Effects of different doses of Arak on liver and kidney microstructures in Swiss albino mice: Histopathological study

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Abstract

Background: Ethiopia is the most culturally diversified country in which a variety of traditional alcoholic beverages are consumed in different cultures and among others Arak is very popular. The effect of Arak on internal organs structures especially liver and kidney is not well studied even through it is consumed by the people in different parts of the country.

Objectives: Therefore, the aim of this study is to assess the effects of different doses of Arak on liver and kidney microstructures in Swiss albino mice.

Methods: To study the effect of effect of Arak on histology of liver and Kidney, twenty eight (fourteen male and female) newly breaded Swiss albino mice were randomly divided into four groups of two male and female in each. Arak was provided for the experimental groups 20%, 40% and 45% at 1ml/BW of mice daily for six weeks and 1ml/BW of distilled water for control once daily for six weeks. At the end of 42 days each animal was anaesthetized with diethyl ether and tissue sample (Kidney and liver) was collected after the mice sacrificed by cervical dislocation and abdominal cavity was opened anteriorly through midline incision of the abdomen to gain access to internal organs notably Liver and Kidneys. Wet organ was accessioned and immersion fixed in 10% neutral buffered formalin for histopathological investigation. The qualitative data including histopathological alterations was investigated through preparing microscopic slides which were examined by under light microscope Anatomist and Pathologist (both single blinded to dose and groups).

Results

In this study, the liver tissue of the control group has a normal histological structure, normal central vein and sinusoidal capillary with no evidence of narrowing, no change in hepatocytes cytoplasm and nucleus. Inflammations and necrosis in the liver tissue of the group of mice administered with 1ml/BW of 20% Arak, 1ml/BW of 40% of Arak and 1ml/BW of 45% of Arak mice was seen under light microscope which degree varies based on concentration of ethanol in the Arak. The kidney tissue of the control group has a normal histological appearance of glomeruli and renal tubules and basement membrane and there was inflammation, swelling, fat accumulation, obscure bowman's space, foamy appearing and necrosis of renal parenchyma in mice administered with 1ml/BW of 20% Arak, 1ml/BW of 40% of Arak and 1ml/BW of 45% of Arak during the study period.

Conclusions

Liver and kidney inflammation, fat drop accumulation and necrosis accompanied by alteration of its functions. The damaging effect was exacerbated as the dose of Arak ethanol concentration was increased. As the world moves towards alcohol control policy, the focus as interventions has to be also given for traditional alcoholic beverages.

1. Introduction

Ethiopia is culturally diversified country in which a variety of traditional alcoholic beverages are consumed in different cultures. These homemade alcoholic beverages are traditionally prepared for local consumption by the communities.(1) Among others, 'Tella', 'Qeribo', 'Geso', 'Borde', 'Tej', and 'Arake' are very common drinks.(2) Throughout the country, 'Arake' is popular traditional drink in most cultures and consumed by most community members. In different regions and cultures, it is known with different vernacular names such as 'Araki' in Amhara, 'Qulammé' in Oromo Illubabor zone 'Qub-lammee' South West Shawa and 'Dibicho'in Sidama. Arake is a colorless conventionally produced alcohol form mass of more concentrated fermentation and commonly used alcoholic drinks by the lower social classes or lower-income particularly in the rural and semi-urban setting of the country.(3) Arake is produced at home from germinated Wheat or Barely, Bread from cereals, water and others through conventional distillation process by using a lot of firewood. Arake is a liquid "prepared from grounded Gesho leave and germinated barley or wheat powder mixed with water 2–3 days before bread (Kita) preparation; locally the mixture is called Xinsisa. Then Kita prepared from Maize or other available cereals added and fermented for 5–7 days locally called as Dififa to be distilled into Arake. The distillation process is carried out by using firewood (traditional means) and on average each distillation times take around 40 minutes particularly in South West Shawa, Oromia regional state of Ethiopia." Key informant on June 20/2020 at 10:00 AM phone interview.

The alcoholic content of Arake is believed to be ranges between 30 and 40% (v/v of ethanol). The alcoholic contents of Ethiopian traditional beverages "Arak" are different and this variation is based on raw materials used for spontaneous fermentation and method of producing; while the PH from 3.5–7.5 but mostly around 4(3) Fermented beverages hold a long history in mankind and used as nutrition in many society and cultures across the world(4). Most traditional alcoholic beverages are believed as a stimulant and facilitate interpersonal relationship as a result it is commonly consumed during holidays, recreation, church festivals, and funerals, invitations of guests, friends, and relatives(5). Arak is a commercially available traditionally prepared alcoholic beverage which can pose health threats due to its high alcoholic strength and undesirable additives by resellers(6). Traditionally Arak classified into two: Tera Arak and Dagim Arak(2). Alcoholic beverages are a part of the human dietary culture and have an inseparable relationship in mankind's history and it is a way of enhancing the nutritional significance and social relationship of human beings(7). In 2016, an estimated 2.3 billion global populations consume alcohol, resulting in 3 million death and 132.6 million DALYs (Daily Activity Living Years)(8) Men consume four-fold of women 18.3L of pure alcohol when compared with Women consume 4.7 L(9). Globally in 2015, unrecorded APC (Alcohol Consumption Per Capital) accounts one quarter 25.5% of total APC 1.6 L pure alcohol(10) Different countries are moving towards controlling the consumption of alcohol globally, particularly, Ethiopia is one of the sub- Saharan African countries that have planned to reduce alcohol consumption by adding excise tax on industrially prepared beverages. The government of Ethiopia initiated an alcohol regulation program in line with African leaders' commitment to achieve prevention
and control harmful alcohol use by 2030(11). Trends in alcohol consumption are moving in an unfavorable direction particularly among women, minorities, and rural residents(12). Liver and kidney are important organs of metabolism, detoxification, storage and excretion of xenobiotics and their metabolites, and are especially vulnerable to damage(13). The Liver is the most vulnerable organ to different insults such as metabolic, toxic, microbial, circulatory, and neoplastic(14). The kidney is one of the vital organs which regulate water intake and outtake, balance minerals and electrolytes, and produce hormones(15). Routine integration of traditional alcohols in alcohol control policy as part of strategies has the potential outcome to make a significant contribution in the fight against alcohol-related health problems. The possibility of continuous consumption of traditional beverages is undetected due to unrecorded production or APC which shows an insignificant understanding the effect traditional alcohols. Therefore, this study aims to determine the effects of different doses of Arak on liver and kidney microstructures in Swiss albino mice.

2. Methods And Materials

Experimental animal preparations and handling

Newly bred adult Swiss albino mice were used in this study. They were obtained from and reared at Jimma University College of Agriculture and Veterinary Medicine (JUCAVM) animal experimentation and biotechnology laboratory. Twenty-eight mice (14 male and 14 female) aged 8–10 weeks and weight of 30–42 gram was used for the study purpose. The male and female mice were housed separately in stainless-steel, aluminum cages bedded with a clean husk. The cage was cleaned with hot water and detergent twice per week to decontaminate from any microbes and husk was changed daily according to standard principles for experimental animal welfare(16). The room temperature was maintained at 20-26°C and a 12 hour light/12 hour dark cycle. The animals were provided with a standard free rat diet (Rodent no soya) ad libitum brought from Alema Koudijs Feed PLC found in Bishoftu, Ethiopia, and clean tap water (SS sipper 250ml plastic water bottle) except when starvation is needed or at end of experiment. Food was changed every 12hr for each group by discarding the residual rodent chow left on the feeding cabinet. Before experimentation, the mice were acclimatized to laboratory conditions for 10 days in order to minimize any non-specific stress as suggested by different scholars in various similar studies(17–20).

Arak Collection and concentration determination

Arak was directly collected from three randomly selected producers to avoid manipulation by resellers found in Bacho woreda Tulu Bolo town of South West Shoa zone located 80km far from Addis Ababa in June 2020. Then its alcoholic contents using an alcoholmeter (Glass electrode with analytical sensor, USA) and P+ were determined by using pH meter (Orion Model SA 72, USA) at Chemical Engineering laboratory of Jimma Institute of Technology (JIT). A calibration routine with pH 4, 7 and 10 buffers was performed prior to using pH meter then before and after using each buffer pH measurement was flushed via abundant distilled water and all tests was conducted at room temperature (2-8°C). The concentration Ethanol of the Arak determined at JIT was 20%, 40% and 45% (v/v) and P+ was found to be 3.8-4.0. The type alcohol was determined as Ethanol using UV-spectrometer at Post graduate Organic Chemistry laboratory of Jimma University.

Experimental Protocol

**Group I**
1ml/Kg distilled water

**Group II**
1ml/Kg/BW of 20% Arak,

**Group III**
1ml/Kg/BW of 40% Arak,

**Group IV**
1ml/Kg/BW of 45% Arak daily for six weeks. The dose or volume administered for the experimental animal 1ml/Kg/BW, 1ml/Kg/BW and 1ml/Kg/BW of 20%, 40% and 45% for I, II and III groups respectively according to Hassan M et al, 2016 daily during the study period try laboratory of Jimma University and it has ethanol(21).

Organ sample collections

After six weeks of Arak administration, overnight starvation was carried out and each mouse was anesthetized with diethyl ether inhalational anesthesia in a glass container. Then liver and kidney of the mice was removed by the midline abdominal incision and finally the mice sacrificed by cervical dislocation before incision to minimum pain or distress.

Processing of Organ samples for Histopathological study
Sample accessioning at the end of 42 days, the hepatic tissue portion was taken via the neck to pubic incision using a sterile surgical blade then preserved in transparent glass container filled with a 10% NBF (neutral buffered formalin) solution and labeled with a specific identification (coded) for gross examination by pathologist and histopathological processing. The after fixation the tissue was washed with water to remove excess fixatives and dehydrated with ascending alcohol of 70%, 90% for 2 hours in each and absolute alcohol I, II, III for one and half hours (I, II) and overnight (III). The dehydrated tissue was cleaned with xylene in two stages for 1 and a half hours and 2 and a half hours. Then tissue was infiltrated with three changes of paraffin wax for one and a half hours, two and a half hours, and overnight.

Finally, the tissues were inserted into paraffin wax in square plastic plates (Cassette) forming tissue blocks, whereby each tissue block will be labeled and preserved at room temperature until sectioned. The tissue blocks was cut into ribbon Microtome (Leica Model: TP 1020, Germany at the pathology lab of Jimma University) at a thickness of 5μm and the section was collected at every 5th then put on to the surface of a warm water bath (Mounting bath Model: MH8523 China) of the temperature of 37–40°C. The floating ribbons over the surface of warm water were placed on to pre-cleaned slides and stretched out with egg albumin. The slides containing paraffin wax (Wax dispenser Electro thermal Model: MH8523, China) was arranged with in the slide holder and placed in an oven with a temperature of 40°C for about 10 minutes to fix the tissue to the slides and allowed to cool at room temperature for 30 minutes and stained with Hematoxylin for 5 minutes and then eosin for 15 second. Two sets of coupling jars were prepared for regular H and E staining; 1) for paraffin removal and hydration and 2) for dehydration and clearing. In order to extract the paraffin the tissue, placed in xylene I for 5 minutes and xylene II for 2 minutes and hydrated with descending concentrations of 95% alcohol for 2 minutes in each, 70% of alcohol for 3 minutes and 50% alcohol for 5 minutes.

The tissue sections were washed with tap water for 5 minutes and stained with Hematoxylin for 6 minutes. The slides were submerged in acidic alcohol for a second for distinction and control over stained hematoxylin then situated in the bluing solution until they became blue. Upon the slides were counterstained with eosin for 15 seconds and then washed in tap water for two minutes. The sections were dehydrated with an increasing alcohol concentration of 50%, 70%, and 95% for two minutes.

For three minutes in each, the dehydrated parts were cleared with xylene I and xylene II, permanently placed on microscopic slides using DPX and cover slips, and then observed under the light microscope to investigate any histological change (histologist and pathologist), thus contrasting the histology of the treated groups with that of the control group. Upon examination the photomicrographs of selected slides of liver and kidney from both the treated and control mice were taken under a Light microscope (Olympus, CX21FS1, Philippines) with magnification power(40X) and the slides Photographs were taken objective using an automated built-in digital photo camera (Camera KRUSS optronic Germany 3.0 MP USB 2.0 Histology lab of Jimma University).

Data Quality Management

Trained Laboratory technician and principal investigator were handled the animal during the study period and provide the Arak according to the protocol. The histotechniques principles (during tissue collection, preservation, processing, and slide preparation) was strictly followed. Experienced histologist and pathologist were single blinded to the groups and the dose to eliminate observer bias before examining the prepared microscopic slides under bright field microscope with different magnification power. All prepared slides were re-examined to assure the consistency of the finding.

Histopathological examination

Microscopic evaluation were qualitative analysis carried out by senior pathologist and Anatomist through preparing microscopic slides for each group and presented in form of photomicrography

3. Results And Discussions

Liver

In this study, the liver tissue of the control group has a normal histological structure, normal central vein and sinusoidal capillary with no evidence of narrowing, no change in hepatocytes cytoplasm and nucleus as shown in Fig. 1(A). There were inflammations and necrosis in the liver tissue of the group II or Fig. 1 (B), III or Fig. 1(C) and Ivor Fig. 1 (D) mice was seen under light microscope which degree varies based on concentration of ethanol in the Arak. This study is comparable with study reported that ethanol affects protein turnover and induces oxidative stress(22, 23). In addition to inflammation and necrosis there were shrinking of hepatocytes and fibrotic tissue observed in group-III Fig. 1(C) and IV Fig. 1 (D) mice. This finding also comparable with study due to small dose for long duration leads to persistence of blood ethanol at high levels and severe lesion development(24). This study revealed that inflammation and necrosis of hepatocytes in mice administered with 1ml/Kg of 20% Fig. 1(B), 1ml/Kg of 40% Fig. 1(C) and 1ml/kg of 45% Fig. 1(D) Arak during the study period as investigated by senior pathologist. The degree of inflammation and necrosis is exacerbated by as the dose of Arake was increased. The finding of this study is consistent with study done in China in 2017 by Tong Zhou et al on alcohol induced liver injury and study done in 2015 in Iraq on Ethanol induced hepatic and renal histopathologic change showed that there were peripheral inflammation and necrosis when prepared slides investigated under bright field microscope(25, 26).

Kidneys

In this study, the kidney tissue of the control group Fig. 2(A) has a normal histological appearance of glomeruli and renal tubules and basement membrane as well. But, there were histopathological changes in the kidney tissues of the experimental groups. There were inflammation, swelling Fig. 2
(C), fat accumulation, obscure bowman's space Fig. 2 (B), foamy appearing and necrosis of renal parenchyma as shown in Fig. 2(D) in mice administered with Arak during the study period. This might be due ethanol in the Arak which leads to sodium and potassium excretion or electrolyte imbalance and causes swelling(27–29). The degree of inflammation and necrosis is exacerbated by as the dose of Arake was increased as pathologist investigates the prepared microscopic slides under different magnification power using Microscope. The degree of inflammation and necrosis is exacerbated by as the dose of Arake was increased. This finding is similar with study done in USA on rats in 2012 by Narin F et al. and Ifudu et al in 2014 in Nigeria alcohol induces alteration in hepatocytes by inducing inflammation and shrinking bowman's space (30, 31). The reason may be due to alcohol induced oxidative stress (ROS) polyunsaturated fatty acid in composition of renal lipids has a degenerative effect on the renal tissue by inducing inflammation and necrosis and acid-base balance abnormality(27, 32, 33).

4. Conclusions

The damaging effect of the Arak consumption was approved through histopathological evaluations. Arak consumption also damages the liver and kidney tissue by inducing inflammation and tissue necrosis which was precipitated by the increased ethanol concentration in the Arak. This study revealed that Arak prepared at home by conventional means and consumed by the community has adverse events on liver and kidney microscopic structures in turn will leads to functional alterations in Swiss albino mice. So, alcohol control policy have to consider a traditional alcoholic beverages specifically Arak.

Abbreviations

APC  Alcohol Consumption per Capital BW: Body Weight
DPX  Dibutylphthalate Polystrene Xylene
IRB  Institutional Review Board
JIT  Jimma Institute of Technology
JUCAVM  Jimma University College of Agriculture and Veterinary Medicine
NBF  Neutral Buffered Formalin
PLC  Private limited Company
UDAW  Universal Declaration of Animal Welfare
USA  United State of America
UV  Ultraviolet

Declarations

Ethical Approval

The study was approved by Institutional Review Board of Jimma University or Institute of Health Research Post graduate Directorate (Ref.No. IHRPGD/835/2020). In any session of this research, Covid-19 prevention was considered by Hand washing, using personal protective equipment (Face mask) and physical distancing.

Detail experimentation procedures involving mice were managed following Ethiopian Public Health Institute (EPHI) animal handling and treatment guidelines. All procedures and principles were strictly reviewed by Doctor of Veterinary Medicine to ensure procedures are appropriate and humane. Adverse effect of Arake was prevented by providing after feeding and dose were adjusted by weighting each mouse before giving. Unnecessary duplication of the experiment were avoided by following the procedure or protocol well. The mouse were handled in a way that improving animal welfare to minimize distress and pain which includes: hunger, thirst, abnormal cold or heat, injury in any time of the study, and anesthesia was provided and checked for pain sensation by pinching tail or over skin before dissection or euthanasia. The principal investigator and animal lab investigator was wear personal protective equipment before performing any procedure on the Swiss albino mice. After completion of the experiment and procedure, any remnant body parts of the mice were discarded/buried in sealed plastic containers according to the Universal Declaration of Animal Welfare (UDAW) to prevent environmental contamination(15,16).

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**Disclosure**

The authors report no conflict of interest in this work.

1. **Consent for publication**: Not applicable.
2. **Availability of data and materials**: Supplementary data mostly photography during each procedure starting from collection, concentration of Arak determination and histopathologic procedures you can request the author rebumasorsa998@gmail.com.
3. **Competing interests**: There is no competing interest for this work.
4. **Author's contribution**: R.S. Wrote the Manuscript T.A. and N.H. Develop the methods and protocol D.A. and Z.B. Participated in supervision of laboratory session. All the authors reviewed the manuscript.
5. **Animal accordance statement**: The study was approved by institutional review board of Jimma University with letter Institute of Health Research Post graduate Directorate (Ref.No. IHRPGD/835/2020) and each experimental procedures involving the mice were managed following Ethiopian Public Health Institute (EPHI) animal handling and treatment guidelines.

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**References**


Figures

Figure 1

The photomicrographs of Liver section taken from mice and stained with H & E and all photomicrographs taken at magnification 40X. A) control group treated with 1ml/Kg/BW distilled water B) 1ml/Kg/BW of 20% of Arak C) 1ml/Kg/BW of 40% Arak (D) and 1ml/Kg/BW of 45% Arak during the experimentation period. The arrow indicates a condition of small fat droplets and Circle shows hepatocyte necrosis in alcohol treated groups. PV: portal vein

Figure 2

The photomicrographs of Kidneys section taken from mice and stained with H & E and all photomicrographs taken at magnification 40X. A) control group treated with 1ml/Kg/BW distilled water B) 1ml/Kg/BW of 20% Arak C) 1ml/Kg/BW of 40% Arak (D) and 1ml/Kg/BW of 45 % Arak during the experimentation period. The arrow indicates a condition of small fat droplets and Red circle shows Bowman's capsule necrosis in alcohol treated groups and Yellow circle shows normal Bowman's capsule.