

**NSAP****47th Annual Conference**
(JOS 2022)**CONFERENCE PROCEEDINGS**THEME
SECURING ANIMAL AGRICULTURE AMIDST GLOBAL CHALLENGES**HAEMATOLOGY, SERUM INDICES AND CHANGES IN WEIGHT OF INTERNAL ORGANS OF RATS FED PROCESSED *JATROPHA CURCAS* L. SEED**

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*Corresponding author's E-mail: oa.abu@mail.edu.ui.ng; alternate E-mail: ohiahmed@yahoo.com**ABSTRACT**

The seed of toxic *Jatropha curcas* L. when processed appropriately is potential plant protein source for farm animals. A total of thirty weanling male Wistar albino rats weighing 80-100g was randomly allocated to five dietary treatments with six replications (n=1) and fed four diets containing 10% crude protein: casein (T1)-control diet, N-hexane extracted, oven-dried dehulled *jatropha* seed (T2), autoclaved and oven-dried raw dehulled *jatropha* seed (T3), solvent extracted, autoclaved, and oven-dried dehulled *Jatropha* seed, while a protein free diet served as a negative control (T5). The rats were allocated to the dietary treatments in a complete randomize design. The feeding trial lasted 10 days. Haematology, serum biochemistry and weight changes in the internal organs were investigated. By the 10th day the rats on hexane extracted oven dried rats had all died. Rats fed *jatropha* based diets show more progressive body weight losses and reduced feed intake compared to rats on protein free diets. Rats fed hexane extracted, autoclaved and oven dried *Jatropha* had poorer Pack Cell Volume, Haemoglobin and Red blood cells values. White blood cells were however not affected in rats irrespective of treatment applied. The aspartate aminotransaminase (AST), alanine aminotransferase (ALT) and cholesterol were also similar for all treatments ($p > 0.05$). Feeding *jatropha* based diets to rats led to lower values of very low density lipoproteins, Triglycerides and Total proteins compare to the control. Unlike the kidney and liver weights the heart and lung weights were not affected by inclusion of *Jatropha* seed cake ($p > 0.05$). Solvent extraction, application of heat either singly or in combination did not improve the value of *Jatropha* in rats compared to the rats fed casein-based and protein free diets.

Keywords: *Jatropha* kernel, processing techniques, blood profile, serum profile, rats**INTRODUCTION**

The pressure on use of soyabean and groundnut makes it imperative to explore other sources of plant protein. Some of these unconventional protein sources such as *Canavalia ensiformis* (Jack bean), *Vigna subterranean* (Bamabara bean) and *Jatropha curcas* have been used in animal feed, although their use is limited by the presence of anti-nutritional factors such as phorbol esters, lectin, curcin, trypsin inhibitor, phytic acid and saponins (Malviya *et al.*, 2011) anti-nutritional factors which can be reduced by chemical, physical and enzymatic methods (Abou-Arab *et al.*, 2019) several processing methods, with much emphasis on dehulling, solvent extraction, sprouting, boiling, soaking, toasting and solid state fermentation. The advent of several processing methods to detoxify the influence of anti-nutritional factors in unconventional and underutilized legumes has increased the possibility of these protein sources as a substitute to conventional legumes. *Jatropha curcas*, the most common of the *Jatropha* spp in Nigeria is one of the possible alternatives. The kernel of *J. curcas* contains 57-63% oil, and about 33.6-34.5% crude protein (Oladele and Oshodi, 2008). This study was carried to investigate autoclaved, solvent extracted and their combinations of *J. curcas* kernels on the haematological and serum biochemical indices and organ weight changes of Wistar rats.

MATERIALS AND METHODS***Experimental site, collection and treatment of seeds***

This study was carried out at the Rat House of the Department of Animal Science, University of Ibadan, Ibadan. *Jatropha curcas* seeds were obtained from University of Ilorin. The seeds were cleaned, sorted, crushed, dehulled and the kernel milled. A batch was subjected to a 6-h extraction using N- hexane as the solvent in a Soxhlet apparatus. The N-hexane extracted cake was autoclaved for 20 minutes at 121°C at 103.35 kPa and then oven dried in hot air oven at 60°C for 12 h. Another batch was autoclaved and then oven dried.



Experimental diets: Four dietary treatments were prepared to supply 10% crude protein on dry matter basis while the fifth diet was protein free (Table 1). A basal diet T1 with casein served as the positive control, the rats on T2 received dehulled *Jatropha* that was solvent extracted and then oven dried, rats on T3 received dehulled *jatropha* raw seed that was autoclaved and then oven dried, and those on T4 received dehulled *jatropha* that was solvent extracted, autoclaved and then oven-dried. Rats on T5 received a protein free diet, which also served as the negative control.

Table 1. Gross composition of processed *jatropha* kernel-based diets fed to Wistar rats (%)

Ingredients	T1	T2	T3	T4	T5
Casein	10.0	-	-	-	-
Oven-dried solvent extracted seed cake	-	47.7	-	-	-
Autoclaved oven dried seed	-	-	30.2	-	-
Autoclaved, oven-dried, solvent-extracted seed cake	-	-	-	24.3	-
Cassava starch	50.0	21.0	29.5	35.4	60.0
Glucose	12.0	7.5	12.0	12.0	12.0
Sucrose	11.7	7.5	12	12	11.7
Groundnut oil	10.0	10.0	10.0	10.0	10.0
Other ingredients+	6.3	6.3	6.3	6.3	6.3
Total	100	100	100	100	100

+ Non nutritive cellulose (5%), oyster shell (0.3%), Vitamin-mineral premix (0.4%), Di-calcium phosphate (0.3%), table salt (0.3%).

*Composition of premix: Vit A 12,500,000IU, Vit D3 2,500,000IU, Vit E 40,000mg, Vit K3 2,000mg, Vit B1 3,000mg, Vit B2 5500mg, Niacin 55,000mg, Calcium pantothenate 11,500mg, Vit B6 5000mg, Vit B12 25mg, Choline chloride 500,000mg, Folic acid 1000mg, Biotin 80mg, Manganese 120,000mg, Iron 100,000mg, Zinc 80,000mg, Copper 8500mg, Iodine 1500mg, Cobalt 300mg, Selenium 120mg, Antioxidant 120,000mg.

Experimental Design and Animal Management: Thirty weanling male Wistar rats weighing 80-100g were collected from Animal House of the Department of Physiology, University of Ibadan. The rats were weighed and were randomly assigned into five dietary treatments of six rats in each. Experimental animal group were housed individually in well-ventilated stainless metabolic cages and allowed access to one of the five experimental diets. The trial commenced after a 7-day acclimatization period and lasted for 10-days. Diets and water were provided *ad lib*. Body weight changes of rats were taken at the beginning and thereafter at three days' interval.

Blood and organ collection: On the 10th day of the experiment, rats were fasted overnight, and they were weighed before blood samples were collected. Whole blood was obtained from a puncture of the retro-orbital sinus using a capillary tube. Blood samples for haematological indices were collected in 8.5% EDTA anticoagulant tubes were immediately mixed with the anticoagulant in the tube, while the samples for serum analyses were collected into sample bottles without EDTA. All blood samples were labeled and immediately carried to the laboratory for analyses. The rats were sacrificed by neck decapitation and the heart, lungs liver and kidney were removed and hot weight of the individual organ was taken.



Statistical analysis: The blood parameters collected were subjected to analysis of variance (ANOVA) procedures (SAS, 2000). Significant differences observed between treatments, were separated using Duncan's Multiple Test (Duncan, 1955).

RESULTS AND DISCUSSION

Haematology and serum profile of the rats are presented in Tables 2 and 3, respectively. Phorbol esters (PE) have been implicated in causing a major toxicity in consuming animals and that a level lower than 3mg/Kg can be tolerated by animals (Makkar and Becker, 2009). From this study the various applied heat treatments and solvent extraction of oil did not seem to reduce the level of PEs to 3mg/kg considering the level of mortality reported in this study. Rats fed processed jatropha kernel showed progress loss in body weights however Belewu and Sam (2010) reported that the solid state fermentation of the seed cake using *Aspergillus niger* and *Mucor mucedo* significantly reduced the antinutritional factors in the jatropha kernel cake.

Table 2. Haematology of Wistar rats fed differently processed jatropha kernel meal

Parameters	T1	T2	T3	T4	T5	SEM	P Value
PCV (%)	48.67 ^a	NA	49.50 ^a	43.50 ^b	50.33 ^a	0.93	0.02
Hb (g/dL)	16.00 ^a	NA	16.50 ^a	15.00 ^b	16.67 ^a	0.23	0.03
RBC (x10 ⁶ μ L)	8.33 ^a	NA	9.00 ^a	7.00 ^b	8.67 ^a	0.26	0.02
WBC (x10 ³ μ L)	3.03	NA	3.65	3.42	3.63	0.17	0.61
Lymphocytes (%)	81.00 ^{ab}	NA	77.00 ^{ab}	74.50 ^b	82.00 ^a	1.25	0.11

^{abc} Means of treatments along a row with different superscripts differed significantly (P<0.05).

Table 3. Serum biochemistry of Wistar rats fed differently processed jatropha kernel meal

Indices	T1	T2	T3	T4	T5	SEM	P value
Alanine aminotransaminase (IU/L)	124.00 ^a	NA	122.50 ^a	87.00 ^b	130.00 ^a	5.98	0.02
Cholesterol (mg/dL)	93.00	NA	81.00	76.00	88.00	3.30	0.33
Total protein (g/dL)	6.33 ^a	NA	5.00 ^b	5.00 ^b	5.67 ^{ab}	0.22	0.05
Total triglycerides (mg/dL)	115.33 ^a	NA	67.00 ^b	67.00 ^b	76.67 ^b	8.37	0.01
VLDL (mg/dL)	23.00 ^a	NA	13.00 ^b	13.00 ^b	15.33 ^b	1.68	0.01

^{abc} Means of treatments along a row with different superscripts differed significantly (P<0.05).

The organ weights are presented in Table 4. Belewu *et al.* (2010) also reported improved growth rates and voluntary feed intake when goats were fed fungus treated jatropha kernels. The cake is reported to be low in lysine and also the phorbol esters are known to promote tumour. The level of mortality reported in this study appears to contradict reports of authors who observed no mortality (perhaps the cake came from non-toxic jatropha) when processed kernel cakes to animals. Protein isolates from jatropha kernel have been recorded impressive results in rats (Makkar and Becker, 2010; Zhao *et al.*, 2018.)



Table 4. Mortality and Relative organ weight of Wistar rats fed differently processed jatropha kernel meal

Parameters (%)	T1	T2	T3	T4	T5	SEM	P-Value
Mortality at day 10	5	0	3	2	5		
Live weight (g)	88.67 ^a	NA	69.00 ^{bc}	60.00 ^c	77.00 ^b	3.89	0.02
Heart	0.35	NA	0.38	0.49	0.48	0.03	0.21
Kidney	0.79 ^b	NA	1.05 ^a	1.13 ^a	0.78 ^b	0.05	0.07
Lung	0.87	NA	0.96	0.98	1.01	0.02	0.25
Liver	4.07 ^a	NA	3.48 ^b	2.76 ^c	4.85 ^a	0.36	0.25

abc Means of treatments along a row with different superscripts differed significantly (P<0.05). T1- Casein (positive control); T2- Oven dried, solvent extracted *J. curcas* seed. T3- Autoclaved and Oven-dried raw *J. curcas* seed. T4-Autoclaved, and oven-dried solvent extracted *J. curcas* seed. SEM- standard error of means, P value-Probability, NA- Not available

CONCLUSION AND RECOMMENDATION

The feeding of processed jatropha kernel to rats was discontinued after 10 days of feeding due to heavy mortality recorded. It was therefore seemingly impossible to determine the protein quality of the processed jatropha kernels. Contrary to some earlier studies solvent extraction, heat treatment (autoclaving) or a combining autoclaving with hexane extraction of jatropha kernels did not seem to be effective in detoxifying the antinutritional factors in the jatropha kernels. Quantifying the levels of residual phorbol esters in the processed jatropha cake should be given a priority to be able to justify inclusion of the processed jatropha kernels in animal feeding trials.

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