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An Assessment of Serum Metabolites, Liver Enzymes Activities and Relative Organ Characteristics in Rabbits Fed Varying Levels of Chromolaena Odorata

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ABSTRACT

Biochemical and relative organ changes of rabbits fed with *Chromolaena odorata* Leaf Meal (COLM) were studied. A total of 32 rabbits consisting of 17 males and 15 females and of mixed breeds, aged 6-8 weeks and weighing 400-800 g were randomized into four diet groups of 0, 10, 20 and 30% in a completely randomized design for 56 days. Each treatment was replicated 4 times with two rabbits per replicate. Samples of blood were collected weekly from the ear vein for biochemical studies and the relative organ changes were determined using an electronic weighing scale. Results of biochemical analysis showed that the serum urea concentration was significantly ($p<0.05$) higher at an increasing level of COLM. Total cholesterol, triglycerides and LDL were found to significantly ($p<0.05$) decreased at increasing rate of COLM with the lowest level at 20 and 30% and corresponding to a value of 55.0 mg dL^{-1} at 20%, 25.0 mol L^{-1} at 30% and 22.5 mol L^{-1} at 20% for total cholesterol, triglycerides and LDL, respectively. As the rate of COLM increases, significant decrease was also observed in serum albumin concentration. Significant ($p<0.05$) increase observed in HDL was higher at 20% (60.5 mol L^{-1}) than at 0% (42.0 mol L^{-1}), 10% (24.0 mol L^{-1}) and 30% (49.5 mol L^{-1}) diet groups. Treatment with *C. odorata* significantly ($p<0.05$) prevented the elevation of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) with the lowest value at 10% for both serum transaminase. Results of relative organ changes showed higher significant ($p<0.05$) difference in the kidney and heart weight. However, significant decrease was observed in lungs and spleen weight at an increasing level of COLM. These results revealed that *C. odorata* may be safe and non-toxic only at a level that does not exceed 10% in the diet of rabbits.

Key words: *Chromolaena odorata*, rabbits, serum metabolites, liver enzymes, organ characteristics

INTRODUCTION

Efforts have been made by the animal nutritionist to reduce the dependence on conventional sources of feedstuffs as their availability and affordability is continually posing a challenge that results from competition of farm animals with human for these materials. Leaves of many plants (*Leucaena leucocephala*, *Microdesmis puberula*, *Ipomoea asrifolia*, *Alchornia mudiflora* etc.) are now being utilized in feeding livestock and this is seen to have tremendously reduced the cost of production. However, it is not all about the reduction of production cost but also the consideration of its safety with regards to the potential toxicities that may be associated with it and also its effective utilization and overall digestibility. This is because although plants generally contain some bioactive properties believed to be responsible for their desirable effects, they also contain some phytotoxins and other heavymetal contaminants whose toxicological actions have always been ignored.

Chromolaena odorata is a perennial shrub which belongs to the family Austeracea. It is native to South and Central America but is presently found throughout the tropics, Nigeria inclusive (Fosberg and Sachet, 1980). It is commonly called "Awolowo", "independence weed", siam weed, triffid weed, bitter bush or jack in the bush (Okon and Amalu, 2003). The phytochemical screening of the leaves of *Chromolaena odorata* revealed the presence of alkaloids, cyanogenic glycosides, flavonoids (aurone, chalcone, flavone and flavonol), phytates, saponins and tannins (Akinmutimi and Akufo, 2006; Ngozi *et al*., 2009).

Elufioye *et al*. (2009) investigated the toxicity of ethanolic extracts of aerial parts of *Tithonia diversifolia* A. Gray (Asterecea) at doses of 400, 800 and 1600 mg g^{-1} in rats and observed that the plant showed acute toxic effects on the liver and kidney and concluded that the observed toxicity at the lowest dose tested raises concern over its safety. In this unfolding scenario, since reports on the safety of *Chromolaena odorata* are scanty and contradictory, the toxicological evaluation of *Chromolaena odorata* would not only justify its use but may also present a low-cost and readily available alternative feedstuff as a means of reducing the cost of animal production. This study was therefore designed to evaluate the effect of *Chromolaena odorata* on biochemical parameters and relative organ changes of the rabbits.

MATERIALS AND METHODS

Collection and preparation of COLM: The leaf meal used for this study was prepared by cutting the stems of nearly matured and just maturing *Chromolaena odorata* plants from the environment of the University of Benin, Benin city with the leaves intact and sun dried for 3-4 days. The dried leaves were hand picked directly into jute sacks and later milled. The leaf meal obtained there was incorporated with other feed ingredients to compound the treatment diets used in this study.

Experimental diet: The test diets fed the rabbits during the period of the experiment contained 4 levels of COLM, 0, 10, 20 and 30% inclusion level as presented in Table 1.

Management of experimental animals: A total of 32 rabbits consisting of 17 males and 15 females and of mixed breeds, aged 6-8 weeks and weighing 400-800 g were used in this study. The rabbits were housed in a dwarf walled rabbitry. The hutches with dimension 60×60×80 cm and raised on wooden stand of about 75 cm high, were arranged on a concrete floor in the rabbitry. They were quarantined for 3 weeks during which they were treated with Ivomec® injection for the control of internal and external parasites. The experimental animals were given *ad libitum* access to water. Commercial diet (15% crude protein and 2500 kcal kg^{-1} metabolizable energy) was supplied throughout the quarantine period before the introduction of the experimental diets.

Experimental design: The rabbits were randomly assigned to four treatment groups. Each treatment was replicated 4 times with two bucks constituting a replicate in a Completely Randomized Design (CRD).

Table 1: Ingredient composition of the experimental diets and their calculated nutrient compositions

Ingredients	Percentage (0%)	Proportion (10%)	Ingredients (20%)	Diets (30%)
Maize	27.50	24.00	19.00	12.50
COLM	-	10.00	20.00	30.00
Groundnut cake	11.00	11.00	10.00	10.00
Dried brewers grain	24.50	17.50	10.50	-
Palm kernel cake	34.00	28.00	17.00	14.00
Wheat offal	-	6.50	20.50	30.50
Oyster shell	1.00	1.00	1.00	1.00
Bone meal	1.50	1.50	1.50	1.50
Salt	0.25	0.25	0.25	0.25
Premix	0.25	0.25	0.25	0.25
Calculated nutrient composition				
Energy (ME kcal kg^{-1})	2460.50	2505.90	2511.60	2508.70
Crude protein (%)	18.23	18.22	18.26	18.18
Crude fibre (%)	10.08	10.09	10.10	10.26

Biological assays: Blood collected from the ear blood vessels under mild chloroform anesthesia was kept for 45 min at 4°C to cloth. Serum was separated by centrifugation at 600 g for 15 min and analyzed for various biochemical parameters. Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alanine phosphatase (ALP) was determined using a chemistry autoanalyser (ciba corning 550 express). Total bilirubin and conjugated bilirubin estimation exploits the use of diazotized sulphanilic acid as described by Pearlman and Lee (1974) and Zoppi *et al.* (1976). The Biuret method as described by Gornall *et al.* (1949) was employed for the determination of total protein concentration in serum and supernatant. Serum albumin and globulin concentration was estimated by the method employing bromocresol green as described by Doumas *et al.* (1971). Sodium, potassium, chloride and bicarbonate were estimated using Flame photometer, serum creatinine determined by the alkaline picrate method (Slot, 1965; Heinegard and Tinderstrom, 1973). Serum urea concentration was by diacetylmonoxime method described by Natelson *et al.* (1951). Plasma Total Cholesterol (TC), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL) and triglyceride (TG) were assayed enzymatically with Randox test kits (Randox Laboratories, Crumlin, England).

At the end of the blood collection period, the rabbits were euthanized and the kidney, heart, lungs, spleen, liver and intestine were harvested and measured.

Statistical analysis: Data obtained on each parameter was subjected to one way analysis of variance (Steel and Torrie, 1980) and differences between treatment means were separated using Duncan's Multiple Range Test (DMRT) (Duncan, 1955).

RESULTS AND DISCUSSION

The mean organ weight of rabbits fed graded levels of COLM is as presented in Table 2. There were significant ($p<0.05$) differences for all the organs examined. Apart from the values obtained for lungs weight and intestine length which decreased significantly ($p<0.05$) with an increased inclusion level of COLM, other measured organ parameters increased at an increasing rate of COLM. The increasing significant ($p<0.05$) difference of kidney weight and serum urea as the level of inclusion of COLM in the diets increased may well buttress earlier studies by Kluwe and Hook (1981) who stated that increased kidney weight (either absolute or relative) and derangement of at least one serum parameter is an indicator of nephrotoxicity. The result of this study is in

Table 2: Relative organ characteristics of rabbits fed graded levels of COLM

Parameters	0%	10%	20%	30%	SEM
Intestine length (cm)	247.10	229.60	175.00	189.10	7.27
Kidney weight (g)	5.68^{b}	6.21^{b}	6.88^{ab}	8.18^{a}	0.48
Heart weight (g)	2.41^{b}	3.69^{a}	3.66^{a}	4.43^{a}	0.34
Lung weight (g)	7.46^{a}	8.06^{a}	6.23^{b}	4.28^{c}	0.21
Spleen weight (g)	0.64^{a}	0.49^{c}	0.53^{ab}	0.35^{b}	0.04
Liver weight (g)	23.20	25.10	29.30	44.00	0.59

^{a,b,c}Means bearing different letters of superscript within the same row differ significantly ($p<0.05$)

Table 3: Serum biochemical components of rabbits fed graded levels of COLM diets

Parameters	0%	10%	20%	30%	SEM
Urea (g dL^{-1})	23.00^{b}	32.00^{b}	41.50^{a}	46.00^{a}	2.91
Sodium (mmol L^{-1})	135.00	138.00	141.00	139.00	2.20
Potassium (mmol L^{-1})	4.10	4.55	4.90	5.55	0.45
Chloride (mmol L^{-1})	116.00	109.00	109.00	117.00	3.44
Bicarbonate (mmol L^{-1})	21.00	24.50	18.00	22.00	2.85
Creatinine (mg dL^{-1})	0.71	0.83	0.96	0.97	0.09
Total cholesterol (mg dL^{-1})	119.00^{a}	123.00^{a}	55.00^{c}	77.50^{b}	6.10
Triglyceride (mol L^{-1})	39.00^{a}	30.50^{ab}	29.00^{ab}	25.00^{b}	3.73
HDL (mol L^{-1})	42.00^{b}	24.00^{c}	60.50^{a}	49.50^{b}	3.76
LDL (mol L^{-1})	70.00^{b}	91.00^{a}	22.50^{d}	52.50^{c}	5.20
Total bilirubin (mg dL^{-1})	0.37	0.37	0.37	0.44	0.06
Conjugated bilirubin (mg dL^{-1})	0.16	0.19	0.17	0.20	0.02
ALP (μ L^{-1})	39.00	35.00	46.00	44.00	4.29
AST (μ L^{-1})	50.50^{a}	26.00^{b}	49.00^{a}	42.00^{a}	3.64
ALT (μ L^{-1})	48.50^{a}	26.50^{b}	38.00^{ab}	34.00^{ab}	4.35
Total protein (g dL^{-1})	6.40	6.15	5.90	5.90	0.48
Albumin (g dL^{-1})	3.55^{a}	3.10^{ab}	3.05^{ab}	2.90^{b}	0.11
Globulin (g dL^{-1})	3.05	3.05	3.00	2.85	0.08

^{a,b,c}Means bearing different letters of superscript within the same row differ significantly ($p<0.05$), HDL: High density lipoprotein, LDL: Low density lipoprotein, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alanine phosphatase, SEM: Standard error of mean

agreement with the findings of Akinmutimi and Akufo (2006) and Asomugha *et al.* (2013) where increased kidney weight were observed following the administration of COLM based diets. The values of spleen weight did not follow a regular pattern that could be attributed to the effect of the test ingredient. However, though the spleen weights differed significantly ($p<0.05$) showing a progressive decline at increasing COLM, the rabbits fed 20% COLM did not significantly ($p>0.05$) differ from the control.

The values for the heart and liver also followed a significant progressive increase in weight following increased COLM in the diets. These observed results could be due to the effect of nitrate, a toxic component of Siam weed. In order to overcome the effect of nitrate, the heart may have pumped more blood, leading to increased metabolic activities in the liver and a resultant increase in the weight of these organs. This result compare with the findings of Akinmutimi and Akufo (2006) and Asomugha *et al.* (2013) who, respectively reported a progressive increase in the relative weight of heart and liver following an increased inclusion rate of *Chromolaena odorata*.

The result of serum biochemical indices of rabbits fed graded levels of COLM is as shown in Table 3. Significant differences ($p<0.05$) were observed in serum urea, total cholesterol, triglyceride, HDL, LDL, AST, ALT and albumin concentrations. Renal function indices such as serum electrolyte, urea and creatinine could be used to evaluate the functional integrity of the kidney, elevated values being an indication of defective functional state (Yakubu *et al*., 2003). Lamb and Price (2012) reported that increased serum urea with normal creatinine concentrations giving rise to high ratios may be seen with a prerenal situation. Thus, the significantly ($p<0.05$) elevated serum urea (at 20 and 30%) and the normal creatinine concentrations observed in this study in the COLM treated rabbits compared to the control could probably imply a prerenal toxicity state posed by *Chromolaena odorata* at these levels. On the other hand, Finco (1989) observed that besides other factors, starvation or stress could contribute to increased protein metabolism thus elevating the serum urea levels. This could as well be the case in this study as animals in the COLM treated groups showed a decrease in the rate of feed consumption. The stress resulting from starvation could have partly contributed to the elevated serum levels of urea through increased protein catabolism. Results obtained for serum electrolytes (sodium, potassium, chloride and bicarbonate) in this study were not significantly different ($p>0.05$) and were within normal concentrations. This combination of electrolyte balance maintained by COLM is of crucial importance which suggests fluid and pH balance, cellular tonicity and regulation of neural and muscular functions of the rabbits used in this study. Increases in conjugated bilirubin and total bilirubin are highly regarded as specific markers of hepatic dysfunction (Lamb and Price, 2012). The values of serum conjugated bilirubin and serum total bilirubin did not differ significantly ($p>0.05$) among the treatment diets. Though the mean values of conjugated bilirubin in the COLM treated rabbits were slightly numerically higher than the control, the observed values were within the normal range for a healthy rabbit and suggests no hepatic dysfunction.

Elevated plasma triglyceride concentrations is both an independent and synergistic risk factor for cardiovascular diseases (Dobiasova, 2004; Martirosyan *et al*., 2007; McBride, 2007; Brunzell *et al*., 2008) and is often associated with abnormal lipoprotein metabolism, insulin resistance and diabetes mellitus (Krauss *et al*., 2006; McBride, 2007; Brunzell *et al*., 2008). Also, raised serum total cholesterol level is a risk factor associated with atherosclerosis and other cardiovascular diseases (Alaupovic, 1971; Ademuyiwa *et al*., 2005). Therefore, the significant reduction of serum triglycerides and total cholesterol levels in the COLM diet groups connotes the ability of the diets to protect against cardiovascular diseases. The cholesterol lowering effect may have been due to the saponin content since saponin has been found to possess hypocholesterolemic properties (Soetan, 2008; Jan Alexander *et al*., 2009). Increases in serum HDL cholesterol have been found to reduce risk in coronary heart diseases (Assmann and Gotto, 2004; Rang *et al*., 2004). This exerts a protective effect by decreasing the rate of entry of cholesterol into the cell and increasing the rate of cholesterol release from the cell (Marcel *et al*., 1980) by enhancing reverse cholesterol transport by scavenging excess cholesterol from peripheral tissues. Elevated HDL also inhibits the oxidation of LDL as well as the atherogenic effects of oxidized LDL by virtue of its antioxidant (Assmann and Gotto, 2004; Brunzell *et al*., 2008) and anti-inflammatory property (Ademuyiwa *et al*., 2005). In this study, the COLM diets significantly ($p<0.05$) increased serum HDL levels. This increase in HDL agrees with the findings of Promise *et al*. (2012) who administered aqueous extract of *Chromolaena odorata* to *Salmonella typhi* infested Wistar rats. Significantly ($p<0.05$) lower serum LDL levels were also observed in the animals that were treated with 20 and 30% of COLM in the diets. This increase in HDL and reduction in LDL and triglycerides confirms the hypolipidemic protective effect of COLM against coronary heart diseases (Rang *et al*., 2004; Shen, 2007). The resultant effect of this could be explained through cholesterol homeostasis which is maintained by the conversion of cholesterol to bile acids and subsequent regulation of bile acid metabolism. Bile acids themselves provide surface-active detergent molecules that facilitate both hepatic excretion of cholesterol and solubilization of lipids for intestinal absorption (Galton and Krone, 1991; Lamb and Price, 2012).

Liver diseases are the most common cause of increased transaminase activity in serum (Lamb and Price, 2012). The significant ($p<0.05$) decrease in the serum AST and ALT activities of the animals treated with COLM could be indicative of an improvement in the liver function due to the hepatoprotective activity of *Chromolaena odorata* (Alisi *et al.*, 2011). ALP values showed no significant ($p>0.05$) difference and were within the normal range (10-96 μL) reported by Mitruka and Rawnley (1977) for clinically healthy rabbits.

Low concentrations of albumin are strong predictor associated with poorer outcomes in kidney, liver dysfunction and cardiovascular diseases (Amaral *et al.*, 2008; Horwich *et al.*, 2008). In this finding, albumin concentration levels are significantly ($p<0.05$) reduced with increase inclusion rate of *Chromolaena odorata* in the diets. Though the observed albumin concentrations were within the normal range of 2.5-4.0 g dL^{-1} (Mitruka and Rawnley, 1977). It will therefore suffice to deduce that the decreasing trend of serum albumin in this study is tending to indicate a prerenal dysfunction. Although, the histological study of the kidney was not investigated, the biochemical results suggest that *Chromolaena odorata* could predispose rabbits to renal dysfunction. Thus, higher inclusion rate of *Chromolaena odorata* above 30% or longer duration of administration would have been implicative of renal failure. The total protein and globulin concentration values were within the normal range of 5.0-7.5 g dL^{-1}, respectively and were not significantly different ($p>0.05$) but followed a numerically decreasing trend with increasing rate of *Chromolaena odorata*. The decreasing trend of these serum proteins (albumin, total protein and globulin), though have no deleterious effect on the rabbits, could be implicated with reduced feed intake and subsequently reduced nutrient digestibility and also the presence of antinutritional factors inherent in the leaf meal.

CONCLUSION

The results obtained from this study shows that *Chromolaena odorata* leaf meal may enhance the physiological status of rabbits at an inclusion rate of not exceeding 10% in diet.

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