.8±6.21	25.2±6.52	44±14	40±14.11	35±9.64
S_3	28.3±10.4	33.7±6.81	30.3±2.25	28.7±7.52	19.3±8.39	39±5.29
S_4	19.8±1.75	36.2±2.04	40±2	28.2±2.75	46±3.6	33.3±7.64
S_5	20.2±0.76	38.3±7.64	48.7±8.08	37.7±2.52	37.5±5.68	36.2±6.29
S_6	28.3±7.64	45±5	35.7±9.81	43.2±7.59	32.7±6.37	42.2±4.91
Lots	Lot_1	Lot_2	Lot_3	Lot_4	Lot_5	Lot_6

Values are expressed as mean ± S.E.M (n=6); P>0.05 compared to control and S_0 level
S_0: Week preceding the first application of treatment/ S1 to S6: Weeks of treatment

The level of total proteins in serum (S_0) was 26.7 ± 7.64 g /L in the untreated group (group 1). This value which varied over time from 19.8 ± 1.75 g/L (minimum S_4) to 28.5 ± 10.21 g/L (maximum S_2), represents a variation of -25.62% (S_3) to 6.87% (S_2) of the initial serum total proteins (table 11). In lot 2 (12.5 mg / kg), serum total protein was 32.2 ± 11.9 g /L before treatment. Over the past six weeks, the rate changed of 23.8 ± 6.21 g/L (minimum S_2) to 45 ±5 g/L (maximum S_6). These values correspond to variations of -25.9% (S_2) to 39.9% (S_6).
Percentage changes recorded in batches 3, 4, 5 and 6 were respectively -10.7% (S_2) to 72.78% (S_5) ; 32.24% (S_4) to 106.6 % (S_2) ; -44% (S_3) to

33.33 % (S_4) to -10% (S_1) to 40.56% (S_6) of the initial serum total proteins (table 11). The statistical analysis shows any change in total cholesterol serum with different doses ($P > 0.05$).

4. Discussion

The initial serum rates of the various enzymes (ALT, AST, γGT, LDH, ALP) and metabolites (glucose, totals proteins, total cholesterol, triglycerides) stored in different batches before treatment and those recorded in the control group (lot1) which has not undergone any treatment are in conformity with the usual values obtained in rabbits by **COULIBALY** [20].

In fact, the liver is a metabolic crossroads with a rich equipment enzyme that plays a very important role in the metabolism of several substances. This is why the assessment of the impact of drugs on its operation is of capital nature.

Statistical analysis of the results indicate that the aqueous extract of Misca don't lead a significant change in catalytic activity of ALT and AST ($P > 0.05$), although there was a slight increase in their serum activities with the dose of 200 mg/kg bw (lot 6), especially in last weeks. These disturbances could be related to transient faults liver tissue or other organs such as heart or skeletal muscle where ALT, AST are also present [14, 15, 16]. The same observation was made with *Morinda morindoides* by **OTIS** [21]. There is no significant change in serum concentrations of glucose, totals proteins, total cholesterol and in serum activity of γGT.

The aqueous extract of MISCA has no influence on transaminase activity in vivo (GOT, GPT). This observation shows that MISCA should not cause liver damage (cholestasis, cytolysis). During processing, the decrease in serum activity of LDH and PAL ($P < 0.05$) may be related to inhibition of their synthesis or action. Clinically, it is considered that the declines in serum activity of PAL reflect nutritional deficiencies, anemia or exposure to radiation. No special meaning is given to decreases in LDH activity [22].

In addition the TG levels decrease. The aqueous extract of MISCA could encourage the setting aside of triglycerides in adipocytes by increasing plasma treatment or by inhibiting their synthesis in the liver. This beneficial effect has also been described with the F2 fraction and would thus protect some vital organs such as the liver, heart and kidney [23]. In fact, there is a strong link between elevated levels of TG and the risk of atherosclerosis.

Finally we can note that even if the dose of 200 mg/kg bw does not seem to cause any hepatic dysfunction, this dose may cause disorders in serum transaminase activity especially during last weeks. Consequently, it would be logical to recommend the use of dose 100 mg/kg bw and reduce processing time to 4 weeks.

5. Conclusion

The use of aqueous extract of Misca at doses ranging from 12.5 to 200 mg/kg bw in rabbits caused a significant decrease in serum activity of LDH, PAL and in serum level of TG. But there is no significant change in the serum activity of transaminase ALT, AST ($P > 0.05$). Decreases in serum activity of LDH, PAL and serum rate of TG ($P < 0.05$), indicate that Misca does not induce specific lesion in the hepatic tissue. Misca is well tolerated by the liver. However, it is necessary to rationalize the traditional use of this plant by reducing the dose (100 mg/kg bw) and time of treatment (4weeks). We note that with this dose of 100 mg/kg bw which is much than the therapeutic dose, Misca always keep a safety margin very interesting. Given the interest that could have Misca in therapeutic management of many diseases, it would be interesting to assess blood electrolytes, to make hematological and histological analyses in order to better understand all aspects of tolerance of this plant.

6. Conflict of interest

The authors declare that they have no conflict of interest.

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