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Chemical and Pathological Evaluation of *Jatropha Curcas* Seed Meal Toxicity With or Without Heat and Chemical Treatment

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Abstract: This study aimed to identifythe suitable detoxification method used to reduce the anti nutritional factors in Egyptian *Ja stropha curcas (J. curcas)* seed meal. For this purpose, moist heat, 0.07% NaHCO3, 4% NaOH and 4% NaOH plus methanol extraction was used. Nutritive values of whole seed were 27% protein, 30% oil, 5% ash, 4% fiber and 28% carbohydrate. Anti nutritional factors were determined in the whole seed to be 11 mg/100g phytic acid, 19 mg/g trypsin inhibitors, 1.5 mg/100g total phenols and 3.5 g/100g total saponins and were also determined in the treated *J.curcas* meals. Diet was treated with 4% NaOH plus moist heat giving the best reduced percentage of anti nutritional factors especially phytic acid, trypsin inhibitors and total phenols. Albino rats were used as an animal model to investigate the nutritional, biochemical and pathological alterations of the untreated and treated *J. curcas* meal. Wide nutritional, biochemical, cellular effects were developed in rats fed on untreated diet. Insignificant alterations were obtained in other tested groups fed on variable treated diets, especially with moist heat and 4% NaOH. This will confirm the efficiency of this detoxification method to decrease the deleterious effects induced by toxins present in untreated meals.

Key words: Jastropha curcas seed meal; anti nutritional factors; hematological; nutritional; biochemical and cellular effects.

INTRODUCTION

Jatropha curcas L. is a plant newly introduced to adapt in Egypt. It is well known as the physic nut, purging nut and Habb-El-Meluk. It belongs to Euphorbiaceae Family. It grows quickly and survives in poor stony soils. It is resistant to drought and diseases, reaches a height of 3-8 m. It can be grown on wastelands, and marginal agricultural lands where no irrigation facility is available (Herrera *et al.,* 2006). In tropical countries, it is used for its medicinal properties as purgative oil. It is also used as a live hedge (not browsed by livestock). It is propagated using branch cuttings or by direct seeding, is yielded up to 5 tons/ha (Heller, 1996). *J. curcas* is a new oil crop in Egypt, used for increasing the local planted production (EL-Gamasy, 2008 and MSEA, 2008). The seeds contain about 30-35 % oil, which can be used as a fuel directly or in its transesterified form as a substitute for diesel (Francis *et al.,* 2005). In Egypt, *Jatropha* is cultivated for the production of biodiesel. The planted area with *J. curcas* was about 100 ha in the period of 2004-2005. It was increased to 700 ha in 2007 by rate of 175 %; about 70 ha were planted with *J. curcas* on treated wastewater in Luxor (MSEA, 2008). At present, the seed meal is used as a fertilizer, the selling price of which is approximately 25% that of the groundnut or soy meal, on protein equivalent basis. The seeds and seed oil are toxic to humans and animals, for that reason nutritional utilization is impossible. A crucial obstacle in the establishment of *J. curcas* as a commercial crop would be overcome by detoxifying the seeds/seed oil (Haas and Mittelbach, 2002). Indirect exposure to such plants take place through consumption of animal products polluted with toxic phorbol esters, such as honey collected by bees (Sosath *et al.,* 1988), meat (fish or game) captured in primitive hunting that is rendered toxic by plant materials. And also meet and milk produced from the animals that feed on diets contaminated with these toxic components (Zayed *et al.,* 1998). The biological effects of these compounds in addition to tumors promotion, bring about a wide range of biochemical and cellular effects, alter cell morphology, serve as lymphocyte mitogens and induce platelet aggregation (Blumberg, 1980 and 1981).

Under the prevailing situations, it is evident that development of a low-cost detoxification process, and use of the detoxified *Jatropha* seed meal as livestock feed will provide economic sustainability to the *Jatropha* based oil productions system (Francis *et al.,* 2005). If the plans for cultivation of *Jatropha* in different countries are realized, *Jatropha* seed meal, after detoxification, has a high potential to be a competitor of the soybean meal in the international feed ingredient markets (Lan, 2006). Phorbol esters (phorbol-12-myristate 13-acetate) have been identified as the major toxic principle in *Jatropha* (Makkar *et al.,* 1997; Makkar and Becker, 1998). Phorbol esters are bioactive diterpene derivatives that have a multitude of effects in cells. Major anti nutrients present in *Jatropha* seed/seed meal are trypsin inhibitors (Goel *et al.,* 2007). The trypsin inhibitors and lectin present in the seed meal from the toxic variety could be inactivated by heat treatment. The challenge is to remove phorbol esters from the seed meal. The removal of phorbol esters would transform *Jatropha* meal into a

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highly nutritious and high value feed ingredient for monogastrics, fish and ruminants (Hass and Mittelbach, 2000). The residual protein-rich seed cake, remaining after extraction of the oil, could form a protein-rich ingredient in feeds for poultry, pigs, cattle and even fish, if it could be detoxified (Martinez-Herrera *et al.,* 2006). There are various physical and chemical ways to destroy these phorbol esters in feeds, which need investigations on optimization of conditions, and testing of the treated material using rat and fish as experimental models. This has to be followed by nutrient utilization, production, animal and product safety studies. Histopathological studies also need to be an integral part of the studies for establishing the non-toxic nature of the detoxified products (Goel *et al.,* 2007).

The present research aims to study the nutritional quality and the efficiency of various detoxification methods; including hydrothermal processing technique and chemical treatment with NaHCO3 or NaOH to inactivate the anti nutritional factors in defatted *Jatropha curcas* meal. As well as studying the biochemical and pathological effect of the untreated and treated meal on albino rats as an experimental model to achieve the best detoxification method of the *Jatropha* seed meal to use in nutrition of monogastric livestock as alternative food.

MATERIAL AND METHODS

Processing of the Toxic Seed for the Meal:

Jatropha curcas seeds were collected from *Jatropha curcas* Forest (Experimental Farm, Faculty of Agriculture, Assiut branch, AL-Azhar University, Egypt). The seeds were ground, defatted using petroleum ether (40-60^{o}C) and referred as the *Jatropha curcas* seed meal **(a)**.

Preliminary Analysis:

Preliminary analysis of *Jatropha curcas* seed meal for chemical composition was carried out according to A. O. A. C. (1990). The collected data were statistically analyzed according to Snedecor and Cochran (1982).

Detoxification Procedures:

The following treatments were carried out according to Aregheore *et al.,* (2003) with some modifications:-

Moist Heat Treatment:

Approximately, 300 g of the defatted meal was mixed with distilled water to 66% moisture. The mixture was made into a paste, covered with aluminum foil and placed in an autoclave at 121^{0}C for 30 minutes. The autoclaved sample was dried at 25^{0}C for 5 h in a fan oven apparatus. It was milled to produce the meal **(b)**.

Treatment with Methanol + Sodium Hydroxide:

300 g of the defatted meal was mixed with 4 % sodium hydroxide (NaOH) and extracted with 80% methanol (3X1L). The residual meal was dried, milled and labeled **(c)**.

Treatment with Sodium Hydroxide + Heat:

300 g of the defatted meal was mixed with 4 % sodium hydroxide (NaOH) to form a paste, heat treated as in (1) and labeled **(d)**.

Treatment with Sodium Bicarbonate + Heat:

The defatted meal was mixed with 0.07% $NaHCO_3$ solution to form a paste. The paste was heat treated, dried, milled as described in treatment (1) and assigned as **(e)**.

Determination of Antinutritional Factors:

Phytic Acid Determination:

Phytic acid was determined by a calorimetric procedure as described by Vaintraub and Lapteva, (1998).

Total Saponin Determination:

Total saponin content was determined using a spectrophotometric method as described by Hiai *et al.,* (1976).

Total Phenol Determination:

Total phenols were determined by colorimetric methods as described by Makkar *et al.,* (1993).

Trypsin Inhibitor Activity Determination:

Trypsin inhibitor activity was determined as described by Smith *et al.,* (1980) except that the enzyme was added in the last, after inhibitor is mixed with the substrate, as suggested by Liu and Markakis, (1989).

Animal and Treatment:

Total of 30 male albino rats were used, weight about 100-150 gm at the start of an experiment. Rats were obtained from the Laboratory Animal House, Faculty of Medicine, Assiut University, Egypt. The animals were used in accordance with guidelines on the care and use of laboratory animals. The ethical committee of Veterinary Medicine Assiut University, Egypt approved the study. They were maintained on a basal diet, given to tap water *ad libitum* and housed in cages with wood chips for bedding in an air-conditioned room at 24-25^{0}C and 55% relative humidity with a 12 h light / dark cycle. They were kept acclimatizing to laboratory conditions two weeks before starting the experiment. Rats were randomly divided into six equal groups (5 each) A, B, C, D,E and control. Rats were given 200 gm / kg ration daily of *Jatropha* seed as meal **(a), (b)**, **(c)**, **(d)** and **(e)**, respectively, for one month. Another five rats were fed on basal diet and kept as the control group. The initial body weights of the rats were recorded at the beginning, end of the experiment and were used to compute weight gain/loss.

Sample Collection:

At the end of the experiment, rats were sacrificed under anesthesia with ether vapor. The whole blood was collected into tubes containing EDTA for different hematological parameters. A second blood fraction was collected without anticoagulant and centrifuged at 4000X g for 10 min for serum separation. Liver, intestine, lung, spleen, kidney and testes were immediately excised, fixed in 10% neutral buffer formalin and were processed for light microscopy.

Determination of Hematological Parameters:

Red blood cells were counted, and other hematological parameters were analyzed by the automated parameter hematology analyzer (MICROS 60-Abx Diagnostics, Montpellier, France).

Biochemical Analysis:

Serum was used to determine total protein and albumin by the colorimetric method according to Doumas, (1971). Gamma glutamate transferase was measured according to Tietz, (1994). Alkaline phosphatase was measured according to Rec, (1972). Serum was used to determined creatinine level according to Sies *et al.,* (1985**)** and uric acid according to Tietz, (1990).

Histopathological Study:

Samples from liver, kidney, intestine, lung, spleen and the testes were obtained from all experimental animals and fixed in neutral buffer formalin. Fixed tissues were processed routinely for paraffin embedding technique. Embedding tissues were sectioned at 3um and stained with hematoxylin and eosin (Bancroft *et al.,* 1996). Slides were submitted for light microscope examination to detect the pathological lesions.

Statistical Analysis:

The data were analyzed by using SPSS 11.0 for Windows. The significance of differences was calculated by using one-way analysis of variance (ANOVA) followed by Tukey's procedure for multiple comparisons. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSIONS

The Physical Characteristics of J. Curcas Seeds:

The seed characters are presented in table (1). The kernel and the shell represent 67.5 and 34.5%, respectively of the seed weight.

Table 1: Physical characteristic of *J. curcas* seeds.

Seed characters	Percentage % ± SD
Seed weight (g)	0.68 ± 0.3
Kernel weight (g)	0.46 ± 0.2
Shell weight (g)	0.22 ± 0.3
Kernel percentage	67.52 ± 2.33
Shell percentage	32.48 ± 1.15

The Preliminary Chemical Analysis of J. Curcas Seeds:

Table (2) shows the chemical structure of *Jatropha curcas* seed. After oil extraction, the seed has high nutritional values of protein, ash, fibers, carbohydrates and oil, which are the main component the shrub is planted for.

Table 2: Preliminary chemical analysis of raw *J. curcas* seed.

Components	Percentage % ± SD		
	Whole seeds	Kernel	Shells
Moisture	5.15 ± 0.02	4.06 ± 0.02	4.50 ± 0.03
Protein	27.55 ±1.08	30.01 ± 1.28	3.42 ± 0.02
Oil	30.82 ±0.18	46.18 ± 0.71	1.31 ± 0.01
Ash	5.60 ± 0.03	4.30 ± 0.20	6.43 ± 0.08
Fiber	3.77 ± 1.15	1.48 ± 0.10	79.77±0.03
Reducing Sugars	14.10 ±0.04	12.00 ± 0.97	1.68 ±0.05
Non-reducing Sugars	10.01 ±1.53	2.00 ± 0.05	2.89 ± 0.04
Total Sugars	28.11 ±0.03	14.00 ± 0.08	4.57 ± 0.03

Determination of Antinutritional Factors:

In Raw Seeds Without Treatment :

Anti nutritional factors of *Jatropha curcas* seed meal (a) of whole kernels and shells from raw seeds were recorded in (Table 3).

Table 3: Anti-nutritional factors of whole, kernels and shells from raw seeds.

Anti-nutritional factors	Values ± SD (on dry weight basis)		
	Whole seeds	Kernel	Shells
Phytic acid (mg/100g)	11.25 ± 0.85	15.37±0.25	7.50 ± 0.03
Trypsin inhibited activity (mg/g)	18.89± 1.02	23.26±0.03	9.83 ± 0.08
Total Phenols (mg/100g)	1.51 ± 0.65	2.13 ± 0.14	1.0 ± 0.42
Saponins (g/100g)	3.50 ± 0.02	3.93 ± 0.04	0.65 ± 0.03

Detoxification by Moist Heat and Chemical Treatments:

Phytic acid content was affected by all applied treatments (b, c, d and e) where their content is 11.15, 11.24, 10.56 and 11.25mg/100g, respectively. Trypsin inhibitor activity content was reduced by all treatments (b, c, d and e) to level of 0.65, 0.82, 0.75 and 1.33 mg/g, respectively. Total phenols content had been reduced by (a, b, c and d) treatments to be 1.948, 1.40, 0.42 and1.5 mg/100g, respectively. Total saponin content was slightly reduced by b, c, d and e treatment to level of 3.33, 3.04, 3.15 and 1.75 g/100g, respectively (Table 4).

Table 4: Anti-nutritional factors (mg/100g) as affected by heat and chemical treatment.

Anti-nutritional factors	Treatments			
	Sample (b)	Sample (c)	Sample (d)	Sample (e)
Phytic acid	11.15	11.24	10.56	11.25
Trypsin inhibitor	0.65	0.82	0.75	1.33
Total phenols	1.48	1.40	0.42	1.51
Total saponins	3.33	3.04	3.15	1.75

Body Weight and Mortality:

There was no mortality during the experimental period. The results of the body weight gain (gm) showed a significant (P <0.05) decrease than control in all tested groups except in group D (Table 5).

Table 5: Body weight at the start and the end of the experiment of exposed groups A, B, C, D,E and control rats.

	Body weight at start gm	Body weight at end gm
A	121±7.4	161±10.25*d
B	120±7.4	165±10.25*d
C	113±15.2	160±12.25*d
D	122±8.4	200±21.21abc
E	115 ±6.7	174±32.09*d
Control	124±13.0	224±25.10

Data are expressed as means ± S.D. of five animals per group.*denotes P < 0.05 as compared to control group, a denotes P < 0.05 as compared to group A. b denotes P < 0.05 05 as compared to group B. c denotes P < 0.05 as compared to group C. d denotes P < 0.05 05 as compared to group D. e denotes P < 0.05 05 as compared to group e. (One- way ANOVA/Duncan).

Hematological Indices:

A significant (P <0.05) decrease in red blood cell count (106 /ml), blood hemoglobin concentration (g/dl), hematocrit (%), total and differential leucocytic count (10 ³ /ml) and (%) was obtained in exposed groups A, B, C, D and E in comparison with control rats (Table 6).

Biochemical Analysis:

A significant (P <0.05) reduction in total protein and albumin concentration (g\dl) was obtained in the serum of rates in groups A, B, C, D and E than the control. A significant (P <0.05) elevation in alkaline phosphatase and gamma- GT concentration (U/I) was recorded in the serum of rates in groups A, B, C and D

than the control. No significant difference of creatinine and uric acids levels (mg\dl) in the serum of tested rats was recorded (Table 7).

Tabl 6: Hematological indices from exposed groups A, B, C, D,E and control rats.

	RBCs (106 /ml)	Hg (g/dl)	HCT (%)	MCV (fl)	MCH (pg)	MCHC
A	6.40 ±2.6*d	10.98±4.48*bcd	31.15±1.51*cde	50.90±2.9*cde	17.96±1.2*b	35.06±2.7*
B	7.00 ±0.6*d	11.55±3.43*cd	32.12±1.42*	51.70±2.5*	18.96±1.0 ac	34.06±0.5*
C	7.14±0.5*d	12.62±0.90 a	32.16±2.83 *ad	53.96±0.6*a	17.92±0.4*	34.14±0.35*
D	7.66 ±0.57*	12.52±1.50 a	35.42±4.75abce	54.52±3.4*abe	19.76±0.9 ac	34.44±0.62*
E	6.82 ±0.62*d	11.72±1.13*	33.26±3.76*ad	57.16±3.3*abcd	20.24±1.0	35.40±0.80*
Control	8.50 ±0.79	13.06±1.31	35.80±0.67	48.72±1.2	19.20±0.8	33.40±2.07

Data are expressed as means ± S.D. of five animals per group.*denotes P < 0.05 as compared to control group, a denotes P < 0.05 as compared to group A. b denotes P < 0.05 05 as compared to group B. c denotes P < 0.05 as compared to group C. d denotes P < 0.05 05 as compared to group D. e denotes P < 0.05 05 as compared to group e. (One- way ANOVA/Duncan).

Table 7: Total and differential leucocytic count (10 ³ /ml) and (%) from exposed groups A, B, C, D,E and control rats.

	Total leucocytes	Lymphocytes	Granulocytes	Monocytes
A	3.62±1.09*bcde	1.90 ±0.22*bcde	1.30±1.62*d	0.30±1.1*de
B	5.62±2.04*a	3.90 ±1.20*ad	1.50±0.82*d	0.30±0.2*de
C	5.70± 3.76*a	3.36 ±2.86*ad	1.82±0 .82*d	0.52±0.3*
D	6.38± 3.69*a	4.86 ±3.67*a	2.00± 0.80	0.68±0.25*ab
E	6.26± 1.44*a	4.56 ±1.34a	1.74±0.55*d	0.58±0.10*ab
Control	7.08± 0.90	5.86 ±1.51	2.20± 0.18	1.68±4.00

Data are expressed as means ± S.D. of five animals per group.*denotes P < 0.05 as compared to control group, a denotes P < 0.05 as compared to group A. b denotes P < 0.05 05 as compared to group B. c denotes P < 0.05 as compared to group C. d denotes P < 0.05 05 as compared to group D. e denotes P < 0.05 05 as compared to group e. (One- way ANOVA/Duncan).

Table 8: Biochemical parameters from exposed groups A, B, C, D,E and control rats.

	TP g\dl	Albumin g\dl	AP U\l	γ- Gt U\ l	Uric Acid mg\dl	Creatinine mg/dl
A	6.88±0.1*d	3.84±0.45*d	88.12±10*d	38.60±4.2*bcd	6.98±1.0	0.52±0.07
B	7.50±0.1*d	4.54±0.45*d	78.12±10*ad	36.80±5.2*	6.38±1.2	0.52±0.08
C	7.40±0.9*d	4.62±0.91d	76.88±21*ad	37.40±4.0	6.64±0.7	0.56±0.05
D	8.59±1.2*abce	4.88±0.31* abce	69.08±10abce	30.20±7.2 abce	6.96±0.3	0.54±0.05
E	7.16±0.5*d	4.02±0.67*d	78.92±10*ad	35.40±8.9*acd	6.76±1.5	0.52±0.10
Control	9.88±0.7	5.87±0.56	55.43±3	23.16±1.0	6.77±0.7	0.52±0.02

Data are expressed as means ± S.D. of five animals per group.*denotes P < 0.05 as compared to control group, a denotes P < 0.05 as compared to group A. b denotes P < 0.05 05 as compared to group B. c denotes P < 0.05 as compared to group C. d denotes P < 0.05 05 as compared to group D. e denotes P < 0.05 05 as compared to group e. (One- way ANOVA/Duncan).

Histopathology:

The main lesions were observed in the group A (without treatment), it is mainly congestion in the internal organ's vasculature. Liver showed congestion of the hepatic vasculature fig (1, 2). There were focal areas of leucocytic proliferation between the hepatic cells fig (3), the hepatic cells' showed vacuolar and fatty degeneration fig (4). Intestine showed an increasing of goblet cells number and degeneration of the intestinal epithelium. The lamina propria showed an increasing number of inflammatory cells specially the macrophages' cell fig (5), also the intestinal blood vessels were congested. Lung showed increase thickness of the alveolar septa with proliferating leucocytes (macrophages and lymphocytes). The alveolar blood vessels were congested with hemorrhage in some areas' fig (6), and the bronchial epithelium was degenerated into fig (7). Kidney showed light pathological changes with a mild increase in a cellularity of the glomeruli due to the increasing number of the mesangeal and endothelial cells with the presence of macrophages' cells fig (8). The tubular epithelium was necrosed, and the renal blood vessels were congested. The testes showed a decrease in the number of the spermatogenic cells in the somniferous tubule fig (9), congestion in the testicular blood vessels and intertubular edema were also observed. Spleen showed necrosis in the lymphocytes in the white pulp which appears as lyses in the lymphocytic area fig (10).

The histopathological lesions in groups B, C and E in which the *J.curcas* treated with (moist heat), (methanol and sodium hydroxide) and (sodium bicarbonate and heat), respectively, were similar to the lesions in the group A. Group D, treated with heat and sodium hydroxide, showed normal hepatocytes appearance fig (11,12). Lung showed normal blood vessels, alveolar septa and normal bronchial epithelium fig (13). The testes showed an increasing number of spermatogenic cells and intact somniferous tubules fig (14). The spleen of the rates in this group also was normal with an increase in the number of lymphocytes in the white pulp fig (15).

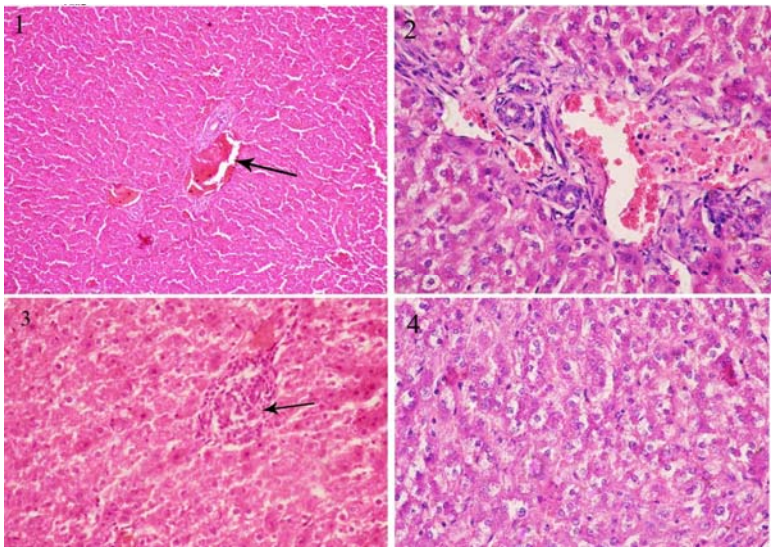

Fig 1: Liver sections of group **(A)** untreated *J.curcas* **(1-4)** showed congestion of the portal blood vessels X10 **(1)**, congested blood vessel X25 **(2**), leukocytic cell infiltration between the hepatic cells X10 **(3)** and hepatic cells with vacuolar and fatty degeneration X25 **(4)**.

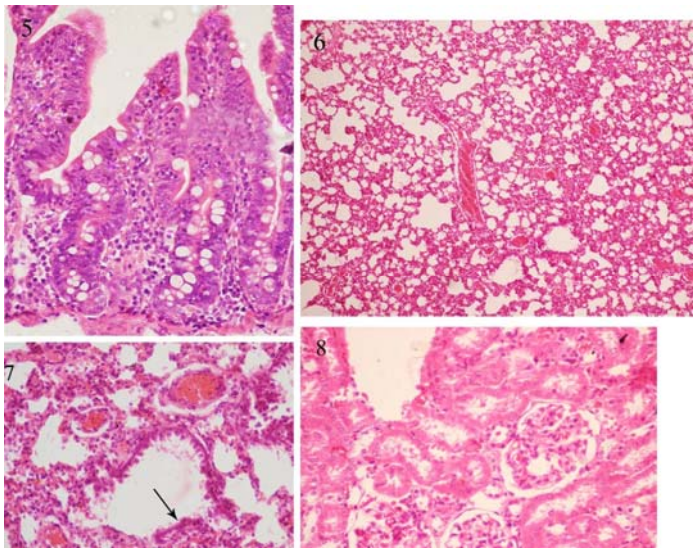

Fig 2: Group **(A)** of the untreated *J.curcas* **(5-8)** intestine showed an increasing number of goblet cells in the intestinal villi and infiltration of the lamina propria with macrophage cells and lymphocyte X25 **(5)**. Lung showed congestion in the pulmonary blood vessels and increasing thickness of the alveolar wall X10 **(6)**, degeneration in the bronchial epithelium X25 **(7)**. Kidney showed increasing a cellularity of the glomeruli with mesangeal, endothelial and macrophage cells X25 (**8)**.

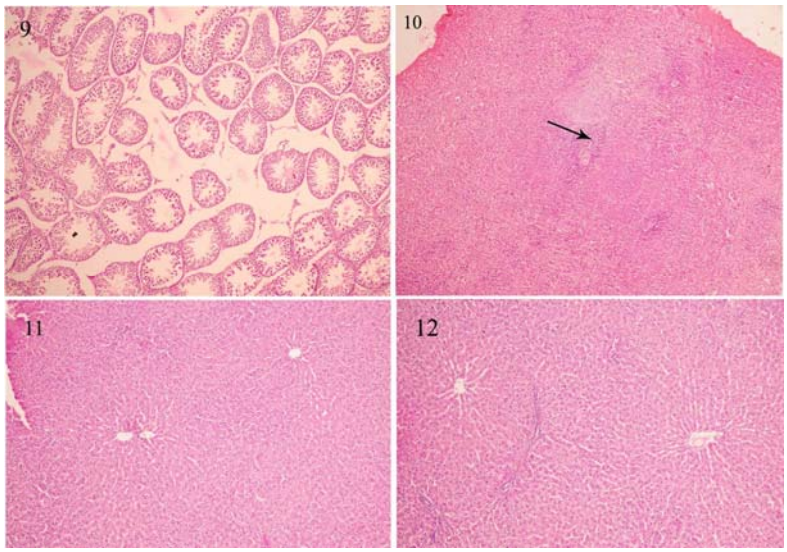

Fig 3: Group **(A)** untreated *J.curcas* **(9-10)** testes showed a decrease in the number of the spermatogenic cells and the somniferous tubule was dissociated from each other X10 **(9)**, Spleen showed lyses in the lymphocytic cells in the white bulb X10 **(10)**. Group **(D)** of *J.curcas* treated with (4% NaoH+ moist heat) showed liver sections with normal hepatic cells and blood vessels X10 **(11)**. Liver of control rats X10 **(12)**.

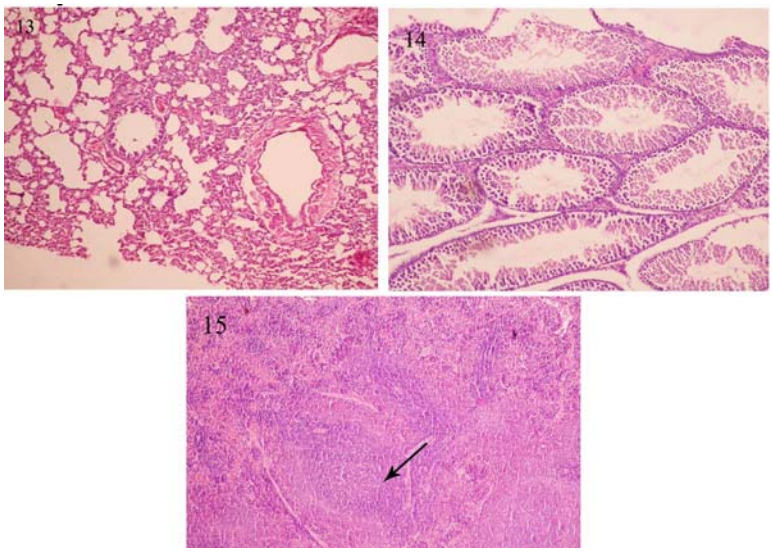

Fig 4: Group **(D)** showed lung with normal alveolar tissues **(13),** testes with normal somniferous tubules **(14)** and spleen with proliferated lymphocytic cells X10 **(15)**.

Discussion:

In the present study, the treatments with moist heat or different chemicals induce variable effects on anti nutritional factors of the defatted diets b, c, d and e. Phytic acid content of diets b, c, d and e was less than the control diet (untreated) by 0.8, 0.08, 6.1 and 0%, respectively. The best reduced percentage achieved in the diet (d) treated with 4% NaOH and heat, but no reduction recorded in the diet (e) treated with NaHCO3 and heat. Phytic acid content (mg/100g) seems to be higher than that of soybean and the recommended content of foods and feed. The high level of the phytate present in *Jatropha* meals might decrease the bioavailability of minerals (especially Ca^{2+} and Fe^{2+}). Phytates have also been implicated in decreasing protein digestibility by forming complexes and as well by interacting with enzymes such as trypsin and pepsin (Reddy and Pierson, 1994). The phytate content of all samples from *J. curcas* was much higher than that of peanut press cake (Aderibigbe *et al.,* 1997). Trypsin inhibitor activity content (mg/g) was decreased with all used treatments in diets (b, c, d and e) as

96.55, 96.02, 95.65% and 92.95%, respectively. These results indicated that efficient ability of all applied treatments to reduce the Trypsin inhibitor activity in all treated diets of J. *curcas.* Trypsin inhibitors interfere with the physiological process of digestion through interference with the normal functioning of pancreatic proteolytic enzymes in non-ruminants, leading to severe growth depression (White *et al.,* 1989). It is possible that the anti nutrient effect of trypsin inhibitors is due to their direct interaction with pancreatic proteolytic enzymes and a corresponding reduction in the digestibility of the proteins of the diet (Hajos *et al.,* 1995). Trypsin inhibitors are heat-labile and can be partially or completely denatured when exposed to elevated temperature. Total phenols' content (mg/100g) had been reduced according to (b, c, d and e) diets' treatments by 1.98, 1.28, 72.18 and 0%, respectively. It is obvious that the treatment with Na OH and heat in the diet (d) give the best reduction percentage of total phenols' in comparison with other diet treatments. Total saponins' content (g/100g) was decreased in b, c, d and e diet treatments by 4.85, 10.0, 13.14 and 50%, respectively. These results explain that saponins' content greatly reduced with sodium bicarbonate and heat treatment in the diet (e) while there is slightly reduction in other treatments. Similar results were reported by Martinez-Herrera *et al.,* (2006) and Abou-Arab and Abu-Salem, (2010). Previous studies documented that the trypsin inhibitor and lectin activities decreased more than 83 and 99%, respectively, after 30 and 45 min of heat treatment. Heat-treated seed meal of the nontoxic variety of *J. curcas* was found to be comparable to the commercially available soybean meal in nutritional quality for common carp. On the other hand, heat treatment followed by solvent extraction to remove phorbol esters could result in an elimination of most of the anti nutrients and toxins from the toxic variety. The meal treated in this manner was found to be innocuous to rats (Makkar and Becker 1997a). The heat treatment in combination with the chemical treatment of sodium hydroxide and sodium hypochlorite has also been reported to decrease the phorbol ester level in *Jatropha* seed meal to 75% (Goel, *et al.,* 2007).

In the present experimental animal model, there was no mortality recorded among the untreated meal group or heat, and chemical treated groups during the experimental period, where their diet contained 20% *J. curcas* seed meal. Similarly, no mortality was recorded on diets containing *J. curcas* seed meal at 5, 10 and 15% relative to reference diet. However, 20-25% dietary *J. curcas* seed meal elicited death of all animal subjects within one week of the experiment (Annongu *et al.,* 2010). This variation in the allowed percentage of *J. curcas* seed may be attributed to the variable concentration of the phorbol esters which has identified as the major toxic principle in *J. curcas* seed (Makkar and Becker, 1997a). There was a variation of phorbol esters content and types based on the countries of origin of Jatropha oil, where Jatropha oil from Indonesia has the highest phorbol esters' content and Jatropha oil from Malaysia has the lowest phorbol esters' content (Ahmed and Salimon, 2009). Where, phorbol ester content depends on the soil and climatic conditions (Martinez-Herrera *et al.,* 2005). The nontoxic Maxican varieties of *J.curcas* were reported to have the negligible or low amount of phorbol esters (0.27 mg/ml of oil) but still contained levels of the other anti nutritive component (saponins, lectins and trypsin inhibitors) similar to those in toxic varieties (Aderibigbe *et al.,* 1997; Makkar and Becker, 1997 a, b, c and Aregheore *et al.,* 1998). They showed that the meal of *J. curcas* has a high activity of trypsin inhibitor and lectin. These substances can be reduced by heat treatment while the high concentration of phorbol esters, which identified as the main toxic agent responsible for its toxicity can't be destroyed by heat treatment because the phorbol esters are heat stable. However, it is possible to reduce their concentration in the meal by chemical treatment. Our result of the body weight gain showed the more significant decrease than control in all tested groups except in the group D. Wherever, the *J. curcas* seed was treated with 4% NaOH and heat. The decrease in body weight gain or growth rate of the rats fed on untreated *J. curcas* seed (group A) may have resulted from the higher concentration of anti nutritional factors in the diet (Aregheore *et al.,* 2003)**.** In contrast, the obtained decrease in body weight gain of the other treated groups (B, C and E) may indicate that the applied treatments were not effective in an elimination of the adverse effects of anti nutritional factors. With a high anti nutritional factors' concentration, the acceptance of *J. curcas* seed meal was always low (Makkar and Becker 1997a). Rats were fed on (d) diet, which treated with heat and 4% NaOH leading to highly reduction of phytic acid, trypsin inhibitors and total phenols levels, previously determined in this study. They showed a significant increase in the body weight gain compared with the other treated groups A, B, C and E. It almost reached the normal control that indicated the effective applied treatment and the higher protein content of *J.curcas* seed meal. Previous works by Makkar and Becker (1999a) showed that *J.curcas* seed meal contains between 56-60 % crude proteins, the higher protein content of *J .curcas* seed meal might have aided the increasing weight gain on the diet in question. They also explored the heat-treated and untreated nontoxic variety of *J. curcas* as animal feed and reported that this variety resulted in a promising protein efficiency ratio (PER) and feed conversion ratio with the simultaneous decrease in trypsin inhibition and lectin activities when tested in rats and fish. The PER for unheated and heated *Jatropha* meal-containing diet was 37% and 86%, respectively, of the casein in rats.

In the present study, rats from the group A, B, C and E showed variable signs of anemia as the significant (P <0.05) decrease in red blood cell count, blood hemoglobin concentration and hematocrit value. These results indicate the presence of anemia and are in agreement with Chivandi *et al.,* (2004). They attributed the obtained anemia to the increase in red blood cell fragility. As well as, these results are in conformity with the preliminary findings of Chivandi *et al.,* (2006) who reported that the hematological profile of *Jatropha* fed pigs to be

characterized by early stage iron deficiency anemia. The anemia could have been hemorrhagic as witnessed by blood loss through the gastrointestinal tract GIT. Further more damage to the GIT as indicated by persistent diarrhea and ulceration, led to lose of nutrients required for erythropoiesis. These findings are in consistent with the obtained intestinal lesions in the present study, including the increasing number of goblet cells and degeneration of the intestinal epithelium.

The gross and microscopical picture of rats fed on untreated *J. curcas* seed meal was mainly congestion of the vasculature of the internal organs, including liver, lung, kidney and intestine. This congestion was attributed to the biological effect of phorbol esters and other phytotoxins on the cellular membrane receptors with modification of their activities led to release of the different inflammatory mediator, including histamine, which led to vascular disturbance inducing edema and congestion of different organs **(**Goel *et al.,* 2007**)**. Congestion in the internal organs was the main sing induced by feeding the diets containing *J. curcas* (Cai Yan *et al.,* 2010).

Histopathological investigation of the hepatic tissue of the untreated J. *curcas* seed meal and treated groups with methanol or $NaHCO_3$ showed necrosis in the hepatocytes with fatty degeneration, degeneration in the biliary epithelium and inflammatory cell infiltration between the hepatocytes. These results, resembled those recorded by Cai Yan *et al.,* (2010). They found sporadic infiltrated lymphocyte in the liver with appearance of fatty vacuoles in the liver cells. Degenerative changes in hepatic tissues were accompanied with enhancement of liver enzymatic activities, which recorded in our biochemical analysis in this study. The significant elevation in alkaline phosphatase and gamma- GT concentration in the serum of rates in groups A, B, C and E than the control were obtained. The significant increase in the liver enzymes activities were attributed to liver damage and cytotoxic effect of *J. curcas* seed meal on the liver cells leading to leakage of alkaline phosphatase from damaged hepatocytes and gamma-GT from the affected biliary epithelium (Tietz, 1994). The hypoproteinemia and hypoalbuminemia observed in the serum of the same group of rats were attributed to the direct toxic effect of phorbol esters led to degeneration and necrosis of hepatocytes (Aregheore *et al.,* 2003). Hepatocytes are considered the main site of albumin and protein synthesis (Eisenbarth, 1986). The interaction of phorbol ester with protein kinase C (PKC) affects activities of several enzymes, biosynthesis of protein, DNA, polyamines, cell differentiation processes, and gene expression (Goel *et al.,* 2007**)**. Lung tissues of rats fed on the untreated J. *curcas* group (A) showed inflammatory cell infiltration in the alveolar septa and congestion of the pulmonary capillaries which turned to hemorrhage in some areas, the similar results were obtained by Cai Yan, (2010). Hemorrhage may be related to rupturing of some congested capillaries (Kumar *et al.,* 1997). Spleen of rats from the same group showed degeneration and lysis of the lymphocytes in the white pulp thus may be the direct cytotoxic effect of several phytotoxins and phorbol esters. Other tested groups B, C, D and E fed on variable treated diets showed slight and insignificant alterations. Kidney tissues from tested groups revealed insignificant lesions, which were confirmed clinically by insignificant variations in the level of creatinine and uric acids in the serum of tested rats. Wide biochemical, cellular effects and alterations of the cell morphology which developed in rats fed on untreated diet of *J.curcas* are due to the primary action of phorbol esters on biological membranes. The phorbol esters are amphiphilic molecules and have the tendency to bind to phospholipid membrane receptors. These receptors are usually the primary targets for the phorbol esters (Ahmed and Salimon, 2009). In conclusion, untreated diet of 20 % *J.curcas* can induce deleterious effects on nutrition, biochemical and histopathology of monogastric animals like rats as observed in this study. The previous alteration and lesions were minimized in the group of rats fed on *J. curcas* seed meal treated with heat and 4%NaOH (group D). This will confirm the efficiency of the detoxification method of heat and NaOH to decrease the hazards induced by toxins present in untreated *J. curcas* seed meal. Further more, it may represent a cheap and suitable source to high quality protein in animal diets, especially in developing countries.

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