An investigation on blood alcohol concentration of Garcinia kola in *Sylvilagus nuttallii* Rabbits

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ABSTRACT

Introduction: Plant based therapy might be relevant in managing ethanol related complications. Alcohol abuse is currently pervasive in societies, hence the search for substances that would enhances its clearance or removal from the blood might be pertinent to reducing blood alcohol concentration (BAC), and hence its associated consequences.

Method: Eight *Sylvilagus nuttallii* rabbits weighing between 1.2kg -1.5kg were divided into two sets (A and B) of four animals each. In the first occasion, animals in both groups were treated with 1.1g (20% ethanol/ kg body weight) once in the first month to generate alcohol oxidation curve. On the second occasion *Garcinia Kola* (G.K)seed extract of 500mg/kg body weight and 2000mg extract/kg body weights were administered to group A and B respectively 20 minutes of ingesting the alcohol. 0.5ml of whole blood collected from the animals' ear vein was used to analyze blood alcohol concentration (BAC) every 20 minutes for 120 minutes. Quantification of blood alcohol concentration (BAC) was performed by the alcohol dehydrogenase method.

Result: Result indicates that the time to zero BAC i.e. the intoxication time was decreased upon co-administration of *Garcinia kola* extract with the ethanol. The intoxication time was dropped from 145.7 minutes to 138.1 minutes upon treatment with 500mg/kg extract and from 158.5 min to 140.1 min upon 2000mg/kg administration of G.K. extract. The treatment value at dose 2000mg/kg was significantly different ($p<0.05$) when compared with the ethanol treatment alone. On assessing the kinetic parameters (β60 and BEER), results indicated a statistically significant difference ($p<0.05$) in β60 and BEER upon the administration of 2000mg/kg extract0.077%/h and 684.31mg/kg/h respectively as to 0.062%/h and 416.67mg/kg/h of ethanol treatment alone. While at 500m/kg extract values obtained were 0.058%/h and 459.13mg/kg/h respectively as against 0.056%/h and 452.67mg/kg/h on ethanol alone application. Dosage at 500mg/kg extract were not statistically different ($p>0.05$) in β60 and BEER upon co-administration with ethanol. However, a reduced Mean ± S.D value was obtained.

Conclusion: The decrease in intoxication time and also increase in the elimination rate are possible indications that*Garcinia Kola* might possess anti-intoxicating properties as postulated by folklore traditional practice. This study tends to corroborate that *Garcinia Kola* might possess some alcohol reducing property.

Keyword: *Garcinia kola* (G. K.), anti-intoxicating, ethanol, blood alcohol concentration (BAC)

INTRODUCTION

The manifold benefit of alcohol as a solvent cannot be over emphasized especially with it usage; industrially and locally. Over decades, both orthodox and folk medicine have relied heavily on alcohol for phytochemical studies as well as the extraction of bioactive ingredients from plants parts such as roots, leaves and stems.[1] This relevance along other purposeful uses of alcohol has created numerous applications for this organic substance. Alcohol popularly perceived as ethanol or ethylalcohol is a chemical substance found in alcoholic beverages such as wine, beer, gin, whisky and other distilled spirit[2]. In Nigerian aside the foreign brands, alcohol is consumed locally as *Pitoh*, *Oti*, *Kia-Kia*, *Burukutu*, *Akepteshi*, *Palmwine*, *Ogogoro*, *Orgoro* etc. Ethanol does not need modification or digestion before absorption. Absorption entails all parts of the GIT with the small intestine being the most efficient region, thus enhancing its increase concentration in the blood.[3] The rate of ethanol absorption is partially determined by genetic[4] factors and also by the amount of alcohol appearing in the systemic circulation.[5]

In humans, alcohol can be metabolized oxidatively[6,7] or non-oxidatively.[8] The oxidative metabolism of alcohol occurs progressively in the liver via acetaldehyde to acetate in the presence of alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) while fatty acid ethyl ester (FAEE) and phosphatidyl ethanol (catalyzed by FAEE synthase and Phospholipase D respectively) are chief products generated from non-oxidative metabolism of ethanol.[8]

The generation of acetaldehyde and acetate from the oxidative metabolism of ethanol elicits cascade of metabolic reactions, leading to the reduction of NAD^+ to NADH[9, 10]. The alteration in the NAD^+/ NADH ratio initiates series of metabolic disturbances that culminate into myriads of health complications amongst alcoholics[6,7]. Furthermore, the release of acetaldehyde into vital organs such as the brain potentially combines with neurotransmitters (such as gamma amino butyric acid - GABA) to form tetrahydroisoquinolines (THIQs)[8, 11]. Hypothetically, THIQs is believed by some researchers to be responsible for alcohol related addiction[12]. Other health challenges associated with alcohol intake is the risk of developing cancer in tissues that converts ethanol into the carcinogenic metabolite such as acetaldehyde[13]. The synthesis of FAEE from nonoxidative metabolism of ethanol exacerbates alcohol induced injury through uncoupling of oxidative phosphorylation and further disrupting ATP generation[14]. Alcohol metabolism shows an interrelationship between the oxidative and non-oxidative pathways. Compound that inhibits ADH, CYP2E1, and catalase during ethanol oxidation results in an increase in the non-oxidative metabolism and corresponding generation of FAEEs in the pancreas and liver[15].

Despite the health risk, social misappropriation and financial frustration connected with chronic, sub-chronic and acute ethanol consumption, alcohol addiction and abuse continues to escalate creating serious societal menace[16, 17]. Therefore the quest for anti-intoxicating substances capable of ameliorating the associated metabolic alterations and enhancing the rate of ethanol removal or its metabolites from the body system would be of great relevance and benefit to regions where ethanol consumption is uprising and in high proportion[17]. It is on this wise that agents capable of enhancing and facilitating alcohol elimination from bloodstream were investigated.

Aside the provision of timber, human nutritional and medicinal needs have being generated from forest products such as shrubs, plant species, flowering plants and trees etc [18]. Since modern therapy is completely not realistic in every part of the world, herbal medicine has augmented orthodox medication in regions where they are either used as main stream treatment or first line treatment before the arrival of modern medication[18].

Garcinia kola (G.K) generally identified as bitter kola (Fig 1) because of its astringent taste, is a tropical plant peculiar to the inhabitants of Western and Central African[19]. Its multi-purpose application in traditional ceremonies and ethno-medicines has earned this plant the name "Wonder plant"[20, 21]. G. Kola has being implicated in the treatment of diseased conditions like benign prostatic hyperplasia, AIDS and multiple sclerosis[22]. Therapeutic and pharmaceutical properties associated with this plant includes, anti-inflammatory, anti-microbial, anti-viral[19], antioxidant, anti-diabetic, anti-genotoxic[23]and aphrodisiac potentials[24]. All these medicinal values have being connected to the presence of the biflavonoid kolaviron complex[18, 22, 23].

Until now, the management of alcoholism and associated complications has recorded minimal progress probably because of their nature, pharmacokinetics and molecular complexities in interacting with target tissues. Thus formulations of therapeutic and pharmaceutical values that will enhance its elimination from the body system might be of immense relevance in managing alcohol toxicosis. Since this plant possesses great therapeutic and pharmaceutical potentials, studies have continued to reveal, unveil and uncovered medicinal properties deposited in this "Wonder" plant.

In addition to the numerous therapeutic and pharmacological potential of *Garcinia kola*, recent scientific observation and documentations has connected the application *Garcinia kola* in the management of alcohol related diseases[23]; hence this research study attempts to investigate the alcohol-clearing or removing potentials of *Garcinia kola* as it relates blood alcohol concentration (BAC).

Fig1: *Garcinia kola* seed displayed for sale

MATERIALS AND METHODS

Wistar **Collection and Preparation of Extract**

Fresh seeds of *Garcinia kola* as depicted in Fig 1 above were purchased from a local market in Ukuani, Delta state Nigeria .The recipe was adopted as described by Akpantah et al., 2005. The brownish coated covering of G. kola were peeled off, the seeds chopped into tiny pieces and air-dried for over a month to a constant weight. The dried seeds were pulverized using simple blender (product of kenwood - Model owBL335014). Extraction was done on the resulting powder, using 70% alcohol in a Soxhlet extraction. Concentration of the yield was performed by evaporation in an Electro - Thermostatic Water bath (Model DK-8A) and dried to solid form. Administered solution was reconstituted with 2.0g of the extract measured out and dissolved in 100mls of distilled water to give 20mg/ml.

Animal Protocol

Eight (8) rabbits (*Sylvilagus nuttallii*) weighing between 1.2kg -1.5kg were used for this study. The rabbits were gotten from the animal Unit in the Faculty of Basic Medical Sciences, Delta State University, Abraka and they were accommodated in improvised metallic gauze cages before allotting them into groups of A and B each containing five *Sylvilagus nuttallii* rabbits per cage. The animals were allowed to acclimatize for7days to the existing laboratory conditions. The animals were fed with rabbit pellets; product of Top Feed, Sapele, Delta State and were not restrained access to portable drinking water and movement. The experiment was designed to

encompass all rabbits in each group given the different doses of alcohol (6 weeks interval) before the extract administration. The research study spanned between the months of March and April before analysis.

Ethical Consideration

Ethical code of conduct with reference to animal care were governed and monitored under strictly observation as stipulated by Ward and Elsea [25]. Furthermore, the experimental procedures entailed in this study were approved and guided by the institutions Sub ethical committee in the Faculty of Basic Medical Sciences, Delsu before the use of laboratory animals.

Determination of *Garcinia kola* seed extract on blood alcohol concentration (BAC)

The determination of GK on blood alcohol concentration was carried out as described by Onyesom and Oriero [26]. Three hours before receiving the ethanol dose, all animals were abstained from feeding. To obtain an ethanol metabolic curve, the two groups A and B received orally, a large dose of 1.1g (20% ethanol/ kg body weight) once in the first month. Administration was carried out orally on two different periods. Firstly alcohol only was consumed, but on the second occasion, *Garcinia Kola* seed extract was given (starting with a lower dose of 500mg/kg body weight and a higher dose 2000mg extract/kg body weights) 20 minutes of ingesting the alcohol. Blood alcohol concentration (BAC) was analyzed using whole blood (0.5ml) collected from the ear vein of the animals at intervals of 20 minutes for 120 minutes.

Equivalent volume of consumed ethanol/kg body weight was applied based on this formula: Volume (ml) = Amount of ethanol (g ethanol/kg body weight) x Animal weight (kg)/ % of ethanol (in decimal) x Density of ethanol.

BIOCHEMICAL ANALYSIS

Alcohol dehydrogenase method as described by Busher and Redetzki[27] was used to quantify Blood alcohol concentration (BAC).Alcohol concentration in the animal blood sample was determined employing the fitted standard curve.

STATISTICAL COMPUTATION

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

RESULTS

Table 1: The alcohol elimination or clearance potential of *Garcinia kola* was demonstrated in this research by establishing oxidation kinetic curve as showing in figure 2 and figure 3. Results from this study showed that administration of the different doses of *Garcinia kola* extract caused a reduction in the peak blood alcohol concentration (pBAC). At a dose of 500mg/kg of the extract, the peak blood alcohol concentration (ρBAC) was reduced from 0.135% to 0.133% (table 1). Likewise, upon treatment with 2000mg/kg of *Garcinia kola*, the pBAC dropped from 0.163% to 0.138% (table 1).

Furthermore, the time to zero BAC which is referred to as the intoxication time was equally decreased upon co-administration of extract with the ethanol. The intoxication time was dropped from 145.7 minutes to 138.1 minutes upon treatment with 500mg/kg extract and from 158.5 min to 140.1 min upon 2000mg/kg administration of *Garcinia kola* extract. The treatment value at dose2000mg/kg *Garcinia kola* extract showed a significant difference ($p<0.05$) when compared with the ethanol treatment alone. Extract dose at 500mg/kg was not statistically significant ($p>0.05$) at the pBAC but was statistically significant ($p<0.05$) at time to zero BAC.

The kinetics of ethanol elimination was demonstrated via the ethanol disappearance rate (β60) and ethanol elimination rate, BEER. The β60 revealed the fall or disappearance of blood ethanol. Graphically, it represents the descending arm of the gradient as shown in Figure 2 and 3. Results from Table 1 indicated a statistically significant difference ($p<0.05$) in β60 and BEER upon the administration of 2000mg/kg extract0.077%/h and 684.31mg/kg/h respectively as to 0.062%/h and 416.67mg/kg/h of ethanol treatment alone. Dosage at 500mg/kg extract was not significant different ($p>0.05$) in β60 and BEER upon co-administration with ethanol, however a reduced Mean ± S.D value was established.

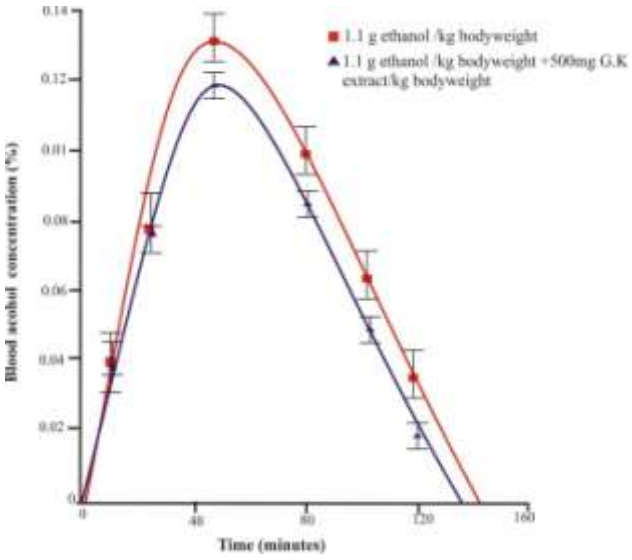

Fig 2: Effect of 500mg/kg of *Garcinia kola* extract on 1.1g ethanol/kg body weight

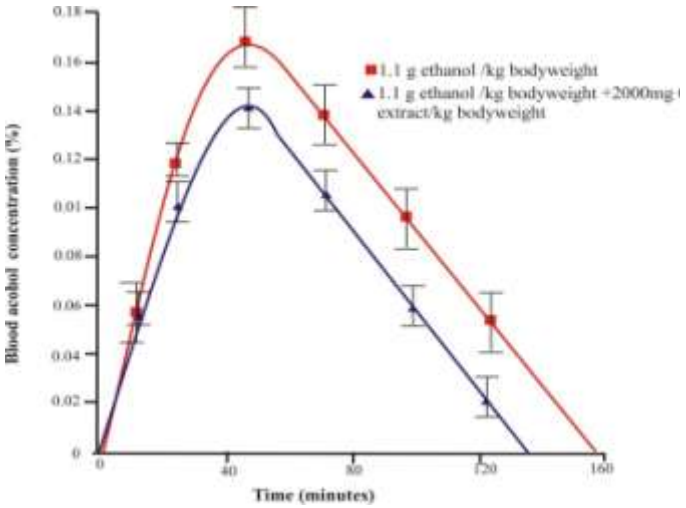

Fig 3: Effect of 2000mg/kg of *Garcinia kola* extract on 1.1g ethanol/kg body weight

Table 1: Oxidation Kinetics Parameter of Ethanol upon *Garcinia kola* administration

Treatment	Peak BAC (%)	Time to Peak BAC (Min)	Time to Zero BAC (Min)	β60 (%/h)	BEER (mg/kg/h)
Group A					
EtOH treatment alone	**0.135±0.67**	**47.7±0.53**	**145.7 ± 2.51**	**0.056±6.91**	**452.67± 7.19**
EtOH + 500mg/kg *G.K.* extract	**0.133±0.44**	**46.5±0.31**	**138.1 ± 1.99***	**0.058±4.33**	**459.13 ± 2.55**
Group B					
EtOH treatment alone	**0.163±1.13**	**59.0±1.82**	**158.5 ± 2.45**	**0.062±4.20**	**416.67 ± 3.17**
EtOH + 2000mg/kg *G.K.* extract	**0.138±0.71***	**59.5±0.72**	**140.1 ± 0.93***	**0.077±3.11***	**684.31 ± 5.22***

n=4, values are expressed as mean ± SD. Values with * are significantly different from each other. Abbreviations: BAC= Blood Alcohol Concentration, β60= Blood Alcohol disappearance rate (%/h), BEER= Blood Ethanol Elimination Rate (mg/kg/h), *G.K.* = *Garcinia kola* EtOH = Ethanol

DISCUSSION

The enormous challenges of alcohol as it relates behavioural, economic, social and physiological consequences have received a wide publicity[3]. Postulations from Center for Disease Control and Prevention (CDC) have rated alcohol abuse as the third leading cause of preventable death[28]. Studies have revealed that complication associated with alcoholism is attributed to its metabolic products[29]. It is on this wise that supportive agents that could further enhance elimination from bloodstream were investigated. Evidences available has indicated that the application of amantadine, naloxone, benzodiazepine receptor, inverse agonist and gelatin- containing 50mg methylene blue have not yielded positive results in humans[30]. In addition, the administration of oral fructose to rabbits enhanced the metabolic clearance of blood alcohol but its use has remained a contentious issue due to associated increase in the level of hyperuricemia and hypertriglyceridaemia [31, 32].

The alcohol clearing or reducing potential of G.K. from blood stream was demonstrated by first establishing an alcohol oxidation kinetic curve (i.e. ethanol treatment alone) which served as the control as in Fig 2 and Fig 3 respectively. The application of *Garcinia kola* both at low (500mg) and high (2000mg) doses caused a reduce peak blood concentration (peak BAC) when co-

administered with ethanol. The peak blood alcohol concentration is a representation of the appearance rate of ethanol in the blood system[31]. The absorption of ethanol through the small intestine into the circulatory system is determined by the rapid gastric emptying and partially by genetic factors[33, 34]. There is every possibility that the administration of G.K extract might have inhibited the rapid transit of ethanol through the stomach or delayed gastric emptying which has culminated into the reduction of the peak BAC (%). The delay in gastric emptying caused an increased first by-pass metabolism thereby decreasing alcohol bioavailability[35, 36].

In a dose dependent manner, the intoxication time that is time to zero BAC as shown in Fig 2 and fig 3 was lowered upon treatment with 500mg/kg and 2000mg/kg *Garcinia kola* extract. The reduction in intoxication time is an indication that the lower the time the faster the clearance of the ethanol from the circulatory system, hence facilitating alcohol catabolism and disappearance[37]. This implies that the extract might possess substances with the ability to increase the metabolism of ethanol thus enhancing ethanol removal faster than ethanol degrading enzymes would perform alone.

Further investigations from this study, revealed some remarkable changes in the oxidation kinetics of ethanol. The oxidation kinetics of ethanol was demonstrated via the ethanol disappearance rate (β60) and ethanol elimination rate, BEER. The β60 reveals the fall or disappearance of blood ethanol upon extract administration. Graphically, β60 represents the descending arm of the gradient as in Fig 2 and Fig 3. The respective values obtained for β60 and BEER upon the administration of 500mg/kg extract was higher than the ethanol treatment alone however, dose at 500mg/kg extract was not statistically significant ($p>0.05$) in β60 and BEER upon co-administration with ethanol (table 1). On the contrary, the administration of 2000mg/kg of the G. K extract statistically elevated the β60 and BEER values to a significant level ($p<0.05$) (0.077%/h and 684.31mg/kg/h respectively) when compared to ethanol administration only (0.062%/h and 416.67mg/kg/h respectively).

Uncertainty exists in the mechanism and pathway in which the extract of *Garcinia kola* accelerates alcohol clearance. It has being 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. Report from previous studies has shown that the ameliorating potential of honey on ethanol toxicosis and intoxication is due to the presence substance like flavonoids, vitamins e.t.c.[31]. This might be attributed to *Garcinia kola* as well, since this herbal plant is endowed with myriads of bioactive agents such as dimeric flavonoid molecules fused together by biflavonoid[38]. Other constituents present in GK that might be of relevance in influencing its alcohol ameliorating potential include xanthones and benzophenones[39, 40].

From this result faster alcohol disappearance rate was observed as the extract dose was increased hence reducing alcohol intoxication in blood.

CONCLUSION

Although the mechanism of *Garcinia kola* extract on alcohol stimulation and acceleration still remains unclear, 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 projections that *Garcinia kola* might possess anti-intoxicating principle.

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