# BIOCHEMICAL AND HISTOLOGICAL INVESTIGATIONS OF ALCOHOL ADMINISTRATION IN WISTAR RATS


## ABSTRACT

Knowledge of biochemical and histological investigation of alcohol administration in Wistar rats is critical for contemporary effort to develop animal models of alcoholism. 20 Male Wistar rats weighing (63.50±3.79g), were divided in to four groups (consisting 15 treated animals and 5 control animals) and administered with varying concentrations of ethanol (5% 15% and 40%) via gavage for a period of 28 days. Probic evaluations, liver biochemical enzymes and alteration in histology profile of GIT and viscera organs were accessed after a period of 28 days ethanol administration. The result of biochemical study of 40% ethanol showed a significant decrease in serum gamma glutamyl transferase (GGT), serum aspartate (AST) and Alanine amino transferase (ALT) when compared to normal study while 5% and 15% ethanol intoxicated rats are within the range with respect to the normal study. The results of probic evaluations such as body weight, water intake and food intake show a percentage decrease in 40% ethanol administrated rat when compared with controls. The pictorial results of liver histopathology organs that received 5% and 15% ethanol did not showed a significant degeneration in histology profile when compared to the normal study while morphology degeneration in histology profile occurred in 40% ethanol administrated rats. Therefore serum aspartate (AST), gamma glutamyl transferase (GGT) and Alanine amino transferase (ALT), probic evaluation (body weight, food intake and water intake) coupled with histopathological investigation may be used as biomarker for the early diagnosis of ethanol toxicity in human beings.



**Keywords**: alcohol, biomarkers, histopathology, growth performance index


## Introduction

Over the centuries, alcohol has become the most socially-accepted addictive drug worldwide (Ohkubo *et al*., 2009). Its use antedates recorded history and may go back as far as the Neolithic age around 8000BC. Ethanol is found associated with varieties of our cultural life, various name have been ascribed to it. Among them are; whisky in Gallic, water of life, Sapele water, gin, ogogoro, ojuna etc has been nomenclature alcohol in various region of spheres. Ethanol is the type of alcohol found in alcoholic beverages (wine, beer and spirit).

Alcohol intoxication is the term used by the toxicologist to describe the point at which alcohol depresses the central nervous system so that mood, physical and mental abilities noticeably change (Sainlan, 2008). Toxicologist used the term "alcohol intoxication to discriminate between alcohols. Intoxication is the consequence of alcohol entering the bloodstream faster than it can be metabolized by the liver, common symptoms of alcohol intoxication include slurred speech, euphoria, impaired balance, loss of muscle coordination, flushed face, dehydration, vomiting, reddened eyes, reduce inhibitions and erratic behaviour. Sufficiently high levels of blood-borne alcohol will cause coma and death from the depressive effects of alcohol upon the central nervous system (Smith *et al*., 2005).

Although the precise mechanism of alcohol intoxication is presently unknown, but studies suggest that its pass directly from the digestive tract into the blood stream in minutes, blood transport the alcohol to all part of the body including the brain which alter their neurons in several ways by changing their membranes as well as their ion channels enzymes and receptors. (Aguayo *et al*, 2002). Long-term use of alcohol in excessive quantities is capable of damaging practically every organ system in the body, (Testino, 2008).

Alcohol biomarkers have important applications in medicine and public safety (Litten and Fertig, 2003). The aim of this study was to investigate the biochemical and histological effects of alcohol administration in Wistar rats.

**Materials and Methods**

**Animal Collection**

Wistar rats ((63.50± 3.79g) were obtained from breeding stock maintained in the animal house of the College of health sciences, Osun State University Osogbo main campus (UNIOSUN) and were authenticated by the farm Director UNIOSUN.

The animals collected were house in well-ventilated wired plastic metabolic cages in the animal facility of the department of Biochemistry, Federal Polytechnic Ede, and Approval was obtained from the Departmental Ethical Committee on animal usage.

The rats were maintained under standard room temperature (25-26$^{0}$C) and humidity of 65-5%. They were allowed unrestricted access to water and rat chow (Tina Livestock feeds Ltd, Oke Gada Ede Osun state Nigeria).

They were allowed to acclimatize for a period of 21days before the commencement of experiments, the weight of the animals were estimated at procurement, during acclimatization, at commencement of the experiment and every day throughout the duration of the experiments using an electronic analytical precision balance.

**Experimental Design**

Twenty male Wistar rats (63.50± 3.79g) were used for this study. They were divided into 4 groups of 5 rats per group, 1st group served as the control administered saline, 2$^{nd}$-4$^{th}$ groups were administered 5%, 15% and 40% ethanol respectively for 28 days by gavage i.e. intra-gastric administration. All animals had access to rat chow and water *ad libitum*.

Animal experiments complied with the ARRIVE guidelines and was carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments, was strictly followed throughout the experiment.

**Animal Sacrifice and Sample Collection and Preparation**

On the 29th day of the experiment, the rats were fasted overnight, sacrificed via cervical dislocation. Blood samples were collected via cardiac puncture, into plain tubes, centrifuged at 3000g for 5min, and serum collected and stored at -20$^{o}$C for further analysis. The liver, kidney, heart and lungs, were excised and fixed in 10% formal-saline for histopathological interpretations.

**Ethical Approval**

Ethical approval for the study was obtained from Ethics Review Committee of College of Medicine University of Lagos, Idi-Araba with CMUL HREC REGISTRATION NUMBER: HREC/15/04/2015

**Estimation of Biochemical Parameters**

The methods of Reitman and Frankel (1957) and Hammed, (2011) was used for the determination of alanine amino transferase (ALT) and aspartate amino transferase (AST), while that of Szasz, (1969), Hyder *et al*., (2013) was used for the determination of gamma glutamyl transferase (GGT) in the serum.

**Histological Procedure and Analysis**

This was done as described by Saalu *et al*., (2008), briefly, the organs were cut on slabs about 0.5cm thick and fixed in 10% formal saline for a day after which they were transferred to 70% alcohol for dehydration the tissues were pass through 90% alcohol and chloroform for different durations before they were transferred into two changes of molten paraffin wax for 20min each in an oven at 57$^{0}$C. Serial selections of 5mm thick were obtained from a solid block of tissues and were stained with haematoxylin and eosin stains, after which they were passed through a mixture of equal concentration of xylene and alcohol. Following clearance in xylene, the tissues were oven-dried; photomicrographs were taken with a colour digital camera mounted on a light microscope.



## RESULTS AND DISCUSSION

**Table 1:** Body weight, feed and water intake of rats administered alcohol for 28 days

| Probic Indices | Control | 5% EtOH | 15% EtOH | 40% EtOH |
|---|---|---|---|---|
| **B.W (g)** | 85.33 ± 11.52 | 89.67 ± 10.04 | 79.33 ± 8.95 | 73.50 ± 6.99 |
| **H.I (ml)** | 90.00 ± 33.15 | 88.67 ± 21.06 | 62.00 ± 27.68 | 53.17 ± 10.33 |
| **F.I (g)** | 90.00 ± 7.46 | 79.33 ± 9.86 | 67.33 ± 16.45 | 37.17 ± 14.93 |

Data are expressed as mean ± standard error of the mean (SEM) of five normal and 15 intoxicated rats.

**Key**: **B.W-**Body Weight, **H.I-** $H_20$ Intake, **F.I-**Feed Intake

**Table 2**: Serum Activities of ALT, AST & GGT of rats administered alcohol for 28 days

| Parameters | Control | 5% EtOH | 15% EtOH | 40% EtOH |
|---|---|---|---|---|
| **GGT(U/L)** | 148.16±4.86 | 151.19±5.11 | 152.36±8.44 | 158.36±8.44 |
| **AST(U/L)** | 433.09±1.66 | 719.70±2.40 | 725.45±8.79 | 733.03±15.95 |
| **ALT(U/L)** | 152.20±3.40 | 256.49±8.01 | 257.43±1.55 | 258.43±9.64 |

Data are expressed as mean ± standard error of the mean (SEM) of five normal and 15 intoxicated rats.

**Key: EtOH**-ethanol, **ALT**- alanine amino transferase, **AST-** aspartate amino transferase, **GGT-** gamma glutamyl transferase**.**

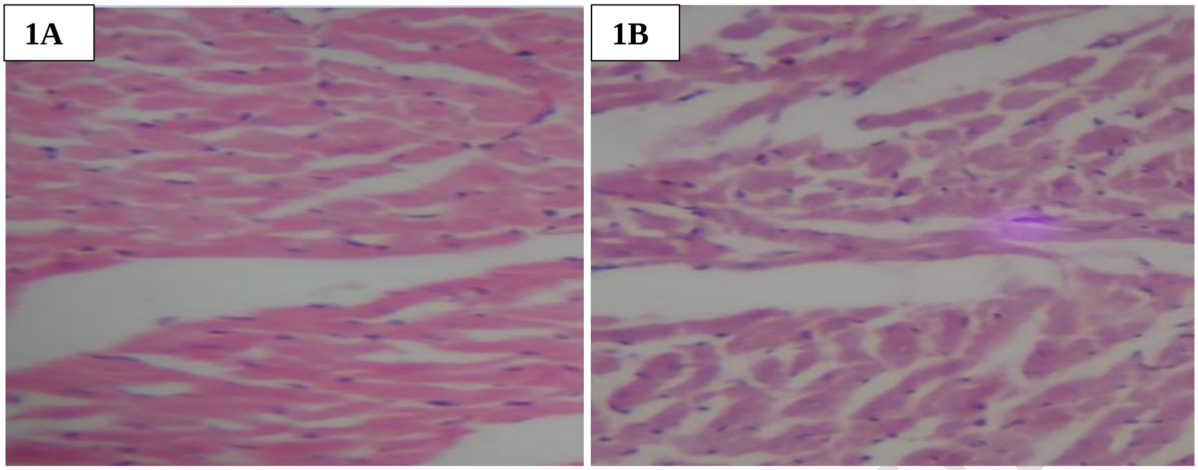


**Plate 1**: Photomicrographs of **Heart** of Alcohol Administered Rats
**1A**: Control rats showing normal study.

**1B**. Rats administered 40% ethanol also showing normal heart architecture.

**Key**: **A**:Control, **B**: 40% EtOH

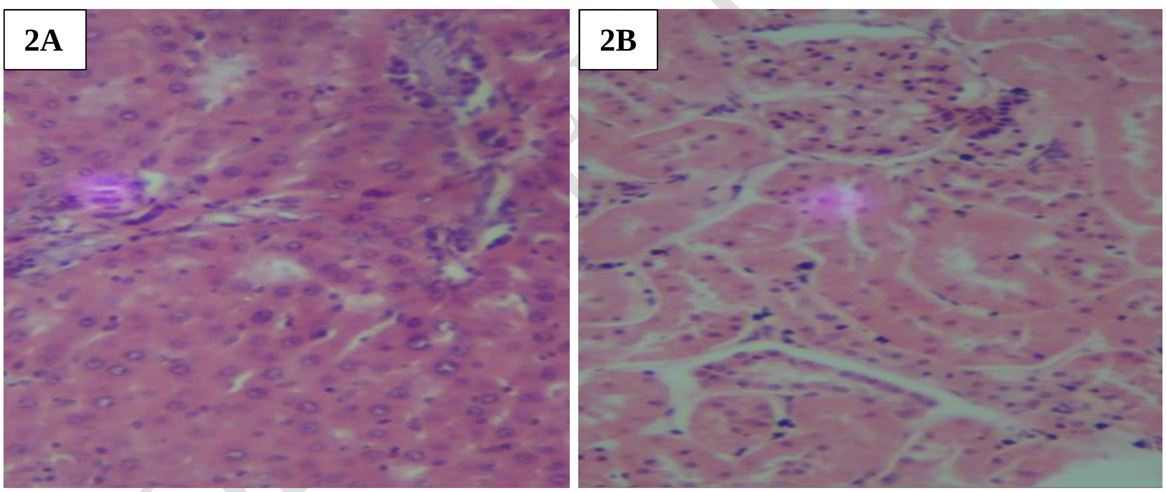


**Plate 2**: Photomicrographs of **Liver** of alcohol administered rats.
**2A**. Well preserved liver architecture showing normal appearing portal tract.

**2B.** Normal study with some ghost appearance of the tubules (Acute tubular necrosis **ATN**).

**Key**: **A**: Control, **B**: 15% EtOH

Alcohol (ethanol) may lead to hepatotoxicity; ALT, AST, and GGT are most commonly used makers of hepatocyte injury, they are more specific enzymes biomarkers in intoxication experiments Palmer, (2004).

Ethanol administrated rats recorded a significant increase in gamma-glutamyl transferase (GGT), Aspartate amino transferase (AST), Alanine amino transferase by GGT (1.2%, 0.2% and 0.5%) AST (0.13%, 0.1% and 5.21%) and ALT (0.11%, 0.21% and 11.49%) followed the administration of various concentrations of ethanol solution (Table 1) as compared to the control group respectively.

In agreement with the present study, Chen *et al*.,(2003) observed a significant increase in AST and ALT after moderate drinkers (at least once per month.,< 210g ethanol/week for men <140g ethanol/week for women). In addition, Onyesom and Anosike (2007) recorded elevation in AST and ALT in rabbit orally given 1.5g ethanol/ kg body weight as single daily dose for a continuous period of fifteen weeks. The increase in enzyme activity was mainly due to the effect of ethanol that interpolate and expands bio membranes leadings to increase membrane fluidity and enzyme release (Yang *et al*., 2005). Following the treatment with alcohol, there were significant elevations in GGT, AST and ALT (Group C, 40% Ethanol) which confirms the likely hepatotoxic effect of alcohol. This finding is in line with the report of Maher (1997).

The evaluation of liver function by measuring serum GGT, AST and ALT of alcohol intoxicated rat can be used for the study of human consumption because most of the alcohol consumed by peoples is metabolized by the liver. Therefore, the liver is constantly saddled with the responsibility of detoxifications of substances ingested. It is documented that a number of potentially dangerous by-product are generated (Maher, 1997) these byproducts especially free radicals are known to cause destruction of the liver cell hence elevation of ALT, AST and GGT Onyesom and Atakuo, (1998).

The effect of ethanol on the body weight was also assessed in this study; Table 1 showed the change in body weight of rats before and after administration of ethanol solutions. Here the weight observed in intoxicated rats recorded a significant decrease by 3.6%, 1.9% and 1.3% as compared to controls. This was in accordance with the study of Rajakrishnan *et al*., (1997) who found out that changes in the body weight of the rat may be due to the deposition of lipids in adipose tissue and fluid accumulation in the organ.

Water intake level was also measured in this study, a notable difference was observed in alcoholic treated rat, water intake was affected at the higher concentration while a less significant increase was observed in the control group.

Food intake level were also measured in this study, significant differences in daily food intake were observed between saline – control and alcohol treated rat utilizing oral administration of alcohol, this is in line with the previous study of Callaci *et al*., (2006) that a significant difference was noticed in between control and alcohol treated rat.

This study examined the slide of H and E stained tissues of the liver, kidney, heart and lungs of all the study animals that received (5%, 15%, 40%) ethanol and the control group that received 0.9% normal saline. The major histopathological changes occurred in organs of animals that received 40% ethanol, there were minimal histopathological changes in the organ tissues of the rat received 5%, and 15% ethanol indicated that high concentration of ethanol is required to caused significant histopathology changes in the liver, kidney, heart and lungs.

In all the experimental groups that received alcohol, none of the heart tissue showed a significant degeneration in histology profile when compared to the normal control groups that received normal saline. The liver tissues of 15% alcohol showed well preserved liver architecture with normal appearance portal tract when compared with the liver tissues of the normal study, this was in agreement with (Adedapo *et al*., 2009) who observed no abnormal features in the histopathology examination of the liver tissue. This could have been caused by low doses used in the study. The kidney tissues that received 15% showed sloughing off of cells that line tubules with some ghost appearance of the tubules causing acute tubular necrosis. This was in accordance with the finding of Kasolo *et al*., (2011) that the kidney tissue showed expanded and congested glomeruli, mononuclear cellular infiltration which are features of mild nephritis that caused kidney tissue damage followed moderate ethanol intake.

Histopathological investigation of lungs that received highest concentration of ethanol 40% showed intense inflammation cells within the interstitium I and congestion C. this present study is in agreement with previous study on alcohol where the acute histopathological change in lungs, kidney and liver were documented at 45% alcohol for 4weeks (Abdelgadir *et al*., 2010).

Gradual mortality was observed following ethanol administration into rat with regard to varying concentration of ethanol. There were progressive toxic signs and symptoms which resulted in pre-terminal death. Gross pathological symptoms were observed in the rat of high concentrated ethanol treated rat. There is an observation of gross abnormality that could be attributed to ethanol administration at the time of autopsy.

**Conclusion and Recommendation**

Though moderate alcohol intake have shown beneficial effect, administration of different concentrations of alcohol in this study caused different alteration to the visceral organs such as liver, kidney, heart and lungs. Varying concentration of ethanol intoxications also leads to a disturbance of certain metabolic parameters that can be used as makers for early detection of ethanol toxicity.

**COMPETING INTERESTS DISCLAIMER:**

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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