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TOXICOLOGICAL EVALUATION OF MANGIFERIN-LOADED POLYMERIC NANOCAPSULES IN WISTAR RATS[1]

AVALIAÇÃO TOXICOLÓGICA DE NANOCAPSULAS POLIMÉRICAS CONTENDO MANGIFERINA EM RATOS WISTAR

Guilherme do Carmo[2], Barbara Dotto[3], Diego Becker Borin[4], Renata Garcez[4], Rodrigo de Almeida Vaucher[5], Matheus D. Baldissera[6], Claiton I. Schwertz[7], Ricardo E. Mendes[7], Virginia Cielo Rech[8] e Renata P. Raffin[9]

ABSTRACT

This study aimed to verify the toxicological profile of nanocapsules containing mangiferin (NCM) in Wistar rats. Two administration routes, oral and intraperitoneal were evaluated. The animals were divided into 10 groups (n=8). The NCM were obtained with particle sizes of 69.1 ± 8.1 nm, polydispersity index of 0.12 ± 0.06 and zeta potential of -32.35 ± 4.03 mV. When administered orally, NCM and unloaded nanocapsules (UNC), as well as free mangiferin, did not alter any biochemical, hematological or histological parameter. The hematological analysis showed a significant increase ($p<0.001$) in total leukocytes in the groups UNC and NCM, and a significant decrease ($p<0.01$) in hemoglobin concentration and hematocrit in the group NCM through intraperitoneal administration. The biochemical analysis showed a significant increase ($p<0.001$) in the serum levels of urea and a significant decrease ($p<0.001$) in the serum levels of uric acid in the groups UNC and NCM administered intraperitoneally. The histological analysis showed an inflammatory process in the abdominal cavity of the groups UNC and NCM intraperitoneally. We observed that oral administration did not cause toxicity according to the evaluated parameters and was considered safe for use during a 24-day period. However, the intraperitoneal route was not appropriate for administering polymeric nanocapsules.

Keywords: Nanocapsules, Mangiferin, Toxicological Profile.

RESUMO

Este trabalho teve como objetivo verificar o perfil toxicológico de nanocápsulas contendo mangiferina (NCM) em ratos Wistar. Duas vias de administração, oral e intraperitoneal, foram avaliadas. Os animais foram divididos em 10 grupos (n = 8). As NCM foram obtidas com tamanho de partícula de 69,1 ± 8,1 nm, índice de polidisperção de 0,12 ± 0,06 e potencial zeta de -32,35 ± 4,03 mV. Quando administrados por via oral, NCM e nanocápsulas não carregadas (UNC), assim como mangiferina livre, não alteraram nenhum parâmetro bioquímico, hematológico ou histológico. A análise hematológica mostrou um aumento significativo (p <0,001)

[1] Pesquisa realizada no Programa de Pós-graduação em Nanociências.

[2] Docente Centro Universitário Univel, Cascavel-PR. E-mail: guilhermebiomedico@gmail.com

[3] Programa de pós graduação em Bioquímica Toxicológica, Universidade federal de Santa Maria. E-mail: barbaradotto@live.com

[4] Laboratório de Nanotecnologia, Universidade Franciscana. E-mail: dbborin@gmail.com

[5] Docente Universidade Federal de Pelotas. E-mail: rodvaucher@hotmail.com

[6] Laboratório de Parasitologia Veterinária, Universidade federal de Santa Maria. E-mail: matheusd.biomed@yahoo.com.br

[7] Laboratório de Patologia Veterinária, Instituto Federal Catarinense, Concordia-SC

[8] Coorientadora, Universidade Franciscana. E-mail: vga.cielo@gmail.com

[9] Orientadora, Universidade Franciscana. E-mail: reraffin@gmail.com

no total de leucócitos nos grupos UNC e NCM e diminuição significativa (p <0,01) da concentração de hemoglobina e do hematócrito no grupo NCM por via intraperitoneal. A análise bioquímica mostrou um aumento significativo (p <0,001) nos níveis séricos de ureia e uma diminuição significativa (p <0,001) nos níveis séricos de ácido úrico nos grupos UNC e NCM administrados por via intraperitoneal. A análise histológica mostrou um processo inflamatório na cavidade abdominal dos grupos UNC e NCM por via intraperitoneal. Observamos que a administração oral não causou toxicidade de acordo com os parâmetros avaliados e foi considerada segura para uso durante um período de 24 dias. No entanto, a via intraperitoneal não foi apropriada para a administração de nanocápsulas poliméricas.

Palavras-chave: *Nanocápsulas, Mangiferina, Perfil toxicológico.*

INTRODUCTION

Mangifera indica L., known as sleeve, is consumed worldwide. In crude mango extract the main compound is mangiferin (1, 3, 6,7-tetrahydroxy-xanthone-C2-bd-glucosylated), which is a natural bioactive. Mangiferin is considered a glycosylated xanthone present in the stem, leaves and bark of *Mangifera indica* (FERREIRA *et al*., 2013).

Mangiferin has many medicinal properties, including antibacterial (SINGH *et al*., 2012), antidiabetic (MIURA *et al*., 2001; MURUGANANDAN *et al*., 2005), antipyretic activity (SINGH *et al*., 2011), antidepressant and anxiolytic (JANGRA *et al*., 2014), anti-inflammatory (GONG *et al*., 2013), antitumor (LI *et al*., 2013), antioxidant (PRABHU *et al*., 2006) and is also used against rheumatoid arthritis (LUCZKIEWICZ *et al*., 2014). However, it presents very low solubility in aqueous medium (FERREIRA *et al*., 2013). Thus, one strategy used to increase its solubility and hence its therapeutic efficacy is nanoencapsulation, since the encapsulation process is so far the best way to preserve the chemical integrity and to increase the bioavailability of low substrate water solubility, as well as extend the final residence time at the destination (AGRAWAL *et al*., 2014; SOUZA *et al*., 2013).

Nanocapsules (NC) are vehicles with a polymeric structure capable of controlling drug release, and present lower toxicity, improved drug stability and reduced side effects (PARK; BALAKRISHNAN; YANG, 2013). The oral administration of drugs is the most desirable form of administration route, due to its high degree of patient compliance (WANG; ZHANG, 2012). Thus, Eudragit drug delivery systems are widely used in the pharmaceutical industry (NAEEM *et al*., 2014) by increasing the bioavailability when administered orally (DAI *et al*., 2004). The suspensions of mangiferin-loaded nanocapsules (NCM) have been developed and characterized previously (MOURA *et al*., 2014). This study aims to evaluate the toxicology profile of NCM in rats.

SUBJECTS AND METHODS

MANGIFERIN-LOADED NANOCAPSULES (NCM)

The suspensions were prepared by adaptation (MOURA *et al.*, 2014) of the initially described technique of interfacial deposition of preformed polymer (FESSI *et al.*, 1989). The suspension composition is described in Table 1. The components of the oil phase and aqueous phase were weighed separately and heated to 40 °C under moderate stirring until complete dissolution of the components. Then, the oil phase was poured into the aqueous phase and stirred for 10 minutes. Ethanol and water were removed in a rotary evaporator until a final volume of 25 ml (concentration of mangiferin 0.25 mg/ml). As a comparison, unloaded nanocapsules (UNC) were prepared under the same conditions in the absence of mangiferin.

Table 1 - Composition of the suspension of nanocapsules containing mangiferin, for the end volume of 25 ml.

oil phase		**aqueous phase**	
Mangiferin	0.00625g	Polysorbate 80	0.385g
Eudragit ® S100	0.25g	Water	125ml
Sorbitan monostearate	0.0962g		
isopropyl adipate	0.395g		
DMSO	200µl		
Ethanol	62.5ml		

Fonte: elaborado pelos autores.

PHYSICOCHEMICAL CHARACTERIZATION OF NANOCAPSULES

Particle size

Mean particle size was measured using Zetasizer® nano-ZS model ZEN 3600, Malvern. For particle size, samples were diluted 500x v/v in water. The results were expressed in nanometers (nm) from the reading of three different suspension batches.

Zeta potential

Zeta potential was measured using Zetasizer® nano-ZS model ZEN 3600, Malvern. For zeta potential, samples were diluted 250x v/v in water. The results were expressed in millivolts (mV) from the reading of three different suspension batches.

Determination of pH

The pH of the nanocapsule suspensions was directly measured using Digimed® DM-20 potentiometer. The results were expressed from the reading of three different suspension batches.

Mangiferin content

The amount of mangiferin was determined by high-performance liquid chromatography. For this, the colloidal dispersions were dissolved in methanol filtered and then injected into an HPLC Prominence (Shimadzu, Japan), equipped with pump model LC-20AT, UV/VIS detector model SPD-M20A, column C18 (150 x 4.6 mm, 5 µm) Shim-pack CLC-ODS (Shimadzu, Japan). An injection volume of 20 µl and flow of 0.8 ml/min and detection at 254 nm were used. The results are expressed as mean values of three different batches of mangiferin nanocapsule suspensions. The amount of mangiferin is described in Table 2.

Animal model

Eighty male Wistar rats (60 days, 200 - 250 g) from the Central Animal House of the Federal University of Santa Maria were used in this experiment. The animals were maintained at a constant temperature (23 ± 1 ºC) on a 12 h light/dark cycle with free access to food and water. All animal procedures were approved by the Animal Ethics Committee from the Universidade Franciscana (protocol 003/2014). All animals were submitted to a period of 15 days for adaptation;

Evaluation of toxicity

Two routes of administration were used for chronic treatment, oral and intraperitoneal. After 24 days, the rats were fasted for 12 hours and killed by decapitation. Blood was separated into anti-coagulant tubes for complete blood count and tubes without anticoagulant to obtain serum. For this treatment the rats were randomly divided into 10 groups (n = 8) described below:

Group 1: Control Group (C), saline was administered orally;

Group 2: Vehicle (V), where it was given orally 0.1 mg/kg of rats;

Group 3: Mangiferin (M), where it was given orally 0.1 mg/kg of rats;

Group 4: Unloaded nanocapsules (UNC), where it was given orally 0.1 mg/kg of rats;

Group 5: Nanocapsules of mangiferin (NCM), where it was given orally 0.1 mg/kg of rats;

Group 6: Control Group (C), saline was administered intraperitoneally;

Group 7: Vehicle (V), where it was given intraperitoneally 0.1 mg / kg of rats;

Group 8: Mangiferin group (M), where it was given intraperitoneally 0.1 mg/kg of rats;
Group 9: Unloaded nanocapsules (UNC), where it was given intraperitoneally 0.1 mg/kg of rats;
Group 10: Nanocapsules of mangiferin (NCM), where it was given intraperitoneally 0.1 mg/kg of rats.

Biochemical analysis

The blood was collected after decapitation in serum-separating tubes to determine the biochemical parameters. Then the tubes were centrifuged at 3,000 rpm for 15 minutes and the supernatant, corresponding to the serum, was fractionated into Eppendorf® plastic tubes and stored at -20 ºC for biochemical analysis. Serum levels of albumin, creatinine, urea, uric acid, total protein (TP), alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), were analyzed using Labtest Kits (Labtest Diagnóstica SA) with the automatic analyzer CELM SBA 200® (CELM, Barueri/SP, Brazil). All tests were carried out in duplicate.

Hematologic analysis

Hematological parameters were assessed in whole blood collected in tubes containing EDTA (Vacutainer®) using an automatic counter COULTER T890® (Coulter Electronics, Inc, Hialeach, FL, USA). Total leukocytes (WBC), total erythrocytes (RBC), hematocrit (Ht), hemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and platelets were determined. Blood smears were fixed in methanol and stained with Instant-Prov (NewProv®) stain for determination of the differential WBC count. At least 200 WBCs were counted for differential WBC determinations.

Histological analysis

At necropsy, the liver tissue, spleen, kidney, stomach were collected and fixed in 10% buffered formalin and embedded in paraffin wax. Tissue sections were stained with hematoxylin and eosin (HE) for histopathological examination. The sections were evaluated by a specialist in Veterinary Pathology at the Federal Institute of Santa Catarina in a blinded fashion and lesions were scored as absent, mild, moderate, severe and very severe.

Statistical analysis

Data were expressed as mean ± standard deviation (SD). Statistical analysis was performed by analysis of variance (ANOVA) followed by Tukey test when appropriate. Values $P < 0.05$ were

considered statistically significant. All data were analyzed using the *Statistical Package for Social Sciences* - SPSS.

RESULTS

PHYSICOCHEMICAL CHARACTERIZATION OF NANOCAPSULES

The results of the physicochemical characterizations of nanocapsules are shown in Table 2.

Table 2 - Physicochemical characterization of nanocapsules of mangiferin.
Results are expressed in mean values ± standard deviation.

Parameters	NCM
Mean particle size (nm)	69.15 ± 8.05
Polydispersity índex	0.12 ± 0.06
Zeta potential (mV)	-32.35 ± 4.03
Ph	4.3 ± 0.3
Drug loading (%)	102.47 ± 2.65

Fonte: elaborado pelos autores.

BIOCHEMICAL ANALYSIS

Table 3 shows the effect of chronic treatment on liver function and kidney injury related to ALT, AST, ALP, PT, albumin, urea, creatinine and uric acid in rats treated orally. No significant differences were observed among the groups.

Table 3 - Effect of chronic oral treatment on biochemical parameters.

	C	V	M	UNC	NCM
AST (U L^{-1})	241± 53.1	315± *64.6*	324 ± 84.7	311 ± 61.6	299 ± 70.1
ALT (U L^{-1})	48 ± 5.5	56 ±10.2	59 ± 14.6	49 ± 7.9	53 ± 10
FAL (U L^{-1})	118 ± 53.7	164 ± 61.8	118 ± 30.3	133 ± 37.6	132 ± 53.5
PT (mg/dL)	6.5 ± 0.3	6.4 ± *0.2*	6.5 ± 0.2	6.5 ± 0.2	6.6 ± 0.2
Albumin (mg/dL)	2.7 ± 0.1	2.6 ± 0.1	2.6 ± 0.1	2.6 ± 0.1	2.6 ± 0.1
Urea (mg/dL)	*37.8 ± 8.8*	*38.5 ± 2.0*	*39.2 ± 3.1*	*38.7 ± 5.1*	*39.8 ±5.5*
Creatinine (mg/dL)	*0.5 ± 0.05*	*0.48 ± 0.03*	*0.51 ± 0.03*	*0.51 ± 0.06*	*0.5 ± 0.05*
Uric acid (mg/dL)	*1.43 ± 0.4*	*1.48 ± 0.2*	*1.46 ± 0.2*	*1.38 ± 0.4*	*1.31 ± 0.2*

Data are expressed as mean ± SD and each treatment was compared to the control.
Fonte: Elaborado pelos autores.

Data on ALT, AST, ALP, PT, and albumin in rats treated intraperitoneally are shown in Table 4. No significant differences were observed among the groups. Table 4 presents the parameters related to kidney injury in rats treated intraperitoneally. A significant difference was identified among groups ($P<0.001$). An increase in the serum levels of urea was observed in the UNC and NCM groups when

compared to the control. Furthermore, a decrease was observed in the serum levels of uric acid in the V, UNC and NCM groups when compared to the control.

Table 4 - Effect of chronic intraperitoneal treatment on biochemical parameters.

	C	V	M	UNC	NCM
AST (U L^{-1})	277 ± 98	221± *31*	303 ± 74	215 ± 48	231 ± 61
ALT (U L^{-1})	52 ± 12	48 ±8.0	53 ± 8.0	43 ± 14	54 ± 13
FAL (U L^{-1})	166 ± 70	145 ± 55	123 ± 17	135 ± 49	122 ± 16
PT (mg/dL)	6.3 ± 0.4	6.1 ± *0.4*	6.3 ± 0.3	5.7 ± 0.2	5.8 ± 0.6
Albumin (mg/dL)	2.6 ± 0.1	2.6 ± 0.2	2.7 ± 0.2	2.5 ± 0.1	2.6 ± 0.3
Urea (mg/dL)	*35 ± 3.5*	*35.6 ± 5.0*	*37.1 ± 4.3*	*49 ± 7.4****	*49.1 ± 4.7****
Creatinine (mg/dL)	*0.4 ± 0.03*	*0.4 ± 0.06*	*0.4 ± 0.04*	*0.4 ± 0.08*	*0.4 ± 0.09*
Uric acid (mg/dL)	*2.4 ± 0.6*	*1.5 ± 0,3****	*2.1 ± 0.2*	*1.7 ± 0.2****	*1.6 ± 0.2****

Data are expressed as mean ± SD and each treatment was compared to the control. $^{**}p < 0.01$; $^{***}p < 0.001$ compared to control.

Fonte: Elaborado pelos autores.

HEMATOLOGIC ANALYSIS

In Table 5, we can observe the effect of oral treatment on hematologic parameters. A significant increase of CHCM was identified in the UNC (P<0.05) and NCM (P<0.001) groups when compared to the control.

Table 5 - Effect of subchronic treatment by oral route on blood red and white cell series.

	C	V	M	UNC	NCM
RBC (x 10^6/ µL)	7.8 ± *0.26*	7.5 ± *0.39*	7.9 ± *0.42*	7.9 ±*0.41*	7.8 ±*0.43*
HGB (g/dL)	12.3 ± *5.0*	13.3 ± *1.0*	13.4 ± *2.2*	14.5 ± *0.8*	14.5 ± *2.5*
HCT (%)	41.6 ±*1.7*	39.9 ±*1.7*	43.3 ± *2.5*	40.1 ± *5.8*	40.4 ± *2.1*
PLT (x 10^3/µL)	786 ± *193*	845 ± *110*	802 ± *76*	971 ± *141*	987 ± *196*
VCM (fl)	53.0 ± *1.4*	52.9 ± *1.7*	53.6 ± *0.8*	52.2 ± *1.3*	51.4 ± *0.9*
CHCM (%)	33.8 ± *0.7*	33.5 ± *1.2*	33.9 ± *0.8*	35.2* ± *0.5*	35.9*** ± *0.4*
WBC (x 10^3/µL)	10.8 ±*1.7*	8.6 ± *2.7*	10.6 ± *2.6*	10.2 ±*1.8*	10.1 ±*2.7*
Lymphocytes (%)	67.8 ± *1.7*	70.2 ± *7.1*	68.5 ± *2.1*	73.5 ± *4.9*	70.5 ± *6.9*
Neutrophils (%)	26.5 ± *3.0*	24.2 ± *6.8*	25.8 ± *2.6*	22.1 ± *5.3*	22.6 ± *8.7*
Rods (%)	1.1 ± *0.3*	0.8 ± *0.4*	1.3 ± *0.5*	0.7 ± *0.4*	0.6 ± *0.5*
Eosinophils (%)	0.4 ± *0.5*	1.0 ± *0.7*	0.5 ± *0.5*	0.8 ± *0.4*	0.6 ± *0.5*
Monocytes (%)	3.8 ± 1.2	3.8 ± 1.3	3.8 ± 1.3	4.0 ± 2.0	5.3 ± 2.4

Data are expressed as mean ± SD and each treatment was compared to the control.

Fonte: Elaborado pelos autores.

In Table 6, we can observe the effect of intraperitoneal treatment on hematologic parameters. A significant decrease (P<0.01) of HGB, HCT, and CHCM was identified in group NCM. A significant increase (P<0.001) of WBC, neutrophils and rods was also identified in the UNC and NCM groups compared to the control.

Table 6 - Effect of subchronic treatment by i.p. on blood red and white cell series.

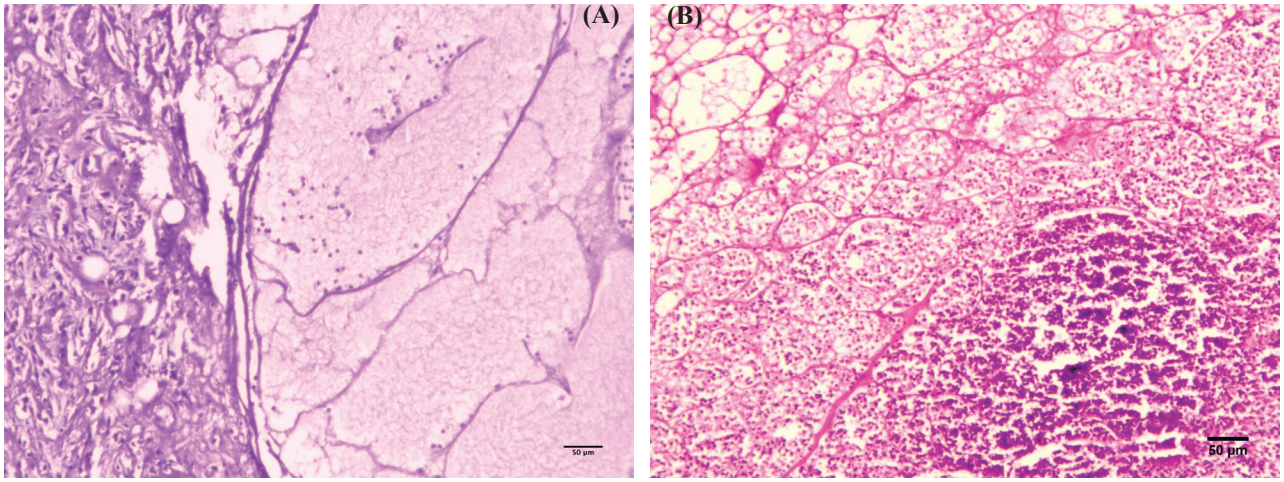
	C	V	M	UNC	NCM
RBC (x 10^6/µL)	7.7 ±*0.44*	7.3 ± *0.35*	7.9 ± *0.24*	7.7 ± *0.40*	7.4 ± *0.67*
HGB (g/dL)	14.2 ± *1.03*	13.9 ± *0.60*	14.3 ± *0.41*	14.3 ± *1.39*	11.3** ± *2.32*
HCT (%)	41.1 ± *2.7*	40.5 ± *1.7*	42.1 ± *1.1*	41.0 ± *2.8*	34.4** ± *5.1*
PLT (x 10^3/µL)	1197 ± *182*	1019 ± *121*	1031 ± *104*	1274 ± *50*	1154 ± *177*
VCM (fL)	53.1 ± *1.8*	55.2 ± *1.1*	53.1 ± *0.9*	52.8 ± *1.6*	54.1 ± *5.3*
CHCM (%)	34.6 ± *0.5*	34.5 ± *0.8*	33.9 ± *0.5*	34.8 ± *1.3*	32.0** ± *1.7*
WBC (x 10^3/µL)	9.9 ± *3.1*	10.6 ± *1.1*	9.7 ± *1.5*	46.5*** ± *12.6*	49.4*** ± *7.3*
Lymphocytes (%)	69.3 ± *4.9*	67.5 ± *3.6*	68.0 ± *2.3*	51.7*** ± *14.0*	49.5*** ± *7.6*
Neutrophils (%)	25.5 ± *5.1*	26.7 ± *2.0*	26.4 ± *2.9*	42.7*** ± *13.5*	44.3*** ± *6.7*
Rods (%)	0.6 ± *0.5*	0.8 ± *0.6*	0.7 ± *0.48*	2.0*** ± *0.5*	2.6*** ± *0.5*
Eosinophils (%)	0.3 ± *0.5*	0.5 ± *0.5*	0.5 ± *0.5*	0.4 ± *0.5*	0.5 ± *0.5*
Monocytes (%)	4.0 ± 1.3	4.2 ± *1.4*	4.2 ± *1.3*	2.5 ± 1.4	3.0 ± 1.2

Data are expressed as mean ± SD and each treatment was compared to the control. $p < 0.01$ and . ***$p < 0,001$ compared to control.
Fonte: Elaborado pelos autores.

HISTOLOGICAL ANALYSIS

There were no lesions in the liver, spleen, kidney and stomach after the treatments, both oral and intraperitoneal. After 18 days of treatment, it was observed that the animals in the BNC and NCM group had an abscess on the wall of the peritoneum at the sites of application which persisted until the last day of treatment, and this material was sent for histological analysis. Figure 1 shows the micrographs of the abdominal wall after intraperitoneal subchronic treatment. In the dermis and the abdominal wall, a severe inflammatory infiltration of neutrophils was observed. It was focal and weakly associated with the accumulation of amorphous eosinophilic material, surrounded by the proliferation of connective tissue and inflammatory infiltrates, with a moderate amount of multifocal lymphocytes and macrophages. In the deeper layers a replacement of muscle fibers by connective tissue was observed.

Figure 1 - Micrograph of the abdominal wall after intraperitoneal subchronic treatment (increased 160x), unloaded nanocapsules (A), mangiferin-loaded nanocapsules (B)



DISCUSSION

After production, the physiochemical characterization of nanocapsule suspension with mangiferin was carried out, and it was observed that the parameters found in this study were similar to those found (MOURA et al,. 2014). The results in Table 2 show that the technique used for production allowed the formation of nanometric particles. These were obtained with particle sizes of 69.1 ± 8.1 nm and low polydispersity index (0.12 ± 0.06), indicating a homogeneous and well-distributed sample. The pH was found to be acid (4.3 ± 0.3), the mangiferin content was 102.47 ± 2.65%, and the zeta potential was -32.35 ± 4.03 mV, showing that the nanocapsules have a negative charge.

The hematopoietic system is the most sensitive target for toxic compounds and is considered an important parameter in evaluating the physiological and pathological state in humans and animals (WANG *et al*., 2014). Thus, it can be inferred that the oral subchronic treatment was not able to cause hematological abnormalities. These results demonstrate that free nanocoated mangiferin caused no toxic effects on the hematopoietic system.

Leucocytosis (increase in the total number of leukocytes) is a physiological response of the hematopoietic system and may be caused by an acute or chronic infection, inflammation, cancer or hematological drugs (CERNY; ROSMARINWHY, 2012). After intraperitoneal treatment, leukocytosis was observed in the UNC and NCM groups (Table 6). This is explained by the presence of inflammation in the abdominal wall of the animals in these groups (Figure 1).

Through these results, we can say that in the groups treated with compositions containing Eudragit ® S-100 polymer, leukocytosis and inflammatory processes were observed in the abdominal wall after IP treatment. Therefore, we suggest that intraperitoneal treatment with NCM would not be appropriate because the synthetic polymer used was able to induce inflammation and leukocytosis.

The NCM group showed a significant decrease in hemoglobin (Table 6) concentration, and consequently there was a decrease in hematocrit. It is characterized that this formulation caused anemia in the group after IP administration This, along with the changes in white cell series shows that mangiferin associated with the polymer Eudragit ® S-100 is capable of affecting the hematopoietic system only when administered by IP route.

The increase in serum urea levels in UNC and NCM groups (Table 4) after the IP subchronic treatment may indicate possible kidney damage (BAYNES; DOMINICZAK, 2010), even if the plasma creatinine levels have not increased. Because according to (MOTTA, 2009) creatinine levels are not in excess of the reference values until impairment of renal function reaches between 50-70%. This justifies the reduction of serum levels of uric acid in these two groups because it occurs after renal impairment (LIMA *et al*., 2008). However, after the oral subchronic treatment no changes were observed in serum urea, creatinine and uric acid (Table 3) and this may suggest that treatment with both free and nanocoated mangiferin was not able to cause kidney damage.

It may be evident in Table 4 that plasma levels of PT and albumin did not change after oral and intraperitoneal treatment. Knowing that protein synthesis occurs in the liver (SMITH *et al.*, 2007) and that the treatments did not injure the hepatocytes, this confirms the results of the PT and albumin.

Eudragit® S-100 is a synthetic non-biodegradable, biocompatible polymer widely used in the pharmaceutical industry for the oral administration of drugs (NAEEN *et al.*, 2014). This polymer demonstrates safety management and increases the oral bioavailability of drugs (DAI *et al.*, 2004). This safe drug administration was observed in the present study. However, it probably showed an accumulation of the UNC groups and NCM in the peritoneal cavity after IP administration, causing an inflammation of the abdominal wall (Figure 1), and consequently an increase in the total number of leukocytes (Table 6).

Based on these results we conclude that the polymeric nanocapsules with mangiferin proved safe when administered orally, and did not cause any biochemical, hematological and histological changes. Their intraperitoneal use is not advised.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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ANIMAL ETHICS COMMITTEE

The present study was approved by the Animal Ethics Committee of Universidade Franciscana (protocol number: 003/2014).

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