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Effect of Philippine Rice Wine (*Tapey*) in Sodium Nitrite Induced Methemoglobinemia in Sprague-Dawley Rats: Input for Management of Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency

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ABSTRACT

Glucose-6-phosphate dehydrogenase (G6PD) is essential for the activity of NADPH-flavin reductase, an enzyme responsible for reducing methemoglobin to its oxygen-carrying form thus patients deficient with this enzyme can lead to methemoglobin accumulation, impairing oxygen transport and causing tissue hypoxia. This study evaluated the effectiveness of Philippine rice wine (*Tapey*) in reducing methemoglobin levels in Sprague-Dawley rats induced with sodium nitrite (NaNo2). The proximate analysis of the rice wine has a 8.11% ethanol concentration and a 1.82% crude protein content, suggesting superior protein content and acceptable ethanol level. Afterwards, the experiments followed an in vivo pre-test and post-test design. Spectrophotometric results showed that rice wine causes a 4.86% decrease of methemoglobin concentration compared to positive control (ascorbic acid) with only 3.61%, exhibiting a more effective methemoglobin reducing capacity, implying a statistical and clinical significance. Histopathology demonstrate a hepatic congestion, diffuse hepatocellular vacuolation, and multifocal micro necrosis which was consistent with the toxicological effects of sodium nitrite to the liver, however, the absence of extensive necrosis indicates a partial hepatoprotection of rice wine via converting methemoglobin to functional hemoglobin for oxygen transport. Thus these findings suggested that Philippine rice wine possesses potent antioxidant properties and may serve as a viable alternative or complementary treatment for methemoglobinemia, in relation to the management of G6PD deficient patients, especially in resource-limited settings or among populations with contraindications to conventional therapies.

Keywords: *Methemoglobinemia, G6PD deficiency, Philippine rice wine, Tapey, Sodium Nitrite (NaNo$_2$), Sprague-Dawley Rats*

INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) deficiency affects an estimated 400 million people worldwide, with the greatest burden in the Middle East, Africa, and Southeast Asia. In the Philippines, especially Cagayan Valley, its impact is pronounced: 1 in 63 newborns screens positive (Newborn Screening Reference Center, 2023), and the region recorded the nation's highest incidence from 2010 to 2015 (Nicholas et al., 2021). G6PD catalyzes the first step of the pentose-phosphate pathway; in erythrocytes this pathway is the sole source of NADPH, which keeps glutathione in its reduced, antioxidant form (Roper et al., 2020). When G6PD is deficient, red cells cannot neutralize oxidants, ferrous iron (Fe^{2+}) is converted to ferric iron (Fe^{3+}), and methemoglobin accumulates, precipitating hemolytic anemia and tissue hypoxia. Because NADPH-flavin reductase relies on NADPH to reconvert methemoglobin (MetHb) to functional hemoglobin, any shortfall worsens oxidative injury.

The clinical risk grows with age as affected individuals encounter more oxidizing drugs, chemicals, and environmental toxins. Such exposures accelerate the conversion of Fe^{2+} to Fe^{3+}, making methemoglobinemia, a disorder defined by elevated MetHb and impaired oxygen transport, a frequent consequence of G6PD deficiency (Alanazi, 2017).

Beyond pharmaceuticals, methemoglobinemia can result from nitrate or nitrite poisoning, paradichlorobenzene-based household products, drugs of abuse, and certain food preservatives. The standard antidote, methylene blue, is imperfect: in high doses it can itself oxidize hemoglobin, trigger hemolysis in G6PD-deficient patients, stain the skin, cause gastrointestinal distress orally, and produce severe local reactions after intravenous infusion (David et al., 2018).

The ascorbic acid is often used when methylene blue is contraindicated because it chemically reduces MetHb. Yet therapeutic efficacy demands large doses that generate hydrogen peroxide and ascorbate radicals, shortening the survival of G6PD-deficient erythrocytes (Quinn et al., 2017) and increasing urinary oxalate, a risk factor for kidney stones (Malik, 2018). These limitations have prompted interest in safer, antioxidant-rich natural products.

One promising candidate is Philippine rice wine (*Tapey*), a traditionally fermented beverage enriched in polyphenols and flavonoids. Fermentation enhances these bioactive compounds, which can scavenge free radicals, up-regulate endogenous antioxidant enzymes, and potentially improve oxygen delivery under oxidative stress. Accordingly, *Tapey* could serve as an adjunct for drug-induced or toxicant-induced methemoglobinemia, including cases arising during substance-abuse rehabilitation. Thus, this research aims to evaluate and test the antioxidative property of Philippine Rice Wine (*Tapey*) in treating Methemoglobinemia in the laboratory and in experimental animals.

Objectives of the Study

The general objective of this study determines the effectiveness of Philippine rice wine (*Tapey*) as a potential reducing agent in managing methemoglobinemia induced in an in vivo animal model. Specifically, it sought to address the following objectives: (1) to determine the composition of locally available rice wines in terms of: (a) Ethanol concentration (b) Crude protein content (2) to determine methemoglobin concentrations before and afterthrough spectrophotometric absorbance readings. (3) to compare the reduction of methemoglobin concentrations in rats treated with rice wine to those treated with the positive control (ascorbic acid) and the negative control (saline). (4) To determine the potential toxicological effects of rice wine on the liver.

MATERIALS AND METHODS

Research Design

The study utilized an experimental pre-test and post-test design to test the activity of Philippine rice wine (*Tapey*) in sodium nitrite-induced methemoglobinemia in Sprague Dawley rats. It sought to study the effectiveness of the commercial rice wines by treating the test animal with the different Rice wines, comparing it with the commercially available treatment for methemoglobin levels.

Sampling Technique

Commercially available rice wines (Oryza sativa) were used in this study. Female Sprague-Dawley rats, aged uniformly and weighing 100–150 g, served as test subjects. A total of 24 rats were utilized: 15 for acute toxicity testing, 9 for the sodium nitrite-induced methemoglobinemia model, and 1 for histopathological examination. Rats were randomly selected and assigned to groups based on their respective tests.

Locale of the study

The study was conducted in the Cagayan Valley region. Philippine rice wines were sourced from Tabuk-Kalinga and Quezon City, with specific brands withheld for anonymity. Experimental rats were obtained from Northern Animalia, Tuguegarao City, and observed at the Philippine Institute of Traditional and Alternative Health Care (PITAHC), Tuguegarao City. Methemoglobin concentration analysis was performed at the Central Analytical Laboratory, Cagayan State University, Andrews Campus.

Research Instruments

The nine (9) female Sprague-Dawley rats were divided into three groups following the study conducted by Kang et al. (2018). To induce methemoglobinemia, all groups will be administered sodium nitrite at a dosage of 50 mg/kg. In the subsequent hours, the groups will receive the following treatments:

Setup A (Negative Control): Rats were administered normal saline solution.

Setup B (Treatment): Rats were treated with Philippine rice wine.

Setup C (Positive Control): Rats were treated with ascorbic acid.

The extract was administered at a concentration of 1.5 mL. All treatment concentrations were prepared consistently to ensure reliability and reproducibility throughout the experiment.

Data Gathering Procedure

A. Pre-Experiment Procedures

A brand of Philippine Rice Wine (*Tapey*) was used in the study, but its specific identity would be withheld to maintain anonymity. In addition, the reagents and materials used are sodium nitrite ($NaNO_2$), ammonium chloride (NH_4Cl), phosphate buffer, Ethylenediaminetetraacetic acid (EDTA) tubes, syringes, needles, and UV-Vis Spectrophotometer.

Animal Laboratory Testing Equipment

Rice wine was administered via oral gavage using a 16–18 gauge, 2–3 inch feeding tube, following IACUC Standard Procedure (2023). For blood collection from the rat's tail, essential equipment included an appropriately sized animal restrainer and access to an anesthetic machine with an induction chamber. Sterile 25–27 gauge hypodermic needles (5/8 inch or smaller) and 1–3 mL sterile syringes were used. The tail was disinfected with 70% isopropyl alcohol using 2" x 2" gauze or cotton-tipped applicators. A weigh scale was used to monitor the animals, and a sharps container was provided for needle disposal, in accordance with UBC Animal Care Committee guidelines (2021).

For toxicologic analysis, precise surgical instruments were necessary to ensure accurate tissue collection and minimal damage, as described by Fiette and Slaoui (n.d.). Blunt-ended forceps were preferred to minimize trauma, while serrated forceps were avoided. Dissection required various scissors—dissecting, surgical, and microsurgical (e.g., ophthalmologic)—along with scalpels fitted with new blades for clean incisions.

B. Experiment Proper

The researchers used the following steps and procedures to conduct their study.

Animal Preparation

Twenty-four (24) female Sprague-Dawley rats, aged uniformly and weighing 100–150 grams, were selected. They were housed in standard polypropylene cages under controlled conditions (20 ±1 °C, 45–55% humidity, 12-hour light/dark cycle) and given a standard rodent diet with ad libitum access to distilled water (National Research Council, 2011; Suckow et al., 2012). The rats acclimated to the laboratory environment for two weeks, during which routine health checks confirmed their good health (Baker et al., 2018). Baseline body weights were recorded for group consistency, and daily handling was performed to minimize stress and improve researcher familiarity, enhancing data reliability (Hurst & West, 2010).

Animal bite Management

Animal bite management should follow the guidelines set by UNC-Division of Comparative Medicine (DCM) Basic Rat Handling and Technique Guide (2022). If bitten by a rat during handling, remain calm and avoid reacting aggressively to the rat, as biting is a natural defensive response that can occur when the animal feels threatened or stressed. Carefully and securely return the rat to its cage to minimize additional stress or injury. Immediately wash the bite wound with antibacterial soap and warm water to reduce the risk of infection and carefully inspect the area for signs of deeper injury. Apply a sterile bandage or dressing to control any bleeding and protect the wound. It is essential to inform the supervisor about the incident as soon as possible to ensure that appropriate safety and training protocols are followed.

Contact any near animal bite treatment center for professional medical evaluation, wound documentation, and follow-up care. Rabies post-exposure treatment is generally not required for bites from rodents, rabbits, or domestic animals other than dogs and cats, unless the biting animal is confirmed to be rabid, however, anti-tetanus prophylaxis should still be provided and immediate action as per DOH Administrative Order No. 164, s. 2002.

Preparation of Reagents

The Sodium Nitrite solution was prepared by dissolving 500 mg of $NaNO_2$ in 10 mL of distilled water (50 mg/mL). While the ammonium chloride lysis buffer were prepared by adding 8.023 g of ammonium chloride (NH4Cl), 1.001 g of potassium bicarbonate (KHCO3), and 0.0372 g of Ethylenediaminetetraacetic (EDTA) disodium salt into 800 mL of distilled water. The pH was adjusted to 7.2-7.4 using Hydrochloric acid (HCl) or Sodium hydroxide (NaOH). In addition, distilled water was added to have a total volume of 1 L (NovoPro Bioscience Inc., 2025).

Handling of Sodium Nitrite

Sodium nitrite is a mildly corrosive and oxidizing chemical that requires careful handling to ensure safety and prevent damage. According to the Safety Data Sheet of Sodium Nitrite (2024), dust deposits should be regularly removed and the product must be kept away from flammable or combustible materials to reduce the risk of fire and limit the formation of aerosols or dust. For storage, sodium nitrite should be kept in a dry place at a temperature between 15°C and 25°C. Containers should also be securely closed and stored in well-ventilated areas, using both general and local ventilation systems.

When handling sodium nitrite, the use of appropriate personal protective equipment (PPE) is essential (OSHA Personal Protective Equipment Standard (29 CFR 1910.132). This includes nitrile or neoprene gloves, chemical safety goggles or a face shield, and a lab coat or protective apron. Good hygiene practices, such as washing hands thoroughly after handling and avoiding inhalation or ingestion, also help minimize health risks.

In case of exposure to the chemical, New Jersey Department of Health Hazardous Substance Fact Sheet (2016) requires the following procedure for safety. If sodium nitrite gets in the eyes, immediately rinse with lots of clean, running water for at least 15 minutes. Make sure to hold the eyelids open to flush all areas, lifting upper and lower lids. Remove contact lenses also if present. For skin contact, quickly remove any clothing that has the chemical on it. Wash the affected skin thoroughly with soap and water. If someone breathes in the chemical, move them to fresh air. Start rescue breathing if their breathing has stopped and begin CPR if their heart action has stopped. Transfer them to a medical facility as soon as possible.

Acute Toxicity Testing

Acute toxicity was assessed following OECD Guideline No. 420 using stepwise fixed doses of 1, 3, and 5 mL/kg of rice wine. Initial dosing was based on a preliminary sighting study to induce mild toxicity without severe effects or death. Subsequent doses were adjusted based on observed toxicity or mortality until a toxic dose or maximum one death was identified, no effects occurred at the highest dose, or fatalities appeared at the lowest dose. Fifteen Sprague-Dawley rats of uniform age and weight were acclimated for 14 days and divided into three groups (five rats each) corresponding to the tested doses. The test substance was administered once by oral gavage; if a single dose was not possible, it was fractionated over 4 hours. Animals were fasted prior to dosing (rats overnight food deprivation, mice 3–4 hours, with water allowed), weighed before administration, and food was withheld for 3–4 hours post-dosing unless fractionated dosing was used. Post-administration, animals were observed individually starting within 30 minutes, frequently during the first 4 hours, then periodically over 24 hours, and daily for 14 days. Observation periods were adjusted according to toxicity onset, severity, and duration. Humane endpoints were applied if needed, and detailed records of clinical signs and outcomes were maintained for each animal.

Sodium Nitrite (NaNO2) Induction on Sprague-Dawley Rats

Nine female Sprague-Dawley rats were divided into three groups following Kang et al. (2018). Rats were fasted for 8 hours with water ad libitum before the experiment. Sodium nitrite was administered subcutaneously according to Canadian Council on Animal Care (2012) guidelines. Each rat was restrained with a towel; loose skin was tented, and a syringe with the needle bevel up was inserted at the base of the tent. Negative pressure was confirmed by pulling back the plunger before injecting the full dose. The sodium nitrite dose used in this study was patterned after Beutler and Mikus (2002), with each 150 g rat receiving 7.5 mg (0.15 mL of 10 mg/mL solution) to achieve a 50 mg/kg dose.

Philippine Rice Wine Administration

Rice wine samples were procured from local markets in Tabuk-Kalinga and Quezon City and selected based on proximate analysis. Administration was performed via oral gavage following a protocol adapted from San Diego State University and approved by the Institutional Animal Care and Use Committee. Gavage volumes did not exceed 1% of body weight; in this study, volumes ranged from 1.0 to 1.5 mL for rats weighing 100–150 g. Rats were restrained using a single-handed technique that immobilized the head without obstructing the airway. The gavage needle was inserted along the roof of the mouth, over the base of the tongue, and gently advanced into the esophagus toward the stomach. If resistance or struggling occurred, the needle was withdrawn and repositioned. After administration, the needle was removed without aspiration, and animals were closely monitored for adverse effects.

Spectrophotometry

Methemoglobin concentration was determined using a spectrophotometer. The device was calibrated with a control solution at specified wavelengths before measuring samples. Fresh blood (70 µL) was collected from the rat's tail vein 30–60 minutes after sodium nitrite induction and placed in EDTA microtubes (Beutler & Mikus, 2002). Following a modified Vertregt (2000) protocol, blood was diluted in 4.7 mL phosphate-buffered saline (pH 6.9) and mixed with 235 µL ammonium chloride lysis buffer, then incubated 5–10 minutes at room temperature until hemolyzed. Absorbance was measured at 630 nm for methemoglobin and 540 nm for total hemoglobin using a 1 cm path length. Methemoglobin percentage was calculated as $(A_{630}/A_{540}) \times 100$ per Vertregt's method.

Toxicological Analysis of Liver Tissue

Rats were euthanized via intraperitoneal sodium pentobarbital following ethical guidelines. A midline abdominal incision exposed the liver, which was carefully excised, rinsed with physiological saline, and sectioned into 3–5 mm slices for fixation in 10% neutral-buffered formalin for 24–48 hours. After fixation, tissues were processed through dehydration, clearing, and paraffin embedding. Thin sections (4–6 µm) were cut with a microtome, mounted on slides, stained with hematoxylin and eosin (H&E), and examined microscopically to evaluate histological features (Bancroft & Gamble, 2008).

Post-research: Extermination of Rats

At the end of the experiment, the rats used in the study was humanely euthanized in accordance with established ethical guidelines and institutional protocols. Euthanasia was carried out using cervical dislocation, a method that rapidly separates the cervical vertebrae from the skull, resulting in immediate loss of consciousness and swift death due to disruption of the brainstem and spinal cord (Cheng, 2021). According to the AVMA Guidelines for the Euthanasia of Animals (2020), this technique is performed by placing the thumb and index finger on either side of the neck at the base of the skull, or by using a rod at that location. The base of the tail or the hind limbs is then pulled quickly with the other hand to achieve the dislocation. The procedure was performed by the Veterinarian who was experienced and skilled in this technique.

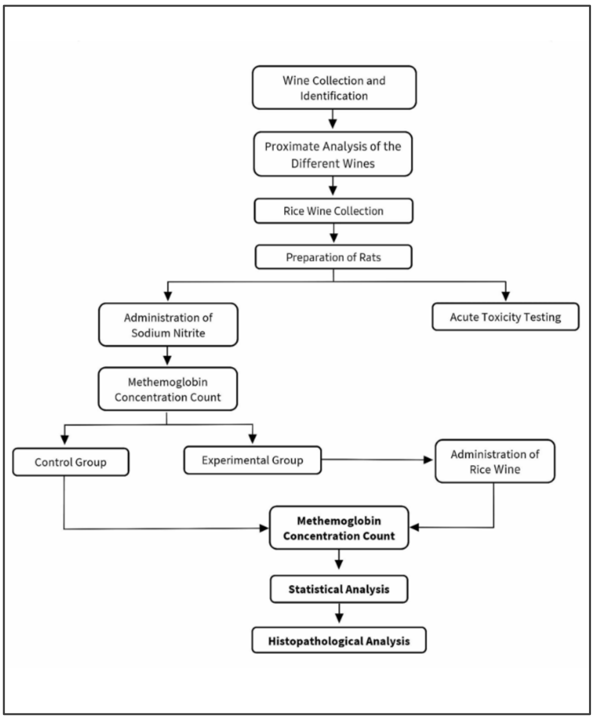


Figure 1. Schematic Diagram of the Process Followed in the Study

Analysis of the Data/ Statistical treatment

The data analysis employed Descriptive Analysis, T-Test, One-Way ANOVA, and Post Hoc Tukey Multiple Comparisons Test to assess the antioxidant activity of Philippine Rice Wines. Descriptive Analysis identified the antioxidative components in commercial rice wines capable of reducing methemoglobin. The T-Test determined the significance of the reduction in methemoglobin concentration before and after treatment, indicating the treatment's effectiveness. One-Way ANOVA compared the effects of different treatments, including rice wine, ascorbic acid, and saline, and when significant differences were found, the Post Hoc Tukey Test identified which specific treatments differed significantly.

RESULTS AND DISCUSSION

Proximate Analysis of Philippine Rice Wine

Table 1 presents the percent ethanol content of each rice wine sample, determined using either steam distillation or the pycnometer method, and their corresponding crude protein content, assessed through block digestion or steam distillation.

Table 1. Results of Ethanol Concentration and Crude Protein in Proximate Analysis

Rice Wine Sample	Percent Ethanol Concentration (g/100g)		Crude Protein (g/100g)	
	Proximate	Standard Range	Proximate	Standard Range
Rice Wine 1	10.37	14-19	Not detected	0.41
Rice Wine 2	8.11		1.82	
Rice Wine 3	12.56		0.30	

Among the three samples, Rice Wine 2 exhibited the lowest ethanol concentration at 8.11% but the highest crude protein content at 1.82%. Although all samples fell below the standard ethanol range of 14–19%, only Rice Wine 2 exceeded the standard crude protein threshold of 0.41%. This suggests a potentially higher concentration of antioxidant peptides, making Rice Wine 2 the most suitable candidate for antioxidant-related studies. In contrast, Rice Wine 3 showed a lower protein content (0.30%), and Rice Wine 1 contained neither sufficient ethanol nor detectable protein levels, rendering them less suitable for further investigation.

The selection was also supported by several studies and literature. According to Malingan (2016) as well as Dela Rosa and Medina (2022), a lower ethanol concentration of a rice wine made from Ballatinao black rice has a higher antioxidant activity. It is further validated by the Australian Wine Research Institute (2020) that an 11.6-13.6% ethanol concentration increases the hotness, metallic, bitterness, roughing, and drying sensation on the palate. Crude protein content can also be a useful indicator of the potential presence of antioxidant bioactive peptides that may help in reducing oxidative stress associated with methemoglobinemia (Rhee et. al, 2004). Moreover, the study of Du, et. al (2020) has shown that antioxidant peptides exhibit strong free radical scavenging abilities and enhance cellular antioxidant defenses, thereby reducing oxidative damage to hemoglobin. Therefore, based on its superior protein content and acceptable ethanol level, Rice Wine 2 is the ideal choice for the study.

Effectiveness of Philippine Rice Wine in Reducing Methemoglobinemia

Table 2. Results of Methemoglobin Analysis Done in Test Animals

TREATMENT	REPLICATES	Percentage of Methemoglobin Concentration	
		Pre-Test	Post-Test
Negative Control (Normal Saline Solution)	1	0.6433	0.6889
	2	0.6300	0.6895
	3	0.6335	0.6754
	Mean	**0.6357**	**0.6846**
Experimental Treatment (Philippine Rice Wine)	1	0.6338	0.6095
	2	0.6585	0.6156
	3	0.6153	0.5899
	Mean	**0.6359**	**0.6050**
Positive Control (Ascorbic Acid)	1	0.5793	0.5566
	2	0.6518	0.6358
	3	0.6709	0.6411
	Mean	**0.6341**	**0.6112**

Table 2 presents the percentage of methemoglobin concentrations observed in the nine test animals. A 4.86% reduction in methemoglobin levels was recorded in the experimental group following the administration of Philippine rice wine, while a 3.61% decrease was observed in the positive control group treated with ascorbic acid, relative to their respective pre-treatment values. In contrast, a continuous increase in methemoglobin concentration was noted in the negative control group. These findings suggest that both Philippine rice wine and ascorbic acid facilitated the reduction of sodium nitrite-induced methemoglobinemia, as evidenced by the decline in methemoglobin concentrations post-treatment.

Table 3. Paired T-test Results Comparing the Percentage of Methemoglobin Concentration Before and After Treatment

	Variable 1	Variable 2
Mean	0.635940464	0.605001139
Variance	0.000469457	0.000181346
df	2	
P(T<=t) two-tail	0.035623638	
t Critical two-tail	4.30265273	

Table 3 presents the results of the paired t-test comparing the percentage of methemoglobin concentrations before and after treatment administration. The mean methemoglobin concentration decreased from 0.6359 (pre-treatment) to 0.6050 (post-treatment). The statistical analysis yielded a p-value of 0.0356, which is below the conventional significance threshold of 0.05. This indicates that the observed reduction is statistically significant and not likely due to random variation. Therefore, the null hypothesis of no difference in methemoglobin levels is rejected, suggesting that the treatment exerted a significant effect on methemoglobin concentration.

These findings are supported by existing literature on the antioxidant properties of rice wine, which is rich in phenolic compounds, bioactive peptides, and other antioxidants. Its radical scavenging and reducing activities—primarily attributed to phenolics—play a key role in mitigating oxidative stress in erythrocytes (Chay et al., 2018; Dizon et al., 2017). These antioxidants facilitate the reduction of methemoglobin to functional hemoglobin, aligning with evidence from studies on red wine polyphenols that prevent oxidative agent-induced methemoglobinemia (Nakamura et al., 2000). Collectively, this supports the observed reduction in methemoglobin concentrations and highlights the therapeutic potential of antioxidant-rich substances in restoring hemoglobin function and maintaining redox balance (Chay et al., 2018; Lee et al., 2013; Nakamura et al., 2000).

Comparison of Philippine Rice Wine, Ascorbic Acid, and Normal Saline

After collecting the data, a One-Way Analysis of Variance (ANOVA) was conducted at a 0.05 significance level to evaluate whether the change in the percentage of methemoglobin concentration differed significantly among the treatment groups.

Table 4. One-Way Analysis of Variance (ANOVA) Comparing Post-Treatment Methemoglobin Levels Among Groups

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.011766642	2	0.005883321	7.105336327	0.02616444	5.143253
Within Groups	0.004968087	6	0.000828014			
Total	0.016734729	8				

The analysis yielded an F-value of 7.1053 and a p-value of 0.0262. Since the p-value was below 0.05, the null hypothesis of equal means was rejected, indicating a statistically significant difference in post-treatment methemoglobin concentrations among the groups. Thus, at the 95% confidence level, there was sufficient evidence to conclude that at least one treatment had a significantly different effect, warranting further analysis using post hoc tests such as the Tukey HSD.

After obtaining the results of the One-Way Analysis of Variance, the researchers then proceeded to use the Tukey Multiple Comparisons Test with a significance level of $\alpha = 0.05$. The test aimed to determine which among the treatments showed significant differences with each other.

Table 5. Tukey Multiple Comparisons Test of Treatments

Pair	Difference	SE	Q	Lower CI	Upper CI	Critical Mean	p-value
x1-x2	0.0796	0.01661	4.7913	0.007511	0.1517	0.07209	0.03393
x1-x3	0.07343	0.01661	4.4201	0.001344	0.1455	0.07209	0.04661
x2-x3	0.006167	0.01661	0.3712	-0.06592	0.07826	0.07209	0.963

x1 – Saline (Negative Control)
x2 – Rice Wine (Treatment)
x3 – Ascorbic Acid (Positive Control)

The results of the test are shown in Table 5. The mean difference is significant at the 5% level for saline vs treatment and saline vs ascorbic acid. As seen, the treatment showed a significant difference against the negative control, saline, with respect to the methemoglobin concentration change for all rats after giving the treatments. On the other hand, even though the said treatment was more effective than the negative control, there was no significant difference between rice wine and the positive control, ascorbic acid. This suggests that the performance of those treatments, in terms of reducing methemoglobin concentration, did not vary significantly. It also indicates that the studied treatment is as effective as the positive control, ascorbic acid, in decreasing methemoglobin concentration. These findings are supported by growing evidence on the antioxidant effects of rice wine, which come from its bioactive compounds like polyphenols and peptides. Studies have shown that polyphenols and polypeptides in Chinese rice wine help protect the heart by reducing oxidative stress, inflammation, and cell death—processes closely linked to lowering methemoglobin levels (Zhao et al., 2022). Research on Hongqu rice wines also shows increased antioxidant enzyme activity and decreased markers of oxidative damage in animal models, indicating they can reduce oxidative stress similarly to ascorbic acid (Zhang et al., 2022). Additionally, antioxidant peptides from rice wine have been found to support immune function and neutralize harmful free radicals, which may explain their role in decreasing methemoglobin (Kim et al., 2013). However, the antioxidant strength of rice wine can vary depending on the type, how it's made, and the dose given, which might explain some differences in effectiveness seen across studies (Zhang et al., 2022).

Toxicological Analysis of Sprague-Dawley Rat Liver

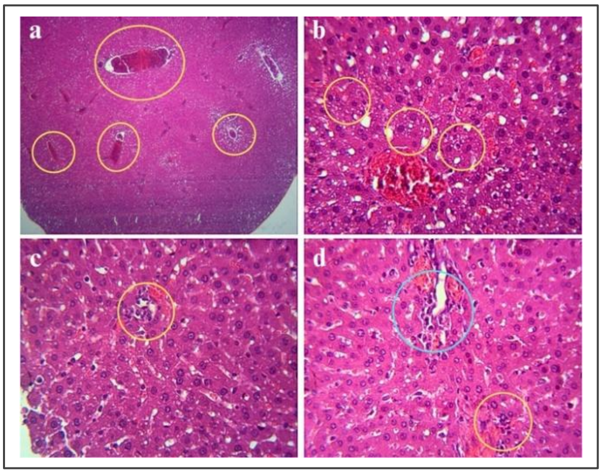


Figure 2. Microscopic view of the experimental rat's liver. (**a**) Hepatic congestion was observed as the presence of red blood cells in the hepatic vessels (**b**) Diffuse hepatocellular vacuolation was observed as the presence of multiple micro-vacuoles in the cytoplasm of hepatocytes (yellow circle) giving them a moth-eaten appearance (**c**) Micro Necrosis was observed as focal accumulation of inflammatory cells (**d**) Micro Necrosis was observed as focal accumulation of inflammatory cells, periportal (blue circle) and mid zonal (yellow circle)

Figure 2 shows the histological evaluation of rat liver tissue post-treatment. Toxicological findings—hepatic congestion, diffuse hepatocellular vacuolation, and multifocal micro-necrosis in periportal and mid-zonal hepatocytes—align with the biochemical effects of sodium nitrite used to induce methemoglobinemia. Sodium nitrite oxidizes ferrous (Fe^{2+}) hemoglobin to ferric (Fe^{3+}) methemoglobin, impairing oxygen transport and causing hypoxia and oxidative stress. The liver, a key organ for detoxification and oxidative metabolism, is particularly vulnerable to such damage. Hepatic congestion indicates circulatory changes due to hypoxia, while vacuolation and micronecrosis reflect oxidative and inflammatory injury, consistent with findings by Ansari et al. (2015).

Building on this, the diffuse vacuolation in hepatocytes further reflects the liver's response to increased oxidative stress and cellular injury caused by reactive nitrogen species generated from sodium nitrite. The multifocal micronecrosis localized in the periportal and mid-zonal regions corresponds to areas more susceptible due to their intermediate oxygen supply and metabolic activity. Hassan and Yousef (2010) have demonstrated that sodium nitrite induces hepatotoxicity through mitochondrial damage, membrane disruption, and inflammatory cell infiltration. Their work has thoroughly documented these effects, confirming the toxic impact of sodium nitrite in inducing methemoglobinemia.

Despite these toxic effects, the administration of Philippine rice wine appeared to confer hepatoprotection and systemic benefits, particularly in reducing methemoglobin levels. Dela Rosa (2021) reported that black rice wine is rich in anthocyanins and phenolic compounds—potent antioxidants capable of scavenging free radicals and enhancing endogenous antioxidant enzymes. These antioxidants help neutralize reactive species produced by sodium nitrite, thereby reducing oxidative stress and preserving liver integrity. Although some toxic lesions persisted, the absence of extensive necrosis or liver failure suggests that the rice wine provided partial protection. Supporting this, Sun et al. (2025) demonstrated that anthocyanins from black rice protect hepatic tissue from nitrite-induced oxidative damage and help restore antioxidant balance.

CONCLUSIONS

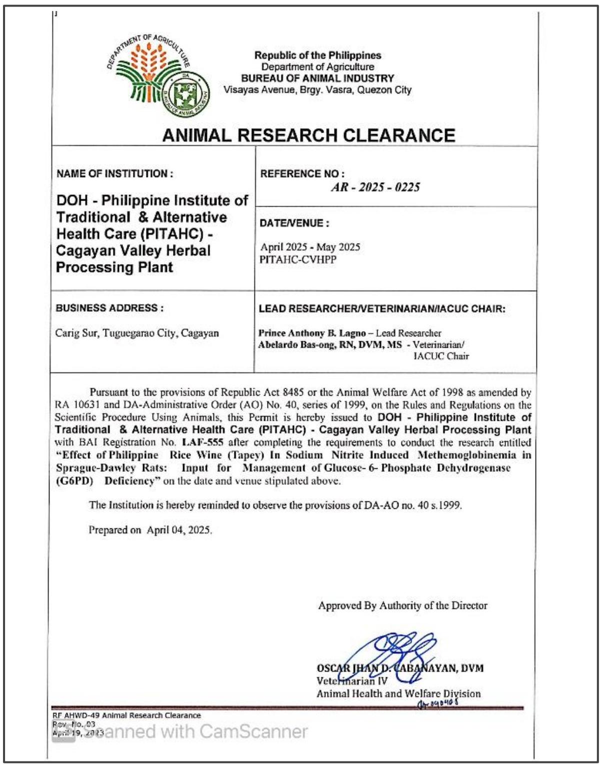
Philippine rice wine (*Tapey*) is effective in reducing sodium nitrite-induced methemoglobinemia in Sprague-Dawley rats, demonstrating both statistical and clinical significance. Unlike vitamin C, which requires high doses and carries the risk of kidney stone formation as well as the promotion of hydrogen peroxide and ascorbate radical production, *Tapey* offers a potentially safer alternative for managing methemoglobinemia, particularly in individuals with G6PD deficiency.

RECOMMENDATION

This research study is available to aspiring researchers seeking to enhance and further develop the scope of the investigation. The researchers highly recommend: (1) Conducting thorough animal studies to advance the preclinical phase of testing. (2) Employing more specific and sensitive equipment and methods for measuring methemoglobin levels to improve accuracy and precision. (3) Analyzing and monitoring key biomarkers, such as oxidative stress indicators, to assess efficacy and safety. (4) Conducting acute toxicity tests specifically for sodium nitrite to better understand its toxic effects and establish accurate dosing. (5) Utilizing five replicates per treatment group to improve the reliability and statistical power of the results. (6) Exploring the effects of other types of wine to compare their potential in reducing methemoglobinemia. Lastly, the researchers are urging relevant agencies and institutions to conduct in-depth research and to innovate methods and protocols to further improve and develop this study.

Appendices

Appendix A. Department of Agriculture Animal Clearance



Republic of the Philippines
Department of Agriculture
BUREAU OF ANIMAL INDUSTRY
Visayas Avenue, Brgy. Vasra, Quezon City

ANIMAL RESEARCH CLEARANCE

NAME OF INSTITUTION :	REFERENCE NO :
DOH - Philippine Institute of Traditional & Alternative Health Care (PITAHC) - Cagayan Valley Herbal Processing Plant	*AR - 2025 - 0225*
	DATE/VENUE : April 2025 - May 2025 PITAHC-CVHPP
BUSINESS ADDRESS : Carig Sur, Tuguegarao City, Cagayan	LEAD RESEARCHER/VETERINARIAN/IACUC CHAIR: **Prince Anthony B. Lagno** – Lead Researcher; **Abelardo Bas-ong, RN, DVM, MS** - Veterinarian/ IACUC Chair

Pursuant to the provisions of Republic Act 8485 or the Animal Welfare Act of 1998 as amended by RA 10631 and DA-Administrative Order (AO) No. 40, series of 1999, on the Rules and Regulations on the Scientific Procedure Using Animals, this Permit is hereby issued to **DOH - Philippine Institute of Traditional & Alternative Health Care (PITAHC) - Cagayan Valley Herbal Processing Plant** with BAI Registration No. **LAF-555** after completing the requirements to conduct the research entitled **"Effect of Philippine Rice Wine (Tapey) In Sodium Nitrite Induced Methemoglobinemia in Sprague-Dawley Rats: Input for Management of Glucose- 6- Phosphate Dehydrogenase (G6PD) Deficiency"** on the date and venue stipulated above.

The Institution is hereby reminded to observe the provisions of DA-AO no. 40 s.1999.

Prepared on April 04, 2025.

Approved By Authority of the Director

OSCAR JHAN D. CABANAYAN, DVM
Veterinarian IV
Animal Health and Welfare Division

RF AHWD-49 Animal Research Clearance
Rev. No. 03
April 19, 2023

Appendix B. Acute Oral Toxicity Test Results

Dosage of Rice Wine		Rat 1	Rat 2	Rat 3	Rat 4	Rat 5
1 ml/kg	1st hour	Alive	Alive	Alive	Alive	Alive
	Day 5	Alive	Alive	Alive	Alive	Alive
	Day 10	Alive	Alive	Alive	Alive	Alive
	Day 15	Alive	Alive	Alive	Alive	Alive
3 ml/kg	1st hour	Alive	Alive	Alive	Alive	Alive
	Day 5	Alive	Alive	Alive	Alive	Alive
	Day 10	Alive	Alive	Alive	Alive	Alive
	Day 15	Alive	Alive	Alive	Alive	Alive
5 ml/kg	1st hour	Alive	Alive	Alive	Alive	Alive
	Day 5	Alive	Alive	Alive	Alive	Alive
	Day 10	Alive	Alive	Alive	Alive	Alive
	Day 15	Alive	Alive	Alive	Alive	Alive

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