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Research Article

## Hepato-Renal Activities of Hydro-Methanol Leaf Extract of *Cnidoscolus aconitifolius* in Adult Male Wistar Rats

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### Abstract

**Introduction:** Medicinal plants such as *Cnidoscolus aconitifolius* (CA) have been studied over the years for their protective and curative potentials against a myriad of common global health challenges such as hepatorenal injuries.

**Objectives:** To ascertain the effects of the hydromethanol leaf extract of CA (HMLECA) on hepatorenal parameters in adult male wistar rats.

**Method:** A total of 18 adult male wistar rats were divided into 3 groups of six rats each. Group I served as the negative control which received distilled water while groups 2 and 3 served as extract treatment groups which received 200 and 400 mg/kg BW of the HMLECA respectively. The administration was daily for a period of 58 days while blood sample for the biochemical analysis was drawn via cardiac puncture at the end of the study following light chloroform anaesthesia.

**Results and Discussion:** The results showed that the administration of both doses of the extract produced no significant ( $P > 0.05$ ) effect on the levels of serum liver enzyme (AST, ALT, ALP), TP, ALB, electrolytes ( $K^+$ ,  $Na^+$  and  $HCO_3^-$ ), Creatinine and Urea while that of the 400 mg/kg BW produced significant ( $P < 0.05$ ) decrease in the level of TB and  $Cl^-$ . Hence, the administration of the leaf extract in this study did not elicit any toxic effect on both the liver and kidney functions and could be effective as hepatorenal protective agent.

**Keywords:** *Cnidoscolus aconitifolius*, Liver function, Liver enzymes, Renal function, Serum biochemicals, Serum electrolytes

## INTRODUCTION

The use of medicinal and edible plants in developing countries for both healthcare and dietary needs has been in practice since antiquity. <sup>1</sup> In addition, Adebisi *et al.*, <sup>2</sup> stated that they have been used individually and in combination in the treatment of different ailments by traditional medical healers for a very long time. This phenomenon and practice has witnessed a resurgence in recent times with about 80% of the world's population currently relying on natural remedies from medicinal plants for their healthcare needs, due to its availability, efficacy, safety and cost effectiveness. <sup>3</sup> This occurrence has been attributed to the enormous presence of pharmacologically active compounds embedded in the plants with proven efficacy against some well-known diseases or medical conditions and their ability to protect humans against disease conditions. <sup>5</sup> Among the several plants identified with such potential is the *Cnidoscolus aconitifolius* (CA). It is an ornamental evergreen drought deciduous shrub which was taxonomically recognized by I. M. Johnston in 1923. <sup>6,7</sup> It is a large, fast growing leafy perennial shrub that belongs to the family *Euphorbiaceae* and native to the Yucatan Peninsula of Mexico where it is popularly known as "tree spinach" or "chaya" by natives of Mexico and Central America. <sup>6-8</sup> In Nigeria, it is commonly grown in the western parts and named according to the communities where it is cultivated, such as; catholic vegetables, hospital too far, "Iyana-Ipaja" or "Iapalapa" by Yorubas, "Ncheobo" or "Ugu-

oyibo" by Igbos and "Bindazugu" by Hausas. <sup>9-11</sup> This plant can grow up to 6 m even in arid conditions and has large, alternate palmate lobed leaves, succulent stems filled with milky fluid (sap) and small white flowers. <sup>8,12</sup> It is mainly cultivated for food because of its important medicinal value and consumed as vegetable in soups, salads. <sup>13,14</sup> It has also been used therapeutically for a number of ailments such as arteriosclerosis, diabetes, gallstone, kidney stones and high cholesterol and in folkloric medicines for the treatment of tropical diseases and other infections. <sup>14,15</sup> This can be attributed to the presence of a great amount of various nutritive substances in the plant such as; ascorbic acid,  $\beta$ -carotene, calcium, iron, potassium, protein, and vitamins, <sup>17,18</sup> phytochemicals such as; alkaloids, anthraquinones, cardiac glycosides, flavonoids, saponins, terpenoids and tannins as well as antioxidants such as Flavonoids, Vitamin C and E. <sup>19-21</sup> These bioactive compounds have been shown in several studies to be able to exhibit some protective effects on both the kidney and liver as well as other vital organs and systems of the body.

### Aim and objectives

This study was designed to ascertain the possible protective effect of the hydromethanol leaf extract of *C. aconitifolius* on both the hepatic and renal parameters.

## MATERIALS AND METHODS

The research experiment was performed in the animal house of the department of Human Physiology, Faculty of Basic Medical sciences, University of Port Harcourt, Nigeria following approval by the Research ethics committee of the Centre for Research Management and Development, University of Port Harcourt.

### Plant Material and Extraction

The *C. aconitifolius* leaves used for this study was obtained from the Botanical Garden of the University of Port-Harcourt and subsequently identified by the taxonomist in the Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria. Voucher specimen of the plant was deposited in the herbarium. The leaves were air dried for two weeks and ground into powder prior to its extraction at the Pharmacognosy department of the University of Port Harcourt, using Soxhlet extractor, with hydromethanol (80% methanol) as solvent. The solution was filtered using Whatman filter paper before being concentrated under reduced pressure in vacuum at 45°C. The extract yield was transferred to a hot oven where they were dried to a constant weight at 45°C and stored at 4°C. The extract was resuspended in distilled water before administration.

### Experimental Animal and Design

A total of 18 adult male wistar rats were procured from the animal house of Department of Human Physiology, University of Port Harcourt and used as the experimental model. The animals which had an average weight of 140g, were divided into 3 groups of 6 animals each and handled under the laboratory conditions, in accordance to National and Institutional guidelines for animal usage in experimental purposes. They were allowed to acclimatize for 14 days before the experimental processes began. According to the grouping, animals in group 1 served as the Negative Control group and only received distilled water while those in groups

2 and 3 which served as the Extract Treatment groups received 200 and 400 mg/kg BW of the HMLECA respectively. The entire administration was by oral gavage once daily for 58 days, while the dose of the extract used was based on that of Iyke et al.,<sup>22</sup> and the toxicity (LD50) based on the findings of Adebisi et al.<sup>6</sup>

### Sample Collection and Determination

At the end of the administration, the animals were sacrificed under light chloroform anaesthesia. Blood was drawn via cardiac puncture into appropriate sample bottles for biochemical tests.

The hepato-renal activity of the extract was assessed by determining the activities of some serum enzymes (alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP)), Total bilirubin (TB), serum proteins (Albumin (ALB) and Total Protein (TP)) and serum excretory metabolites (Urea (UR), Creatinine (CR), Chloride (CL<sup>-</sup>), Potassium ions (K<sup>+</sup>), Sodium ions (Na<sup>+</sup>) and Bicarbonate (HCO<sub>3</sub><sup>-</sup>)). The analysis was carried out with the aid of the appropriate kits and in accordance with methods previously used by Oyagbemi and Odetola,<sup>23,24</sup> and Nasiru et al.<sup>25</sup>

### Statistical Analysis

Statistical analysis was done using SPSS vs 23.0 (SPSS incorporated, Chicago, Illinois, USA) and Microsoft excel. Data are expressed as mean ± standard error of mean (SEM) and presented in tables. Significant differences were determined using one-way analysis of variance (ANOVA), while a p-value of less than 0.05 ( $p < 0.05$ ) was considered statistically significant.

## RESULTS AND DISCUSSION

### Effect of HMLECA on Liver Function

**Table 1:** Effect of hydromethanol leaf extract of *C. aconitifolius* on Liver Function (U/L).

Groups	ALP	AST	ALT
Control	30.80 ± 1.32	55.40 ± 5.95	15.30 ± 1.81
200 mg/kg HMLECA	27.60 ± 2.34	48.40 ± 1.21	13.10 ± 0.95
400 mg/kg HMLECA	24.80 ± 2.92	38.80 ± 2.13	12.90 ± 1.53

Data are expressed as mean ± SEM, n=6

A test of the liver function as reported by Dufour et al.<sup>26</sup> is commonly employed in clinical practices for investigating liver diseases, monitoring the progress of a known disease and determining the effects of administration of potentially hepatotoxic drugs. The liver function as analysed in this study was determined by the activities of enzymes (ALT, AST and ALP) which serve as biochemical markers of liver damage.<sup>27</sup> These reliable markers are released into circulation above the normal threshold and identified after serum examination to indicate inflamed or injured liver cells.<sup>28</sup> Overall, when these enzymes are increased in circulation, it shows a state of damage to the structural integrity and loss of functional integrity of the liver as well as hepatotoxicity and cellular leakage.<sup>29,30</sup> In this present study, the administration of the extract reduced the serum liver enzymes non-significantly ( $P > 0.05$ ) with increasing dose, in comparison with the control group (Table 1). This decrease is an indication that the leaf extract does not exhibit any negative or toxic activity on the hepatocytes. This agrees with the finding of Mordi and Akanji<sup>31</sup> which showed that the administration of only the aqueous leaf extract did not alter

the activities of ALT, AST and ALP, while Akachukwu et al.,<sup>32</sup> showed that both the ethanol and aqueous leaf extracts produced similar result. On the contrary, the result from the study of Chukwu et al.,<sup>30</sup> showed significant ( $P < 0.05$ ) increase in the level of ALT, AST and ALP when the animals were administered with aqueous leaf extract of *C. aconitifolius* while administration of the ethanol leaf extract in the same study produced no pathological change in the liver and may be said to have hepato-protective potentials. This potential could be linked to the presence of flavonoids which has been reported to exert a membrane-stabilizing action that protects the liver cells from injury.<sup>34</sup> Also, an early study by Uhegbu et al.,<sup>35</sup> added that non-significant changes in the level of these enzyme serves as an indication that there may not be enzyme leakage into the bloodstream due to liver damage.<sup>36</sup> Hence, the decrease in liver enzymes concentration in the serum of the experimental rats in this study indicates that administration of HMLECA may not cause liver damage and could be effective as hepato-protective agent.

### Effect of HMLECA on Serum Total Bilirubin and Proteins

**Table 2:** Effect of HMLECA on Serum Total Bilirubin and Proteins.

Groups	TB ( $\mu\text{mol/l}$ )	ALB (g/l)	TP (g/l)
Control	10.60 $\pm$ 0.76	42.20 $\pm$ 1.02	70.6 $\pm$ 1.43
200 mg/kg HMLECA	9.92 $\pm$ 0.24	41.80 $\pm$ 1.16	66.0 $\pm$ 2.63
400 mg/kg HMLECA	7.14 $\pm$ 0.34*	39.20 $\pm$ 3.43	67.2 $\pm$ 3.06

Data are expressed as mean  $\pm$  SEM, n=6

\*significantly different from control group (P<0.05)

Another common biochemical parameter for detecting liver injury and assessing its excretory function is serum total bilirubin (TB), which is the yellow breakdown product of normal heme catabolism released into circulation following cellular damages to indicate the development of hepatotoxicity.<sup>37-39</sup> When excess in circulation, it serves as an indicator to excessive destruction of heme, blockage of biliary tract and release of unconjugated bilirubin from damaged and dead hepatocytes.<sup>16,40</sup> Also, Akachukwu *et al.*,<sup>32</sup> stated that alteration in the concentration of both Total Protein (TP) and Albumin (ALB) which can be used to indicate the integrity of glomeruli, may also serve as indicators for cirrhosis of liver, nephritic syndrome, malnutrition, and malignancy. As seen in this study (Table 2), administration of the different doses of HMLECA brought about a non-significant (P>0.05) decrease in ALB and TP while the 400 mg/kg of HMLECA resulted in a significant (P<0.05) decrease in the level of TB, all in comparison with the control group. Other previous studies have also demonstrated the ability of plants to exact similar effect such as that of Nasiru *et al.*,<sup>25</sup> Iroanya *et al.*,<sup>39</sup> and Ogbe *et al.*,<sup>16</sup> which showed that the administration of aqueous leaf extract of *Moringa oleifera*, ethanolic leaf extract of *Jatropha tanjorensis* and aqueous leaf extract of *Lophira lanceolata*

respectively, caused a reduction in serum TB. Also, Akachukwu *et al.*,<sup>32</sup> showed that the administration of the aqueous leaf extract of *C. aconitifolius* produced no significant change in the level of the proteins while a contrary report by Chukwu *et al.*,<sup>30</sup> showed a significant (P<0.05) decrease for TP and ALB following the administration of the aqueous leaf extract of *C. aconitifolius*. However, the administration of the ethanol leaf extract in the same study produced a non-significant (P>0.05) increase for TP and ALB. According to Nasiru *et al.*,<sup>25</sup> the reduction of bilirubin levels after treatment with extract suggests the potency of extract to protect the red blood cell membrane against damage, while Ogbe *et al.*,<sup>16</sup> added that it may indicate a possible hepatocyte membrane stabilization and hepato-protective effect, thereby preventing elevation of this biomarker. Also, Saba *et al.*,<sup>4</sup> stated that a significant decrease in serum ALB is associated with active cirrhosis and biliary liver damage, while Oyagbemi and Odetola,<sup>25</sup> reported that the serum ALB in paracetamol intoxicated animals were significantly elevated following the administration of the ethanol leaf extract of *C. aconitifolius*. Hence, this confirms the non-hepatotoxic potentials of HMLECA.

### Effect of HMLECA on Renal Function Indices

**Table 3:** Effect of HMLECA on Serum Renal function indices.

Groups	K <sup>+</sup> (mmol/l)	Na <sup>+</sup> (mmol/l)	HC03 <sup>-</sup> (mmol/l)	Cl <sup>-</sup> (mmol/l)	UR (mmol/l)	CR ( $\mu\text{mol/l}$ )
Control	5.50 $\pm$ 0.29	138.0 $\pm$ 3.78	26.00 $\pm$ 1.41	56.60 $\pm$ 0.93	3.60 $\pm$ 0.39	73.40 $\pm$ 6.59
200 mg/kg HMLECA	5.68 $\pm$ 0.38	147.4 $\pm$ 5.62	26.8 $\pm$ 1.02	54.6 $\pm$ 0.93	3.94 $\pm$ 0.52	79.0 $\pm$ 10.17
400 mg/kg HMLECA	5.30 $\pm$ 0.25	137.0 $\pm$ 2.98	23.6 $\pm$ 0.75	51.2 $\pm$ 0.37*	2.98 $\pm$ 0.29	60.0 $\pm$ 5.65

Data are expressed as mean  $\pm$  SEM, n=6

\*significantly different from control group (P<0.05)

The administration of HMLECA on the kidney function indices was also determined in this study by analyzing the levels of the excretory metabolites; Creatinine, urea and electrolytes. Both Creatinine (a non-protein nitrogenous substance formed from creatine and phosphocreatine during muscle metabolism) and Urea (the major nitrogen containing metabolic product of protein catabolism) which serve as biochemical markers of renal injuries, renal function and evaluation of the functional capacity of the nephrons, are seen to be elevated in cases of kidney dysfunction.<sup>16,42,43</sup> The rate of excretion of these substances are influenced by glomerular filtration rate (GFR), hence, any abnormality that leads to a decrease in the GFR will result in an increased serum creatinine and Urea.<sup>33</sup> Also, Oyagbemi and Odetola,<sup>24</sup> added that increase in blood urea nitrogen (BUN) and creatinine is an indication of impairment in kidney function. The result from this study as shown in Table 3 demonstrates

that the administration of the 200 and 400 mg/kg of the extract both produced a non-significant (P>0.05) increase and decrease in CR and UR levels respectively, when compared with the control group, while the electrolyte levels were seen to decrease with increasing dose of the extract. However, only chloride anions (Cl<sup>-</sup>) showed a significant (P<0.05) decrease upon administration of the 400 mg/kg extract in comparison with the control group. The non-significant effect of the administration of the extract on Serum CR and UR in this present study disagrees with the finding of Akachukwu *et al.*,<sup>32</sup> which showed that the administration of the aqueous leaf extract of *C. aconitifolius* significantly (P<0.05) altered the serum CR contents, while that of the 200 mg/kg significantly (P<0.05) decreased the serum UR contents. This may be attributed to the different extraction solvent used in the two studies. However, Adebayo *et al.*,<sup>2</sup> stated that an insignificant change in serum



creatinine and urea concentration suggests that the kidneys are not compromised by the administration of the extract.

However, its effect which is seen to be more in the higher dose, may be attributed to its ability to protect the nephrons and increase GFR. <sup>33</sup> Pertaining to the electrolyte levels which are implicated in both homeostatic and metabolic functions, only  $Cl^-$  showed a significant ( $P < 0.05$ ) change upon administration of 400 mg/kg of the extract. In agreement with this finding, Oyagbemi and Odetola<sup>23</sup> showed that the administration of the ethanolic extract of *C. aconitifolius* following paracetamol induced toxicity reduced the elevated levels of these electrolytes to near normal in dose-dependent manner. According to Ohwokevwu and Ogunka-Nnoka, <sup>5</sup> the two most important group of electrolytes that can be used to access renal functions are; serum chloride and bicarbonate ions. Hence, their low level of concentration in this study indicates that the extract possesses a renal protective potential and did not induce any pathological effects on the tubular and glomerular functions.

## CONCLUSION

The liver is essential in detoxifying and transforming chemicals and drugs while the Kidney is involved in the homeostasis and excretion of drugs. Hence, these organs are both exposed to any accompanying toxic effects of both chemicals and drugs to which the body is exposed to. The findings of this study have shown that the administration of HMLECA did not induce any toxic effect on both organs but may rather possess protective effect against the occurrence of hepato-renal injuries. This gives credibility to the efficacy of the presence of several bioactive substances in the plant and its use in traditional medicine for healthcare and dietary needs as well as the treatment of various tropical diseases. Further studies may be conducted to isolate and characterize the bioactive compounds responsible for these activities as well as their mechanism of action.

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