

Biochemical and Histologic Effect of Dietary Substitution with Solvent Extracted Neem Seed Cake of Albino Rats (wistar strain)

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Abstract: The performance of 20 albino rats (wistar strain) ages 3 weeks was investigated by feeding neem seed cake (NSC) after treatment with solvents. Group 1 and those of group II and III were fed experimental diets containing water, 75% methanol and 75% ethanol processed neem seed cake respectively as a replacement for soya bean for a period of 28 days. Replacement of soya bean by water processed neem seed depressed the growth at the end of 4th week while for the other solvent processing 4th week was a period of stunted growth. There was no significant ($p > 0.05$) difference in the PCV and Hb of animals fed MNSC diet compared to animals fed with standard protein diet. All the animals fed with processed neem seed cakes showed significant ($p < 0.05$) increases in serum albumin compared to standard. Of all the serum marker enzymes determined only the level of SGOT for animals fed with ethanol processed neem seed cake was significantly ($p < 0.05$) higher and the histopathology studies revealed fatty degeneration and necrosis of hepatic tissue and glomerular and renal necrosis compared to the standard feed and other solvents treated.

Key words: Neem seed, Processed cake, Substitution, Solvent extraction, Albino rats.

INTRODUCTION

Since low production of a protein is directly connected with the cost of animal feed, under these circumstances, most of the animal production system in the tropics are generally relying more on the uncovectioal feed sources. Neem (*Azadirachta indica*) seed cake, is a byproduct showing great potential for livestock feeding (Musalia *et al.*, 2000 and Rao, *et al.*, 2003). Recent study (James *et al.*, 2007) shows that solvent extracted neem cake can be fed to animal. However vital information is lacking on any side effect which may be associated with solvent processing. This study was carried out to evaluate the effect of processing on some biochemical parameters and histological Studies of solvent treated neem seed cake

MATERIAL AND METHODS

Ripe neem (yellowish) fruits were collected from the tree in Zaria environ in the month of August, they were depulped, dried in a hot air circulating oven at about 45 °C for 48 hours, and then decorticated to obtain seed kernel. These kernels were then stored in screw-capped bottles until required for processing and subsequent analyses.

Oil Extraction: The well ground sample (10g) was accurately weighed into a pre-weighed fat-free thimble. About 350cm³ petroleum ether (40°C - 60°C) was poured into a previously weighed 500cm³ round bottom flask, containing boiling chips. Soxhlet extractor was then fitted

and the extraction carried out for 6 hours. The neem cake was in an oven temperature of 45°C for 48 hours, and kept in an air tight container for further extraction.

Extraction of the Residual Bitter Component (neem seed cake alcoholic extract): Five grams of ground neem seed cake were weighed into three conical flasks and 100cm³ portions of 75% methanol, 75% ethanol (%v/v) and distilled water were added separately into the flasks. These were set inside the mechanical shaker at 120 r.p.m at 60°C for 6 hours as described by modified method of Mitra, (1963) to remove the residual bitter component, the extracts were collected through filtered paper and the debitterised neem cake was dried at 45°C for 2 hours. It was then stored in a screwcapped bottle until needed.

Animal Housing and Management: Healthy wistar albino rats of both sexes weighing between 100-130g were purchased from Animal unit of Pharmacology Department, Ahmadu Bello University, Zaria, Nigeria and kept in well aerated laboratory cages in Biochemistry Department Ahmadu Bello University, Zaria, Nigeria. The rats were divided into five groups, One week were allowed for the animals to adapt to experimental condition. Clean drinking water was provided *ad libitum*. At the end of conditioning periods, animals weighing between 100g to 130g were randomly distributed into 5 groups (I, II, III, IV and V) of 4-rats each of both sexes and were housed by groups in well ventilated cages.

Feeding Schedule: Rats in group I, II and III were offered neem seed cakes processed with 75% methanol,

Table 1: Dietary component (w/w) of the diet containing processed neem seed cake.

Ingredient	WNSC 1	MNSC 2	ENSC 3	SFD 4	CFD 5
Yellow maize ¹	46.62	51.10	52.19	44.41	
Soya bean meal	-	-	-	32.38	
Processed Neem cake	30.18	25.69	24.60	-	
Corn Pomace	10	10	10	10	
Limestone	0.50	0.50	0.50	0.50	
Salt	0.50	0.50	0.50	0.50	
Vitamin Premix ¹	0.20	0.20	0.20	0.20	
Corn Oil	10	10	10	10	
Bone Meal	2.00	2.00	2.00	2.00	
TOTAL	100	100	100	100	
Crude Protein ²	19.65	19.93	19.75	19.50	15.00
Crude Fibre ²	6.62	4.22	6.60	5.02	7.00

WNSC= Water processed neem seed cake; MNSC= Methanol processed neem seed cake; ENSC= Ethanol processed neem seed cake; RFD= Reference diet containing soya bean meal; CFD = Commercial feed diet. ¹The vitamin-premix used in this study is a product of Pfizer Nig.Ltd. Each contains Vit.A (I.U), 10,000; Vit.D³ ²Determined, (I.U); 2,000;Vit.E(I.U); 2.5;Vit.K (mg); 20 Choline (mg),300; Riboflavin (mg),4.2; Folic acid (mg), 0.5; Methionine (mg), 0.225; Mn (mg), 56.0; I(mg), 1.0; Fe (mg), 20.0; Cu (mg),10.0; Zn (mg), 1.25; and Co (mg), 1.25.

75% ethanol and water, group IV were given diet containing soya meal as a protein source, group V was fed commercial diet (Pfizer Nigeria Limited). The different proportions of the feed ingredients are shown in Table 1.

At the end of 28-days of feeding trials, the animals were anaesthetised by using chloroform, and were bled by cardiac puncture, the blood were collected in specimen bottles and a lateral section was cut through each. Liver, kidney and spleen were excised. Tissue samples were fixed in 10% formalin and histopathological studies was carried out by the method of (Igbokwe, 1989). Part of the whole blood was used for PCV and Hb (Dacie, and Lewis, 1991) parameters. The remaining blood was allowed to clot and serum separated using pasture pipette into clean and labeled sample bottles for determination of some biochemical parameters. Serum transaminase (ALT and AST) was determine by method of Reitman-Frankel, 1957, serum protein by Plumer, 1978 and serum albumin (Doumas *et al.*, 1971).

Statistical Analysis: The data were subjected to two-way analysis of variance and differences between the means of diets were tested (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

The effect of processed neem cake on live weight are shown in Table 2 and Figure 1. The body weight of rats fed with water- processed neem seed cake decreased significantly ($p < 0.05$) compared to other processed diet and the standard.

Biochemical parameters (Table 3 and Figure 2) show no significant ($p > 0.05$) differences in the levels of serum marker enzymes (SGPT and SGOT) in animals fed water and methanol processed neem cake and that of standard protein diet. However, the level of SGOT in rats fed with ethanol processed neem cake significantly ($p < 0.05$) increased compared to the standard protein diet. hematological parameter (Table3) indicate no significant difference between the PCV and Hb on methanol processed neem seed cake (MNSC) compared to the

Table 2: Effect of processed neem seed cake on change in weight of rats.

Diet	Average Initial Weight	Average Final Weight	Weight Change
		(% body weight)	
WNSC	70.63	65.23	- 4.90 ^c
MNSC	75.25	94.00	18.75 ^b
ENSC	76.63	97.25	20.62 ^b
SFD	80.00	101.81	21.81 ^b
CFD	96.69	129.22	32.53 ^a
Pooled			2.21
-(SEM)			

WNSC=Water processed neem seed cake MNSC=Methanol processed neem seed cake SFD=Standard protein feed diet; SEM=Standard error of the means CFD=Commercial feed diet; Values are means of four determinations. Means with different superscript in a row differ significantly ($p < 0.5$).

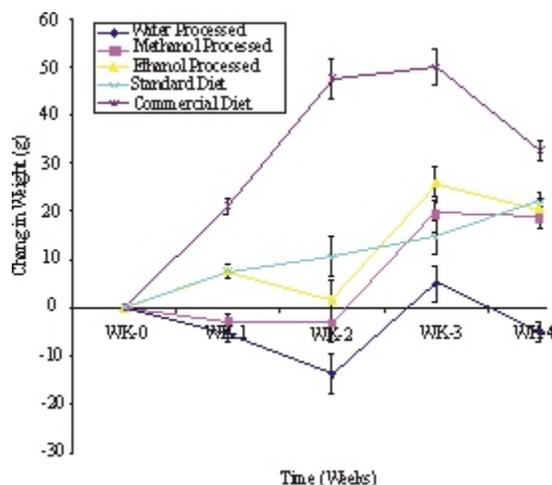


Fig 1: Body Weights Change (G) Of Rats Fed Diets Containing Processed Neem Seed Cake

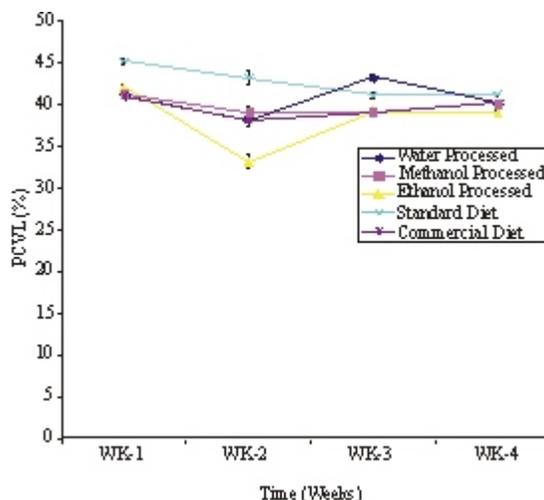


Fig 2: Change in PCV Level of Rats Fed With Processed Neem Seed Cake

standard protein diet, however significant reduction in PCV and Hb was observed in animals fed with ethanol processed neem seed cake (ENSC).

There was no significant ($p > 0.05$) difference in the level of serum protein (Table 3) in all processed neem seed cake compared with standard protein diet and commercial diet. The serum albumin in standard protein diet and commercial are significantly ($p < 0.05$) lower than all neem processed products.

Table 3: Effect of processed neem seed cake on some hematological and biochemical parameters.

Diet	PCV	Hb (g/100ml)	Serum Albumin (g/100ml)	Serum Protein (g/100ml)	SGPT U/L	SGOT U/L
WNSC	40 ^a	13 ^b	4.38 ^a	6.78 ^a	38.00 ^a	21.00 ^b
MNSC	41 ^a	14 ^a	4.40 ^a	6.60 ^a	37.50 ^a	23.50 ^b
ENSC	39 ^b	13 ^b	4.05 ^a	6.73 ^a	40.25 ^a	32.00 ^a
SFD	41 ^a	14 ^a	3.80 ^b	6.89 ^a	38.50 ^a	19.00 ^{bc}
CFD	39 ^b	13 ^b	3.87 ^b	6.77 ^a	42.50 ^a	15.00 ^c
Pooled Sem	0.49	0.18	0.10	0.13	0.91	1.26

WNSC=Water processed neem seed cake; ENSC=Ethanol processed neem seed cake; MNSC=Methanol processed neem seed cake; SFD=Standard protein feed; CFD=Commercial feed diet SEM=Standard error of the mean. Values are means of triplicate determinants. Means with different superscripts in column differ significantly (p<0.05).

The histopathological studies showed no cellular infiltration or observable tissue damage in the animals fed water processed and methanolic processed neem cake (Plate I, II, III, V, VII and VIII). However, those fed on ethanol processed neem cake (Plate IV and VI) showed fatty degeneration and necrosis of the hepatic cells as well as glomerular and renal tubular necrosis in the kidney.

Average growth rate of the rats indicated a general decrease at the second week of the experiment for processed neem seed cake, while at the third week, there was a general increase. The fourth week was a period of stunted growth, and a decrease in weight of animal fed water processed neem seed cake. This could be as a result of reduction of feed conversion with increase in the age of the animals (Omole and Onwudike, 1981).

Biochemical indices show sub-molecular effect of nutrients. Data on hematological indicate no significant difference between the PCV and Hb of animals fed MNSC compared to the standard protein diet while there was significant (p<0.05) decrease in animals fed on ENSC, this shows that animals that feeds on ENSC might develop anaemia. Anaemia is a reduction in the number of erythrocytes, haemoglobin, or both in the circulating blood. It results from excessive red blood cell (RBC) destruction, RBC loss, or decreased RBC production and is a manifestation of an underlying disease process; therefore, the response to treatment of anaemia is transient unless the underlying disease process is addressed (Straus, 1998).

There was no difference between the standard and the neem processed seed cakes results for serum protein, however all the processed neem seed cake showed significant (p<0.05) higher serum albumin level compared to the standard protein diet. Inclusion of processed neem seed cake especially MNSC have no reducing effect on the mobilization of nutrients from the diets, hence there is no reduction effect on serum levels of biomolecules. In fact processed neem seed cake in this study has been found to significantly (p<0.05) increase the level of serum albumin compared to standard diet. Low serum albumin has also been associated with low protein intake. Animals that grow at a faster rate than others sometimes have higher serum albumin, Hb, glucose and low concentration of potassium (Mitchell and Macleod, 1983; McDonald *et al.*, 1994). The processed neem seed cake in this study can be said to be of good protein content, however it has been shown that feeding trial of 10, 15 and 20% neem seed cake included in concentrate mixtures to lactating buffaloes shows lower RBC, WBC, and haemoglobin

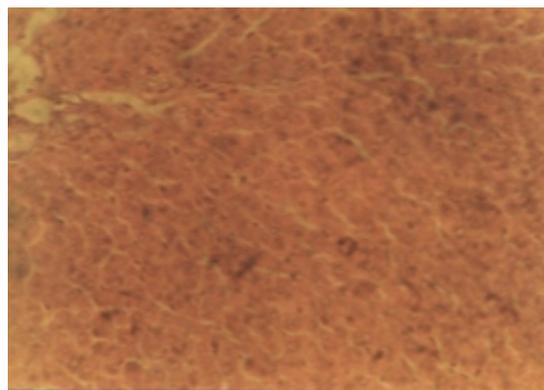


Plate I: Photomicrograph of a section of liver from a control rat fed with standard feed Note: The normal microscopic appearance of the liver section. H & E. X 400

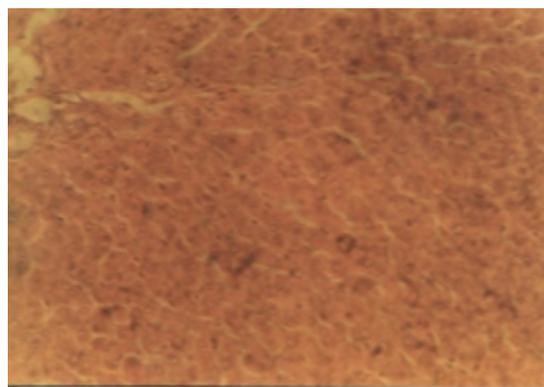


Plate II: Photomicrograph of a section of liver from a rat fed with water processed neem seed cake Note: The normal microscopic appearance of the liver section. H & E. X 400

levels (Pyne *et al.*, 1979). The difference may be as result of processing done in this study.

The level of serum marker enzymes (SGPT and SGOT) of the animal fed water and methanol processed neem seed cake compared well with standard protein diet, however the level of SGOT for ethanol processed neem seed cake significantly (p<0.05) increased compared to the standard. Both SGPT and SGOT are excellent marker of liver damage caused by exposure to toxic substances (Ranjna, 1999), SGOT is not specific for the liver only but is also located in other organs like the heart, brain, kidney and skeletal muscle. SGPT is more liver specific

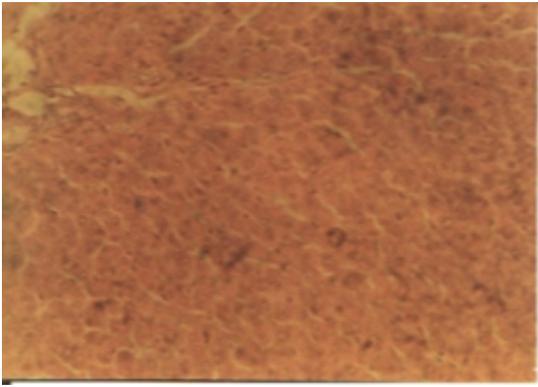


Plate III: Photomicrograph of a section of liver from a rat fed with methanol processed neem seed cake. Note: The normal microscopic appearance of the liver section. H & E. x 400

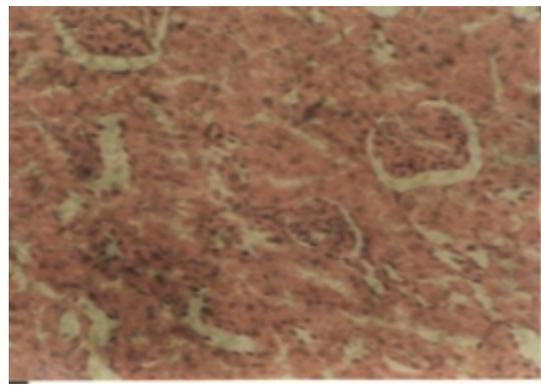


Plate VI: Photomicrograph of a section of kidney from a rat fed with water processed neem seed cake. Note: The normal microscopic appearance of the kidney section. H & E stain x 400

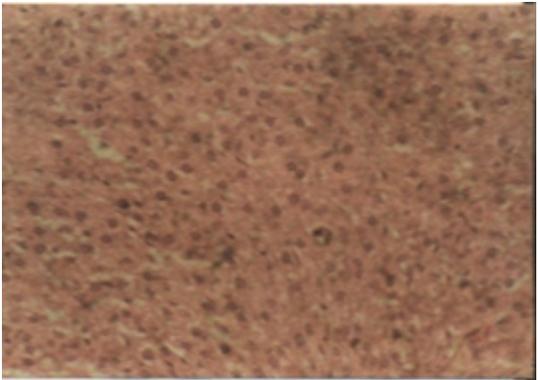


Plate IV: Photomicrograph of a section of liver from a rat fed ethanol neem seed cake (ENSC). Note: Fatty degeneration (F) and necrosis (N) of the hepatic cells. H & E. x 400

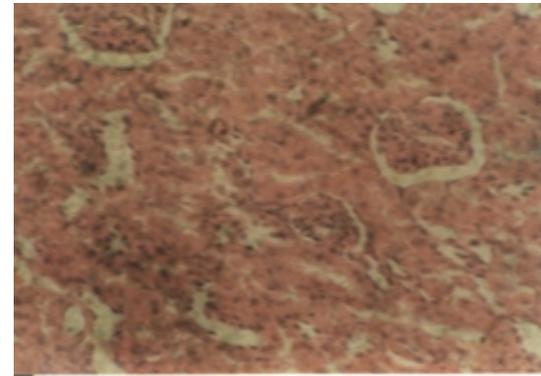


Plate VII: Photomicrograph of a section of kidney from a rat Fed with methanol processed neem seed cake. Note: The normal microscopic appearance of the kidney section. H & E stain x 400

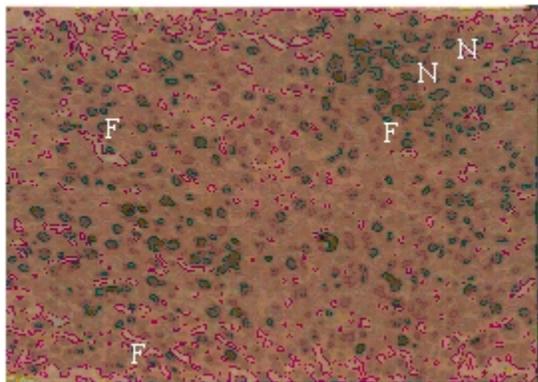


Plate V: Photomicrograph of a section of kidney from a control rat fed with standard feed. Note: The normal microscopic appearance of the kidney section. H & E stain x 400

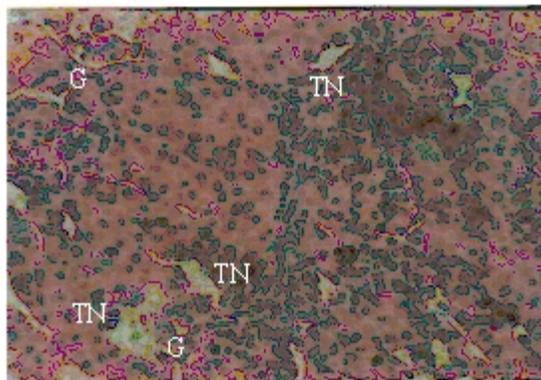


Plate VIII: Photomicrograph of a section of kidney from a rat fed with ethanol neem seed cake (ENSC). Note: The glomerular (G) and renal tubular necrosis (TN). H & E stains x 40

enzyme for diagnostic use when the integrity of the hepatocellular membrane is compromise, there is extrusion of the enzyme into the plasma (Moss and Henderson, 1999). Elevation in the activity of SGOT in

ethanol processed neem cake can be associated with cell necrosis of many tissues. Pathology involving the skeletal or cardiac muscle and/or the hepatic parenchyma, allows leakage of large amounts of this enzyme.

The histopathological studies showed no cellular infiltration or observable tissue damage for the animal fed with water, and methanol processed neem seed cake. Those on ethanol processed, showed fatty degeneration and necrosis of the hepatic cells, as well as glomerular and tubular necrosis in the kidney thus suggest that the inclusion of ethanol processed neem seed cake on the diet of rats is not safe. Similar findings (Subbarayuda and Reddy, 1975) have reported fatty changes in liver, and sluggish spermatogenesis in Bobcock cockerels fed deoiled neem seed cake.

CONCLUSION

Data on hematological and biochemical parameters indicate that the PCV, Hb and serum protein of animals on MNSC processed neem seed cake showed no significant ($p < 0.05$) change compared with standard diet and the histopathological studies of the organs showed no cellular damaged hence it safe when incorporated in to animal feed.

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