Hepatoprotective and nephroprotective effects of *Cnidoscolus aconitifolius* in protein energy malnutrition induced liver and kidney damage

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ABSTRACT

Introduction: This study was designed to evaluate the ameliorative and hypocholesterolemic effects of dietary supplementation of *Cnidoscolus aconitifolius* leaf meal (CALM) on hepatic injury and kidney injury associated with protein energy malnutrition (PEM). **Materials and Methods:** In this study, PEM was induced in weaning male Wistar albino rats by feeding them with low protein diet for 2 weeks. The effects of several recovery diets containing 20% soya protein or 20% *C. aconitifolius* in place of soya protein or 10% soya proteins with 10% *C. aconitifolius* or commercial rat feed were assessed in PEM rats. Plasma biochemical parameters were assessed as well. **Results:** After the induction of PEM, results obtained showed significant increase in alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total proteins (T.P), total bilirubin (T.Bil), triglycerides, total cholesterol, low density lipoproteins (LDL), blood urea nitrogen (BUN), and creatinine with significant reduction in plasma high density lipoproteins (HDL), albumin, sodium (Na^+), potassium (K^+), chloride (Cl^-), bicarbonate (HCO_3^-), and phosphate ($P04^{2-}$) in PEM rats. Upon introduction of recovery diets containing 20% soya protein or 20% *C. aconitifolius* in place of soya protein or 10% soya proteins with 10% *C. aconitifolius* or commercial rat feed for 4 weeks caused significant ($P < 0.05$) reduction in plasma values of ALP, ALT, AST, T.bil, T.P., LDL, total cholesterol, triglycerides, BUN, creatinine, and significant increase in HDL and complete restoration of plasma electrolytes. **Conclusions:** *C. aconitifolius* in protein deficient diets has a protective role against hepatic injury and renal damage associated with PEM.

Key words: *Cnidoscolus aconitifolius* leaf meal, hepatic injury, protein energy malnutrition, plasma biochemistry

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INTRODUCTION

Protein energy malnutrition (PEM) has been a great source of concern to the developing countries and Sub-Saharan Africa in particular. Poverty, war, famine, and lack of good quality proteins have continued to play a staggering role in the pathogenesis of PEM. This has been reported as a common condition in most developing countries especially Africa including Nigeria, Senegal and in most of the war ravaged countries such as Somalia and Sudan in Africa.[1-2] PEM has been reported to be associated with anaemia, peroxidation of unsaturated bonds in the erythrocytes membrane, decreased osmotic fragility, disruption of cholesterol phospholipids ratio due to deficiency of scavenger receptor class B type, hepatic injury, impaired blood coagulation, fatty liver, and renal insufficiency.[3-10] PEM retard growth, cause wasting and suppress host defense in humans and animals.[11-13]

This current research is aimed at determining the restoration of hepatic injury and kidney damage associated with PEM by the consumption of *Cnidoscolus aconitifolius* leaf meal. *C. aconitifolius* is a perennial shrub that belongs to the family Euphorbiaceae. It is commonly found in the tropics and sub-tropical region worldwide. It is commonly eaten as soup condiment in southwestern Nigeria specifically Lagos and Oyo States, where it is nick-named as "Iyana Ipaja." Its nutritional and antibacterial activities have recently

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been reported.[14,15] In our laboratory, the phytochemical screening, hepatoprotective, anti-inflammatory, and analgesic properties and the effect of *C. aconitifolius* against multi-drug resistant microorganism have been reported.[16] This study therefore illuminates the therapeutic significance of *C. aconitifolius* in liver and renal damage.

MATERIALS AND METHODS

The plant was harvested fresh between October and December 2007 in Ibadan, Oyo State, Nigeria and air-dried at room temperature. It was later identified and authenticated at the herbarium of the Department of Botany and Microbiology, University of Ibadan.

Animals and diets

Forty weaning male Wistar albino rats (*Rattus norvegicus*) were obtained from the experimental animal house of Department of Veterinary Physiology, Biochemistry and Pharmacology, Faculty of Veterinary Medicine, University of Ibadan and were maintained under controlled conditions of light (12-h light/dark cycle) and temperature (30 ± 1°C). PEM was induced according to a standard method.[17]

The composition of the malnourished diet is shown in the Table 1. After 14 days of inducing PEM, seven rats from each group were sacrificed. The remaining rats in the PEM groups were grouped into three and fed with recovery diets [Table 2] for 4 weeks while the forth group constitutes the animals that did not go through the PEM effects. Group A were fed with diet containing 10% of *C. aconitifolius* plus 10% soya meal, group B with 20% of *C. aconitifolius* without soy, group C with 20% of soya meal alone, and group D with commercial feed preparation from Ladokun feeds Nigeria Limited [Table 1].

Table 1: Different recovery diets

Recovery Diets (grams)	(g)	B(g)	C(g)
Soya meal	100	-	200
Cornstarch	660	660	660
Vitamix	60	60	60
Groundnut oil	80	80	80
Cnidoscolus aconitifolius	100	200	-

Animal ethics

All animals received humane care according to the criteria outline in the Guide for the Care and the Use of Laboratory Animals prepared by the National Academy Science and published by the National Institute of Health (USA). The ethic regulations have been followed in accordance with national and institutional guidelines for the protection of animals' welfare during experiment.[18] The experiment was conducted at Biochemistry Laboratory, Department of Veterinary Physiology, Biochemistry and Pharmacology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria. The experimental animals were kept at the experimental unit of the faculty.

Blood sample collection and analysis

Blood was collected from the rats through the retro–orbital venous plexus into lithium heparinised tubes. The rats were first anaesthetized with ether to make blood collection easier. The blood was centrifuged at 4,000 revolutions per minute (rpm) for 10 min. The plasma was later separated with Pasteur pipette for analysis of plasma enzymes that included alkaline phosphatise (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, total protein, total bilirubin, total cholesterol, HDL, LDL, and triglyceride.[19-26] Plasma sodium (N^+), potassium (K^+), and phosphate ($P04^{2-}$) ions were determined by flame photometry. The concentration of K^+ was calculated using the standard calibration method of Kolthoff and Elving.[27] Bicarbonate ($HC03^-$) and Chloride (Cl) anions were measured as described by Van Slyke and Aullen and Schales, respectively.[28-30]

Statistical analysis

The data were expressed as mean ± standard error of means (SEM). The test of significance between treated groups and the control was determined by the student t-test at $P < 0.05$.[31]

RESULTS

The effects of *C. Aconitifolius* on malnourished rats

Initial results obtained before the introduction of recovery diets showed significant $P < 0.01$ increase in ALP, AST,

Table 2: Plasmas enzymes and proteins post-treatment of PEM rats with different recovery diets. (n = 5)

Group	ALP (U/L)	AST (U/L)	ALT (U/L)	Total protein (g/dl)	Total bilirubin (μmol/L)	Albumin (g/dl)
A	312.00 ± 42.56	320.00 ± 58.94	66.80 ± 8.81	6.30 ± 1.37	0.44 ± 0.11	3.52 ± 0.15
B	429.40 ± 30.72**	434.20 ± 7.29 **	74.60 ± 3.32	10.88 ± 1.60 **	1.00 ± 0.21 ***	1.92 ± 0.62 ***
C	327.60 ± 3.64***	248.20 ± 4.92***	40.20 ± 3.11***	6.06 ± 2.08	0.48 ± 0.13*	3.33 ± 0.40
D	316.80 ± 2.59 ***	244.20 ± 7.80***	35.40 ± 5.59***	5.74 ± 2.01	0.36 ± 0.05***	3.06 ± 1.01
E	339.20 ± 2.88 **	257.60 ± 9.91**	44.40 ± 2.35***	6.24 ± 0.81	0.46 ± 0.09**	3.46 ± 0.54

Superscripts within each column are statistically significant. Asterisk *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$. **Group A** commercial feed preparation (control), **Group B** rats subjected to protein energy malnutrition, **Group C** were fed with diet containing 10% of Cnidoscolus aconitifolius plus 10% soya meal, **Group D** 20% of Cnidoscolus aconitifolius, and **Group E** 20% of soya meal. Malnourished rats on recovery diets were compared with rats on commercial feed preparation.

T.P, and Alb. of malnourished rats as compared to the control [Table 2]. There was increase in values of T.Bil and ALT, but the increase was not significant. There was also significant increase ($P < 0.01$) in total cholesterol, triglyceride, and LDL and significant reduction ($P < 0.01$) in HDL of malnourished rats as compared to normal control [Table 3].

The plasma electrolytes obtained from malnourished rats showed significant reduction ($P < 0.01$) in Na^+, Cl^-, and significant increase ($P < 0.05$) for creatinine and ($P < 0.01$) for urea, respectively, of malnourished rats as compared with normal control [Table 4]. The reduction in the valued of K^+, $HC0_3^-$, and $PO4^{2-}$ obtained from malnourished rats compared with the control was not statistically significant [Table 2].

The effects of recovery diets on plasma enzymes and electrolytes

The inclusion of 20% and 10% CALM recovery diets of *C. aconitifolius* without soy and with 10% soy produced significant reduction ($P < 0.001$) in ALP, ALT, and AST when compared with the commercial feed preparation. Supplementation of 20% soy also produced significant reduction ($P < 0.01$) in both ALP and AST and significant reduction ($P < 0.01$) in ALT values. There was no significant difference in the values of T.P and albumin between the groups on recovery diets and the control [Table 2].

Also, 20% of CALM inclusion without soy produced significant reduction ($P < 0.01$) in T. C., TAG, and LDL as compared with the values obtained from animals on commercial feed preparation, and 10% CALM inclusion with 10% soy significantly reduced ($P < 0.01$) in TAG and LDL as compared with the values obtained from animals on commercial feed preparation. Moreover, 10% CALM inclusion with 10% soy also significantly increased ($P < 0.05$) HDL and 20% of CALM without soy significantly increased ($P < 0.01$) HDL when compared with other recovery diets, respectively [Table 3].

There was increase in plasma of Na^+ values of the groups that received 20% of CALM without soy and soy without *C. aconitifolius*, but these values were not significant. There was also both significant reduction ($P < 0.001$) in plasma values of K^+ and HCO_3^- ($P < 0.05$) in animals that were administered 20% CALM without soy and 10% CALM inclusion with 10% soy $HCO3^-$ ($P < 0.05$) when compared to rats that received commercial feed preparation. There was also significant increase ($P < 0.05$) in both plasma values of $PO4^{2-}$ of animals on 20% CALM inclusion without soy and 10% CALM with 10% soy supplementation when compared with commercial feed preparation, respectively. Both 20% of CALM without soy and 10% CALM with 10% soy supplementation completely restored the plasma electrolytes to normal, with the exception of plasma Na^+ and K^+ plasma concentration. Plasma creatinine values were significantly reduced with the supplementation of 20% CALM inclusion without soy and 10% CALM with 10% soy and 10% soy alone at $P < 0.05$, $P < 0.01$, and $P < 0.01$, respectively [Table 4]. Results obtained show significant reduction in the plasma values of urea at $P < 0.001$ for 20% CALM inclusion without soy and 10% CALM with 10% soy at $P < 0.001$ [Table 4]. Moreover, 10% CALM,

Table 3: Plasma lipid profiles post treatment of PEM rats with different recovery diets ($n = 5$)

Groups	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	High Density Lipoproteins (mg / dl)	Low Density Lipoproteins (mg / dl)
A	78.60 ± 11.35	41.4 ± 5.81	46.00 ± 3.39	21.20 ± 8.35
B	94.40 ± 7.86 *	145.80 ± 8.64***	29.60 ± 1.14***	43.40 ± 3.05***
C	63.00 ± 5.15a	36.60 ± 3.97**	73.60 ± 7.37**	16.80 ± 2.05a***
D	38.80 ± 2.03***	21.60 ± 3.29***	67.50 ± 7.57*	8.80 ± 2.17***
E	63.00 ± 3.80	50.80 ± 4.27	55.60 ± 5.32	57.00 ± 2.35*

Superscripts within each column are statistically significant. Asterisk *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. **Group A** commercial feed preparation (control), **Group B** rats subjected to protein energy malnutrition, **Group C** were fed with diet containing 10% of *Cnidoscolus aconitifolius* plus 10% soya meal, **Group D** 20% of *Cnidoscolus aconitifolius*, and **Group E** 20% of soya meal. Malnourished rats on recovery diets were compared with rats on commercial feed preparation

Table 4: Plasmas electrolytes levels post-treatment of PEM rats with different recovery diets ($n = 5$)

Group	Na^+ (mmol/l)	K^+ (mmol/L)	CL^- (mmol/L)	$HC03^-$ (mmol/L)	$P04^-$ (mg/dl)	Creatinine (mg/dl)	Urea (mg / dl)
A	122.20 ± 5.3	6.18 ± 0.60	95.40 ± 3.29	18.40 ± 1.41	12.32 ± 3.08	0.64 ± 0.11	48.20 ± 6.98
B	107.40 ± 6.1*5	5.70 ± 1.42	49.60 ± 8.21***	18.40 ± 2.51	9.42 ± 1.63	0.84 ± 0.09*	67.80 ± 10.76**
C	120.00 ± 23.74	10.00 ± 2.24***	108.20 ± 8.26 ***	23.80 ± 8.26 *	12.10 ± 4.41*	0.42 ± 0.23*	26.60 ± 7.50***
D	150.00 ± 16.65b	11.20 ± 2.28	103.00 ± 8.69	22.20 ± 1.79*	10.34 ± 0.13*	0.38 ± 0.13**	27.60 ± 2.30***
E	139. 20 ± 6.06b	11.00 ± 1.22	95.20 ± 5.06 *	29.80 ± 4.21	7.10 ± 0.03	0.50 ± 0.07**	50.40 ± 2.28

Superscripts within each column are statistically significant. Asterisk *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Superscripts within each column are statistically significant. Asterisk *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. **Group A** commercial feed preparation (control), **Group B** rats subjected to protein energy malnutrition, **Group C** were fed with diet containing 10% of *Cnidoscolus aconitifolius* plus 10% soya meal, **Group D** 20% of *Cnidoscolus aconitifolius*, and **Group E** 20% of soya meal. Malnourished rats on recovery diets were compared with rats on commercial feed preparation

20% CALM, and 10% soy alone also significantly reduced plasma creatinine at $P < 0.05$, $P < 0.01$, and $P < 0.01$, respectively [Table 4].

DISCUSSION

The significant increase in the values of ALT, AST, ALP, total protein, and total bilirubin and significant reduction in albumin obtained in malnourished rats in this work is an indication of extensive damage to the liver and kidney due to induction of PEM. Biochemical parameters ranging from hypoalbuminaemia, anaemia with significant increase in serum ALT, and AST in PEM have been extensively discussed.[9] Hypoalbuminaeinia alone, as observed in the PEM rats, has also been reported.[32-35] Taken together, these results therefore corroborate the aberrations in liver enzymes, total protein, and albumin that are obtained in PEM rats. The three recovery diets show similar hepato-protective effects in malnourished rats after supplementation.

According to this work, PEM also resulted in significant increase in total cholesterol, triglycerides, and lower density lipoproteins and significant reduction in HDL. The alteration in these lipid profiles caused by PEM shows that PEM could be a predisposing factor to coronary heart disease (CHD) in malnourished individuals. The aberration in lipid profiles was corrected with supplementation of 20% CALM inclusion without soy and 10% CALM with 10% soy. Inclusion of soya meal in protein deficient diet slightly corrected the elevated plasma lipid, but could not ameliorate increased LDL.

The increase in blood urea nitrogen (BUN) and creatinine indicates the impairment of kidney function because of PEM. The impairment of kidney function as shown by elevated BUN and creatinine, which are associated with PEM has also been reported.[36] Different recovery diets were able to ameliorate the abnormally increased BUN and creatinine, but pronounced effect was observed from the animals that received 20% CALM inclusion without soy and 10% CALM with 10% soy.

The plasma electrolytes were also significantly reduced by induction of PEM. The electrolytes depleted are Na^+, K^+, Cl^-, $HC03^-$, and $P04^{2-}$. These electrolytes are implicated in homeostasis and metabolic function and their depletion can result in grave metabolic disorders. The depletion in serum electrolytes has been reported to be associated with PEM.[9,32]

The depletion observed in plasma electrolytes was adequately restored post-treatment with various recovery diets as compared to control animals. The plasma sodium, chloride, and phosphate ions were completely replenished with 10% and 20% CALM inclusion and soy meal supplementation. On the other hand, potassium ions could not be completely restored by any of the recovery diets; this might be due to the duration of experiment. Soy meal was much effective in restoring plasma bicarbonate to near normal values. Therefore, inclusion of 10% and 20% CALM alone or with 20% soy meal could be used to ameliorate and rejuvenate electrolyte loss or imbalance that is associated with gastrointestinal disorders such as vomiting and diarrhoea. Our laboratory worked extensively medicinal properties and toxicities that might be associated with this plant in question.[37-43]

In conclusion, the current research demonstrated that the hepatic and kidney damage that are associated with PEM could be ameliorated with 10% and 20% CALM alone or with 20% soy meal inclusion in protein deficient diets especially in poverty ravage countries in Sub-Saharan Africa. Similarly, 10% and 20% CALM alone or with 20% soy meal could also be substituted in the diets of patients suffering from gastrointestinal disturbances. The cholesterol lowering properties of *C. aconitifolius* could be potentially utilized to ameliorate CHD in malnourished individuals. This benefit is outstanding in this work. The concentration of plasma creatinine and BUN was also maintained at low level with the supplementation of 10% and 20% CALM or with 20% soy meal. Hence, different recovery diets as shown in this research have nephro-protective effect. Further studies are needed to confirm the antioxidant activities of *C. aconitifolius* and the possible underlying mechanisms that are associated with the hepato and nephro-protective effects. This plant is cheaper and serves as an alternative source of novel plant protein to soy bean.

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