

Vitamin C and alcohol interaction in adult male Wistar rats; effect on selected biochemical and haematological indices

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ABSTRACT

Alcohol interaction with medication is often harmful and it may increase the risk of body organ malfunction. The objective of this study was to investigate the effect of vitamin C and alcohol on biochemical and hematological indices in male adult Wistar rats. After fourteen days of acclimatisation period, twenty adult male Wistar rats weighing between 150 g-200 g were randomly divided into four groups; control group A (distilled water), group B (9 mg/kg b.w of vitamin C), group C (2000 mg/kg b.w of alcohol), group D (9 mg/kg b.w of vitamin C and 2000 mg/kg b.w of alcohol). Alcohol and vitamin C was administered orally for twenty-one days. Blood samples were collected from the retro-orbital sinus for biochemical and haematological analysis using the standard procedure: 1—level of significance set at $P < 0.05$. Observation from the results indicates a significant increase in the values of serum cholesterol, total protein, and liver enzymes (alanine aminotransferase and alkaline phosphate) in group D rats when compared with the control group A. No significant differences were recorded in body mass index, fasting blood sugar, white blood cells and blood urea nitrogen. However, significant decreases were recorded in the value of packed cell volume, platelet count, haemoglobin concentration, neutrophil and eosinophil count in group D rats when compared with group A. In conclusion,

alcohol taken in combination with vitamin C may have a deteriorating effect on the body and the use may be discouraged.

Keywords: alcohol, biochemical, haematology, vitamin C.

Introduction

Alcohol intake has been recognised as a leading risk factor for certain diseases, impairment of body parts, and death (Rehm *et al.*, 2009a). Alcohol intake is a serious healthcare problem throughout the world and in Nigeria. Although alcohol intake varies significantly worldwide, the health and mortality impact remains significant in most regions (Eze *et al.*, 2017; Rehm *et al.*, 2010). According to several studies, alcohol is one of the most often used psychoactive drugs in Nigeria's young people and adults (Gureje *et al.*, 2007; Makanjuola *et al.*, 2004). Alcohol intake has a detrimental impact on health throughout life. Studies have associated the intake of alcohol with various conditions, such as cancer, gastroenteritis, alcoholic liver disease, pneumonia, diabetes mellitus, malignancies, psychological morbidity, and trauma (Schütze *et al.*, 2011; Rehm *et al.*, 2009b).

Vitamins are essential in the human body for several biochemical and physiological processes. However, since the body does not

generate most vitamins, they must be obtained through diet (Chambial *et al.*, 2013). In addition, vitamin C metabolism is highly regulated in healthy individuals, resulting in a complex relationship between the steady-state levels of many physiological systems and tissues. The availability of vitamin C in the food, as well as the unique "configurations" and expression levels of saturable sodium-dependent vitamin C transporters (SVCTs) in the tissues, all, play a role in this relationship (Lykkesfeldt and Tveden-Nyborg, 2019).

Many medications interact with alcohol, causing the metabolism or effects of both the alcohol and the medication to be significantly changed. Even at moderate alcohol consumption levels, several of these interactions can occur, resulting in adverse health effects for the consumer. When taken with alcohol, numerous over-the-counter and herbal medications can have harmful side effects. Medications and alcohol may have pharmacokinetic relationships in which alcohol interrupts the medication's metabolism in one of two ways: Because the medications must compete with alcohol for cytochrome P450 breakdown, the breakdown and excretion of the affected medications are delayed, and the metabolism of the affected medications is accelerated because alcohol improves the action of medication-metabolising cytochromes. Enhanced cytochrome action leads to a greater elimination rate for medications that these enzymes metabolise when alcohol is not present to compete for the cytochromes

(Weathermon and Crabb, 1999). Alcohol can disrupt vitamin C homeostasis, resulting in harmful consequences or altering vitamin C metabolism (Lykkesfeldt and Tveden-Nyborg, 2019).

In Nigeria, Vitamin C is majorly sold as an over the counter drug and also used as a food supplement. Due to the high consumption of alcohol in Nigeria, there may have been cases in which vitamin C and alcohol have been used together. This study will evaluate the effect of the combination of alcohol and vitamin C on selected haematological and biochemical indices in male Wistar rats.

MATERIAL AND METHODS

Animal care

Twenty healthy adult male Wistar rats weighing between 150g and 200g were used for this experiment. The rats were bred in plastic and wire gauze cages in the animal house of the Obafemi Awolowo College of Health Sciences, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria. The rats were allowed to acclimatise for two weeks; they were fed with a standardised pellet diet and allowed free access to water. The care and handling of the animals were following the internationally accepted standard guidelines for animals' use by the National Research Council (2011)

Animal grouping

The rats were randomly divided into four groups of five rats, each as follows:

Table 1: Animal grouping and treatment administered

Group	A	B	C	D
Treatment	Distilled water	9 mg/kg b.w of Vit C	2 mg/kg b.w of ALC	9 mg/kg / Vit C & 2 mg/kg / ALC (VCA)

Vit C represents vitamin C; ALC represents alcohol; VCA represents vitamin c and alcohol, treatment group. The administration was done orally for twenty-one days.

Preparation and administration of alcohol

The alcohol used was manufactured by Guangdong Guanghuasci-Tech co. Ltd and bought in Nigeria. First, 2 ml of absolute alcohol that contains 200 mg of ethanol was dissolved in 0.5ml of distilled water (200mg/0.5ml); this was administered to 100 g weight of rats (2000 mg/kg) body weight (Husain *et al.*, 2001).

Preparation and administration of Vitamin C

100 mg commercial-grade vitamin C tablet (Kunimed pharmachem ltd) was crushed and 0.9mg dissolved in 0.5ml of distilled water, and was administered to 100 g of rats (9 mg/kg body weight of rat).

Procedure for blood collection

Blood was collected from the retro-orbital sinus, the rat was restrained, the neck gently scruffed, and the eye bulged. Then, a capillary tube was inserted medially into the eye and blood was allowed to flow by capillary action through the capillary tube into EDTA and lithium heparin sample bottle (Hounkpatin *et al.*, 2012).

Haematological Analysis

White blood cell count (WBC), percentage lymphocyte, percentage neutrophils, monocyte, eosinophil, haemoglobin concentration (HGB), packed cell volume (PCV) and platelet count (PLT) were estimated using an automated Beckman coulter auto-analyser according to the method of Kakel (2012).

Determination of fasting blood sugar concentration (FBS)

The glucose concentration in the blood was determined after enzymatic oxidation in glucose oxidase; the hydrogen peroxide

formed reacted under the catalysis of peroxidase with phenol and 4-aminophenazone forming a red-violet quinine imine dye as an indicator (Barham and Trinder, 1972).

Determination of fasting lipid profile (FLP)

The blood was analysed for serum cholesterol by using standard methods.

Serum total cholesterol was analysed by BIOTRON BTR 820 Auto Blood Analyser using the enzymatic method (Richmond, 1973).

Blood urea nitrogen (BUN) and Creatinine level determination

Enzymatic colourimetric assays were used to measure serum creatinine and blood urea nitrogen (BUN) levels according to the methods described by Amadi *et al.* (2018).

Liver enzymes determination

The activities of liver enzymes such as Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and Alkaline Phosphatase (ALP) were determined using diagnostic kits. Serum total protein (TP) content was estimated by Lowry *et al.* (1951). Serum albumin (ALB) was determined by the bromocresol green method, using bovine serum albumin as a standard (Doumas *et al.*, 1971). Alanine and Aspartate aminotransferases (ALT and AST) were determined based on the colourimetric measurement of hydrazine formed with 2, 4 dinitrophenyl hydrazine (Sellers *et al.*, 2007), alkaline phosphatase (ALP) by the phenolphthalein monophosphate method (Babson, 1965).

Statistical Analysis

All analysis was done using the statistical package for the social sciences (SPSS) software package (NORUSIS, 1998). The

data were presented as mean \pm Standard Error of Mean (SEM) (n=5), and statistical analysis was carried out using the Student t-test and analysis of variance (ANOVA). Values were considered to be statistically significant when $p < 0.05$.

Results

Table 2 shows the effect of the interaction between alcohol and vitamin C on selected blood parameters in male Wistar rats. There was a significant decrease in PCV level compared with the control group and Vit C groups; haemoglobin concentrations decreased significantly in VAC compared with the Vit C treated group. VCA showed a significant decrease in PLT count compared to other test groups and the control group. In addition, there was a significant decrease in neutrophil and eosinophil count in VAC compared to the control group, and in the ALC treated group, monocyte and WBC decreased in VAC groups.

The effect of the interaction between alcohol and vitamin C on selected biochemical

parameters and body mass index in male Wistar rats is shown in table 3. According to statistical analysis, there was no significant difference in body mass index, blood glucose level, or body urea nitrogen level (BUN) compared with the control group or other test groups.

In the VCA groups, there was a significant increase in cholesterol and total protein levels compared to the control group. In addition, the VCA groups demonstrated a significant increase in AST and ALP compared to the control group and a significant decrease when compared to the other test groups. When comparing the ALT content of the ALC and Vit C groups to that of the control group, the ALC and Vit C groups' ALT content increased significantly. Compared to Vit C groups, ALC groups exhibited a significant increase in creatinine levels. In contrast, the Vit C groups showed a significant increase in creatinine levels compared to the control group. Compared to the Vit C group, the VCA group's albumin content increased significantly.

Table 2: Effect of the administration of vitamin C, alcohol and the combination of both on selected haematological parameters in male wistar rats

Group	A	B	C	D
Treatment	Distilled water	0.9 mg/kg b.w of vitamin C	0.2 mg/kg b.w of alcohol	0.9 mg/kg / vitamin C & 0.2 mg/kg / alcohol
PCV (%)	42.00 \pm 1.2.00	40.00 \pm 2.00	41.40 \pm 2.13	35.60 \pm 1.87 ^{ab}
HBG(g/dL)	14.40 \pm 1.12	13.56 \pm 0.71	13.78 \pm 1.19	12.10 \pm 0.67 ^b
PLT (10 ³ / μ L)	6960 \pm 112	6900 \pm 100	7300 \pm 400	6140 \pm 120 ^{abc}
Neutrophil (%)	56.60 \pm 1.68	56.60 \pm 2.40	50. 20 \pm 0.53 ^{ab}	51.20 \pm 2.27 ^a
Leucocyte (%)	42.00 \pm 0.40	42.40 \pm 2.07	49.00 \pm 1.00 ^{ab}	48.40 \pm 2.80
Eosinophil (%)	0.40 \pm 0.64	0.60 \pm 0.60	1.00 \pm 0.33	0.20 \pm 0.27 ^c

Monocyte(%)	0.60 ± 0.48	0.40 ± 0.40	0.00 ± 0.00	0.20 ± 0.27
WBC (10³/μL)	6.34 ± 0.07	6.28 ± 0.12	6.66 ± 0.21	5.32 ± 0.23

*a, b, c: shows Significant differences compared to groups A, B, C and D, at P < 0.05.
Data are expressed as mean±SEM; ^a shows significant from control group A;
^b shows Significant from control group B; ^c show significant from group C*

Table 3: Effect of the administration of vitamin C, alcohol and the combination of both on body mass index, and selected biochemical parameters

Group	A	B	C	D
Treatment	Distilled water	0.9 mg/kg b.w of vitamin C	0.2 mg/kg b.w of alcohol	0.9 mg/kg / vitamin C & 0.2 mg/kg / alcohol
BMI	1.93±0.60	2.26±0.63	1.63±0.23	1.77±0.02
FBS	77.80±5.44	59.00±12.33	67.00±4.00	81.60±5.20
Cholesterol	64.20±3.36	90.40±0.93 ^a	68.00±2.00 ^{ab}	76.20±1.20 ^{abc}
ALB	3.84±0.48	2.98±0.03 ^a	4.06±0.04 ^{ab}	3.82±0.51 ^b
TP	5.84±0.11	5.30±0.20 ^a	6.26±0.05 ^{ab}	6.60±0.07 ^{abc}
ALT	20.60± 1.12	24.60± 1.07 ^a	25.20± 0.53 ^a	17.40± 1.47
AST	58.60 ± 1.68	66.20 ± 2.13 ^a	65.00 ± 0.67 ^a	62.20± 0.27 ^{ac}
ALP	72.20 ± 1.52	85.00± 3.00 ^a	81.00 ± 0.80 ^a	77.00 ± 0.77 ^{ab}
Creatinine(Mg/dl)	0.34 ± 0.048	0.48 ± 0.03 ^a	6.66 ± 0.21 ^b	5.32 ± 0.27
BUN (Mg/dl)	16.40 ± 1.28	18.00 ± 0.66	17.80 ± 0.53	18.20 ± 0.53

a, b, c: shows Significant difference in comparison with the group A,B,C and D, at P < 0.05.

Data are expressed as mean±SEM; ^a shows significant from control group A;

^b shows Significant from control group B ; ^c show significant from group C

Discussion

Observations from table 2 show a decrease in selected hematological parameters of rats administered with ALC and VCA, indicating the destruction of blood cells, which could cause anaemia. The reduction could be attributed to the production of free radicals during alcohol metabolism in microsomes via cytochrome P450. Free radicals are known to deplete antioxidants, and antioxidant depletion makes blood cells

exceedingly vulnerable, resulting in rapid blood cell disintegration and eventual death (Van Antwerpen *et al.*, 1995). Therefore, the result of this study suggests that consumption of alcohol and vitamin C may cause changes in Complete Blood Counts (CBC) (Jain *et al.*, 2020). This is also in agreement with the study of Das *et al.* (2011) and Igboh *et al.* (2015).

According to this study, vitamin C had an antihyperglycemic effect by reducing blood

sugar when monitored for 21 days. This corresponds with the study of Afkhami-Ardekani and Shojaoddiny-Ardekani (2007). However, there was an increase in the level of blood sugar in rats administered with alcohol and vitamin C; this indicates that the combination of alcohol and vitamin C can cause hyperglycemia, and this may be due to the obstruction of the metabolism of the vitamin by alcohol or the decrease in the effectiveness of insulin which may also be caused by alcohol-vitamin C interaction. Furthermore, the reduction in body mass index may indicate that the combination of vitamin C and alcohol may cause reduced appetite, which will lead to decreased carbohydrate intake and malnutrition, thereby reducing the body weight (Colditz *et al.*, 1999).

The consumption of alcohol and vitamin C may lead to an increase in the blood's cholesterol level, thereby causing hyperlipidemia; this is because alcohol inhibits gluconeogenesis and fat metabolism. As a result, the production of specific molecules called very-low-density lipoprotein (VLDL) particles is increased (Weathermon and Crabb, 1999). A decrease in albumin level in the VCA test group indicates decreased production in the level of albumin in the liver due to liver damage. In severe liver disease, low plasma albumin concentrations are common. They are frequently associated with decreased plasma albumin concentrations, most likely due to disrupted synthesis of the liver's inability to secrete albumin into the circulation (Lieber, 2017). The increase in the total protein level indicates the abnormal accumulation of protein in the blood of rats administered with alcohol and a combination of alcohol and vitamin C, which may be due to disruption in liver function. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are the most commonly used enzymes to assess

hepatocellular injury (AST). Both are helpful hepatocellular injury markers (Cattley and Cullen, 2013). In liver damage, there is an increase in serum liver enzymes. In the rat, total ALP measured in serum is a poor indicator of hepatocellular or biliary injury (Cattley and Cullen, 2013), but an increase in the level of ALP indicates liver diseases.

Across all groups, there was an increase in the level of creatinine and BUN; this increase is a sign of alteration and could result from the effects of the substance administered, thereby altering the rate at which the kidney balances the body electrolyte (You and Crabb, 2004). Dehydration generally causes BUN levels to rise more than creatinine levels. This causes a high BUN-to-creatinine ratio. In addition, kidney disease or blockage of the flow of urine from the kidney causes both BUN and creatinine levels to increase, indicating kidney damage.

Conclusion

This study shows that the consumption of vitamin C and alcohol caused a decrease in haematology indices, which indicated the destruction of blood cells. In addition, it caused an increase in liver enzymes, total protein, serum cholesterol, and creatinine and blood urea nitrogen. Therefore, the consumption of vitamin C and alcohol may cause malfunction of the liver and kidney.

Conflict of interest

The authors declare no conflict of interest

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