MERIT RESEARCH JOURNALS
www.meritresearchjournals.org

Merit Research Journal of Food Science and Technology Vol. 1(2) pp. 023-030, June, 2013
Available online http://www.meritresearchjournals.org/fst/index.htm

Full Length Research Paper

Biological evaluation of *Jatropha curcas* seed as a new source of protein

Elsayed H. Rahma, *Esam H. Mansour and Shirin T. Hamoda

Department of Food Science and Technology, Faculty of Agriculture, Menufiya University, Shibin El-Kom, Egypt.

Accepted June 03, 2013

The effects of autoclaving, roasting, germination and chemical detoxification (treating with ethanol 90% for 2 hours followed by autoclaving in 0.07% sodium bicarbonate at 121°C for 20 min) of *Jatropha curcas* seeds on the protein quality, liver function, kidney function, organ weight and histology in rats were evaluated. Roasting, autoclaving and germination treatments did not affect the toxicity of Jatropha seed flour. However, detoxification treatment improved the protein quality of Jatropha seed flour. The protein efficiency ratio, true digestibility and biological value of detoxifying Jatropha seed flour were 87.33, 92.33 and 86.11% of the casein. Rats fed detoxified Jatropha seed flour had higher heart and kidney weights and lower lung weight than those fed casein. These differences were not enough to cause any side effects on the tissues of these organs. However, detoxified Jatropha seed flour did not affect in liver and spleen weights. Liver and kidney functions in rats fed detoxified Jatropha seed flour were within the normal and safe range. Gross examination and histopathological of vital organs of rats fed detoxified Jatropha seed flour did not show any atrophy compared to rats fed casein.

Keywords: Detoxified Jatropha seed flour, Protein quality, Liver and kidney functions, Organs weight, Histopathology examinations.

INTRODUCTION

Jatropha curcas, a tropical plant introduced in many Asian and African countries is presently used as a source of biodiesel plant with 35–40% of oil depending on the variety. After the extraction of oil from the *Jatropha curcas* seeds the residual cake contains 61.84% crude protein. Except for lysine all other essential amino acids in *Jatropha curcas* flour protein have been reported to be in higher concentrations than those of the FAO/WHO (1990) reference pattern suggested in the pre-school children of 2-5 years old (Mansour et al., 2010; Rakshit et al., 2008). The nutritive value of *Jatropha curcas* has been limited due to the presence of saponin, phytate, trypsin inhibitor, glucosinolates, amylase inhibitors, cyanogenic glucosides, curcin and phorbol esters (Mansour et al., 2010). The *Jatropha curcas* seeds are toxic in mice and rats (Stripe et al., 1978; Adam, 1974). Adam and Magzoub (1975) reported that the Nubian goats fed *Jatropha curcas* seeds at levels ranging from 0.25 to 10 g/kg/day, were toxic with mortality occurring between 2 and 21 days. It was also observed that there was a decrease in glucose and marked rise in the concentration of arginase and glutamate oxaloacetate tranasaminase in the serum. Increase in aspartate amino transferase, ammonia, potassium and decrease in total protein and calcium in serum were observed in Jatropha fed calves (Ahmed and Adam, 1979). Carp (*Cyprinus carpio*) fed diets containing the non-toxic, defatted Jatropha seed meal (23% by weight of feed) showed a lower weight gain than the Jatropha meal heat-treated at

*Corresponding Author E-mail: esam_mansour@yahoo.com

121°C for 15 min (Makkar and Becker, 1999). Makkar and Becker (1997) and Makkar et al. (1997) reported that phorbol esters in *the Jatropha curcas seed meal* have known as main toxicants and heating at 160°C for 30 min did not destroy the phorbol esters. However, Rakshit and Bhagya (2007) have shown the possibility of destroying phorbol esters up to 90% by treating defatted meal with chemicals. *Rakshit* et al. (2008) Reported that phorbol ester and lectin contents were reduced up to 89% by treating defatted Jatropha meal with 2% NaOH or $Ca(OH)_2$ followed by autoclaving at 121°C for 30 min. Rakshit and Bhagya (2008) reported that toxic phorbol esters and cyanogenic glucosides were removed almost completely by extraction of Jatropha protein at alkaline pH and isoelectric precipitation followed by steam injection and washing.

The objective of this study was to evaluate the effect of autoclaving, roasting, germination and chemical detoxification (treating with ethanol 90% for 2 hours followed by autoclaving in 0.07% sodium bicarbonate at 121°C for 20 min) of *Jatropha curcas* seeds on the protein quality, liver function, kidney function, organ weight and histology in rats.

MATERIALS AND METHODS

Materials

Jatropha curcas seeds were obtained from Aswan farm, Egypt. The seeds were hand sorted to remove wrinkled, moldy seeds and foreign materials then stored in polyethylene bags in the refrigerator (4°C) until used.

Raw flour

The seeds were dehulled, ground and defatted at room temperature using petroleum ether (40-60°C). Petroleum ether was removed by filtration and then dried at 50°C for 5 hours in an electric air draught oven (VEB MLW Medizinische Geräte, Berlin, Germany). The dried flour was packed in airtight jars and kept in refrigerator at 4°C until used.

Autoclaving

The defatted flour was placed in a conical flask. After adding distilled water (2 ml/g flour) the mixture was autoclaved at 121°C for 45 min. The autoclaved flour was dried at 50°C for 20 hours in an electric air draught oven, packed in airtight jars and kept in refrigerator at 4°C until used.

Detoxification

The detoxified flour was prepared according to Martínez-Herrera et al. (2006). The defatted flour was extracted with 90% ethanol for 2 hours at room temperature. The flour to solvent ratio was 1:10 (w/v). The solvent was removed by filtration and the residue was mixed with 0.07% $NaHCO_3$ solution in the ratio of 1:5 (w/v) and autoclaved at 121°C for 20 min. The autoclaved flour was dried at 50°C for 20 hours in an electric air draught oven, packed in airtight jars and kept in refrigerator at 4°C until used.

Roasting

Jatropha seeds were put in a frying-pan. The seeds were roasted on a hot plate (160°C) with continuously stirring for 15 min.

Germination

Jatropha seeds were soaked in tap water at room temperature (25°C) for 12 hours. The soaked seeds were kept between thick layers of cotton cloth and allowed to germinate in the dark for 9 days. Germinated seeds were frozen for 12 hours to stop the germination process. After thawing at room temperature, the seeds were dried in an electric air draught oven at 50°C for 20 hours.

The roasted and germinated Jatropha seed were ground, defatted, packed in airtight jars and kept in refrigerator at 4°C until used.

The total protein content (N × 6.25) of raw and treated Jatropha seed flours was determined according to AOAC (1995).

Biological evaluation of protein quality

Weanling male albino rats of Sprague Dawley strain, 4 weeks old, with an average body weight 50-55g were used to estimate apparent digestibility (AD), true digestibility (TD), biological value (BV), and net protein utilization (UPU) using techniques described by Eggum (1973). Six rats were used per test diet and an additional six for the nitrogen-free diet (control). The preliminary period lasted 4 days and the balance period 5 days. The urine and feces of each rat were collected during the balance period and their nitrogen content was determined according to AOAC (1995).

Under the same experimental conditions, the weight changes and protein consumption over the 28 days were used to estimate the protein efficiency ratio (PER), corrected protein efficiency ratio (CPER) and a net protein ratio (NPR).

The basal diet consisted of 79.7% corn starch, 10% corn oil, 5% fiber, 4% salt mixture and 1% vitamin mixture (AOAC, 1995) and 0.3% DL-methionine. The casein and tested proteins were incorporated into the basal diet at the expense of corn starch to give a final 12% crude

Table 1. Feed intake, protein intake, weight gain, PER, CPER and NPR of raw and treated *Jatropha curcas* seed flours as compared to casein.

Treatments	Feed Intake (g/28 day)	Protein intake (g/28 day)	Weight gain (g)	PER	CPER	NPR
Casein	245.67[a] ± 6.03	29.48[a] ± 2.43	58.40[a] ± 5.03	1.98[a] ± 0.11	2.50[a] ± 0.12	1.61[a] ± 0.06
Detoxification[1]	212.08[b] ± 4.11	25.44[b] ± 2.21	44.02[b] ± 3.41	1.73[b] ± 0.07	2.18[b] ± 0.08	1.30[b] ± 0.04
Raw	-	-	-	-	-	-
Roasting	-	-	-	-	-	-
Autoclaving	-	-	-	-	-	-
Germination	-	-	-	-	-	-
LSD	4.534	1.836	2.439	0.023	0.161	0.016

[1]Treating with ethanol 90% for 2 hours followed by autoclaving in 0.07% sodium bicarbonate at 121°C for 20 min.
Mean in the same column with different letters are significantly different ($p \leq 0.05$).

protein in all formulated diets. The total protein content of raw and treated *Jatropha curcas* seed flours were 61.84 raw flour; 61.66 roasted flour; 57.79 autoclaved flour; 64.59 detoxified flour and 59.75% germinated flour (Mansour et al., 2010). The tested proteins were added at the following level 14.90 casein; 19.40 raw flour; 19.46 roasted flour; 21.78 autoclaved flour; 22.81 detoxified flour and 21.91% germinated flour.

At the end of the experimental period (28 days), animals were fasted overnight, anesthetized with diethyl ether and sacrificed. Blood samples were collected in a clean dry centrifuge tube containing 10g EDTA/Liter prior to sacrifice. Plasma was separated by centrifugation at 4000 rpm for 10 min at room temperature.

The liver, kidney, heart, lungs, and spleen of each animal were removed, dried by filter paper and weighed and kept in 10% formaldehyde solution until used in histopathology analysis.

Liver and kidney functions

Alanine amino transferase (ALT) and aspartate amino transferase (AST) enzymes were measured according to the methods described by Reitman and Frankel (1957). Albumin was determined calorimetrically by the method described by Dumas et al. (1971) Serum urea and creatinine were measured calorimetrically according to the methods of Fawcett and Scott (1960) and Siest et al (1985), respectively.

Histopathology examinations

Small specimens of the organs liver were taken from each experimental group, fixed in neutral buffered formalin, dehydrated in ascending concentration of ethanol (70, 80 and 90%), cleared in zylene and embedded in paraffin. Sections of 4–6µm thickness were prepared and stained with hematoxylin and eosin according to Harris (1988).

Statistical analysis

The data were analyzed using a completely randomized factorial design (1988) when a significant main effect was detected, the means were separated with the Student-Newman-Keuls Test. Differences between treatments at 5% ($p \leq 0.05$) level were considered significant.

RESULTS AND DISCUSSION

Protein quality of casein and *Jatropha curcas* seed flour as measured by protein efficiency ratio (PER), corrected protein efficiency ratio (CPER), net protein ratio (NPR), apparent digestibility (AD), true digestibility (TD), biological value (BV) and net protein utilization (NPU) are presented in Tables (1 and 2). The mortality was occurring in all rats fed Jatropha seed flour diets with the exception of the rats fed detoxified diet. This indicated that roasting, autoclaving and germination treatments did not affect the toxicity of *Jatropha curcas* seed flour. However, detoxification treatment improved the protein quality of Jatropha seed flour. This improvement could be attributed to eliminate the antinutritive and indigestible factors and / or denaturation of protein.

Data presented in Table (1) showed that rats fed the casein diet had higher ($p \leq 0.05$) food intake, protein intake, weight gain, PER, CPER and NPR than rats fed detoxified Jatropha seed flour diet. The weight gain was higher ($p \leq 0.05$) in the rats fed casein diet (58.40g) than rats fed detoxified Jatropha seed flour (44.02g). The

Table 2. Protein digestibility, biological value and net protein utilization of raw and treated *Jatropha curcas* seed flours as compared to casein.

Treatments	AD (%)	TD (%)	BV (%)	NPU (%)
Casein	92.24[a] ± 8.03	93.32[a] ± 8.21	91.71[a] ± 6.30	85.58[a] ± 3.44
Detoxification[1]	83.93[b] ± 3.01	86.16[b] ± 3.22	78.97[b] ± 2.03	69.47[b] ± 1.62
Raw	_	_	_	_
Roasting	_	_	_	_
Autoclaving	_	_	_	_
Germination	_	_	_	_
LSD	3.614	3.436	2.028	1.930

[1]Treating with ethanol 90% for 2 hours followed by autoclaving in 0.07% sodium bicarbonate at 121°C for 20 min.
Mean in the same column with different letters are significantly different at ($p \leq 0.05$).

reduction in weight gain of rats fed detoxified Jatropha seed flour could be not due to lower feed intake but to lower protein utilization (Tables 1 and 2). The weight gain of detoxifying Jatropha seed flour was in agreement to soybean flour (46.67g/28 day) reported by Machado et al (2008).

The PER of detoxifying Jatropha seed flour was 87.33% of the casein. This value is higher than those reported by Machado et al (2008) for soybean flour (72.59%) and Mansour (1996) for germinated chick pea flour (83%). These results agree well with those reported by Makkar and Becker (1999). They found that the PER for heated (autoclaving at 121°C for 45 min) Jatropha seed flour of a non-toxic provenance containing diet was 86% of the casein diet. Similar trends were observed for CPER and NPR.

Data in Table (2) showed that rats fed the casein diet had higher ($p \leq 0.05$) AD, TD, BV and NPU than rats fed detoxified Jatropha seed flour diet. The TD of detoxifying Jatropha seed flour was comparable with heat treated (autoclaving at 121ºC for 25 min) soybean flour (89.56%), (Machado et al., 2008) and germinated chick pea flour (83.11%), (Mansour, 1996). The TD of detoxifying Jatropha seed flour was 92.33% of the casein, and higher than that reported by Machado et al (2008) for heat treated soybean flour.

The biological value of detoxifying Jatropha seed flour was comparable with germinated chick pea flour (75.20%) reported by Mansour (1996). The BV of detoxifying Jatropha seed flour represented 86.11% of casein. Mansour (1996) reported that the biological value of germinated chick pea flour was 90.78%. The NPU of detoxifying Jatropha seed flour (69.47%) was much higher than that reported for heat treated soybean (40.78%) by Machado et al (2008) and comparable with germinated chick pea flour (64.60%) reported by Mansour (1996).

Data in Table (3) shows the mean values of organ weights of rats fed casein diet and rats fed detoxified Jatropha seed flour. There were no significant ($p > 0.05$) differences in liver and spleen weights between rats fed casein diet and those fed detoxified Jatropha seed flour diet. Rats fed detoxified Jatropha seed flour had higher ($p \leq 0.05$) heart and kidney weights and lower lung weight than those fed casein diet. These differences were not enough to cause any side effects on the tissues of these organs (Figures 2, 3 and 4) and kidney functions (Table 5).

The higher heart and kidney weights observed in this study may be due to the deficiency of essential amino acids in Jatropha seed flour proteins (Rakshit et al., 2008; Rakshit and Bhagya, 2008). Similar observations were made in the case of detoxified castor proteins (Puttaraj et al., 1994) However, high kidney weights in rats fed with field bean and navy bean diets have been attributed to low availability of essential amino acids (Ramamani, 1976)

Mean in the same column with different letters are significantly different at ($p \leq 0.05$).

Data in Table (4) showed the mean values of serum albumin, ALT and AST in rats fed casein and rats fed detoxified Jatropha seed flour. Serum albumin of casein group was slightly higher than detoxified Jatropha group but no significant ($p > 0.05$) differences were observed between them.

Rats fed casein had lower ($p \leq 0.05$) ALT and AST

Table 3. Body, lungs, heart, kidney, liver, and spleen weights of rats fed casein and *Jatropha curcas* seed flours for 28 days.

Treatments	Organ weights (g)					
	Body	**Lungs**	**Heart**	**Kidney**	**Liver**	**Spleen**
Casein	63.5[b] ± 0.92	2.89[a] ± 0.14	0.68[b] ± 0.012	0.27[b] ± 0.009	0.51[a] ± 0.011	0.38[a] ± 0.011
Detoxification[1]	67.5[a] ± 0.97	2.37[b] ± 0.13	0.73[a] ± 0.015	0.31[a] ± 0.011	0.51[a] ± 0.013	0.37[a] ± 0.015
Raw	–	–	–	–	–	–
Roasting	–	–	–	–	–	–
Autoclaving	–	–	–	–	–	–
Germination	–	–	–	–	–	–
LSD	1.252	0.113	0.036	0.023	0.024	0.036

[1]Treating with ethanol 90% for 2 hours followed by autoclaving in 0.07% sodium bicarbonate at 121°C for 20 min.
Mean in the same column with different letters are significantly different at ($p \leq 0.05$).

Table 4. Liver functions of rats fed casein and *Jatropha curcas* seed flours.

Treatments	Serum albumin (mg/dl)	ALT (u/l)	AST (u/l)
	Normal range (3.8 - 4.7)	**Normal range (25 - 45)**	**Normal range (50 - 90)**
Casein	3.9[a] ± 0.02	25[b] ± 0.11	69[b] ± 0.17
Detoxification[1]	3.8[a] ± 0.04	30[a] ± 0.10	76[a] ± 0.20
Raw	-	-	-
Roasting	-	-	-
Autoclaving	-	-	-
Germination	-	-	-
LSD	0.226	1.133	3.584

[1]Treating with ethanol 90% for 2 hours followed by autoclaving in 0.07% sodium bicarbonate at 121°C for 20 min.
Mean in the same column with different letters are significantly different ($p \leq 0.05$).

than those of rats fed detoxified Jatropha seed flour. ALT and AST in rats fed detoxified Jatropha seed flour were within the normal and safe range. This indicates no danger or risk for using Jatropha seed flour treated by detoxification in the human diet from the health point of view.

Data in Table (5) showed no significant ($p > 0.05$) differences between rats fed casein and rats fed detoxified Jatropha seed flour on the content of serum creatinine and blood urea. Urea is the end product of protein metabolism; an increasing in blood urea level usually indicates renal failure although it may also result from dehydration, gastrointestinal bleeding, congestive heart failure, high protein intake and insufficient renal blood supply (Lee and Nieman, 2003). Elevated blood urea is referred to azotemia. Decreased in blood urea can result from liver disease.

Measurement of serum creatinine, like measurement of blood urea is used for evaluating renal functions, elevated serum creatinine levels are seen when 50% or more of the kidneys nephrons are destroyed. There was not any significant ($p \leq 0.05$) difference between diets containing casein and that containing detoxified Jatropha seed flour on the functionality of kidney of both groups of rats (Table, 5).

Figures (1, 2, 3, 4 and 5) represent the gross examination and histopathological of vital organs of rats fed casein diet and rats fed detoxified Jatropha seed flour. Gross examination of vital organs of rats fed detoxified Jatropha seed flour did not show any atrophy compared to rats fed casein. Liver tissues were normal showing, normal hepatic parenchyma cells with distinct nuclei and normal eosinophilic cytoplasm with normal sinusoids (Figure 1). Kidney displayed normal renal architecture with normal glomerulus, proximal tubules and collecting ducts (Figure 2). The appearance of the heart muscle was normal (Figure 3). Lungs showed no histopathological changes (Figure 4). Spleen revealed no

Table 5. Kidney functions of rats fed casein and *Jatropha curcas* seed flours.

Treatments	Serum creatinine (mg/dl)	Blood urea (mg/dl)
	Normal range (0.5 - 1.0)	Normal range (15 - 25)
Casein	0.5[a] ± 0.02	22[a] ± 0.21
Detoxification[1]	0.7[a] ± 0.06	24[a] ± 0.18
Raw	-	-
Roasting	-	-
Autoclaving	-	-
Germination	-	-
LSD	0.227	3.473

[1]Treating with ethanol 90% for 2 hours followed by autoclaving in 0.07% sodium bicarbonate at 121°C for 20 min.
Mean in the same column with different letters are significantly different at ($p \leq 0.05$).

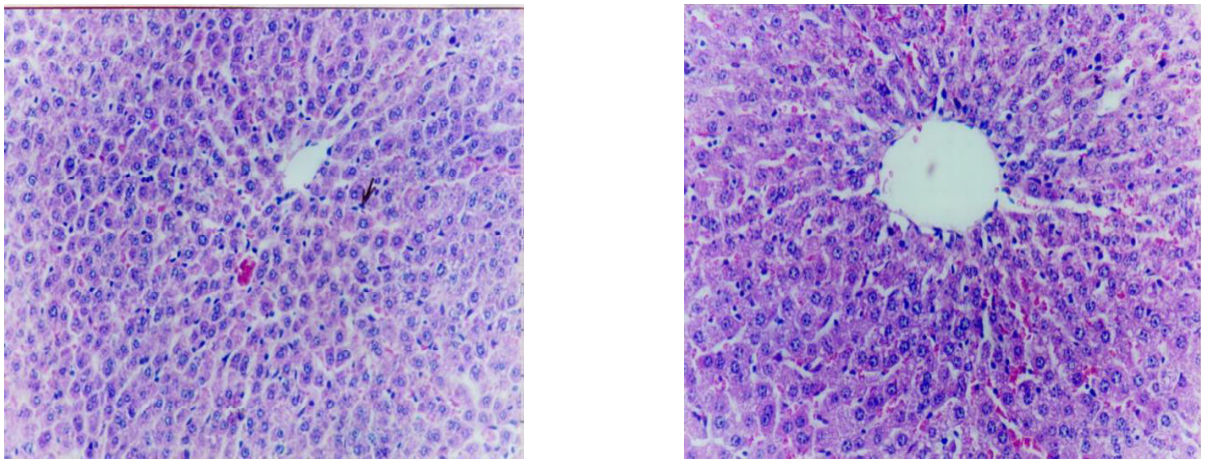

Figure 1. Effect of detoxification treatment on the histology structure of liver of rats.

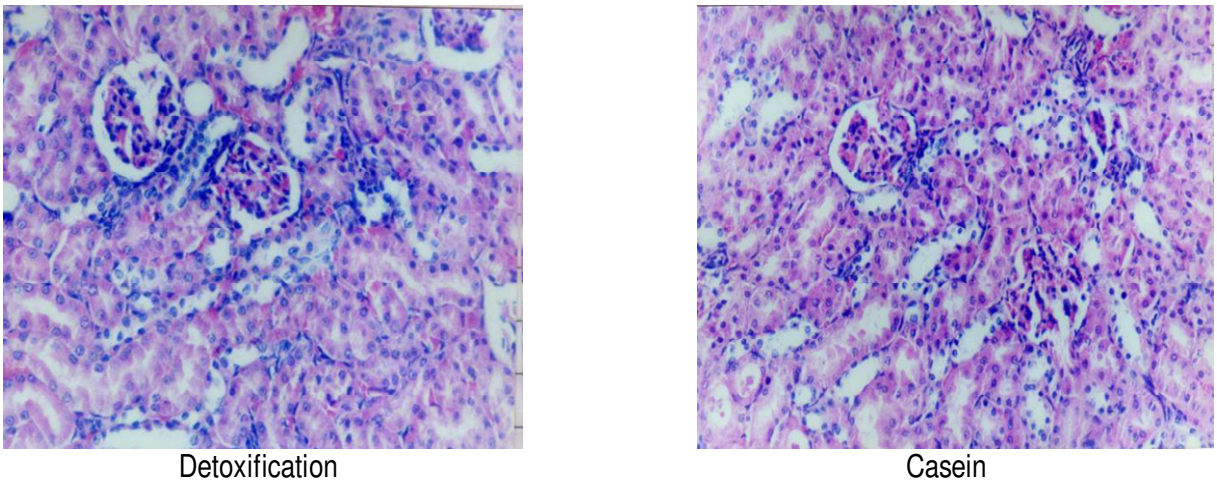

Figure 2. Effect of detoxification treatment on the histology structure of kidney of rats.

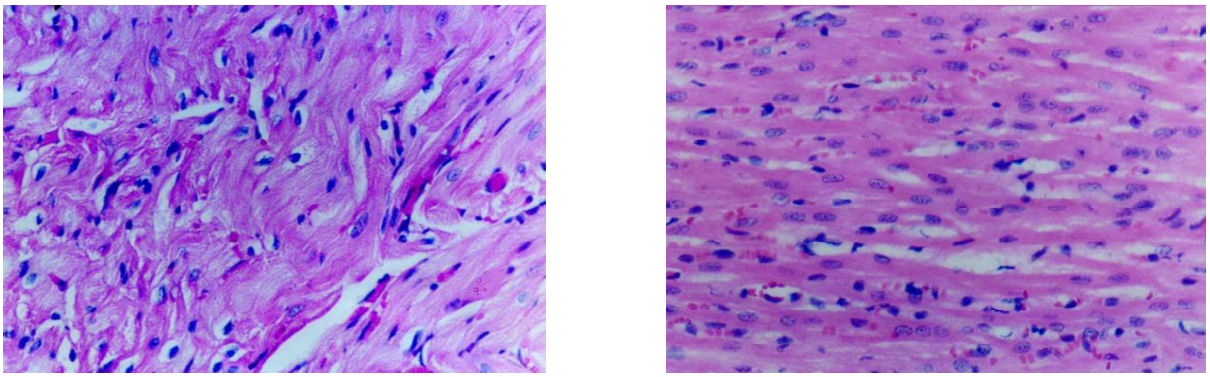

Figure 3. Effect of detoxification treatment on the histology structure of heart of rats.

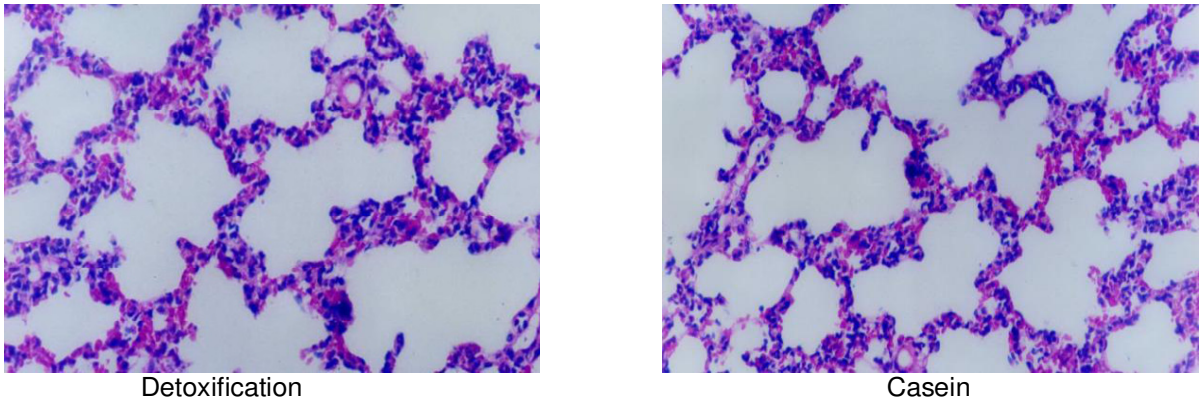

Figure 4. Effect of detoxification treatment on the histology structure of lung of rats

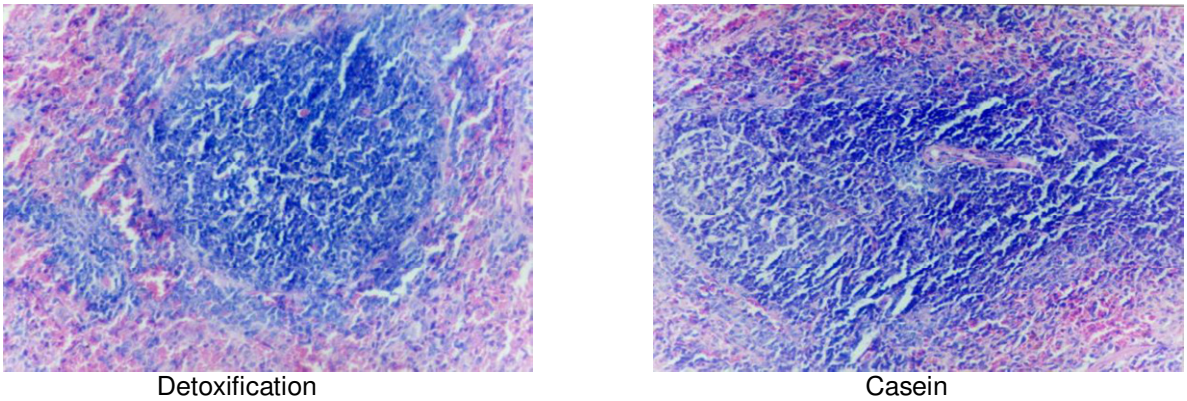

Figure 5. Effect of detoxification treatment on the histology structure of spleen of rats

histopathological changes with normal hymphoid follicles (Figure 5).

According to our findings regarding to the histopathology examination which proved that all organs and tissues showed no related microscopic changes suggesting that the mortality of rats occurred due to lack of food intake, diarrhea and emaciation. Rakshit et al. (2008) have reported that microscopic findings of various tissues/organs of rats fed Jatropha flour did not exhibit any related microscopic changes. Adam (1974) has reported histological changes in various tissues/organs in rats fed 5–50% *Jatropha curcas*. They have further reported that inclusion of Jatropha in the diet at 5% level had no significant effect on various tissues/organs, and inclusion of meal at higher levels produced degenerative changes in various tissues/organs.

From the above results, it could be concluded that detoxified Jatropha seed flour had high quality protein comparable to chick pea and soybean which increase its potential uses as a protein supplement in several food items.

REFERENCES

Adam SEI (1974). "Toxic Effects of *Jatropha curcas* in Mice," J. Toxicol., Vol. 2, No. 1, pp. 67-76.

Adam SEI, Magzoub M (1975). "A toxicity of *Jatropha curcas* for Goats," J. Toxicol, Vol. 4, No. 3, pp. 347–35.

Adolf W, Opferkuch HJ, Hecker E (1984). "Irritant Phorbol Derivatives from Four Jatropha Species," J. Phytochem., Vol.23, No. 1, pp. 129–132.

Ahmed OM, Adam SE (1979). "Effects of *Jatropha curcas* on Calves," Vet Pathol, Vol. 16, pp. 476–482.

AOAC (1995). "Official Methods of Analysis, 16th edn. Washington, DC: Association of Official Analytical Chemists".

Dumas BT, Watson WA, Biggs HG (1971). "Quantitative Colorimetric Determination of Albumin in Serum or Plasma," Clin. Chem Acta, Vol. 31, pp. 87-91.

Eggum BO (1973). "A study of Certain Factors Influencing Protein Utilization in Rats and Pigs," Copenhagen: National Institute of Animal Science, Report No. 406,

FAO/WHO, "Protein quality evaluation. Report of a joint FAO/WHO expert Consultation FAO/WHO, Rome ", 1990.

Fawcett JK, Scott JE (1960). "Determination of Urea (Urease modified Berthelot reaction)," J. Clin. Pathol, Vol. 13, pp. 156-159.

Harris HE (1988). "Cited by Carleton MA, Drury RA, Wallington EA, Cameron H 4th Ed., Oxford Univ. Press, New York, Toronto".

Machado FP, Queiroz JH, Oliveira MG, Piovesan ND, Peluzio MC, Costa NM, Moreira MA (2008). "Effect of Heating on Protein Quality of Soybean flour Devoid of Kunitz Inhibitor and Lectin," J. Food Chem., Vol. 107, No.2, pp. 649-655.

Makkar HP, Becker K (1999). "Nutritional Studies on Rats and Fish (*Carp cyprinus carpio*) Fed Diets Containing Unheated and Heated *Jatropha curcas* meal of A nontoxic Provenance," Plant Foods for Human Nutrition, Vol. 53, pp. 183-192.

Makkar HPS, Becker K (1997). "*Jatropha curcas* Toxicity: Identification of Toxic Principles," Fifth International Symp., San Angelo, Texas, USA.

Makkar HPS, Becker K, Sporer F, Wink M (1997). "Studies on Nutritive Potential and Toxic Constituents of Different Provenances of *Jatropha curcas*," J. Agric Food Chem., Vol. 45, No. 11, pp. 3152–3157.

Mansour EH (1996). "Biological and Chemical Evaluation of Chick Pea Seed Proteins As Affected by Germination, Extraction and α-Amylase Treatment," Plant Foods for Human Nutrition, Vol. 49, , pp. 271-282.

Mansour EH, Rahma EH, Shirin MH (2010). "Physico-Chemical Properties and Evaluation of *Jatropha curcas* Seeds," Minufiya J. Agric. Res., Vol. 35, pp. 911-926.

Martínez-Herrera J, Siddhuraju P, Francis G, Davila-Ortiz G, Becker K (2006). "Chemical Composition, Toxic/Antimetabolic Constituents, and Effects of Different Treatments on their Levels, in Four Provenances of *Jatropha curcas L.* from Mexico," J. Food Chem., Vol. 96, No. 1, pp. 80-87.

Puttaraj S, Bhagya S, Narasimha MK, Singh N (1994). "Effect of Detoxification of Castor Seed (*Ricinus communis*) Protein Isolate on Its Nutritional Quality," Plant foods for Human Nutrition, Vol. 46, pp. 63-70.

Rakshit KD, Bhagya S (2007). "Effect of Processing Methods on the Removal of Toxic and Antinutritional Constituents of Jatropha meal: A potential Protein Source," J. Food Sci. Technol., Vol. 3, No. 1, pp. 88–95.

Rakshit KD, Bhagya S (2008). "Biochemical and Nutritional Evaluation of Jatropha Protein Isolate Prepared by Steam Injection Heating for Reduction of Toxic and Antinutritional Factors," J. Sci. Food Agric., Vol. 88, No.3, pp. 911–919.

Rakshit KD, Darukeshwara J, Rathina RK, Narasimhamurthy K, Saibaba P, Bhagya S (2008). "Toxicity studies of Detoxified Jatropha meal (*Jatropha curcas*) in Rats," J. Food Chem. Toxicol., Vol. 46, No. 12, pp. 3621-3625.

Rakshit KD, Madhvi D, Bhagya S (2006). "*Jatropha curcas* – A potential Source of Protein in Food/Feed," Rural Food and Nutrition Symp., Organized by Sole Company and Central Food Technological Research Institute, Mysore, India.

Ramamani S (1976). Studies on the Factors Affecting the Nutritive Value of Field Bean (Dolichos lablab). Ph.D. Thesis," India, Mysore: University of Mysore.

Reitman S, Frankel S (1957). "A colorimetric Method of the Determination of Serum Glutamic. Oxaloacetic and Glutamic Pyruvic Tranasaminase," Am. J. Clin. Path, Vol. 28, No. 1, pp. 57-63.

SAS (1985). SAS Users Guide: Basics Version 5th edn Cary. NC: SAS Institute Inc, USA.

Siest G, Henny J, Schiele F, Young DS (1985). Kinetic Determination of Creatinine," Interpretation of clinical Laboratory Tests, pp. 220-234.

Stripe F, Pession-brizzi A, Lorenzoni E, Stroccji L, Montanaro L, Sperti S (1976). Studies on the Proteins from the Seeds of *Croton tiglium* and of *Jatropha curcas*," J. Biochem., Vol. 156, pp. 1–6.