

Research Article

Effect of Simultaneous Administration of Alabukun and Ethanol on Hematological Parameters and Liver of Adult Wistar Rats (*Rattus norvegicus*)

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Abstract: Alabukun is a local drug containing 760mg acetylsalicylic acid and 60mg caffeine, this drug has been highly abused by Nigerians as analgesic and majorly for delaying intoxication when simultaneously taken with alcohol. However very scanty or no literature is available especially with the simultaneous combination of these drugs despite their high degrees of abuse and misuse. The present investigation was therefore undertaken to study the effect of ethanol and alabukun on the hematological parameters and liver of adult wistar rats. In this study, a total of 25 adult wistar rats of average weight 160 ± 20.5 g acclimatized for two weeks were divided into four treatment groups T1, T2, T3, T4 and one control group (N=5). Group C serves as control which were given only distilled water while group T1 received 31 mg /kg of alabukun only, group T2 received 25% ethanol in 2% sucrose solution for 14 days as their drinking water, group T3 received 31mg/kg of alabukun and 25% ethanol in 2% sucrose solution as their drinking water for 14 days while group T4 received 31mg/kg of alabukun and 25% ethanol in 2% sucrose solution as their drinking water for 7 days and withdrew for another 7days. The animals were sacrificed by cervical dislocation and their blood sample were collected directly from the heart for hematological parameters while their liver were dissected out and processed for routine histological techniques. The drug reduced the RBC count, HGB and HCT values of the treated group compared to the control group. The histological result showed that there is liver damage.

Keywords: Alabukun, Alcohol, hematological parameters, brain, heamatoxylin, eosin.

Introduction

Alabukun powder is a popular drug in Nigeria; it is a cheap local drug containing acetylsalicylic acid 760mg and caffeine 60mg. This drug is highly abused by Nigerians for the treatment of pains, cold, headache and feverishness and in delaying the effect of drunkenness when taken simultaneously with alcohol. One of the components of Alabukun, aspirin (acetyl salicylic acid, ASA) is a nonsteroidal anti-inflammatory drug (NSAID) probably the most highly consumed pharmaceutical product in the world and works similarly to other NSAIDs but also suppresses the normal functioning of platelets ("Aspirin". Drugs.com. 2016a). Aspirin is a medication used to treat pain, fever, or inflammation ("Aspirin". Drugs.com. 2016b). Specific inflammatory conditions which aspirin is used to treat include Kawasaki disease, pericarditis, and rheumatic fever ("Aspirin". Drugs.com. 2016c). Aspirin given shortly after a heart attack decreases the risk of death ("Aspirin".

Drugs.com. 2016d). Aspirin is also used long-term to help prevent further heart attacks, ischemic strokes, and blood clots in people at high risk ("Aspirin". Drugs.com. 2016e). It may also decrease the risk of certain types of cancer, particularly colorectal cancer (Dews, 1982a). For pain or fever, effects typically begin within 30 minutes (Dews, 1982b).

Numerical clinical observations have associated the use of aspirin with blood disorders like anemia and cytopenia (Rayback, 1992). It has been shown that oral administration of low doses of aspirin significantly reduces circulatory erythrocytes and leukocyte counts suggesting the inhibitory action of this drug on bone marrow hemopoiesis. It has also been shown that high doses of aspirin causes death of the blood vessel cells (Dikshit *et al.*, 2006). One common adverse effect is an upset stomach. More significant side effects include stomach ulcers, stomach bleeding, and worsening asthma ("Aspirin". Drugs.com. 2016f). Acetylsalicylic acid is known to cause GI tract erosion resulting in occult bleeding; it is also reported to reduce iron uptake resulting in iron deficiency (Langman *et al.*, 2003). It is widely distributed in the body, having its highest concentration in the plasma, liver, renal cortex, heart, and lungs. It is metabolized through phase II conjugation reaction in the liver to form acetylsalicylic acid and other metabolites (Marcia, 2007).

The other compound in Alabukun called caffeine has a variety of pharmacological and cellular responses in biological systems. These include stimulation of the central nervous system and cardiac muscle, increased urinary output and relaxation of smooth muscle (Nehlig *et al.*, 1992a). Caffeine is a central nervous system (CNS) stimulant of the methylxanthine class (Liguori, 2001a). It is the world's most widely consumed psychoactive drug. Unlike many other psychoactive substances, it is legal and unregulated in nearly all parts of the world (Nehlig *et al.*, 1992b). When alcohol and caffeine are consumed jointly, the effects produced by caffeine are affected, but the alcohol effects remain the same (Liguori, 2001b). For example, when additional caffeine is added, the drug effect produced by alcohol is not reduced. However, the jitteriness and alertness given by caffeine is decreased when additional alcohol is consumed (Liguori, 2001c).

Alcohol consumption alone reduces both inhibitory and activational aspects of behavioral control. Caffeine antagonizes the activational aspect of behavioral control, but has no effect on the inhibitory behavioral control. Drug users abuse over-the-counter products and prescription medicines when they are unable to obtain their usual illicit street drugs. However, almost any substance can be abused or misuse.

Ethanol, also called alcohol, ethyl alcohol, grain alcohol, and drinking alcohol, it is a volatile, flammable, colorless liquid with a slight characteristic odor. It is a psychoactive substance and is the principal type of alcohol found in alcoholic drinks. As a central nervous system depressant, ethanol is one of the most commonly consumed psychoactive drugs (Ighodaro and Omole, 2012). The inability to modify drinking has also culminated into chronic consumption which is a problem of public health concern. If ingested orally, ethanol is extensively metabolized by the liver, particularly via the enzyme CYP450 (Harger, 1958a). Ethyl Alcohol increases the secretion of acids in the stomach (Harger, 1958b). The metabolite acetaldehyde is responsible for much of the short term, and long term effects of ethyl alcohol toxicity (Wallner and Olsen, 2008).

Study Objectives

To investigate the effect of simultaneous administration of alabukun and ethanol on the hematological parameters and on the histology of liver of adult wistar rats.

Materials and Methods

Chemicals

Alabukun drug contains 760mg acetylsalicylic acid and 60mg caffeine per sachet was obtained from AKOL PHARMACY, Ogbomosho and absolute ethanol was gotten from Sigma Laboratory Ltd, San Francisco, USA.

Experimental Design

Adult wistar rats (body weight ranging between 130-200g) were acclimatized for two weeks to laboratory condition $23\pm 2^{\circ}\text{C}$. They were bred and housed in the animal house of the department of Anatomy, LAUTECH, Ogbomosho, Nigeria.

The treatments for the various groups were administered accordingly, following strictly, as stated in the "Guide to the care and use of Laboratory Animals Resources". National Research Council, DHHS, Pub.No NIH 86-23 (1985) and in accordance with the guideline and approval of Nigeria Medical Ethical Association for Accreditation of Laboratory Animal Care. They were fed with animal chow ad libitum.

Thirty rats were divided into five groups of five rats each:

Control: Animals fed with normal diet and water ad-libitum without drug for a period of seven days.

Treatment Group 1: Animals received a dose of Alabukun of 31mg/kg body weight daily for fourteen days.

Treatment Group 2: Animals received only 2% sucrose in 25% ethanol solution as their drinking water for fourteen days.

Treatment Group 3: Animals received only 2% sucrose in 25% ethanol solution as their drinking water and alabukun of 31mg/kg body weight daily for fourteen days.

Treatment Group 4: Animals received only 2% sucrose in 25% ethanol solution as their drinking water and alabukun of 31mg/kg body weight daily for seven days and are withdrawn for seven days.

Laboratory animals oral LD₅₀ for alabukun is given as 31mg/kg (Momoh Johnson and Manuwa, 2014) and was administered using orally using cannula. The 2% sucrose in 25% were replaced afresh daily at 18:00 hours G.M.T.

Animal Sacrifice and Sample Extraction

Twelve hours after the administration of the last dosage, cervical dislocation was carried out following ethical humane, animal euthanasia which was adopted was carried out using expertise cervical dislocation. The abdominal cavity of each rat was opened up through a midline abdominal incision to expose the liver. The liver was excised and weighed; the liver was weighed with an electronic analytical and precision balance. The liver of each animal was fixed in 10% formol-saline for histological examination [BA 210S, d=0.0001-Sartoriusen GA, Goettingen, GERMANY].

Histological Procedures and Analysis

This was done as described by (Ogunlade *et al.*, 2012). Briefly, the organs were cut on slabs about 0.5 cm thick and fixed in 10% formol saline for a day after which they were transferred

to 70% alcohol for dehydration. The tissues were passed through 90% alcohol and chloroform for different durations before they were transferred into two changes of molten paraffin wax for 20 min each in an oven at 75°C. Serial sections of 5µm thick were obtained from a solid block of tissue and were stained with heamatoxylin 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 JVC colour video digital camera (JVC, China) mounted on an Olympus light microscope [Olympus UK Ltd, Essex, UK] to demonstrate the liver damage.

Determination of Hematological Parameters

The total red blood cell (RBC), hemoglobin concentration (HGB) ,white blood cell count (WBC), Lymphocyte and other hematological parameters were determined using ADVIA 60 Closed Tube (CT) Automated Hematology System in LAUTECH Teaching Hospital, Ogbomoso, Nigeria.

Ethical Approval

All the authors hereby declare that all the experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in line with the ethical procedure laid down by Nigeria Medical Ethical Association for Accreditation of Laboratory Animal Care.

Table 1. Effect of Alabukun on hematological parameters of adult wistar rats

Hematological Parameters	C	T1	T2	T3	T4
White blood count (WBC) mm ³	8.5x10 ³	11.5x10 ³	12.7x10 ³	14.5x10 ³	9.6x10 ³
Red blood count (RBC) mm ³	5.3x10 ⁶	3.3x10 ⁶	2.8x10 ⁶	2.0x10 ⁶	5.1x10 ⁶
Hemoglobin (HGB) g/dl	15.5	12.7	11.5	10.3	14.2
Hematocrit (HCT)%	48.1	43.7	42.9	40.9	45.5
Mean cell volume (MCV)	63.8	65.3	65.2	65.5	65.4
Mean corpuscular hemoglobin (MCH) pg	18.1	18.7	18.5	18.8	18.2
Mean corpuscular hemoglobin concentration(MCHC)g/dl	28.7	29.8	29.0	29.6	29.3
Platelet count (PLT) L	1,245.7 x10 ⁹	1,323.5 x10 ⁹	1,376.1 x10 ⁹	1,330.8 x10 ⁹	1,345.4 x10 ⁹
Lymphocyte (LYM)%	42.5	49.6	46.8	49.7	46.2
Mean platelet volume (MPV) µm ³	7.2	7.3	7.3	7.4	7.3
Monocyte (MON)%	5.2	5.7	6.3	6.2	6.9
Plateletcrit (PCT)%	0.81	0.72	0.78	0.79	0.70
The values are the Means ± SD for five rats in each group.					

Photomicrograph Demonstration of H and E Control

Photomicrograph of the liver of animal fed with normal diet ad libitum for a period of fourteen days.

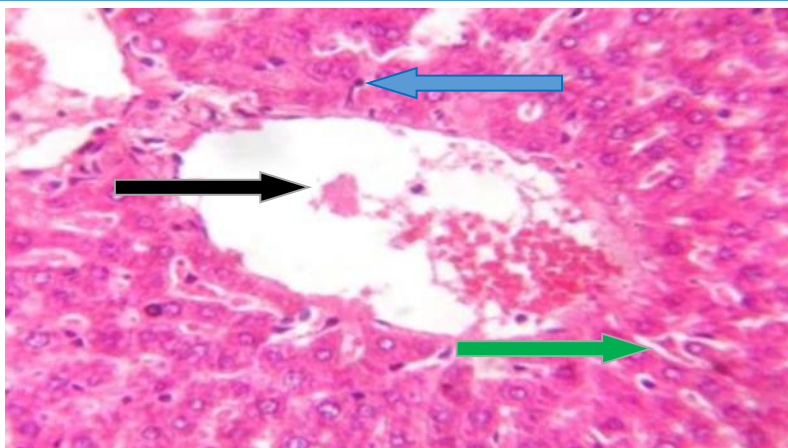


Figure 1. Histological demonstration of the liver using H & E staining techniques [X400] showing normal central vein (CV, black arrow) with normal sinusoid (S, green arrow) around the hepatocyte (H, blue arrow).

Treated Group 1

Photomicrograph of liver of animals administered with dose of Alabukun of 31mg/kg body weight for fourteen days.

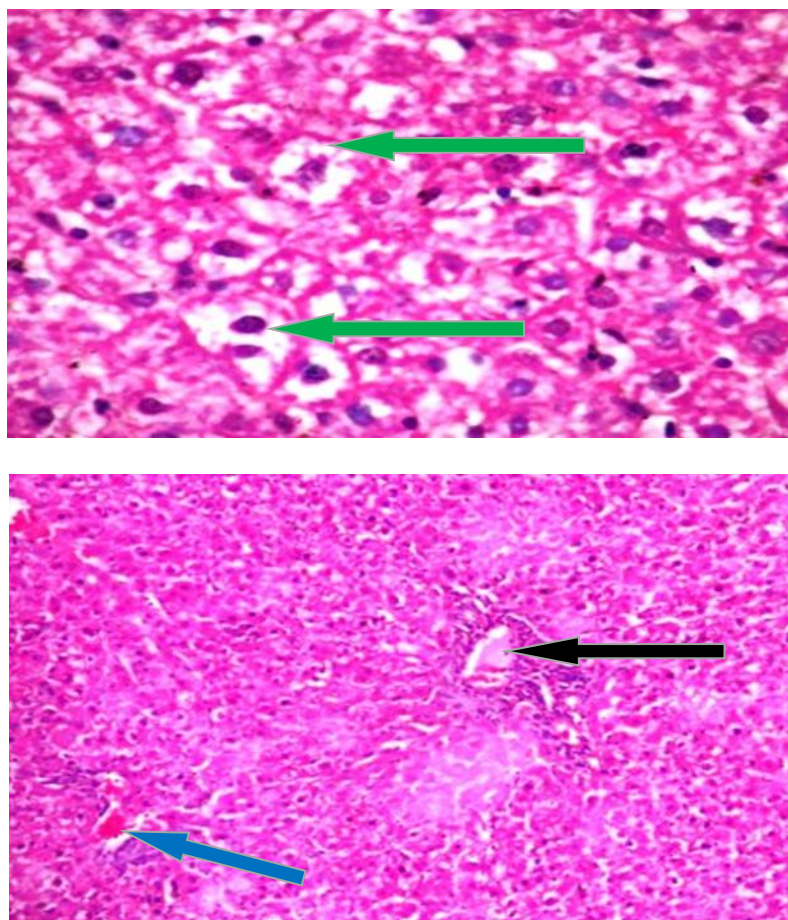


Figure 2. Histological demonstration of the liver using H&E staining technique [X400] showing moderate disseminated microvesicular steatosis (green arrows) mild disseminated periportal infiltration by inflammatory cells (black arrow) and mild disseminated congestion (blue arrows).

Treated Group 2

Animals administered with 2% sucrose in 25% ethanol solution as their drinking water for fourteen days.

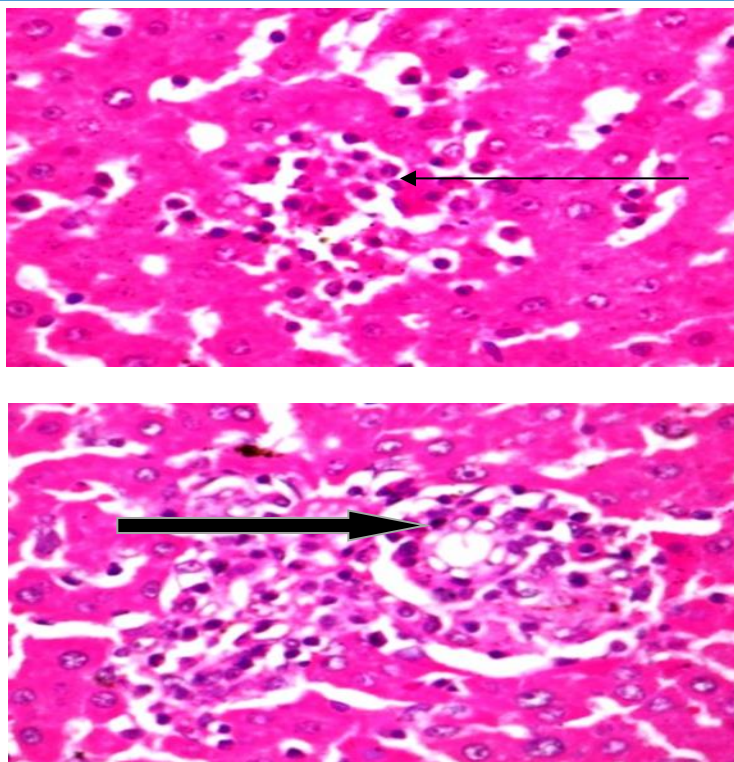


Figure 3. Histological demonstration of the liver using H&E staining technique [X400] showing mild focal congestion (blue arrow), mild disseminated infiltration of zone 2 by inflammatory cells (slender arrows) and mild disseminated periportal infiltration by inflammatory cells (black arrows).

Treated Group 3

Animals received only 2% sucrose in 25% ethanol solution as their drinking water and alabukun of 31mg/kg body weight daily for fourteen days.

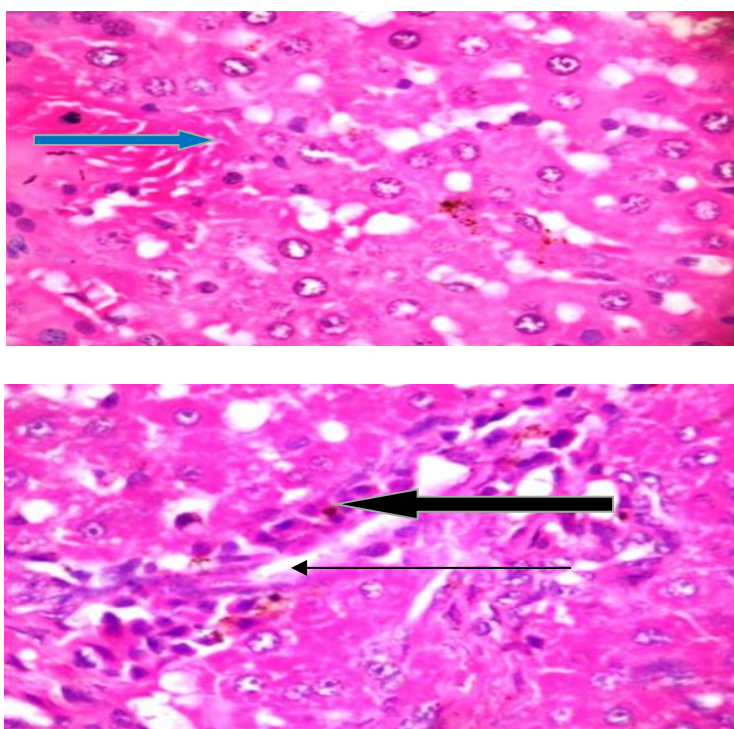


Figure 4. Histological demonstration of the liver using H&E staining technique [X400] showing marked congestion occluding the veins (blue arrows), mild disseminated infiltration of zone 2 by inflammatory cells (slender arrow) and mild disseminated periportal infiltration by inflammatory cells (black arrows).

Treated Group 4

Animals received only 2% sucrose in 25% ethanol solution as their drinking water and alabukun of 31mg/kg body weight daily for seven days and are withdrawn for seven days.

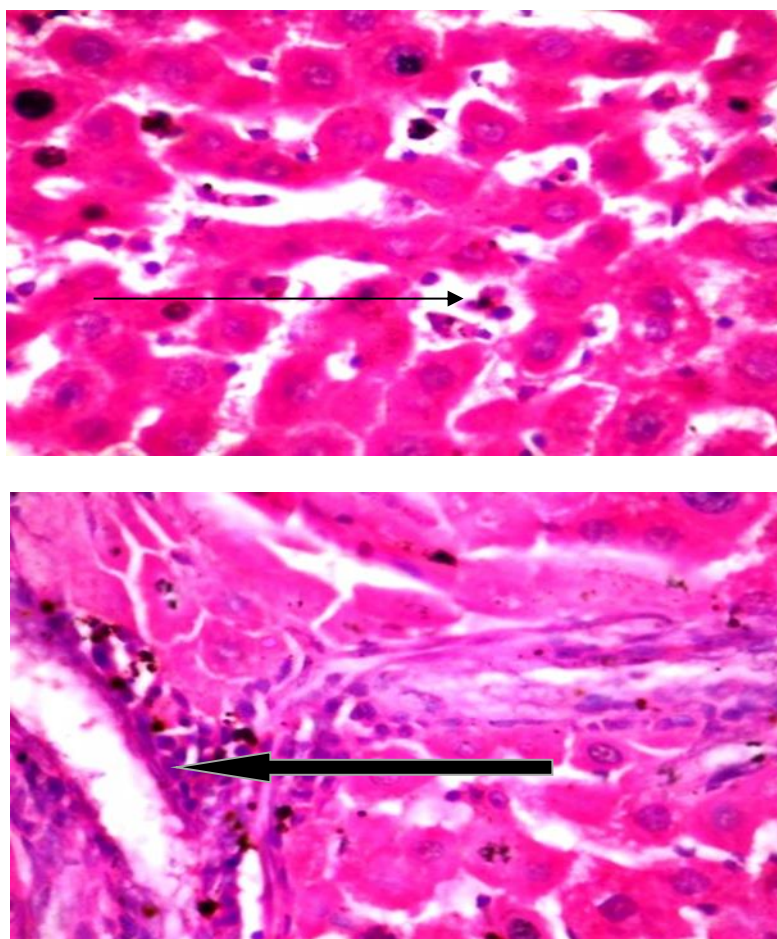


Figure 5. Histological demonstration of the liver using H&E staining technique [X400] showing mild disseminated infiltration of zone 2 by inflammatory cells (slender arrows) and mild disseminated periportal infiltration by inflammatory cells (black arrows).

Results

Results from the hematological parameters of adult wistar rat as shown in the above table. Treated animals (T1, T2, T3 &T4) had significantly ($P<0.05$) higher white blood count (RBC), hemoglobin (HGB) and Hematocrit (HCT) were significantly ($P<0.05$) reduced in the treated groups (T1, T2, T3 &T4) compared to the control group. The mean cell volume (MCV), Mean Corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were not significantly higher in treated animals (T1, T2, T3 &T4) compared to the control group. The PLT, LYM, PMV, MON and PCT had no significant value changes in the treated animals (T1, T2, T3 &T4) when compared with the control group. Results shown from the histology showed that administration of alabukun and ethanol either administered separately or simultaneously induces liver damage by causing inflammation in the liver and even there was no ameliorative effect after the drugs were withdrawn.

Discussion

The present study shows that treatment with alabukun and alcohol administered separately and concurrently and even upon withdrawal significantly reduces the red blood count,

hemoglobin and hematocrit when compared with the control group. These hematological imbalances seen in the treatment group (T1, T2, T3 & T4) is a confirmation that these drugs used either separately or concurrently/simultaneously can cause anemia if abused. Since it has been frequently reported that anemia, agranulocytosis, thrombocytopenia and leucopenia are some of the adverse effects of acetylsalicylic acid (one of the two major component of alabukun) (Raybak, 1992). Aspirin, (the second major component of alabukun) was also reported by Raybak 1992, to cause hemolytic anemia in humans especially in cases with certain hemoglobinopathies. Acetylation of bone marrow macromolecules by aspirin has been suggested as a possible mechanism causing blood disorders (Meischer, 1986).

The WBC plasma values were higher in the treated group. This suggests that treated animals are anemic, leukemic and have tissue damage. Other parameters such as MCV, MCH, MCHC, PLT, LYM, MPV, MON & PCT show no significant changes in the treated groups (T1, T2, T3 & T4) when compared with the control group. Part of this result, the treatment group1 synchronized with the work of Johnson and Manuwa (2014) that alabukun causes hematological disorders.

The histological evaluation showed there is adverse effect of Alabukun and ethanol on the liver. It was observed in the control group that the normal hepatic cytoarchitecture was evident showing normal central vein, with normal sinusoid around the hepatocytes.

Alabukun treated group (T1) showed moderate disseminated microvesicular steatosis, mild disseminated periportal infiltration by inflammatory cells and mild disseminated congestion (Figure 2). Ethanol treated group (T2) showed mild focal congestion, mild disseminated infiltration of zone 2 by inflammatory cells and mild disseminated periportal infiltration by inflammatory cells (Figure 3). Alabukun and Ethanol treated group (T3) showed marked congestion occluding the veins, mild disseminated infiltration of zone 2 by inflammatory cells and mild disseminated periportal infiltration by inflammatory cells (Figure 4). Alabukun and Ethanol withdrawal treated group (T4) showed the liver with mild disseminated periportal infiltration by inflammatory cells.

We therefore conclude from T1, T2, T3 & T4 that administration of alabukun at the dose of 31mg/kg. Body weight has inflammatory effect on the liver, this is in support of Johnson and Manuwa (2014) and that Alabukun induced damage to the liver in rats.

Ethanol induced in rat also causes liver inflammation as shown from this work and this is supported by Mohammed A. Alsaif (2007). Also simultaneous administration of the two drugs causes inflammatory too and the withdrawal of the drugs does not ameliorate the damage done to the liver.

Conclusion

It can therefore be concluded from the present study that alabukun and ethanol administered separately or simultaneously will affect hematological parameters and induced liver damage. This is relevant to the alabukun and alcohol consumers that their health is at risk. I therefore recommend that further work be done on the effect of alabukun and alcohol on other major organs of the body.

Authors' contributions

This work was carried out in collaboration among all authors. Author OOA designed the study and wrote the protocol. Authors OOA, JAO, MAA and AAO managed the animals,

collected all data, performed the statistical analysis and wrote the first draft of the manuscript. Authors OOA, OOO, OOA and JIA did the literature search and also wrote part of the manuscript. All authors read and approved the final manuscript.

Conflicts of interest: There is no conflict of interest of any kind.

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