International Journal of Chemistry Studies
www.chemistryjournal.in
ISSN: 2581-348X
Received: 05-04-2024, Accepted: 27-04-2024, Published: 22-05-2024
Volume 8, Issue 1, 2024, Page No. 23-25

Analysis of SGOT and SGPT levels in Wistar Rats given intake balinese Arak

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Abstract
Arak is one of Bali's typical alcoholic beverages classified as type C. Continuous alcohol consumption can lead to liver damage, as indicated by increased cellular enzyme activity such as SGPT and SGOT, making SGPT and SGOT levels biochemical markers for alcohol-related liver damage. This study aims to analyze ethanol in arak, Wistar rat serum, as well as SGPT and SGOT levels after arak intake. Ethanol levels in arak and serum were determined using validated Flame Ionization Detector Gas Chromatography (GC-FID), while SGPT and SGOT levels were determined using a photometer. The ethanol content in arak was 40.01 ± 0.01%, and the ethanol levels in Wistar rat serum after arak intake at 40% concentration with treatments of 0.5; 1.0; and 2,0 mL were 57.4667±0.70; 79.8083±5.89; and 133.4767±5.58 ppm, respectively. SGPT levels in serum in the arak intake treatment groups increased within the normal range. SGOT levels in serum in the arak intake treatment groups increased, surpassing the normal values.

Keywords: Arak, ethanol, GC-FID, SGOT, SGPT, validation

Introduction

Arak as a traditional Balinese beverage has recently become headline news in Bali. The Governor of Bali issued Governor Regulation No. 1 of 2020 concerning the Governance of Bali's Fermentation and/or Distillation Beverages, because arak is considered a part of the cultural diversity that deserves protection. The arak commonly sold by arak producers comes in several types characterized by producing foam when shaken in a bottle and easily igniting with a bluish hue, often used for medicinal purposes, and not uncommonly consumed by Bali arak enthusiasts with alcohol content up to 30%, while arak with lower alcohol content only reaches 20%. This type of arak is often consumed by Bali arak enthusiasts and serves as a spirit ingredient for cocktails. Meanwhile, the lowest alcohol content, ranging from 5-10% alcohol, is used as equipment or means for religious ceremonies. The National Anti-Alcohol Movement from 2011 to 2016 recorded a death toll of 18,000 people due to counterfeit alcoholic beverages. Cases handled by the Forensic Laboratory Division of Denpasar Branch every month include methanol poisoning cases, averaging 12-14 cases annually covering Bali Regional Police, West Nusa Tenggara, and East Nusa Tenggara. A good analytical result requires method validation including limits of detection (LOD), limits of quantification (LOQ), standard deviation (SD), coefficient factor (KF), and % recovery. Gas chromatography with flame ionization detection (GC-FID) is an appropriate method for determining ethanol levels in arak due to its volatile nature. One of the diseases that can be caused by alcohol consumption is liver dysfunction, such as fatty liver, alcoholic hepatitis, and cirrhosis. Liver function tests that can be examined to assess liver damage include serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), and Gamma-GT. Liver damage levels are usually indicated by an increase in the serum glutamic pyruvic transaminase (SGPT) ratio of more than twice the normal level [1]. Gandasoebrata in 2011 mentioned that the negative impacts of alcohol abuse include an increase in total bilirubin, direct bilirubin, indirect bilirubin, Gamma-GT, serum glutamic pyruvic transaminase (SGPT), and serum glutamic oxaloacetic transaminase (SGOT) [2]. Determining the level of ethanol consumption in an individual is generally done by examining ethanol in the blood. Liver damage can be indicated by increasing cellular enzyme activity. SGPT and SGOT are two types of enzymes often associated with liver cell damage. The level of liver damage is usually indicated by an SGPT/SGOT ratio exceeding twice the normal/reference value. Therefore, SGPT and SGOT values are biochemical markers of liver damage due to alcohol.

Materials and Method

Ingredient

Ethanol and butanol pro-analysis; SGPT (Dyasis) and SGOT (Dyasis) reagents; arak produced by arak producers in Besan Village, Dawan District, Klungkung, Bali, aquadest; Wistar rats

Equipment

red-capped blood tubes, volumetric flask, micropipette, tips, beakers, glass capillary tubes, red-capped blood tubes, a cool box, ice gel, a set of photometer tools, centrifuge, and a GC-agilent Technologies 6890-N Network GC System, with an HP InnoWax column length of 30 m, diameter of 0.32 µm, and flow rate of 0.70 mL/minute with a polyethylene glycol stationary phase, a Flame Ionization Detector (FID), helium carrier gas (He), and nitrogen make-up gas (additional gas).

Procedure

The arak samples are obtained from producers in Besan Village, Dawan District, Klungkung, Bali. The amount of arak taken comes from three arak producers with a sampling volume of 250 mL taken three times, with sampling conducted in the first week, second week, and third week.
The ethanol and butanol solutions have concentrations of

0,2, 5,0, 25,0, 125,0, 250,0, 500, 0 and 1000 ppm. The mixed solutions are prepared by mixing ethanol and butanol solutions in a 1:1 ratio.

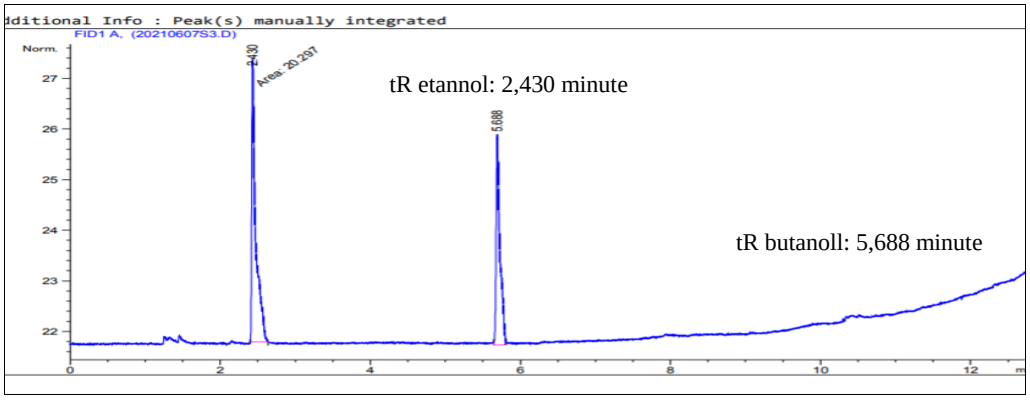
The analysis of ethanol content in arak involves adding an internal standard, butanol, to the arak sample and conducting the analysis using Gas Chromatography with Flame Ionization Detection (GC-FID). The results of the arak analysis are shown on a chromatogram by observing peaks at retention times, followed by determining the ethanol content in the arak using the calibration curve equation for ethanol.

Acclimating the test animals with standard rat feed and ad libitum access to drinking water for 7 days before the study is a common practice to ensure consistent conditions and reduce stress on the animals. To create 1 negative control group and 3 treatment groups, each group consisting of 6 rats. P0 is the negative control group, receiving pellet feed and ad libitum access to water. PI, PII, and PIII are the treatment groups, each receiving different oral doses of arak (0.5 mL, 1.0 mL, and 2.0 mL, respectively). The study involves administering Klungkung arak orally to Wistar rats via a sonde for 30 days. On day 31, blood samples are collected through the retroorbital plexus using a capillary tube to analyze the effect of Klungkung arak on the serum levels of SGPT and SGOT in Wistar rats. The analysis of SGPT and SGOT is conducted using the substrate start method in a kinetic test. The procedure for SGPT examination begins with pipetting 20 µL of serum into a sample container, followed by adding 1000 µL of reagent R1. It is then incubated for 1 minute at a temperature of 37°C. Afterward, 250 µL of reagent R2 is added and incubated for 3 minutes. Subsequently, it is read using a photometer at a wavelength of 340 nm. The procedure for examining SGOT is the same as for analyzing SGPT, except that it is read at a wavelength of 405 nm. For all procedures, three replicates are conducted.

Data obtained from the examination results are subjected to statistical analysis with validation results such as selectivity, linearity, detection limits (SD, KV, LOD, LOQ, and % Recovery), lowest and highest test results to correct errors in the analysis process. The enzyme levels of SGPT and SGOT data obtained are statistically analyzed using One Way Analysis of Variance (ANOVA) with a significance level (α) of 0.05, followed by the Duncan test to observe differences in the effects of treatments among experimental groups. The data is analyzed using SPSS version 21 software.

Result and Discussion

The chromatogram results from gas chromatography provide optimal separation between the peaks of ethanol and butanol. The peak of the standard ethanol solution appears at a retention time of 2.430 minutes, while the retention time for the standard butanol solution is at 5.688 minutes. Ethanol and butanol have closely spaced retention times but exhibit good peak separation. The resolution value (Rs) between two peaks, namely ethanol (tR=2.430 minutes) and butanol (tR=5.688 minutes), is 1.9.



The mixture of standard ethanol and butanol compounds gives a resolution value of 1.9, indicating that Rs ≥ 1.5. According to Skoog *et al*., 1992, a compound will be perfectly separated from other compounds if the Rs value is ≥ 1.5 [3]. The resolution value between the two standards indicates that gas chromatography

has separated the compounds with high selectivity under optimum conditions. The analysis results of the standard ethanol solution yielded the regression equation y=0.9209x with a correlation coefficient value of 0.999. Therefore, the GC-FID chromatogram provides a high linearity response between the peak area and the concentration of the standard solution. The average analysis result is 1.3220 ppm, standard deviation is 0.0009, coefficient of variation is 0.85%, LOD is 0.0312 ppm, and LOQ is 0.1040 ppm. The coefficient of variation for each standard has met the criterion of ≤ 2%, indicating that the measurements with gas chromatography have provided high precision and accuracy with high validity. The % recovery values obtained range from 95% to 105% with an average of 99.77%. From the results obtained, this value is still acceptable in terms of accuracy, with the generally accepted accuracy range being 80%-120%. The % recovery results fall within the acceptable range criteria. Therefore, it can be concluded that this method has good precision in indicating measured values close to the true values [4]. The ethanol content in arak is presented in Table 1.

Table 1: The ethanol content in arak

Sampele code	Ethanol Content (%)	$(x-\bar{x})2$	
A1	40,00	40,01	$1,0 \times 10^{-4}$
A2	40,02		$1,0 \times 10^{-4}$
A3	40,01		0
$\bar{x} \pm SD$	40.01 ± 0.01%		
%CV	0.025		

The arak sample contains 40% ethanol, which falls into category C of alcoholic beverages, with an ethanol percentage ranging from 20-55%. This category can cause

severe symptoms such as severe ataxia, double or blurred vision, fainting, and sometimes convulsions.

The ethanol levels in Wistar rat serum are shown in Table 2. Table 2 shows a significant increase in ethanol levels with the administration of 40% ethanol arak at doses of 0.5, 1.0, and 2.0 mL. The higher the volume of arak administered, the higher the detected ethanol levels in the serum. This occurs because 90% of the ethanol in arak is metabolized in the liver, while the remaining amount is excreted through urine and lungs. That's closely related to the increase in ALDH levels in the body. The role of ALDH in converting acetaldehyde to acetate is also evidenced in its function of ester hydrolysis, antioxidant properties, and detoxification bioactivation [5].

Table 2: The ethanol levels in Wistar rat serum

Treatment groups	Serum ethanol levels (ppm)
P0	0,00
PI	57,4667±0,70
PII	79,8083±5,89
PIII	133,4767±5,58

The statistical analysis shows a significant difference ($p < 0.05$) between the negative control group and the treatment group. The increase in SGPT occurs due to the release of intracellular enzymes into the blood caused by necrosis of liver cells or acute liver damage, leading to plasma membrane damage due to toxic compounds like ethanol. Enzymes originally located in the cytosol then enter the bloodstream due to differences in cell membrane permeability, resulting in an increase in SGPT enzyme levels in the blood plasma [6, 7]. The SGPT levels in Wistar rat serum are presented in Table 3.

Table 3: The SGPT levels in Wistar rat serum given arak intake

Treatment group	Reference value(U/L)	Concentration SGPT (U/L)
P0	45,7-80,8	32.75±1,76
PI		42,97±4,17
PII		66,83±2,73
PIII		80,45±1,30

The SGOT levels for all treatment groups exceed the reference values (17.5-30.2 U/L), both in the negative control group and in the experimental groups given arak at various doses. Elevated SGOT levels are a sign of liver damage. This enzyme is found in mitochondria and is also present in muscles, liver, heart, and kidneys. If those tissues experience acute damage due to arak consumption, their levels will increase [10]. The negative control and treatment group with arak intake obtained a significant value of $p < 0.05$, indicating a meaningful difference between the treatment groups in terms of the SGOT parameter. The administration of 40% ethanol arak with varying doses was able to increase SGOT levels.

Table 4: The SGOT levels in Wistar rat serum are presented

Treatment group	Reference value SGOT (U/L)	Concentration SGOT (U/L)
P0	17,5-30,2	48,17±3,46
PI		61,50±3,56
PII		73,30±3,49
PIII		84,82±3,06

Conclusion

The ethanol content in arak as validated by GC-FID is 40.01%, and the ethanol levels in the serum of Wistar rats in each treatment group PI, PII, and PIII are 57.4667 ± 0.70, 79.8083 ± 5.89, and 133.4767 ± 5.58 ppm, respectively. There is an increase in SGPT levels still within the normal range, while SGOT levels in all treatment groups exceed the normal values. Arak intake in Wistar rats affects the increase in SGPT and SGOT enzyme activities, serving as biochemical markers for liver damage in Wistar rats.

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