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Animal Model for Hypoglycemic Studies Using Albino Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AJA, IUM, AAI and AMW designed the study, carried out literature searches, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AI, AM, AN and IA managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: It could be speculated that hypoglycemia increases level of serum α-amylase and α-glucosidase and their corresponding mRNA, with concomitant increase in plasma level of glucagon. However, the concept of developing hypoglycemic rats' model is associated with number of hitches.
Aim: To successfully induce and sustain hypoglycemia in albino rats using ethanol.
Methodology: Total of twenty four (24) rats used for the study were grouped into four (4) of six (6) rats each. Group I served as normal control, group II, III and IV were respectively administered with

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single dose of 250, 500 and 750 mg/kg body weight of ethanol and observed for 72 hours. Blood glucose was monitored at 0, 30 mins, 1 hr, 2 hrs, 24 hrs, 48 hrs and 72 hours using glucometer. **Results:** Within 30 minutes of ethanol administration, a significant ($p<0.05$) decrease in fasting blood glucose level of group II, III, and IV was observed compared to group I, with no significant weight decrease ($P>0.05$) among groups. Toxicity studies however show damages to vital organs (liver, kidney and pancreas) which could be associated with ethanol administration in a dose dependent pattern.
Conclusion: The present study demonstrated that oral administration of ethanol at 250 mg/kg body weight to experimental rats can aggravates and maintain hypoglycemia with mild toxicity to the vital organs, it may therefore be concluded that 250 mg/kg body weight of ethanol could be used in maintaining an ideal hypoglycemic rat model as research tool.

Keywords: Albino rats; modelling; ethanol; hypoglycemia.

1. INTRODUCTION

Research animals are valuable tools for understanding the pathophysiology and in developing therapeutic interventions for a disease. These animals are used in basic medical and veterinary research. Various animals have been reported as useful models in studying diseases afflicting humans and animals. Research animals include mice, rats, rabbits, guinea pigs, sheep, goats, cattle, pigs, dogs, cats, birds, fish, and frogs [1]. With increasing awareness of animal welfare and research ethics, it is important to obtain accurate results using suitable models while reducing wastage of animals used for testing. Animals are used in biomedical research for number of reasons including the following.

(i) Feasibility: Animal models are relatively easy to manage, as compounding effects of dietary intake and environmental factors (temperature, relative humidity and lighting) can be controlled. Therefore, there is relatively less environmental variation and no incompetence compared to human subjects in which the researcher does have total control over them. Blood vessels and cardiac tissues can be isolated for detailed experimental and biomolecular investigations. Animals typically have a shorter life span than humans, hence they make good models, as they can be studied over their whole life cycle or even across several generations [2].

(ii) Similarities to Human: Moreover, animal species adapted as experimental animals due to their similarity in anatomical basis and physiological functions with humans. For example, chimpanzees and mice share about 99% and 98% of DNA with humans, respectively [3]. As a result, animals have the tendency to be affected by many health problems afflicting humans. These and many more qualify some categories of animals to be models for the study of human diseases.

(iii) Drug Safety: Preclinical toxicity testing, pharmacodynamics, and pharmacokinetics profile of drugs may be investigated on animals before the compounds or drugs are used in humans. This is vital, as prior to testing on humans, the effectiveness of a drug and toxicity profile needs to be evaluated on animals [4]. These are in line of protecting the animals, human, and environment [5].

It is a common sense to induce hypoglycemia by feed withdrawal, however, it may place the animals in unstable metabolic state of low energy tension. Therefore the concept of developing hypoglycemic model animal posits a serious problem to researchers, especially in raising a model without compromise to the functionality of vital organs. This research was aimed at developing and sustaining a hypoglycaemic rat's model using ethanol.

Ethanol is metabolized in the liver by the enzyme alcohol dehydrogenase to acetaldehyde, which in turn reduces nicotinamide adenine dinucleotide (NAD^+) to NADH, a reaction that is facilitated by co-enzyme cytochrome P_{450} E1 (CYPE1). The acetaldehyde is then metabolized by the enzyme aldehyde dehydrogenase to produce acetic acid and NAD^+ is also reduced to NADH + H^+ in this reaction. The acetate produced from breakdown of ethanol leaves the liver for its metabolism in extra hepatic tissues such as skeletal muscle, where it is spontaneously broken down to CO_2 and H_2O. Thus, alcohol metabolism significantly decreases the hepatic NAD+/NADH ratio [6].

The sustained hypoglycemic activity of ethanol could be linked to different mechanisms. Firstly, high [NADH] inhibits gluconeogenesis by preventing the oxidation of lactate to pyruvate. The consequence of this reaction is hypoglycemia, because glucose cannot be generated from other precursors [7]. Secondly, acetate formed is converted to acetyl CoA in the mitochondria, and because of the presence of high [NADH], it cannot be oxidized through the TCA cycle, instead it forms ketone bodies which provide the immediate energy requirements of some vital organs in alcoholic individuals [8]. Thirdly, high [NADH] inhibits fatty acids oxidation, since ethanol metabolism increase the ratio of NADH to NAD^+, thereby creating condition necessary for fatty acid synthesis rather than oxidation in alcoholic individual [9].

Hypoglycemia may be associated with increased expression of genes coding for carbohydrates digesting enzymes (alpha amylase, glucosidase). This model could be a vital tool for identifying plants that elicits its hypoglycemic effect by affecting the expression of these genes.

2. MATERIALS AND METHODS

2.1 Study Animals

Male and female albino rats weighing between 80 g to 150 g were purchased from animal house of Biological Science Department; Bayero University, Kano. The animals were housed in well-ventilated cages in the animal house of Biological Science Department of Bayero University Kano. The rats were allowed to acclimatize for one week prior to the experiment and had access to food and clean water.

2.2 Preparation of Ethanol

The ethanol administered was prepared by 1:1 dilution of 79.3 mg/ml absolute ethanol solution to give a final concentration of 39.65 mg/ml.

2.3 Administration of Ethanol

Rats induced with hypoglycemia were fasted overnight for a period of 12 hours, the induced hypoglycemia was sustained by oral administration of ethanol at dose of 250 mg/kg, 500 mg/kg and 750 mg/kg body weight to different groups of the experimental rats. The volume to be administered to animals was calculated according to the method of [10].

$$\text{Volume to be administered (ml)} = \frac{\text{weight of rats (kg)} \times \text{dose (mg/kg)}}{\text{concentration of the extract (mg/ml)}}$$

2.4 Experimental Protocol

Twenty four (24) rats were used and were grouped into four (4) groups of six (6) rats each. They were housed in plastic cages at an ambient temperature (28℃) and relative humidity, and were allowed free access to pelletized grower mash and water.

Group I: Normal control, no ethanol was administered
Group II: Were administered with 250 mg/kg body weight of ethanol
Group III: Were administered with 500 mg/kg body weight of ethanol
Group IV: Were administered with 750 mg/kg body weight of ethanol

Blood glucose concentrations of rats were determined at an interval of 30 min, one hour, two hours, three hours, 24 hours, 48 hours and 72 hours after ethanol administration. The water intake as well as feed intake of the animals were recorded.

2.5 Statistical Analysis

Results were expressed as mean ± standard deviation and analyzed using ANOVA, with p value <0.05 considered significant followed by Tukey´s post hoc test. A component of GraphPad Instat3 Software [11] version 3.05 by GraphPadInc was used to analyze the data.

3. RESULTS AND DISCUSSION

3.1 Results

Table 1 shows the effect of oral administration of the different doses (250 mg/kg, 500 mg/kg and 750 mg/kg body weight) on blood glucose concentrations of rats taken at the interval of 30 min, one hour, two hours, three hours, 24 hours, 48 hours and 72 hours after ethanol administration. At thirty minutes after ethanol administration, the blood glucose levels of test groups (II, III and IV) decrease significantly ($p<0.05$) compared to normal control (group I) in a non-dose dependent pattern, thus aggravation of fasting induced hypoglycemia. The ethanol sustained the induced hypoglycemia for 48 hours after which the blood glucose begins to rise up.

Table 1. Blood glucose level (mg/dl) of rats before and after ethanol administration, and at an interval of 30 min, one hour, two hours, three hours, 24 hours, 48 hours and 72 hours after ethanol administration

	No ethnl	30 min	1 hour	2 hours	3 hours	24 hour	48 hours	72 hours
G I	91.60 ± 6.69	95.00 ± 8.48	95.20 ± 7.53	110.00 ± 6.12	97.60 ± 7.36	76.20 ± 6.34	105.60 ± 4.33	108.67± 12.097
G II	90.00 ± 8.00^{a,b,c,d,e,f}	63.33 ± 8.73^{a}	64.33 ± 4.93^{b}	58.33 ± 5.85^{c}	52.66 ± 6.42^{d}	51.33 ± 15.82^{e}	61.66 ± 7.63^{f}	109.00± 12.53
G III	92.33 ± 5.68 ^{a,b,c,d,e,f}	72.33 ± 4.50^{a}	74.66 ± 5.68^{b}	63.00 ± 7.21^{c}	57.33 ± 9.07^{d}	57.66 ± 22.50^{e}	54.00 ± 10.14^{f}	96.33 ± 7.23
G IV	92.33 ± 9.01 ^{a,b,c,d,e,f}	68.33 ± 7.50^{a}	65.33 ± 8.38^{b}	60.00 ± 3.60^{c}	53.66 ± 3.78^{d}	43.33 ± 1.15^{e}	49.33 ± 1.15^{f}	121.00± 3.60

Results are expressed as mean ± SD (n=6). Values in the same row bearing similar superscripts are significantly different at P<0.05

Table 2. Body weight (g) of rats before and after ethanol administration, 24 hours, 48 hours and 72 hours after ethanol administration

	Initial weight	24 hour	48 hours	72 hours
G I	90.00±17.32	88.66 ±16.07	89.33 ± 15.04	93.33 ± 15.27
G II	116.67±35.11^{a,b}	106.66±40.41^{a}	109.33±30.02^{b}	112.33± 35.11
G III	100.00±20.00 ^{a,b}	90.33 ± 17.61^{a}	92.33 ± 15.88^{b}	96.00 ± 21.63
G IV	106.67 ± 23.09^{a,b}	100.33 ± 22.30^{a}	103.33 ± 22.94^{b}	105.33± 22.54

Results are expressed as mean ± SD (n=6). Values in the same column bearing similar superscripts are significantly different at P<0.05

Table 3. Water and feed intake of rats administered with varying doses of ethanol

	24 hour		48 hours		72 hours	
	Feed(g)	Water(ml)	Feed(g)	Water(ml)	Feed(g)	Water(ml)
G I	30.21±3.22^{a,b,c}	17.10±2.10	31.62±1.07 ^{a,b,c}	19.23±1.04	25.00±15.27 ^{a,b,c}	15.10±1.98
G II	5.10±0.91^{a}	14.2±0.93	6.6±0.41^{a}	10.3±2.02	21.30±1.11^{a}	15.2±0.85
G III	5.02±0.70^{b}	15.8±1.34	4.33±1.61^{b}	18.30±1.88	20.40±1.63^{b}	17.31±2.01
G IV	4.67±0.79^{c}	17.43±0.30	5.08±0.94^{c}	18.33±2.10	20.65±2.06^{c}	22.54±1.87

Results are expressed as mean ± SD (n=6). Values in the same column bearing similar superscripts are significantly different at P<0.05.

Table 4. Liver function indices of rats administered with varying doses of ethanol for 72 hours

	AST(IU/L)	ALT(IU/L)	ALP(IU/L)	ALB(g/dl)	DB(mg/dl)	TB(mg/dl)	TP(g/dl)
G I	34.33 ± 2.88^{a,b,c}	27.67 ± 2.31^{a,b,c}	57.21 ± 3.36^{a,b,c}	6.42 ± 0.37^{a,b}	0.30 ± 0.05^{a,b}	1.64 ± 0.12^{a,b}	9.64 ± 0.27^{a,b}
G II	38.34 ± 2.43^{a}	33.53 ± 3.21^{a}	94.43 ± 4.93^{a}	8.23 ± 1.02	0.66 ± 0.02	1.93 ± 0.52	10.61 ± 0.63
G III	42.33 ± 4.08^{b}	52.08 ± 4.50^{a}	274.06 ± 5.08^{b}	3.80 ± 0.71^{a}	0.93 ± 0.07^{a}	3.80 ± 0.50^{a}	14.80 ± 0.24^{a}
G IV	50.76 ± 3.00^{c}	55.67 ± 3.22^{b}	310.13 ± 1.02^{c}	3.50 ± 0.77^{b}	1.36 ± 0.20^{b}	4.50 ± 0.67^{b}	16.28 ± 0.39^{b}

Results are expressed as mean ± SD (n=6). Values in the same column bearing similar superscripts are significantly different at P<0.05. Key: AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase, ALB: albumin, DB: direct bilirubin, TB: total bilirubin, TP: total protein

Table 2 shows the weight of rats before and after administration of ethanol. There is a significant ($p<0.05$) decrease in weight of rats administered with varying doses of ethanol at day one and two respectively compared to initial weight.

Table 3 shows the water and feed intake of rats. There is a significant decrease in feed intake of rats administered with varying doses of ethanol in a dose dependent pattern for a period of 48 hours compared to normal control. However there is no significant difference in water intake between groups throughout the period of research.

Table 4 present the liver function indices of rats administered with varying ethanol. There is a significant increase ($p<0.05$) in AST, ALT, ALP in groups administered with 250 mg/kg, 500 mg/kg and 750 mg/kg with a significant increase ($p<0.05$) in DB, TB and TP in groups administered with 500 mg/kg and 750 mg/kg body weight when compared to normal control. A significant decrease in the level of serum ALB was also seen in groups administered with 500 mg/kg and 750 mg/kg compared to the normal control. No significant changes was observed in all parameters between normal control and group administered with 250 mg/kg.

Table 5 present the kidney function indices of rats administered with varying ethanol. A significant increase ($p<0.05$) in K^+ , Cl^- , HCO_3^- urea and Creatinine was observed in all ethanol administered groups compared to normal control, No significant changes was observed in level of serum Na^+ between the groups.

Table 6 shows the lipid profile (TG, TC, LDL-C and HDL-C), lactate dehydrogenase (LDH) and Creatine kinase (CK) of rats administered with varying doses of ethanol. No significant changes ($p<0.05$) was observed in level of serum all parameters between the respective groups. However a significant increase in lactate dehydrogenase and creatine kinase was observed in groups administered with 500 mg/kg and 750 mg/kg respectively.

3.2 Histological Analysis

The results of histopathological study in Plates 1, 2, 3 and 4 shows a cross sections of liver of Group I (control group), Group II (250 m/kg of ethanol), Group III (500 m/kg of ethanol), Group IV (750 mg/kg of ethanol) respectively. Plates 5, 6, 7 and 8 shows a cross sections of kidney of Group I (control group), Group II (250 m/kg of ethanol), Group III (500 m/kg of ethanol), Group IV (750 mg/kg of ethanol) respectively. While Plates 9, 10, 11 and 12 shows a cross sections of pancreas of Group I (control group), Group II (250 m/kg of ethanol), Group III (500 m/kg of ethanol), Group IV (750mg/kg of ethanol) respectively.

3.3 Discussion

Fasting of rats overnight lead to development of symptoms of mild hypoglycemia (Table 1, at no ethanol) which was sustained by administration of varying dosses of ethanol up to 48 hours. Though maintenance of hypoglycemia seems not to follow dose dependent at on set (30 min),

Table 5. Kidney function indices (in mmol/L) of rats administered with varying doses of ethanol

	Urea	Na^+	K^+	Cl	HCO_3	Creatinine
G I	44.42±4.03$^{a.b.c.d}$	135.75±7.62	3.37±0.11^{a}	56.09±4.87^{a}	24.72±3.32^{a}	46.00±2.12^{a,b,c}
G II	48.04±3.43^{a}	133.53±5.10	4.43±0.32	58.23±5.22	26.06±4.02	49.30±5.21^{a}
G III	52.33±2.08^{b}	126.08±6.50	4.06±0.18	63.80±4.71	27.90±4.70	53.80±5.30^{b}
G IV	68.74±6.16^{c}	124.67±2.54	5.60±0.16^{a}	64.78±8.79^{a}	30.10±3.5^{a}	60.72±5.61^{c}

Results are expressed as mean ± SD (n=6). Values in the same column bearing similar superscripts are significantly different at P<0.05

Table 6. Lipid profile (in mmol/L) and cardiac markers in (U/L) of rats administered with varying doses of ethanol for 72 hours

	TG	TC	LDL-C	HDL-C	LDH	CK
G I	1.22±0.22	4.27±0.06	2.42 ±0.15	1.00±0.09	44.21±0.82^{a,b}	143.23±3.1^{a,b}
G II	1.10±0.10	4.11±0.07	2.24±0.21	1.08±0.13	50.23±1.32	149.61±2.16
G III	1.12±0.13	4.08±0.11	2.05±0.14	1.05±0.16	76.87±1.07^{a}	154.20±3.41^{a}
G IV	1.02±0.15	3.75±0.08	1.89±0.16	0.95±0.10	82.32±2.12^{b}	182.21±2.43^{b}

Results are expressed as mean ± SD (n=6). Values in the same column bearing similar superscripts are significantly different at P<0.05

It could be attributed to metabolic alteration/ shift induced by the ethanol. These may include increase production of energy equivalence, deprivation of feed intake and increase fatty acid biosynthesis. All these may vary with pre inducement physiological condition of the rat. However, the dose dependent hypoglycemic effect of ethanol was shown at 24 and 48 hours after ethanol administration.

Hypoglycemia is as a result of elevated levels of NADH during ethanol metabolism, which affect a number of critical dehydrogenase in the liver required for gluconeogenesis. The high amount of NADH inhibits the conversion of lactate to

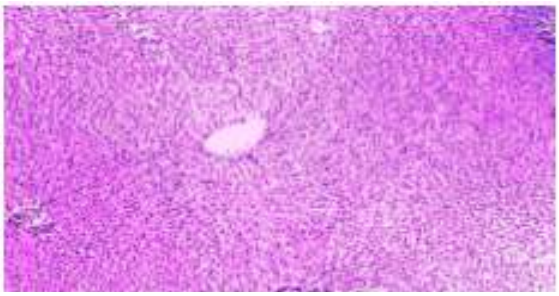

Plate 1. Section shows normal hepatocyte arranged as radiating corps forming hexagonal units containing a central venules (H and E, mag.×100)

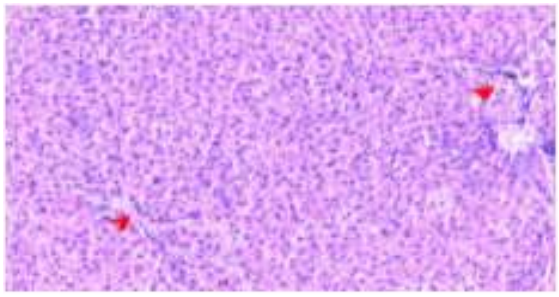

Plate 2. Section of liver administered orally with dose of 250 mg/Kg of ethanol showing areas of mild fibrosis. (H and E, mag×100)

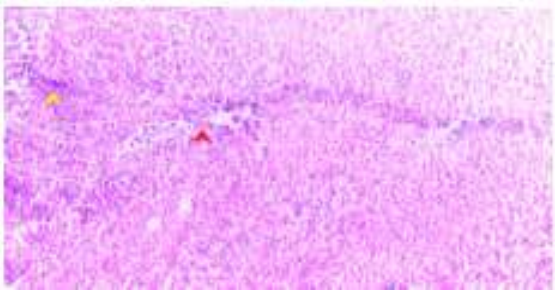

Plate 3. Section of liver administered orally with dose of 500 mg/Kg of ethanol showing areas of severe fibrosis and inflammation (H and E mag×100)

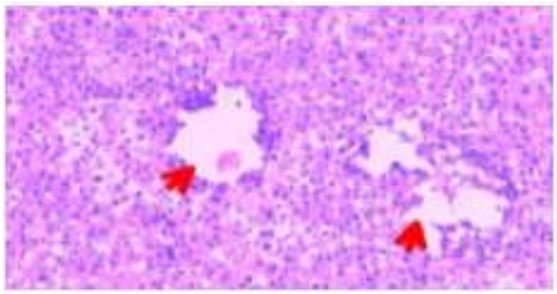

Plate 4. Section of liver administered orally with dose of 750 mg/Kg of ethanol showing areas of severe fibrosis and inflammation with loss of liver architecture (H and E mag×100)

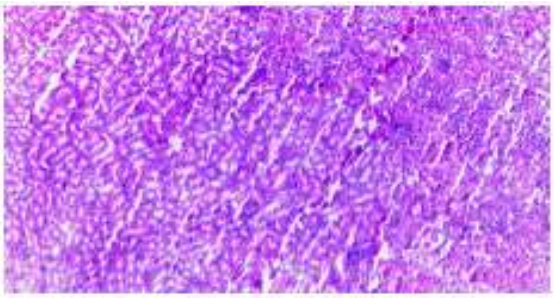

Plate 5. Section of kidney of control rat showing no significant pathology (H and E, mag.×100)

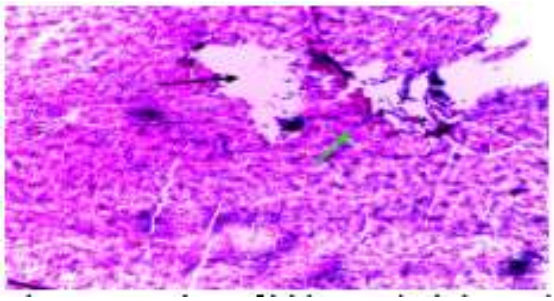

Plate 6. Section of kidney administered with dose of 250 mg/Kg of ethanol showing area pathology and renal damage (H and E, mag.×100)

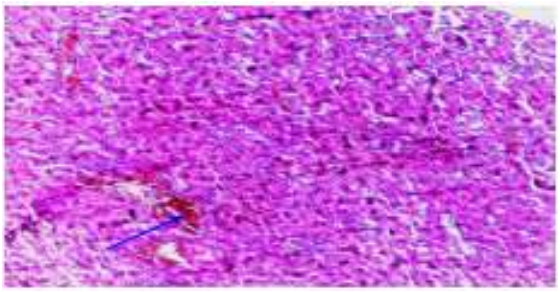

Plate 7. Section of kidney administered with dose of 500 mg/Kg of ethanol showing area of congestion (H and E, mag.×100)

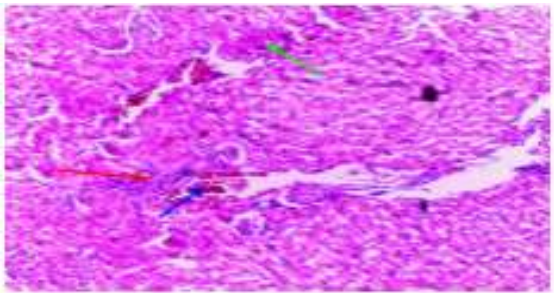

Plate 8. Section of kidney administered with dose of 750 mg/Kg of ethanol showing area of fibrosis, inflammation and congestion (H and E, mag.×100)

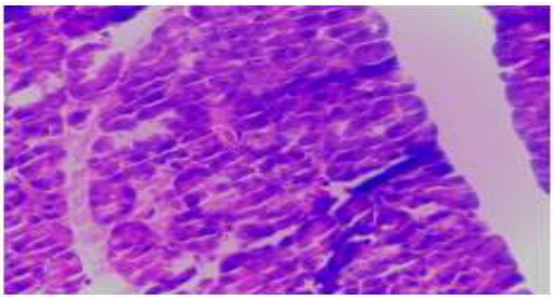

Plate 9. Section of pancreas control rats showing no significant pathology (H and E, mag.×100)

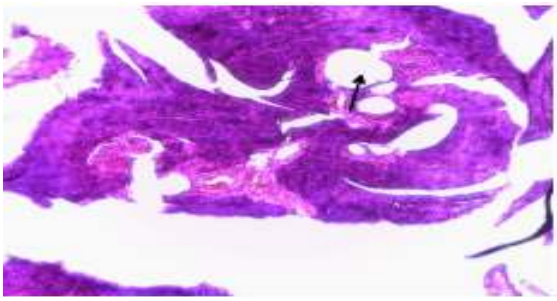

Plate 10. Section of pancreas of rats administered with dose of 250 mg/Kg of ethanol showing area of pancreatic damage (H and E, mag.×100)

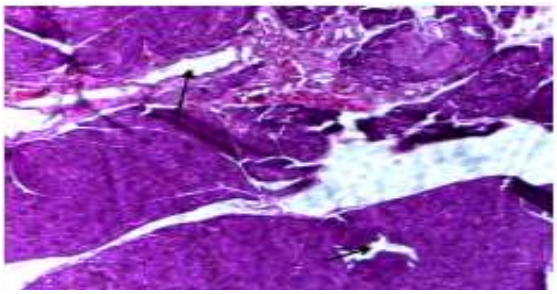

Plate 11. Section of pancreas of rats administered with dose of 500 mg/Kg of ethanol showing area of pancreatic damage (H and E, mag.×100)

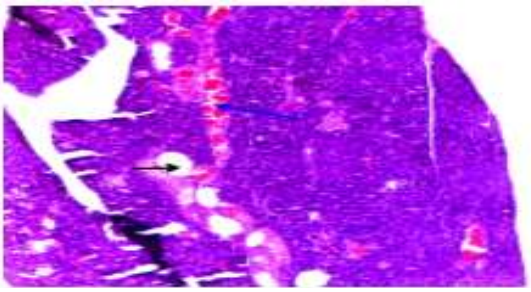

Plate 12. Section of pancreas of rats administered with dose of 750 mg/Kg of ethanol showing area of pancreatic damage and congestion (H and E, mag.×100)

pyruvate by the enzyme Lactate dehydrogenase, malate to oxaloacetate by enzyme Malate dehydrogenase which causes decreases availability of pyruvate and oxaloacetate for gluconeogenesis to take place [12]. As ethanol inhibits gluconeogenesis, hypoglycemia therefore result very fast after glycogen stores are exhausted. The slight increase in blood glucose levels of groups II, II and IV was notice after 48 hours and a significant increase in the normal control group was equally notice and can be attributed to the resumption of feed intake.

Ethanol can also induce an anion gap due to an increase in lactic acid and ketoacids. The increase in ketoacids is thought to be due to increased release of free fatty acids from adipose tissue [13]. An increased anion gap correlated

Table 7. Hematological parameters of rats administered with varying doses of ethanol for 72 hours

Groups	Control	250 mg/kg	500 mg/kg	750 mg/kg
WBC(10^3/µL)	4.7± 1.76[a,b,c]	6.3± 0.21[a]	7.3± 1.46[b]	11.1± 1.59[c]
RBC (10^6/µL)	7.11± 0.46[a]	4.54± 1.42[a]	6.26± 1.18	6.28± 0.18
HGB (g/dL)	12.4± 0.28[a]	8.4± 1.76[a]	10.3± 0.10	11.2± 0.26
PCV (%)	40.4± 2.45[a]	27.2±1.11[a]	37.6± 2.37	39.3± 2.39
MCV (f L)	56.8± 0.62	59.9± 0.23	60.1± 1.05	63.6± 1.05
MCH(pg)	17.4± 0.23	18.5± 0.26	16.5± 0.30	17.8±0.98
MCHC (g/dL)	30.7± 0.39	30.9± 1.23	27.4± 1.56	25.4± 1.01
PLT(10^3/µL)	641± 7.5[a,b,c]	641± 7.5[a]	789± 6.23[b]	801± 5.87[c]
LYM (%)	74.2± 0.82	59.8± 0.14	65.5± 0.62	76.4± 0.25
RDW-SD (f l)	44.2±1.30	36.9±0.97	37.7±0.43	41.4±1.62
RDW-CV (%)	24.9± 0.82	16.6± 1.27	16.6± 1.27	18.4± 1.76
PDW (f l)	8.26±0.87	10.01±0.48	12.9±0.76	13.34±0.92
MPV(f l)	5.4±0.45 [a,b,c]	7.9±1.08[a]	8.2±1.31[b]	9.1±0.98[c]

Results are expressed as mean ± SD (n=6). Values in the same row bearing similar superscripts are significantly different at P<0.05

with ethanol-induced hypoglycemia in the study by [14]. Another mechanism of ethanol hypoglycemia is that Alcohol exerts substantial influences on pancreatic microcirculation by evoking a massive redistribution of pancreatic blood flow from exocrine into endocrine (insulin-producing) part via mechanisms mediated by the messenger molecule nitric oxide and the vagus nerve, augmenting late phase insulin secretion leading to hypoglycemia [15].

Liver is an important body organ and actively involved in different metabolic functions [16]. Hepatic damage caused by chemicals or infectious agents is associated with distortion of these metabolic functions and may lead to progressive liver fibrosis and ultimately cirrhosis and liver failure. From Table 2, a significant increase ($p<0.05$) in the maen serum activities of liver function indices was observed between ethanol administered groups (II, III and IV) was observed in a dose dependent pattern compared to normal control. These results are in agreement with the work of [17] who reported that alcohol causes server liver damage. The histopathological changes observed in liver tissue supported the biochemical parameters (Plates 1-4).

Kidneys are the major organs in eliminating toxic compound metabolized by the liver. It receives about 1200 ml of blood per minute [18], containing a lot of chemical compounds. Therefore damage to the kidneys can be determined by measuring the level of urea, electrolyte and creatinine in blood as an indicator of kidney damage. Urea is a byproduct from protein breakdown. About 90% of urea produced is excreted through the kidney [19]. Meanwhile, the creatinine is a waste product from a muscle creatinine, which is used during muscle contraction. Creatinine is commonly measured as an index of glomerular function [20], it is excreted exclusively through the kidney [21]. From the results obtained, administration of ethanol to experimental rats lead to kidney damage ranging from mild to severe in a dose dependent pattern. The mechanisms by which ethanol induces kidney damage may be associated with the metabolism of ethanol which is a probable contributor to the ketotic state.

The pancreas is an endocrine organ in vertebrates containing α-cells, β-cells, δ-cells and γ-cells; secreting glucagon, insulin, somatostatin and peptide proteins respectively. It also function as an exocrine organ producing α-amylase, lipases, peptidases and ribonuclease which catalyze the hydrolysis of starch, fats, peptides and ribonucleic acids in the duodenum [22]. Administration of ethanol lead to changes in the architecture of the pancreatic tissue ranging from mild to severe depending upon the dosage administered.

Hematological complications consist mainly of abnormalities in the functions, morphology and metabolism of erythrocytes, leukocytes and platelets [23]. The primary reasons for assessing the red blood cell (RBC) is to check anemia and to evaluate normal hematopoiesis [24]. Packed cell volume (PCV) represent the volume of red blood cell in 100 ml of blood and helps to determine, and diagnose states of hydration,

polycythemia, and degree of anemia [25]. There was significant decrease ($p>0.05$) in RBC, PCV and HGB levels of rats administered with 250 mg/kg of ethanol (group II) compared to normal control, contrary to what is observed in group III and IV administered with 500mg/kg and 750 mg/kg respectively. These may be as a result of anemia or the onset of glycosylation process because reactive oxygen species (ROS) may be generated upon administration of ethanol.

Platelets are fragment of cells that participates in blood clotting, and initiate repair of blood vessels, and are also considered as acute phase reactant to infection or inflammation [26]. Platelet count (PLT) show cases the precise method of determining the degree of acute blood loss while white blood cell count (WBC) measures the total number of white blood cells which defend the blood against opportunistic infection. Administration of ethanol to rats indicated a significant ($P<0.05$) increase in WBC when compared to normal rats. Ethanol may have cause perturbation in the bone marrow stem cells which lead to increase in WBC. Significant ($p<0.05$) increase in platelet count was also observed in groups experimental groups. Generally, a reactive thrombocytosis due to abnormal increase in platelet is associated with an increased thrombotic risk when it is accompanied with overproduced red blood cells and white blood cells to some degree [27].

Mean platelet volume (MPV) is used for investigating the ability of a drug to enhance blood clotting [28] and to determine platelet function, as its increase is a newly emerging indicator for atherothrombosis [29]. Ethanol administration leads to increase in the level of MPV in a dose dependent pattern, a finding supporting the increase in PLT and WBC observed.

Mean cell volume (MCV), platelet distribution width (PDW), mean corpuscular hemoglobin (MCH), red blood cell distribution width coefficient of variation (RDWCV), red blood cell distribution width standard deviation(RDW-SD), and mean corpuscular hemoglobin concentration (MCHC) in all groups were not significantly varied.

4. CONCLUSION

Although successful induction of hypoglycemia was achieved using ethanol, however toxicity studies on key organs show mild damages to the organs even at the lowest dose administered for this study. Researches on hypoglycemia using ethanol should therefore use the lowest dosage that can induce hypoglycemia.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that Principle of laboratory animal care [30] and ethical guidelines for investigation of experimental pain in conscious animals [31] were observed during experimentation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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