Jatropha cake (*Jatropha curcas*): hepatotoxic implications

Torta de pinhão-manso (Jatropha curcas): implicações hepatotóxicas

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Abstract

Jatropha has been highlighted as an oleaginous potential for the production of biofuel. The cake, produced by oil extraction, could be used in animal feed. However, some varieties of jatropha are toxic by limiting their incorporation into animal diets. The objective of this study was to evaluate the hepatotoxicity of diets added with jatropha cake – JC (*Jatropha curcas*) in rats. Thirty-five (35) male Wistar adults rats (*Rattus norvegicus*) with initial weight of 352.1 ± 26.8 g were used. The animals were fed for 21 days with the diets: control, 10, 25, 40 and 50% JC. In the feeding with 50% JC the animals presented themselves prostrate and with piloerection. Development and survival decreased, since the inclusion of JC in diets increased. In rats submitted to 10 and 25% JC there was an increase of 17.52% in the hepatosomatic index in relation to the control group. Increase of JC in the rat diet promoted an increase in the activity of ALT and AST enzymes. Anatomic-histopathological evaluation demonstrated that, regardless of the levels tested, JC in rat diet causes hypertrophy of the hepatocytes, with a reduction in energy reserves. This study demonstrated that the use of JC resulted in decreased food intake, associated with weight loss due to the clinical pattern of toxicity, demonstrated by biochemical and histopathological changes in the liver. It was concluded that the inclusion of jatropha cake in rat feeding presents high hepatotoxic potential leading to lesions in the liver parenchyma.
Keywords: Toxic. The hepatotoxicity. Animal feed.

Resumo

O pinhão-manso tem se destacado como oleaginosa potencial para a produção de biocombustível. A torta, coproduto da extração do óleo, poderia ser utilizada na alimentação animal. No entanto, algumas variedades de pinhão-manso são tóxicas, limitando sua incorporação em dietas animais. Objetivou-se neste estudo avaliar a hepatotoxicidade de dietas acrescidas de torta de pinhão-manso (*Jatropha curcas*) em ratos. Foram utilizados trinta e cinco (35) ratos Wistar (*Rattus norvergicus*) machos adultos com peso inicial de 352,1 ± 26,8 g. Os animais foram alimentados por 21 dias com as dietas: controle, 10, 25, 40 e 50% TPM. Na alimentação com 50% TPM os animais apresentaram-se prostrados e com piloereção. O desenvolvimento e a sobrevivência apresentaram diminuição conforme o aumento da inclusão de TPM nas dietas. Em ratos submetidos a 10 e 25% TPM houve aumento de 17,52% no índice hepatossomático em relação ao grupo controle. O aumento de TPM na dieta de ratos promoveu aumento da atividade das enzimas ALT e AST. A avaliação anatomo-histopatológica revelou que, independentemente dos níveis testados, a TPM na alimentação de ratos provoca hipertrofia dos hepatócitos, com redução das reservas energéticas. Este estudo demonstrou que a utilização de TPM resultou em diminuição do consumo de alimento associado à perda de peso devido ao quadro clínico de toxicidade demonstrado pelas alterações bioquímica e histopatológica no fígado. Conclui-se que a inclusão de torta de pinhão-manso na alimentação de ratos apresenta alto potencial hepatotóxico levando a lesões no parênquima hepático.
Palavras-chave: Tóxico. À hepatotoxicidade. Alimentação animal.

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Introduction

Jatropha (*Jatropha curcas* L.) is a perennial and monoic species, belonging to the family Euphorbiaceae, originating in Central America (OVANDO-MEDINA et al., 2011), but it vegetates spontaneously in several regions of Brazil (LAVIOLA; DIAS, 2008). Currently, jatropha grains with high oil content (35 to 45%) are used for the biofuel industry (ACHTEN et al., 2008) and generate cake as a co-product.

There is interest in the use of these co-products originated in agricultural production and agribusiness in animal feed in concentrated feed (GOMES, 2007). However, there are records of the use of *Jatropha curcas* as purgative by humans and animals causing intoxication (OLIVEIRA et al., 2008). The limitation of the use of this co-product as food is attributed to the presence of toxic, allergenic and anti-nutritional components (ABDALLA et al , 2008; MENDONÇA, 2010) such as curcina, diterpene esters (phorbol ester) and proteins with allergenic potential (OLIVEIRA; AKISUE, 2005). The toxic activity of Jatropha seeds and oil is due to the presence of phorbol esters (MAKKAR; BECKER, 1999). These are molecules derived from tetracyclic diterpenes, predominant in the majority of accessions, in the range of 0.82 to 3.85 mg/g albumen (MAKKAR et al., 1997), which activates the protein kinase C (PKC) leading to its hyperactivation (GOEL et al., 2007). PKC regulates several cellular processes including proliferation, apoptosis, survival, cell migration, tumors (GRINER; KAZANIETZ, 2007) and inflammatory activity (GOEL et al., 2007). Toxicity of extracts obtained from seed, oil, root, latex, bark, fruit and leaf of the *J. curcas* is related to molluscicidal, ictiocidal, insecticidal, rodenticidal, antimicrobial and cytotoxic activities and have adverse effects on animals, including rats, poultry and ruminants (DEVAPPA et al., 2010).

A study on jatropha cake evaluation in animal feed investigated the effects of adding increasing quantities of this co-product on the digestibility and performance of sheep (OLIVEIRA, 2012). However, studies on the mechanisms involved in the pathological conditions observed have not yet been reported. In the liver, histological (GARGIULO et al., 1998) and biochemical (MARIZ et al., 2006) changes should be evaluated to understand the effects of intoxication, since this is the central organ of animal metabolism.

Thus, the objective of this study was to evaluate the hepatotoxicity of diets added with jatropha cake (*Jatropha curcas*) in rats.

Materials and Methods

The jatropha cake (JC), resulting from the extraction of oil by mechanical press, was acquired in an industrial crushing plant of Fazenda Paraíso, Dourados (MS), Brazil. Grains and JC were analyzed for moisture content, crude protein, ethereal extract and ashes (AOAC INTERNATIONAL, 2005). Moisture content was determined by subjecting the samples to 105°C in an oven to constant mass. Crude protein content was determined by the Kjeldhal method, where the nitrogen content obtained is multiplied by the factor 6.25. Ethereal extract levels were determined using the Soxhlet extraction apparatus, petroleum ether (p.e. 30-60°C) as the solvent, with continuous reflux for 6 hours. Ash concentration was determined by incinerating the sample in muffle at 600°C, until constant mass. The analysis of phorbol esters was carried out in the laboratory of Embrapa Agroenergy, using a methodology suggested by Makkar et al. (1997) with modifications regarding the stage of sample extraction, as reported by Ribeiro et al. (2010).

Thirty-five (35) male Wistar rats (*Rattus norvergicus*) from the vivarium of the Universidade Federal do Mato Grosso do Sul (UFMS) were used, aging 9 weeks of life and with initial mean weight of 352.1 ± 26.8 g. The animals were housed in individual cages, in a room with a temperature of 26°C (day: night 12 hours cycle) and with access to feed and water *ad libitum* for 21 days.

The animals were randomly weighed and distributed in five groups with seven animals per experimental group. Treatments were: control – standard diet without inclusion of JC and four groups with diets containing 10, 25, 40 and 50% JC (Table 1). The following parameters were evaluated: weight gain, diet consumption, feed conversion index and survival.

Table 1 – Experimental diet of rats plus jatropha cake – Dourados, MS – 2014

	Control	% jatropha cake			
		10	25	40	50
Commercial diet	100	90	75	60	50
Jatropha cake	-	10	25	40	50
Humidity	12.5	11.8	10.7	9.7	9.0
Crude Protein	22.0	22.7	23.8	24.8	25.5
Ethereal Extract	4.5	5.4	6.7	8.0	8.9
Mineral Matter	10.0	9.7	9.2	8.7	8.4
Fibrous Matter	8.0	11.2	16.0	20.8	24.0
Calcium	1.4	1.3	1.1	0.8	0.7
Phosphor	0.8	0.7	0.6	0.5	0.4
Non-nitrogenous extraction	55.5	51.0	44.3	37.6	33.1

At the end of the feeding period, animals were anesthetized for blood collection to perform the biochemical analyses. Later, they were euthanized by inhalation anesthesia with halothane (Committee of Ethics in Animal Experimentation – 261/11) and the liver was withdrawn.

The liver of the rats was weighed to calculate the hepatosomatic index (IHS) = organ weight/animal weight. Subsequently, fragments of this organ were fixed in formalin solution buffered for 24 hours and washed in alcohol 70%, dehydrated, diaphanized and included in paraffin with Histosec plastic polymer (Merck). The microtomy (2 to 5 µm thick), stained with Hematoxylin-Eosin, and the histochemical PAS-H method were performed. Microscopic analyses, histomorphometry and material documentation were performed in an Olympus BX41 photomicroscope. For the morphometry of the material were selected two slides per treatment in which seven sections were photographed. The diameter of the hepatocytes was subsequently measured; core diameter of the hepatocytes and calculated hepatocyte and core ratio in software Image Pro plus version 4.5 Media Cybernetics.

In blood, the activity of the enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) determined by the method of Reitman and Frankel (1957) with 430 nm reading were analyzed.

The experimental design was the randomized design with five treatments (control and inclusion levels of jatropha cake). Results were submitted to analysis of variance and, when significant ($p < 0.05$), compared by Tukey test at 5% of probability.

Results

Jatropha grains presented 6.6% moisture and 32.61% of ethereal extract. Jatropha cake presented 5.49 ± 0.74 of water content, 13.37 ± 1.21% of ethereal extract, 29.04 ± 0.96% of crude protein and 6.85 ± 0.73% of mineral matter. The jatropha cake used for the test had a content of 1.13 $mg.g^{-1}$ of phorbol ester.

Among the animals in the control group (no inclusion of JC), no physiological changes were observed, and the biochemical parameters evaluated were within the reference values for animals raised in vivariums. Regarding the animals treated with food containing above 25% JC, piloerection was observed after three days of feeding. In feed with 50% JC, we observed that these animals were prostrated and with evident piloerection. The development of the rats fed with different levels of JC addition is shown in Figure 1. Weight gain, dietary intake and feed conversion decreased gradually as the inclusion of JC in diets increased (Figure 1, A, B, C).

Survival was proportionally lower with increased JC inclusion (Figure 1D). Feeding with 50% JC caused death of the animals with 18 days of feeding, and in the control group and fed with the 10% JC diet there was no mortality. Animals showed a gradual loss of weight (Figure 1a), although consumption was similar between 10% JC and control (Figure 1B). The feed conversion showed the same behavior as diet consumption of the rats (Figure 1C). Feed intake was altered by the inclusion of 25% of JC in the diet, causing a 67% decrease in consumption compared with the control (Figure 2A).

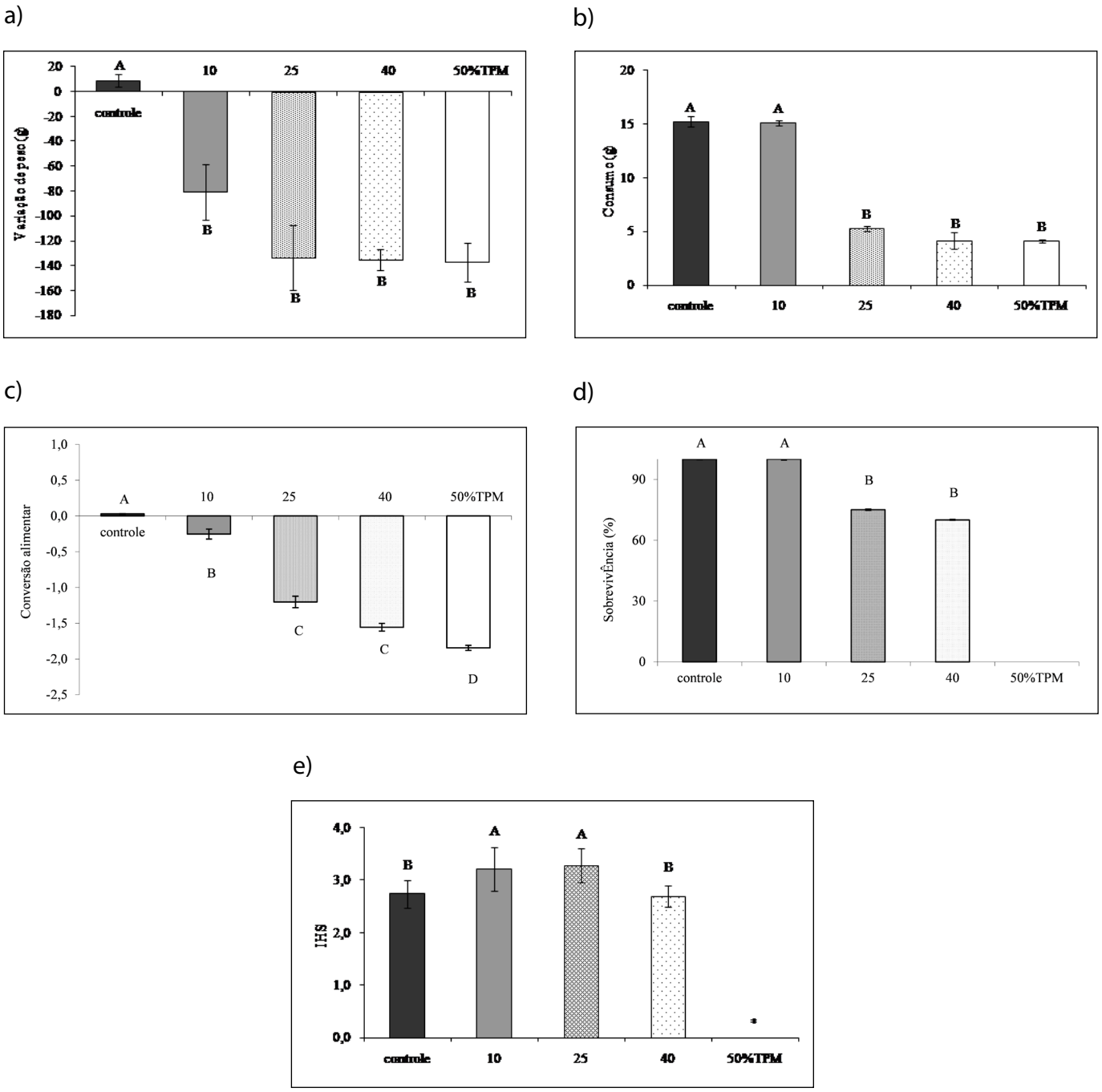

Figure 1 – Rats fed with diets with increased jatropha cake (A) weight after test, (B) daily feed intake (C) feed conversion, (D) survival, and (E) hepatosomatic index (IHS) of rats fed with diets plus jatropha cake. (*) *the animals of this treatment died and the livers had no pattern for collection*

In rats submitted to 10 and 25% JC, there was an increase of 17.52% in IHS in relation to the control group (Figure 1E).When using 40% JC, IHS decreased. A normal histological pattern of the hepatic parenchyma of the control group was observed at the end of 21 days of feeding (Figure 2A). A reduction of glycogen content was observed as the increase of the JC inclusion was accompanied by an increase in the lobular parenchyma, thus demonstrating hepatic glycogen depletion. In the liver of rats fed with 10% of JC, hepatocellular necrosis and steatosis of the hepatic parenchyma were present, with a slight inflammatory reaction (Figure 2B). In the 25% JC diet, the liver showed hepatocellular necrosis and steatosis with a slight inflammatory reaction and septal fibrosis (Figure 2C). In the animals fed with 40% JC, the liver showed hepatocellular necrosis and steatosis with an inflammatory reaction and septal fibrosis (Figure 2C). With the increase of JC inclusion (25%), there was an intensification of cordonal derangement, presence of melanomacrophagus, cholestasis and dilatation of the hepatic sinusoids.

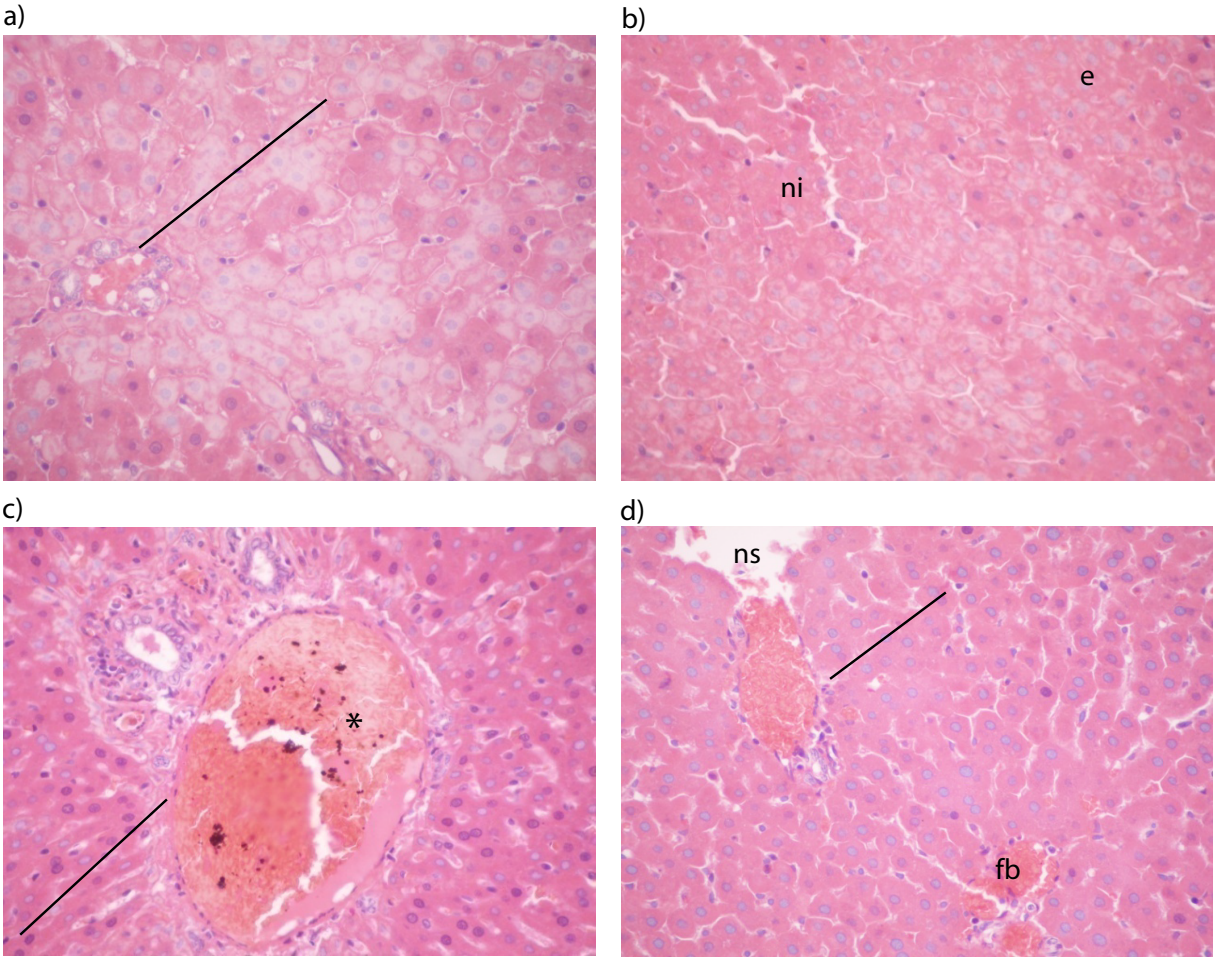

Figure 2 – Photomicrography of liver of Wistar rats fed with diets increased with jatropha cake a) control group – Detail of the liver without histopathological alterations, where a cordonal arrangement of hepatocytes is observed; b) 10% JC, presence of initial necrosis (ni) and steatosis (e), c) 25% JC, detail of the occurrence of necrosis (ns), disarrangement of the cord structure (fine trace), presence of melanomacrophagus* and blood congestion (cg); d) 40% JC, occurrence of necrosis (ns) and fibrosis (fb), disarrangement of the cord structure (fine trace). Coloring with HE, 200X

The morphometry of hepatocytes showed decreased cytoplasm and nucleus, as increased inclusion of JC (Table 2). The increase of jatropha cake in the diet of rats promoted an increase in the activity of ALT and AST enzymes (Table 3). The changes observed in rat liver were found to be dose-dependent to the percentage of inclusion of PMS.

Table 2 – Morphometry of the hepatocytes of rats fed with diets increased with jatropha cake – Dourados, MS – 2014

Parameters (μm^3)	Control	% jatropha cake		
		10	25	40
Cytoplasm area	16.97 ± 0.30a	16.18 ± 0.33a	14.2 ± 0.32b	15.15 ± 0.30b
Core area	5.25 ± 0.09	5.36 ± 0.11	5.32 ± 0.10	5.17 ± 0.11
Cytoplasm/core	3.23 ± 0.16a	3.02 ± 0.15a	2.67 ± 0.16b	2.95 ± 0.14b

Different letters in the line report statistical difference by the Tukey test ($p > 0.05$)

Table 3 – Activity of AST enzymes – aspartate aminotransferase, ALT – alanine aminotransferase in rats fed with diets increased with jatropha cake – Dourados, MS – 2014

Parameters	Control	% jatropha cake		
		10	25	40
AST (U/L)	145.00 ± 8.30	180.00 ± 26.46	196.52 ± 31.63	202.40 ± 26.65
ALT (U/L)	41.65 ± 1.65b	49.97 ± 6.65ab	36.55 ± 0.05b	53.25 ± 6.65a
ALT/AST	0.29 ± 0.05	0.28 ± 0.04	0.19 ± 0.06	0.26 ± 0.04

Different letters in the line report statistical difference by the Tukey test ($p > 0.05$)

Discussion

Jatropha cake presents phorbol ester in its composition, which restricts the inclusion, since it is a toxic component (MENDONÇA, 2010), and presents carcinogenic activity and inflammatory action (OLIVEIRA; AKISUE, 2005).

Rats fed with 50% JC showed more marked clinical signs of morbidity than the other treatments. Similar inhaled descriptions were found in rats treated with leaf extract of *Jatropha gassypiifolia* (MARIZ et al., 2006). In sheep submitted to pericarp feeding of *J. Curcas,* it was verified that the animals presented intoxication, characterized by cachexia, diarrhea, hypocortical mucosae, dehydration, alopecia, bradypnea, crackling respiration (FERREIRA et al., 2012). Developmental effects of rats fed with diet containing *Jatropha* defatted grains promoted serious pathological symptoms causing death with the concentration of phorbol esters of 47.31 $mg.kg^{-1}$ body mass (RAKSHIT et al., 2008). Among the toxic or anti-nutritional compounds, saponins stand out as inhibitors of consumption and reducers in the absorption of nutrients due to changes in the permeability of cell membranes (FRANCIS et al., 2002). The inclusion of the pericarp of *J. Curcas* in the sheep diet promoted dose-dependent reduction in feed intake and was attributed to the association of several toxic compounds present in the plant (FERREIRA et al., 2012). However, in an experiment where only the most toxic compound (phorbol ester) was absent, these other substances did not affect consumption or performance in sheep (OLIVEIRA et al., 2013).

The decrease in food consumption is associated with the presence of toxins and also the decrease of the palatability of the diet plus JC. Decrease in dietary intake and weight loss were attributed to severe intestinal inflammation in rats (RAKSHIT et al., 2008). Symptoms of intoxication in chickens fed with jatropha grains comprise reduced growth, hepato and nephrotoxicity, hemorrhages and congestion (SANTOS et al., 2008). It was also found that changes in IHS may be associated with liver atrophy (LI et al., 2010).

Hepatocellular necrosis and steatosis with discrete inflammatory reaction and septal fibrosis in rats fed diets containing JC were observed in our study. Congestion of hepatic sinusoids was observed in rats submitted to the dose of 26.4 $mg.kg^{-1}$ of jatropha (LI et al., 2010). Similar changes, such as congestion, lymphoplasmacytic and histiocytic periportal infiltrate, centrilobular vacuolar hepatic degeneration, and mild to moderate cholestasis, were observed in sheep liver (FERREIRA et al., 2012). Histopathological changes were observed by the reduction of the cytoplasm and nucleus, according to the increase of the inclusion of JC. Decreased hepatocyte nucleus size is related to chromatin condensation and cellular apoptosis (SEGNER et al., 1988). Histological changes show that *J. Curcas* has a hepatotoxic effect. These histological changes in hepatic cells were observed in cassava leaf (MELO et al., 2008) and cyanide (SOUZA et al., 2002) usage in the rat's diet. In rats submitted to acute treatment with ethanolic extract of aerial parts of *Jatropha gossypiifolia*, inflammatory responses were found in the liver (MARIZ et al., 2008).

Hepatic injury was confirmed by increased activity of ALT and AST enzymes. ALT and AST are enzymes present in hepatocytes and their elevation is associated with acute liver damage. ALT and AST ratio are useful in differentiating the causes of liver injury. The AST enzyme is preferentially found in mitochondria, while the ALT is a hepatic cytosolic enzyme. These changes are indicative of hepatic injury suffered by animals fed with JC. Increased concentrations of these enzymes from protein catabolism corroborate results reported by Mariz et al. (2006) and Ferreira et al. (2012).

Conclusion

This study demonstrated that the use of JC resulted in decreased food intake, associated with weight loss due to the clinical pattern of toxicity, demonstrated by the biochemical and histopathological changes in the liver. It was concluded that the inclusion of jatropha cake in rat feeding, at the doses studied, presents high hepatotoxic potential leading to lesions in the liver parenchyma.

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