BIOCHEMICAL AND HEMATOLOGICAL RESPONSES OF RATS FED WITH DETOXIFIED *JATROPHA CURCAS* SEED MEAL.

S. Sharif, F. Masood, R. Naseer, [*]S. Khawar, S. Naveed[**], L. Ahmad[***] and G. Saleem[****]

Institute of Biochemistry and Biotechnology, [*] Services institute of medical Sciences Lahore [**]Department of Animal Nutrition, University of Veterinary and Animal Sciences, Lahore, Pakistan,[***] Department of statistics, UVAS,[****] Department of Pathology, UVAS Lahore
Corresponding author's email: rahat.naseer@uvas.edu.pk

ABSTRACT: *Jatropha curcas,* has a great potential to replace soya bean. The main problems are the anti-nutritional factors, which hinder its use as an alternative protein source in the animal feed. The present study was focused on the detoxification of *J. curcas* seed. Detoxification was carried out using a mixture of aqueous methanol (9:1) and NaOH (0.15%) at 60°C for 30 min. subsequent washing with methanol and then oven dried. Results showed significant detoxification (P>0.05) of *J. curcas*. The impact of feeding detoxified *J. curcas to* rats was studied. The detoxified *J. curcas* seed meal (DJM) at the rate of 25, 50, 75 and 100% was offered to albino rats (n=36) of almost equal weights (45±0.76gm). On blood and serum analysis the white blood cells (WBC) (7.23±0.29, 10.56±0.16, 8.29±0.30, 5.94±0.17, 5.6±0.177 and (7.07±0.27), red blood cells (RBC) count (6.73±0.109, 7.61±0.18, 7.00±0.056, 6.68±0.96, 6.28±0 and 5.83±0.18), hemoglobin (11.94±0.11, 12.29±0.19, 12.66±0.27, 11.78±0.14, 10.97±0.177 and 10.97±0.177), total protein (5.33±0.09, 5.17±0.86, 4.6±0.096, 4.41±0.109, 4.1±.096 and 3.82±0.13), uric acid (32.25±0.82, 34.83±0.32, 54.83±0.84, 61.75±0.68, 62.36±0.7135 and 26±0.34), creatinine (0.841 ±0.017, 801± .018, 0.841±0.027, 0.833±0.032, 0.801± 0.14 and 1.44±0.049) and LFT (liver function test) including alkaline transaminase (29.2± 0.42, 24.13±0.37, 21.133±0.48 , 32.006±1.39, 75.93± 2.16 and 24.75±0.377), Aspartate transaminase (3.20±1.01 2.08±1.03, 1.25±1.4, 1.33±0.71, 1.82±0.72 and 1.81±1.47) and Alkaline phosphatase (4.96±1.09, 4.14±0.71, 4.79±1.47, 5.30 ±1.5, 5.6±2.3 and 2.08±2.3) didn't differ significantly among the experimental groups. A significant increase (P>0.05) in red blood cells and hemoglobin level was observed in 25 (7.61±0.18) and 50% (7.0±0.056) DJM fed groups as compared to control group (6.73±0.0.109). There was an increase in the value of PER (Protein efficiency ratio), TD (True digestibility), NPU (±Net protein utilization) and BV (Biological value) of 25% (0.32±.009, 77.66±.21, 65.33±.17 and 83.42±0.16) and 50% DJM *fed* rats (0.42±.007, 88.08±.26, 75.5±0.18, 85.10±0.073) as compared to control group (0.36±.004, 90.5±0.22, 75.25±0.17, 83.0±0.14) . Present study indicated that 50% DJM protein fed group has the same performance as the control group and better than other experimental groups, that showed a better efficiency of detoxified *J. curcas* meal as an alternative livestock feed.

Keywords*: Jatropa curas,* Anti-nutritional factors, Detoxification, Serum and Digestibility.

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INTRODUCTION

Jatropha curcas, commonly known as physic nut, is a tropical plant of the *Euphorbiaceae* family. It is native to Mexico and Central America, however widely distributed in wild and semi cultivated lands of Latin America, Africa, India and South East (Pandey *et al*., 2012). *J. curcas* is a small tree or a large shrub with a height of 8 to 10 meters (Kumar and Sharma, 2008).

The *J. curcas* seed weighs from 0.53 - 0.86 g (Singh *et.al* 2008) and the kernel contains 57 - 63% oil. Meal obtained after oil extraction contains 60–66 % crude protein. Conventionally, *J. curcas* seed is used in fertilizers, briquettes, candles, soaps, lubricants and for illumination. The seed kernels are used as fuel or as direct substitute to diesel. The seed cake after extraction is used as a good source of protein for humans as well as livestock (Abou-Arab and Abu-Salem, 2010).

It has been investigated that increasing value byproducts of oil extraction are important for the viability of the crop for the farmers (Francis *et al.,* 2013). The *J. curcas* plant can yield upto 4 tons seed annually (Kumar *et al.,* 2010).

Major toxic compounds present in *J. curcas* seeds are cursin and phorbol ester. Anti-nutritional factors such as Trypsin inhibitor, lectin, phytate, tannis, phenols, and saponins are also present, posing a major hindrance in utilizing *curcas* seeds in animal feed (Makkar *et al.,* 2008). The *J. curcas* seeds can be

detoxified using combination of heat and chemical treatments (Azzaz *et al.,* 2011).

Major purpose of detoxification is to prevent the plausible health hazards thought to be associated with its toxic compounds (Kumar *et al*., 2010b). So, the present study was to determine the potential of heat and chemicals to detoxify *J. curcas* (Azzaz *et al.,* 2011) and to evaluate the biochemical and hematological responses of albino rats fed with detoxified *J. curcas* seed meal.

MATERIALS AND METHODS

Raw material: Apparently healthy *J. curcas* seeds were repeatedly washed, shade dried at high temperature (above 40°C) and powdered by using mechanical grinder (Akbar *et al.,* 2009).

Proximate analysis: Proximate analyses of seeds were carried out to determine the percentage of moisture, ash, crude protein, nitrogen free extract, crude fiber and crude fat (AOAC, 1990).

Estimation of the anti-nutritional factors: The phytate contents of seeds were determined by spectrophotometric procedure using phytic acid sodium salt as a standard (Makkar *et al.,* 2008).

Total saponin contents were determined by spectrophotometric method using diosgenin as a standard (Hiai *et al.,* 1976). Total phenolics and tannins were determined by spectrophotometric methods using Folin–Ciocalteu reagent as described by (Harinder *et al.,* 2008). Lectin contents of *curcas* seed were analyzed by haemagglutination assay using trypsinized cattle washed erythrocytes in a phosphate buffer saline (10%) (Makkar *et al.,* 2008). Trypsin inhibitory activity was determined by colorimetric method using trypsin enzyme and Azocasein as a substrate (Robin *et al*., 2011).

Detoxification: Defatted seed sample (10g) was mixed with aqueous methanol (90ml methanol+10 mL distilled water) and NaOH (0.4g) in a 250 ml flask. Flask was capped and placed in water bath at 60°C for 30 min. Filtrate residue was washed with 20ml methanol (2 times of the weight of the initial defatted seeds). The filtrate was dried in the oven (50°C) and stored in air tight jar. Pre and post detoxification the nutritional and anti-nutritional contents were determined according to the methods described by Aregheore *et al*., (2003).

Feed formulation: The diet formulated contained skimmed milk, ground nut oil, vitamin mixture, salt mixture, corn starch and sucrose. The experimental diets were prepared by replacing 25%, 50%, 75% and 100% skimmed milk casein with detoxified *curcas* seed as shown in table. In addition, a negative control diet was also prepared without protein. The diets were offered randomly to the rats (Rakshit *et al.,* 2008).

Table-1. Feed formulations for trial on rats.

Ingredients	Control Group	Group A 25% J.C	Group B 50% J.C	Group C 75% J.C	Group D 100% J.C	Group E Protein Free
Sucrose	15%	15%	15%	15%	15%	15%
Canola oil	10%	10%	10%	10%	10%	10%
Potato starch	5%	5%	5%	5%	5%	5%
Vitamin & minerals	2%	2%	2%	2%	2%	2%
Casein protein	10%	7.5%	5.0%	2.5%	5.0%	-
Corn starch	58%	58%	58%	58%	58%	68%
Jatrpha Curcas	-	2.5%	5.0%	7.5%	5.0%	-

Experimental design: Thirty six (n=36) weanling rats were divided into 6 groups randomly. One group was positive control and the other as negative control. The experiment were conducted in duplicate (Azzaz *et al.,* 2011). The experimental parameters studied were body weight, feed intake, protein efficiency ratio (PER), true digestibility (TD), net protein utilization (NPU) and biological evaluation (Miller and Bender 1955).

Biochemical and hematological analysis: Liver function tests (LFTs), lipid profile, TP (total protein), glucose, urea and creatinine were determined. The total erythrocyte count (RBC's) and the total leucocyte count (WBC's) were determined using method described by Azzaz *et al.,* (2011)

Statistical analysis: To determine the significant differences in each experiment analysis of variance (ANOVA) was applied using SPSS version 17.0

RESULTS AND DISCUSSION

Proximate analysis: Proximate composition refer to crude protein, total fat, total fiber and ash. The proximate composition of defatted and detoxified *J. curcas* seeds was calculated according to A.O.A.C (1990) and is presented (Table 2).

Table-2: Proximate composition and Anti-nutritional Factors present in defatted and fatted *Jatropha curcas* seed meal

Parameters	Defated		P-value	Detoxified		P-value
	Mean	Std. Error		Mean	Std. Error	
Dry matter	96.7000	.32146	.000	96.2233	.12706	.000
Moisture	5.9333	.08819	.000	4.0633	.03180	.000
Crude protein	34.9800	.07937	.000	31.8200	.09866	.000
Crude Fiber	37.0333	.14530	.000	35.3233	.05925	.000
Total Fat	5.7933	.02028	.000	3.2000	.05774	.000
Ash	3.3000	.05774	.000	3.4833	.04410	.000
NFE	12.4333	.05608	.000	22.0500	.01732	.000
Saponin	.1700	.00577	.001	.1400	.00577	.002
Tanin	.0530	.00115	.000	.0177	.00088	.002
Trpsin	.1800	.00577	.001	.0500	.00577	.013
Phytic acid	.3467	.00882	.001	.153	.03977	.019
Phytate	.3100	.00577	.000	.1100	.00577	.003
Phenolics	.1767	.00882	.002	.0467	.00333	.005
Lectin	4.2633	.02404	.000	2.5167	.01764	.000

By proximate analysis estimated contents of crude protein, crude fiber and total fat were 34.98±0.07, 37±0.014 and 5.79±0.02, respectively. The saponins and tannins were found to be 0.17±0.00 and 0.053±0.00 respectively. *Curcas* seed had phytic acid (0.34±0.008), phytate (0.31±0.005), total phenolics (0.17±0.008) and lectin (4.26±0.024). The result of this analysis was in line with the previous studies conducted by Abou-Arab and Abu Salem (2010) in the kernel meal, with a little difference in crude protein contents (32.8%). However, contradictory results were reported by Azzaz *et al.,* (2010) and Francis *et al.,* (2013). Moreover, the fiber contents reported by Azzaz and Francis were 3.4 and 4.9 %, whereas in present study were 37% and 35.32% in defatted and detoxified seed samples. Dry matter percentage was found to be 96.7% and 96.2% respectively in defatted and detoxified seed samples of present study which were found to be similar as reported by Chivandi *et al.,* (2004).

In the present study, the detoxification of *J. curcas* seeds were carried out with 4% NaOH, aqueous methanol and moist heat treatment. After detoxification the amount of anti-nutrients present in *J. curcas* seed were determined (Table 2). In samples of defatted *J. curcas* the amount of Phytic acid and Phytate was 0.34 mg/mL and 0.31 mg/mL and reduced to 0.15 mg/mL and 0.11 mg/mL respectively in detoxified samples. This reduction in the Phytic acid amount suggested that the combination of 4% NaOH and moist heat was effective to reduce the level. The total phenolics were determined using standard curve of phenolics. The amount was 0.17mg/mL in defatted and reduced to 0.04mg/mL in detoxified *J. curcas* seeds. Phenols interfere with the normal process of digestion either binding directly to digestive enzymes and indirectly to feed components like proteins and minerals and impaired the process of digestion.

The amount of saponin in defatted and detoxified *J. curcas* seed sample was found to be 0.17 μg/g and 0.14μg/g. Saponin is secondary metabolite of plants that cause the hemolysis of RBCs and release of hemoglobin. The results indicated that the saponin can be degraded by combination of NaOH and heat treatment as reported by Martinez *et al.* (2006) and Abou-Arab and Abu Salem (2010).Trypsin inhibitory activity of defatted and detoxified *curcas* seed samples were found to be 0.18mg/mL and 0.05mg/mL, respectively.

Lectin hemagglutination Assay was performed for both the defatted (A) and detoxified (B) samples the results showed no agglutination (Fig. 1) which confirmed the absence of lectins. In similar study by Aregheore *et al.* (1998) using cattle blood have shown the presence of lectin activity but the results of present study were in contrast. Lectin is heat labile and might be destroyed by the moist heat (Aregheore *et al*., 1998).

Biological evaluation: The detoxified *J. curcas* seed meal was fed to rats and biochemical parameters determined are presented (table 3).

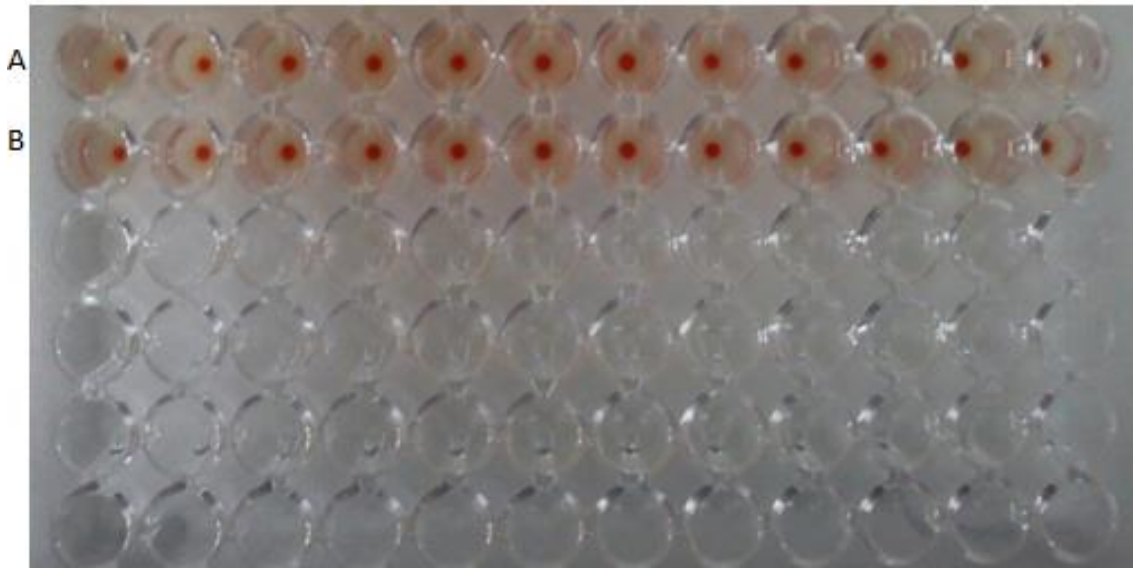

Figure-1: Lectin hemagglutination Assay, row labelled as A is defatted and B is detoxified *Jatropha curcas*, showing no activity of lectins

In present study, biological evaluation of rats fed with detoxified *J. curcas* seed meal showed no significant ($P<0.05$) differences with control (fed with casein protein). There was non-significant difference in the growth rate of detoxified *J. curcas* seed meal (DJM) fed rats. The average weight gain of DJM (25%) and DJM (50%) was increased compared to the control group (Table 3). The protein efficiency ratio (PER), true digestibility (TD), net protein utilization (NPU) and biological evaluation (BV) of detoxified *J. curcas* seed meal (DJM) 25%(B) and DJM50% was also increased. In detoxified *J. curcas* seed meal DJM (75%) and DJM (100%) these value were decreased, which may be due to poor utilization of protein. Zero weight gain in protein free group indicated that protein enables rats to grow properly as it's a necessary component of the diet and is the building block of the life and no protein intake in this group showed no PER, NPU and BV ratios. The results are in agreement with the results of Azzaz *et al.,* (2011) and Rahma *et al.,* (2013). The studies of Herrera *et al.,* (2012) and Martinez Ayala. (2013) observed a decrease in PER and NPU value compared to control group. Friedman and Brandon, (2001) and Rozan *et al.,* (1997) reported little increase in weight gain using soya bean meal.

The biochemical parameters showed no significant change ($P<0.05$) between rats fed with DJM and control group (Table 4). The amount of glucose and total protein was decreased in six groups. While there was an increase in the concentration of creatinine, urea, ALT, AST, ALP of 75% and 100% DJM fed rats. Similar results were interpreted by Awasthy *et al.,* (2010); Wang *et al.,* (2011); and Kumar *et al.,* (2010a). Although a slight reduction in blood sugar and protein level was observed in the Nubian goats fed with DJM (Adam and Magzoub, 1975). The reduced blood sugar and protein levels of rats may be due to impaired digestion of carbohydrate because of the presence of anti-nutrient amylase inhibitor (tannins/polyphenols) in the seeds (El-Sayed, 1997; Glick and Joslyn, 1970). The trypsin inhibitor interferes with digestion of dietary protein and the tannins complex with it and inactivates proteins in general, thus reducing the total protein of rats as also described by (Awasthy *et al.,* 2010).

The increase in plasma urea, ALT, AST and ALP of group DJM (75%) and DJM (100%) was an indication of impairment of liver function while normal level of these parameters in groups DJM (25%) and DJM (50%) showed that the percentage of detoxified *J. curcas* can't be exceeded from certain limits.

In present study, non-significant difference ($P<0.05$) for the hematological parameters (Table 5) among all the six groups including control was observed. Although an increase in the RBCs count of DJM25% and DJM50% was observed when compared to the control group fed with casein protein. Similar results were observed by (Kumar *et al.,* 2010). An increase in the RBCs count of DJM fed groups may suggest that the plant ingredients or DJM may have caused an immature release of erythrocytes that increased the RBC count (Hemre *et al.,* 2005). The Hb levels of different groups (Table 5) did not differ significantly and were within the normal range. Although there was an increase in the Hb level of DJM (25%) and DJM (50%) groups suggested the normal health of the rats. These results were in agreement with Kumar *et al.,* (2010) and Akinleye *et al.,* (2012) whereas contradicted with Azzaz *et al.,* (2011) and Katole *et al.,* (2011).

Table-3: Biochemical Parameters of rats fed with detoxified *Jatropha curcas* meal.

Biochemical parameters	Control	25% DJM	50% DJM	75% DJM	100% DJM	Protein free
Alanine Transaminase	29.2± 0.42 ^{abd}	24.13±0.37 ^{acd}	21.133±0.48 ^{abcd}	32.006±1.39 ^{abd}	75.93± 2.16^{abcd}	24.75±0.377 ^{abcd}
Aspartate transaminase	3.20±1.01 ^{abcd}	2.08±1.03 ^{bcd}	1.25± 1.4 ^{acd}	1.33±0.71 ^{abd}	1.82±0.72 ^{abc}	1.81±1.47 ^{abc}
Alkaline phosphatase	4.96±1.09 ^{abcd}	4.14±0.71 ^{bcd}	4.79±1.47 ^{acd}	5.30 ±1.5 ^{abd}	5.6±2.3 ^{abc}	2.08±2.3 ^{abcd}
Serum Total protein	5.33±0.09 ^{bcd}	5.17±0.86 ^{bcd}	4.6± 0.096^{abc}	4.41± 0.109 ^{abc}	4.1± .096 ^{abc}	3.82±0.13 ^{abcd}
Serum Glucose	115±0.38 ^{abcd}	112±0.60 ^{bcd}	97.41± 0.46 ^{ad}	95.51±0.71 ^{ad}	75.866±1.15 ^{abc}	44.60±0.66 ^{abcd}
Urea	32.25±0.82^{bcd}	34.83±0.32 ^{bcd}	54.83±0.84 ^{acd}	61.75±0.68 ^{ab}	62.36±0.71 ^{ab}	35.26± 0.34^{bcd}
Creatinine	0.841± 0.017**	.801± .018**	0.841± 0.027**	0.833± 0.032**	0.801± 0.14**	1.44±0.049 ^{abcd}

Table-4: Analysis of the rats fed with detoxified *Jatropha curcas* seed meal.

Parameters	Control	25% DJM	50% DJM	75% DJM	100% DJM	Protein free
Weight gain	10.33±1.3 ^{abcd}	13.66±0.33 ^{bcd}	12.33±0.66 ^{bcd}	6.8±1.4^{abc}	5.0±0.70 ^{abc}	0±0 ^{abcd}
Protein efficiency ratio	0.36±0.004 ^{abcd}	0.323±0.009 ^{abcd}	0.42±0.007 ^{acd}	0.283±0.009 ^{ab}	0.27± 0.02^{ab}	0.036±0.036^{abcd}
True Digestibility	90.5±0.22 ^{abcd}	77.66±0.21 ^{bcd}	88.08±0.26 ^{acd}	31.108±0.44 ^{abd}	18.56±0.33 ^{abc}	.00±.00 ^{abcd}
Net protein Utilization	75.25±0.17	65.33±0.17 ^{bcd}	75.5±0.182 ^{abcd}	17.35±0.16 ^{abd}	10.16±0.08 ^{abd}	.00±.00^{abcd}
Biological Value	83±0.14 ^{abcd}	83.42±0.16 ^{bcd}	85.10±0.073 ^{acd}	57.00±0.22 ^{abd}	53.37±0.07 ^{abc}	.00±.00^{abcd}

* The mean values with the different superscripts are significantly different

Table-5: Hematological Analysis of the rats fed with detoxified *Jatropha curcas* seed meal.

Parameters	Control	25% DJM	50% DJM	75% DJM	100% DJM	Protein free
Red blood cells	6.73±0.109 ^{abcd}	7.61±0.18 ^{bcd}	7.00±0.056 ^{bcd}	6.68±0.96 ^{abc}	6.28±0.0 ^{abc}	5.83±0.18 ^{abcd}
White blood cells	7.23±0.29 ^{abcd}	10.56±0.16 ^{bcd}	8.29±0.30 ^{acd}	5.94±0.17 ^{ab}	5.6±0.177 ^{ab}	7.07±0.27 ^{abcd}
Haemoglobin	11.94±0.11 ^{abcd}	12.29±0.19 ^{abcd}	12.66±0.27 ^{acd}	11.78±0.14 ^{abcd}	10.97±0.177 ^{abcd}	10.97±0.177 ^{abcd}

*The mean values with different superscripts are significantly different

Conclusion: The results of present study indicated that the detoxified *J. curcas* meal using combination of heat and chemical treatment can efficiently replace 50% of the ssoybean meal in the diets of poultry and small ruminants without negative effects on health or production.

REFERENCES

Abou-Arab, A.A. and F.M. Abu-Salem. (2010). Nutritional quality of *Jatropha curcas* seeds and effect of some physical and chemical treatments on their anti-nutritional factors. Afr. J. Food Sci. 4(3): 93-103.

Adam, S. and M. Magzoub. (1975). Toxicity of *Jatropha curcas* for goats. Toxicol. 4(3): 388-389.

Akbar, E., Z. Yaakob , S.K. Kamarudin, M. Ismail and J. Salimon. (2009). Characteristic and Composition of *Jatropha Curcas* Oil Seed from Malaysia and its Potential as Biodiesel Feedstock. Euro. J. Sci. Res. 29(3): 396-403.

AOAC (1990). Official methods of analysis (14th ed.) Arlington, VA. Association of Official Analytical Chemists 2.

Azzaz, N.A., N.A. El-Nisr, E.E. Elsharkawy and E.A. Elmotleb. (2011). Chemical and Pathological Evaluation of *Jatropha Curcas* Seed Meal Toxicity With or Without Heat and Chemical Treatment. Aust. J. Basic Appl. Sci. 5(12).

Akinleye, A., V. Kumar, H. Makkar , M. Angulo-Escalante and K. Becker . (2012). *Jatropha platyphylla* kernel meal as feed ingredient for Nile tilapia (*Oreochromis niloticus L.*): growth, nutrient utilization and blood parameters. J Anim Physiol Anim Nutr. 96(1): 119-129.

Aregheore, E.M., H.P.S. Makkar and K. Becker . (1998). Assessment of lectin activity in a toxic and a non-toxic variety of *Jatropha curcas* using latex agglutination and haemagglutination methods and inactivation of lectin by heat treatments. J. Sci. Food Agric. 77(3): 349-352.

Aregheore, E.M., K.Becker and H.P.S. Makkar . (2003). Detoxification of a toxic variety of *Jatropha curcas* using heat and chemical treatments, and preliminary nutritional evaluation with rats. S. Pac. J. Nat. Sci. 21(1): 51-56.

Awasthy ,V., V. Vadlamudi, K. Koley, B. Awasthy and P. Singh. (2010). Biochemical changes after short-term oral exposure of *Jatropha curcas* seeds in wistar rats. Toxicol. Int. 17(2): 67.

Chivandi, E., J. Mtimuni, J. Read and S. Makuza. (2004). Effect of processing method on phorbol ester concentration, total phenolics, trypsin inhibitor activity and the proximate composition of the Zimbabwean *Jatropha curcas* provenance: a potential livestock feed. Pak. J. Biol. Sci. 7(6): 1001-1005.

El-Sayed MM, R.M., H.M. Ali, E.A. El-Morsi. (1997). Biochemical studies on alpha-amylase inhibitors in some legume seeds. Ann.Agric.Sci. 35(1): 357-374.

Francis, G., J. Oliver and M. Sujatha. (2013). Non-toxic *Jatropha* plants as a potential multipurpose multi-use oilseed crop. Ind. Crops Prod. 42: 397-401.

Friedman, M. and D.L. Brandon. (2001). Nutritional and health benefits of soy proteins. J. Agric. Food Chem. 49(3): 1069-1086.

Glick, Z., and M. Joslyn. (1970). Food intake depression and other metabolic effects of tannic acid in the rat. J. Nutr. 100: 509-515.

Harinder, P.S.M., F. George and B. Klaus. (2008). Protein concentrate from *Jatropha curcas* screw-pressed seed cake and toxic and antinutritional factors in protein concentrate. J. Sci. Food. Agric. 88:1542–1548.

Hiai, S., H. Oura and T. Nakajima. (1976). Color Reaction Of Some Sapogenins and Saponins With Vanillian and Sulfuric acid. Planta Med. 29(02): 116-122.

Hemre, G-I., M. Sanden , A. Bakke-Mckellep, A. Sagstad and A.Krogdahl. (2005). Growth, feed utilization and health of Atlantic salmon Salmo salar L. fed genetically modified compared to non-modified commercial hybrid soybeans. Aquac. Nutr. 11(3): 157-167.

Martinez Ayala, A.L. (2013). Evaluation of the nutritional quality of nontoxic kernel flour from *Jatropha curcas* l. in rats. J. Food Qual. 35(2): 152-158.

Martinez-Herrera, J., P. Siddhuraju , G. Francis, G. Davila-Ortiz and K. Becker. (2006). Chemical composition, toxic/antimetabolic constituents, and effects of different treatments on their levels, in four provenances of *Jatropha curcas* L. from Mexico. Food chem. 96(1): 80-89.

Martinez Herrera, J., C. Jimenez Martinez , A. Martinez Ayala A, L.G. Siciliano, R.M. Escobedo, G.D. Ortiz , G.C. Cevallos, H. Makkar, G. Francis and K. Becker. (2012). Evaluation of the nutritional quality of nontoxic kernel flour from Jatropha curcas L. in rats. J. Food Qual. 35(2): 152-158.

Miller, D. and A.E. Bender. (1955). The determination of the net utilization of proteins by a shortened method. Br. J. Nutr. 9(04): 382-388.

Makkar , H.P., J. Martínez-Herrara and K.Becker. (2008b). Variations in Seed Number per Fruit, Seed Physical Parameters and Content of Oil, Protein and Phorbol Ester in Toxic and Non-Toxic and Non-Toxic Genotypes of *Jatropha curcas*. J. Plant Sci. 3(4).

Kumar, A. and S. Sharma . (2008). An evaluation of multipurpose oil seed crop for industrial uses (*Jatropha curcas* L.): A review. Ind. Crops Prod. 28(1): 1-10.

Katole, S., S. Saha, V. Sastry, M. Lade and B. Prakash. (2011). Intake, blood metabolites and hormonal profile in sheep fed processed *Jatropha* (*Jatropha curcas*) meal. Anim. Feed Sci. Technol. 170(1): 21-26.

Kumar, V., H.P. Makkar and K. Becker. (2010a). Dietary inclusion of detoxified *Jatropha curcas* kernel meal: effects on growth performance and metabolic efficiency in common carp, Cyprinus carpio L. Fish Physiol. Biochem. 36(4): 1159-1170.

Kumar, V., H.P. Makkar, W. Amselgruber and K. Becker K. (2010b). Physiological, haematological and histopathological responses in common carp (Cyprinus carpio L.) fingerlings fed with differently detoxified *Jatropha curcas* kernel meal. Food Chem. Toxicol. 48(8): 2063-2072.

Pandey, V.C., K. Singh, J.S. Singh, A. Kumar, B. Singh and R.P. Singh. (2012). *Jatropha curcas*: A potential biofuel plant for sustainable environmental development. Renewable Sustainable Energy Rev. 16(5): 2870-2883.

Rahma, E.H., E.H. Mansour and S.T. Hamoda. (2013). Biological evaluation of *Jatropha curcas* seed as a new source of protein. Merit Res. J. Food Sci. Technol. 1(2): 23-30.

Rakshit, K., J. Darukeshwara , R.K. Rathina , K. Narasimhamurthy, P. Saibaba and S. Bhagya. (2008). Toxicity studies of detoxified *Jatropha* meal (*Jatropha curcas*) in rats. Food Chem.Toxicol. 46(12): 3621-3625.

Rozan, P., R. Lamghari, M. Linder, C. Villaume, J. Fanni, M. Parmentier and L. Méjean. (1997). In vivo and in vitro digestibility of soybean, lupine, and rapeseed meal proteins after various technological processes. J. Agric. Food Chem. 45(5): 1762-1769.

Robin, E.J.S., J.G. Pieter, M. Carina and L.F.G. Marco. (2011). Quantitative Determination of Trypsin Inhibitory Activity in Complex Matrices. Open Food Sci. J. 511(12): 42-46.

Singh,R.N., Vyas, D.K., Srivastava N.S.L.,Narra, M. (2008). SPRERI experience on holistic approach to utilize all parts of Jatropha Curcas fruit for energy.Renew.Ener .33(8) 1868-1873.

Wang, H., Y. Chen, Y. Zhao, H. Liu , J. Liu , H.P. Makkar and K. Becker K. (2011). Effects of replacing soybean meal by detoxified *Jatropha curcas* kernel meal in the diet of growing pigs on their growth, serum biochemical parameters and visceral organs. Anim. Feed Sci. Technol. 170(1): 141-146.