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## Amethystic properties of the aqueous leaf extract of *Cnidoscolus aconitifolius* on different alcohol dosage in *Sylvilagus nuttallii* Rabbits

Mordi J. C.

Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Delsu, Abraka,

### ABSTRACT

Alcohol abuse and its associated complications have lead to the search of scientific formulations and herbal drugs that would enhance alcohol clearance and its management. In this study, anti-intoxicating principle of *C. aconitifolius* extract was demonstrated. Twelve *Sylvilagus nuttallii* rabbits weighing between 1kg -1.2kg were used. The animals were divided into three groups of four rabbits each. On two different sessions, administration was carried out orally. During the first occasion, alcohol alone was consumed, but during the second occasion different extract doses were used (0.5, 1.5 and 2g extract/kg body weight). The ingestion of 1.1g ethanol/kg body weight produced a peak blood alcohol level (pBAL) of 0.105%, 0.133% and 0.092% respectively for the three groups. The administration of 0.5g/kg of *C. aconitifolius* extract generated a pBAL of 0.093% as against 0.105%, while 1.5g/kg of *C. aconitifolius* extract produced a pBAL of 0.129% as against 0.133% ethanol alone. Also at a dose of 2g/kg of *C. aconitifolius* extract, the peak BAL obtained was 0.090% as compared to 0.092% ethanol. Subsequently, the blood ethanol disappearance rate ( $\beta_{60}$ ) was enhanced from 0.052%/h on ethanol administration to 0.059%/h upon 1.5g/kg *C.a* treatment, with a significant decrease ( $p<0.05$ ) in time to zero BAL of 150.9mins for ethanol administration alone and 131.5mins on 1.5g/kg treatment of *C.a*. The same ethanol kinetic change was observed when the extract dose was increased to 2g/kg. The time to zero BAL was reduced from 139.6mins to 119.8mins when 2g/kg was administered. The result from this claim corroborates folk claims that *C. aconitifolius* might posses anti-intoxicating properties, however, this claim needs to be substantiated in terms of establishing a clear-cut mechanism as to how the extract facilitates and accelerates alcohol clearance from blood system.

**Keywords:** *Cnidoscolus aconitifolius* (C.A), anti-intoxicating, ethanol, blood alcohol level (BAL)

### INTRODUCTION

The search for anti-intoxicating, alcohol clearing and alcohol reducing agents in the body would be of greatly relevance to the people of Niger Delta and precisely Nigeria at large. Alcohol consumption has caused various biochemical disturbances such as alterations in the cytosolic and mitosolic  $NAD^+$ /  $NADH$  ratio [1]. These metabolic alterations can contribute to myriad of biochemical events that result in diverse health complications common among alcoholics [2]. Beside the stigmatization associated with ingesting large quantities of alcohol, alcohol oxidation can generate products like acetaldehyde and acetate in the liver that can be injurious to body tissues [3]. More so, these disturbances and complications can further lead to alcohol dependence upon long-term heavy consumption of alcohol [4]. In spite of the health challenges implicated with prolong alcohol consumption and use, its abuse and addiction has continued to heighten to alarming levels that can be problematic to many communities [5].

Substances like naloxone, amantadine, and gelatin composed of 50mg methylene blue have not shown positive amethystic properties in humans [6]. Oral fructose which has shown to enhance the metabolic clearance and removal of ingested alcohol has alongside revealed side effects that could be detrimental to animal health [7, 8].

The redirection of medical practice to herbal therapy and use of plant product has contributed positively to the management of ailments in recent times. Not only because they are accessible locally but also cost effective [9]. *Cnidoscopus aconitifolius* popularly known by the Yoruba ethnic community in Western Nigeria as “*Efo Iyana Ipaja*”, and nick-named “*Hospital Too Far*” by the Urohbo people of Niger Delta, has propagated that the plant possesses anti-intoxicating properties and other medicinal efficacy [10]. More so, drunks were given some quantity of the extract in glass cups called “shorts” to lower blood alcohol level. Possible claims by these traditional practitioners have associated the plant to the treatment of alcohol related diseases. *Cnidoscopus aconitifolius* has received wide publicity in the management of diabetes, obesity, kidney stones, hemorrhoids, acne, and eye problems [11]. Several studies have shown the plant to possess antioxidant properties against paracetamol toxicity [12] while other school of thoughts has clearly elucidated and recommended the aqueous leaf extract of *Cnidoscopus aconitifolius* as female contraceptive to prevent conception [13].

This present research therefore attempts to investigate the effect of the aqueous leaf extract of *Cnidoscopus aconitifolius* on blood alcohol level by administering various doses to male rabbits to ascertain its alcohol clearance ability or amethystic properties.

## MATERIALS AND METHODS

### Collection of Plant material

Fresh leaves of *Cnidoscopus aconitifolius* were collected from the vicinity of the Delta State University Health Center, Abraka, Nigeria and were authenticated by Mr Michael Onadeji of the Herbarium Unit of Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. Samples were preserved for future references and a voucher number was obtained.



Fig 1: *Cnidoscopus aconitifolius* in its original habitat at Abraka

### Preparation of the aqueous plant extract

The leaves were detached from the stalk of the plant; air dried for two weeks and then blended into powder. The weighed of the powdered sample obtained was 300g. The powder was soaked in 500ml of distilled water for 48hrs. The extract was obtained using a rotary evaporator and the solvent was extracted at a temperature of 45°C. Paste-like crude extract obtained was stored cold in desiccators and used when required. The extract was reconstituted into appropriate volumes with distilled water to obtain the desired concentration.

### Ethical Consideration

The ethical procedures governing experimental code of conduct with life animals were strictly observed as stipulated by Ward and Elsea, [14]. Experimental procedures to this study were approved and guided by the institutions Sub ethical committee in the Faculty for the use of laboratory animals.

### Animal and Experimental Design

Twelve *Sylvilagus nuttallii* rabbits weighing between 1kg -1.2kg were used for this study. The animals were purchased from Greham's Farms in Warri, Delta State and were taken to the animal Unit of the Delta state University where they were kept in improvised metallic guazed cages before separating them into three groups (A, B and C) containing four *Sylvilagus nuttallii* rabbits per cage. The animals were allowed to acclimatization for a time frame of about 10 days to the laboratory conditions before commencement of this study. The animals were allowed to roam about freely accessing drinking water and rabbit chows feeds; a product of Top Feed, Sapele, Delta State. The experiment was designed in such a way that all four rabbits were administered the different doses of alcohol alongside the aqueous extract of *Cnidoscopus aconitifolius*. Administration was carried a month interval before the next set of treatment. The research study spanned a period of two months before analysis (from June to August, 2015).

### Determination of aqueous leaf extracts of *Cnidoscopus aconitifolius* on blood alcohol level:

After feeding, all twelve rabbits were deprived feed for 3hours before receiving the ethanol doses. All three groups A, B and C received a large dose of 1.1g (20% ethanol/ kg body weight) once orally in the first month to obtain ethanol metabolic curve. The dose of alcohol given was in accordance with the report [15]. On two different periods, administration was carried out orally. During the first period, alcohol only was consumed, but during the second occasion, *Cnidoscopus aconitifolius* extract were used (starting with a lower dose of 0.5g/kg b. wt, then to 1.5 and 2g extract/kg body weight) 20 minutes of ingesting the alcohol. Blood alcohol level (BAL) was analyzed every 20 minutes for 120 minutes using 0.5ml whole blood obtained from the ear vein of the animals. The equivalent volume of ethanol/kg body weight ingested was determined using the formula adopted in previous study: Volume in ml = Amount of ethanol (g ethanol/kg body weight) x Body weight (kg)/ % of ethanol (as a decimal) x Density of ethanol [15].

### Biochemical analysis of sample

Blood alcohol level (BAL) was quantified using the alcohol dehydrogenase method as described by Busher and Redetzki, [16]. The concentration of alcohol in the blood sample was determined using the fitted standard curve.

### Statistical Computation

The values obtained from the standard alcohol calibration curve were presented as mean  $\pm$  SD. The mean values of the various treatment groups were compared using ANOVA. The significance level was set at  $p < 0.05$ .

## RESULTS AND DISCUSSION

The excessive consumption of ethanol has been reported to promote as well as initiate enormous problems that culminate in the death of several alcohol addicts aside the social stigmatization it procures for them [17]. Although no accurate statistic to describe the rate of occurrence of such deaths in Nigeria, estimations indicates an elevated percentage [18].

Since alcohol consumption and abuse cannot be completely eradicated from the society, the research for substance that would facilitate its disappearance or clearance from the blood might be useful. Many substances like oral fructose and honey, methylene blue has demonstrated to enhance the metabolic clearance of ingested alcohol but their use still remains contentious until date [19]. Previous studies has further buttressed and established the relationship between chronic doses of ethanol and its associated complications in rabbits [15], hence the aqueous extract of *Cnidoscopus aconitifolius* was used in this study as an anti-intoxicating substance upon such established dose in rabbit.

Report from this study shows that administration of the different doses of *Cnidoscopus aconitifolius* extract caused a reduction in the peak blood alcohol level (pBAL). At a dose pf 0.5g/kg of the extract, the peak blood alcohol level (pBAL) was reduced from 0.105% to 0.093% (table 1). Likewise, upon treatment with 1.5g/kg of *Cnidoscopus aconitifolius*, the pBAL dropped from 0.133% to 0.129% (table 2), and while at 2g/kg extract, pBAL was lowered

from 0.092% to 0.090% (table 3). Supporting the above results obtained, previous works have demonstrated as well as establishes the relationship between pBAL and intoxication. The higher the pBAL the more the individual is intoxicated; however this has no bearing with behavioral disorder [20]. It appears from this work that the extract might have contributed to the reduction in the peak BAL as observed in Tables 1, 2 and 3.

Furthermore, the time to zero BAL which is referred to as the intoxication time was equally decreased upon co-administration with the ethanol. The intoxication time was dropped from 130.5 minutes to 121.3minutes upon treatment with 0.5g/kg extract and from 150.9 min to 131.5min upon 1.5g/kg administration *Cnidoscopus aconitifolius* extract. Result in table 3also reveals a reduction in intoxication time (time to zero BAL) from 139.9min to 119.8min when 2g/kg extract was co-administered with ethanol. This observed reduction in intoxication time might connote that the lower the time the faster the clearance of the ethanol from the animal body, hence facilitating alcohol catabolism and disappearance [21]. It can be implied from this report that the reason for the reduction in time by the extract might be due to the ability of the extract to increase the metabolism of ethanol which is time dependent thus enhancing its removal faster than ethanol degrading enzymes would perform. Doses at 1.5g/kg and 2.0g/kg extract showed significant difference ( $p < 0.05$ ) when compared with the ethanol treatment alone. Extract dose at 0.5g/kg was not statistically significant ( $p > 0.05$ ). From this investigation, remarkable changes were seen in the metabolic kinetics of ethanol. The kinetics of ethanol was demonstrated via the ethanol disappearance rate ( $\beta 60$ ) and ethanol elimination rate, BEER. The  $\beta 60$  reveals the fall or disappearance of blood ethanol. Graphically, it represents the descending arm of the gradient [21]. Results from Table 2 indicated a statistically significant difference ( $p < 0.05$ ) in  $\beta 60$  and BEER upon the administration of 1.5g/kg extract 0.059%/h and 684.31mg/kg/h respectively as to 0.052%/h and 437.38mg/kg/h of ethanol treatment alone. The same result trend was revealed in Table 3 when 2g/kg of the extract was given (0.045%/h and 997.01mg/kg/h respectively) when compared to ethanol administration only (0.039%/h and 472.10mg/kg/h respectively). Dosage at 0.5g/kg extract did not reveal statistically any significant difference ( $p > 0.05$ ) in  $\beta 60$  and BEER upon co-administration with ethanol.

**Table 1: Effect of 0.5g/kg of *C. aconitifolius* extract upon 1.1g ethanol/kg body weight**

Subjects (Group A)	BAL After 20min (%)	BAL After 40min (%)	BAL After 60min (%)	BAL After 80min (%)	BAL After 100min (%)	BAL After 120min (%)	Peak BAL (%)	Time to Peak BAL (Min)	Time to Zero BAL (Min)	$\beta 60$ (%/h)	BEER (mg/kg/h)
Mean $\pm$ SD of EtOH treatment alone	0.035 $\pm$ 0.01	0.082 $\pm$ 0.02	0.103 $\pm$ 0.02	0.078 $\pm$ 0.01	0.047 $\pm$ 0.02	0.022 $\pm$ 0.01	0.105 $\pm$ 0.02	38.8 $\pm$ 1.5	130.5 $\pm$ 3.5	0.048 $\pm$ 0.01	505.75 $\pm$ 7.1*
Mean $\pm$ SD of EtOH + 0.5g/kg <i>C. a.</i> extract	0.028 $\pm$ 0.01	0.067 $\pm$ 0.02	0.090 $\pm$ 0.02	0.065 $\pm$ 0.02	0.034 $\pm$ 0.01	0.012 $\pm$ 0.01	0.093 $\pm$ 0.02	33.5 $\pm$ 1.2	121.3 $\pm$ 4.0*	0.047 $\pm$ 0.01	247.83 $\pm$ 6.5

*n=4, values are expressed as mean  $\pm$  SD. Values with \* are significantly different from each other.*

*Abbreviations: BAL= Blood Alcohol Level,  $\beta 60$ = Blood Alcohol disappearance rate (%/h), BEER= Blood Ethanol Elimination Rate (mg/kg/h), C. a. = Cnidoscopus aconitifolius. EtOH = Ethanol*

**Table 2: Effect of 1.5g/kg of *C. aconitifolius* extract upon 1.1g ethanol/kg body weight**

Subjects (Group B)	BAL After 20min (%)	BAL After 40min (%)	BAL After 60min (%)	BAL After 80min (%)	BAL After 100min (%)	BAL After 120min (%)	Peak BAL (%)	Time to Peak BAL (Min)	Time to Zero BAL (Min)	$\beta 60$ (%/h)	BEER (mg/kg/h)
Mean $\pm$ SD of EtOH treatment alone	0.060 $\pm$ 0.01	0.110 $\pm$ 0.02	0.131 $\pm$ 0.02	0.105 $\pm$ 0.01	0.075 $\pm$ 0.02	0.046 $\pm$ 0.01	0.133 $\pm$ 0.02	58.7 $\pm$ 2.2	150.9 $\pm$ 5.1	0.052 $\pm$ 0.02	437.38 $\pm$ 5.0
Mean $\pm$ SD of EtOH + 1.5g/kg <i>C. a.</i> extract	0.058 $\pm$ 0.02	0.097 $\pm$ 0.02	0.120 $\pm$ 0.02	0.096 $\pm$ 0.02	0.064 $\pm$ 0.01	0.030 $\pm$ 0.01	0.129 $\pm$ 0.02	43.5 $\pm$ 1.2	131.5 $\pm$ 6.6	0.059 $\pm$ 0.01*	684.31 $\pm$ 9.6*

*n=4, values are expressed as mean  $\pm$  SD. Values with \* are significantly different from each other*

*Abbreviations: BAL= Blood Alcohol Level,  $\beta 60$ = Blood Alcohol disappearance rate (%/h), BEER= Blood Ethanol Elimination Rate (mg/kg/h), C. a. = Cnidoscopus aconitifolius. EtOH = Ethanol*

There is still uncertainty in the mechanism and pathway in which the aqueous extract of *Cnidoscopus aconitifolius* accelerates alcohol clearance. It is proposed that it might have delayed gastric emptying and further reduce alcohol

absorption, however first by-pass metabolism might be elevated hence decreasing alcohol bioavailability [9]. From this result faster alcohol disappearance rate was observed as the extract dose was increased hence reducing alcohol intoxication in blood.

Table 3: Effect of 2.0g/kg of *C. aconitifolius* extract 1.1g ethanol/kg body weight

Subjects (Group C)	BAL After 20min (%)	BAL After 40min (%)	BAL After 60min (%)	BAL After 80min (%)	BAL After 100min (%)	BAL After 120min (%)	Peak BAL (%)	Time to Peak BAL (Min)	Time to Zero BAL (Min)	$\beta 60$ (%/h)	BEER (mg/kg/h)
Mean $\pm$ SD of EtOH treatment alone	0.040 $\pm$ 0.01	0.070 $\pm$ 0.02	0.090 $\pm$ 0.02	0.078 $\pm$ 0.01	0.050 $\pm$ 0.01	0.033 $\pm$ 0.01	0.092 $\pm$ 0.01	47.4 $\pm$ 6.2	139.6 $\pm$ 6.0	0.039 $\pm$ 0.02	472.10 $\pm$ 7.0
Mean $\pm$ SD of EtOH + 2.0g/kg <i>C.a.</i> extract	0.038 $\pm$ 0.02	0.069 $\pm$ 0.01	0.087 $\pm$ 0.02	0.069 $\pm$ 0.02	0.050 $\pm$ 0.02	0.025 $\pm$ 0.01	0.090 $\pm$ 0.02	39.5 $\pm$ 5.4	119.8 $\pm$ 4.9	0.045 $\pm$ 0.02*	997.01 $\pm$ 11.3*

*n*=4, values are expressed as mean  $\pm$ SD. Values with \* are significantly different from each other  
Abbreviations: BAL= Blood Alcohol Level,  $\beta 60$ = Blood Alcohol disappearance rate (%/h), BEER= Blood Ethanol Elimination Rate (mg/kg/h), *C. a.* = *Cnidioscolus aconitifolius*. EtOH = Ethanol

### CONCLUSION

In conclusion, although the mechanism of *Cnidioscolus aconitifolius* extract on alcohol stimulation and acceleration still remains unclear, however, the need to ascertain and investigate if the stimulatory effect of the extract on ethanol is by influencing the activities of ethanol –metabolizing enzymes. This study further corroborates traditional claims on the use of the extract as an amethystic substance.

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