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Original Article

The protective effect of Thai rice bran on N-acetyl-ρ-aminophen-induced hepatotoxicity in mice

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Abstract

Background and purpose: N-acetyl-ρ-aminophen (APAP) is a widely used medication with analgesic and antipyretic characteristics. High paracetamol doses can damage the liver. Thai-pigmented rice may treat numerous liver disorders due to its antioxidant, anti-inflammatory, and glutathione-restoring capabilities. This study aimed to evaluate the phenolic components in three Thai rice bran extracts and their antioxidant and hepatoprotective activities in an animal model.
Experimental approach: Fifty male mice were randomly assigned to the control and APAP studies. Each study was divided into 5 groups (n = 5) treated with distilled water, Hom Mali, Hang-Ngok, and Hom Nil (HN) rice compared with N-acetylcysteine with/without 60 mg/kg/day of APAP orally once a day for two weeks. Blood and liver sampling were collected for analysis.
Findings/Results: HN rice bran exhibited higher contents of total phenolic, total flavonoid, total anthocyanin, ferric-reducing antioxidant, and 1,1-diphenyl-2-picrylhydrazyl radical scavenging activities than Hom Mali and Hang-Ngok. Anthocyanin was merely detected in HN. Following APAP administration, mice exhibited significant increases in hepatic enzymes including alanine aminotransferase (ALT) and aspartate aminotransferase (AST), pro-inflammatory cytokines (tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6)), and malondialdehyde (MDA), but lower levels of antioxidant enzymes and glutathione profiles. Amongst the three cultivars, HN rice was the only compound that decreased MDA, ALT, AST, TNF-α, and IL-6 while increasing antioxidant enzyme activity such as superoxide dismutase, catalase, and glutathione peroxidase that was very close to that of N-acetylcysteine groups.
Conclusion and implications: Given the hepatoprotective and antioxidant properties, HN has the potential to be used as a health supplement.

Keywords: Anti-inflammation; Antioxidant; N-acetyl-ρ-aminophen; Thai rice cultivars.

INTRODUCTION

N-acetyl-ρ-aminophen (APAP) is a commonly used medicine that serves as both an analgesic and an antipyretic worldwide. At therapeutic concentrations, it is safe; an adult's typical dose is 10-15 mg/kg, and a maximum daily intake of 3-4 g is recommended (1,2). Despite being classified APAP as a non-steroidal anti-inflammatory drug (NSAID), it has very modest anti-inflammatory activities due to its ability to block the cyclooxygenase enzyme and prostaglandin synthesis (3). For treating fever and both acute and chronic pain, because of its many benefits including its affordable price, easy accessibility, and few side effects, APAP is often chosen as the first medication of choice (4). Nevertheless, current research has connected the negative consequences of APAP therapy to central nervous system disturbances and neuronal death in addition to hepatotoxicity (5).

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Hepatotoxicity is the most significant side effect of paracetamol. N-acetyl-p-benzoquinone imine (NAPQI) is mostly converted in the liver to sulfate and glucuronic acid through the oxidative metabolism involving CYP450 enzyme systems. Glutathione transforms this metabolite form which is produced under normal circumstances into cysteine and mercaptopurine. Conversely, when ingested in excess, hepatocyte glutathione depots decrease and NAPQI levels rise. Glutathione reserves that fall below 30% of the typical level are the first indications of hepatic damage (3,6). Moreover, NAPQI, which is not metabolized, combines with lipids, proteins, and DNA in the cells to induce necrosis. Reactive oxygen species (ROS) production leads to the development of oxidative stress. ROS further reduces glutathione (GSH) reserves by oxidizing GSH to glutathione disulfide (GSSG), which ultimately compromises hepatocyte integrity and function. Thus, protecting against the hepatotoxic effects of paracetamol should focus on restoring GSH (6,7). GSH readily detoxifies the metabolite NAPQI at therapeutic levels of APAP. Conversely, reactive species develop when high concentrations of APAP deplete glutathione and the toxic metabolite links itself to proteins within the cell, especially in the mitochondria (6,8). N-acetylcysteine (NAC) has been used to treat paracetamol intoxication by stimulating the synthesis of reduced glutathione. Nevertheless, there are still disagreements over the optimal dosage and duration of NAC administration due to its severe side effects, including bronchospasm, nausea, vomiting, and anaphylaxis (1,9,10).

Rice is one of the most significant cereal crops farmed globally (*Oryza sativa L.*). Additionally, it is a significant staple crop, especially in Asia. Important bioactive components of rice, including γ-oryzanol, flavonoids, anthocyanins, sterols, and tocopherols have been shown to modulate and ameliorate human health conditions like oxidative diseases and cancer, owing to their diverse biological activities (11,12). Pigmented rice is regarded as an effective source of antioxidants in functional diets and possesses components that promote health (13). Thailand produces and consumes an extensive amount of rice, similar to many other Asian nations. Thailand's Khao Dok Mali 105 (KDML105), also known as Hom Mali (HM) rice, is well known throughout the world for its delicate texture and pleasant aroma. Traditional folklore wisdom is used to make Hang-Ngok (HNg), also known as germinated Hang rice or parboiled germinated brown rice. Its capacity to produce gamma-aminobutyric acid (GABA) has grabbed a great deal of interest from researchers in the field (14). Thai black rice (*Oryza sativa*) known as Hom Nil (HN) has antioxidant-rich polyphenolic compounds (15). The most nutrient-dense portion of the rice grain is the bran. It has high concentrations of essential fatty acids and ɤ-oryzanol. Rice bran contains ɤ-oryzanol, which has been shown to have potent antioxidant properties. Because of its many distinctive qualities, rice bran is suited for niche markets including the pharmaceutical and nutraceutical sectors.

Thus, the key contribution of this study was to determine rice bran fractions of the potential of HN rice bran extract that are appropriate sources of phytochemicals for a nutraceutical development that aims to evaluate Thai rice cultivars' hepatoprotective effects in the treatment of liver damage induced by APAP, specifically compared to other Thai rice bran cultivars, i.e., HM and HNg, in addition to the use of a standard treatment of NAC. Information compiled from our study can validate Thai rice bran's potential use as an effective natural antioxidant that could be used to create food, pharmaceutical, and nutraceutical products.

MATERIALS AND METHODS

Chemicals

Johnson & Johnson Ltd. (Bangkok, Thailand) was the manufacturer of paracetamol. Now Foods (New York City, USA) supplied NAC. Sigma Aldrich (St. Louis, MO) provided the reduced β-nicotinamide adenine dinucleotide phosphate (NADPH), 4-vinylpyridine, 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), nitrotetrazolium blue chloride (NBT), standard malondialdehyde (MDA), bovine serum albumin (BSA), xanthine oxidase, and standard superoxide dismutase (SOD) from bovine erythrocytes. Fisher

Scientific provided hydrogen peroxide (H_2O_2) (Leicestershire, UK). FlukaChemika Co. (Steinheim, Switzerland) provided the thiobarbituric acid (TBA). The supplier of Trizol® was Invitrogen® (Carlsbad, CA). The purified chemicals used in the laboratory were obtained from commercial suppliers.

Preparation of Thai rice bran crude extracts

Northeastern Thailand provided samples of Thai rice, including HM (Khao Dawk Mali 105 variety), HNg (germinated Hang rice or parboiled germinated brown rice of Khao Dawk Mali 105 variety), and HN (non-glutinous purple rice). The bran was extracted by polishing the brown rice. By drying to constant mass at 110 °C, moisture was measured. Three duplicate samples were used for each study, and the results were expressed on a dry matter basis. Before being analyzed, the samples were kept in storage at a temperature of -20 °C. Three Thai rice varieties yielded 1,000 g of bran that was extracted using 80% ethanol at 25 °C for seven days. A rotary evaporator R-II (Buchi Company, Flawil, Switzerland) was used to filter the crude extract before it was evaporated at a reduced pressure. The extracted rice bran was weighed to determine the yield percentage.

Sample preparation for the in vitro assays

For 48 h, 10 g of each sample was steeped in 200 mL of 80% methanol (1:20) and shaken intermittently. The resultant suspension was filtered *via* Whatman No. 1 filter paper. The filtrate was then kept at -20 °C until needed, and it was concentrated at 40 °C in a rotary evaporator R-II (Buchi Company, Flawil, Switzerland).

For one week, 80% ethanol was used to extract the three Thai rice cultivars brans (1000 g), HM, HNg, and HN, at 25 °C. The crude extract was filtered *via* a rotary evaporator R-II and evaporated at a reduced pressure. Weighing the extracted rice allowed us to determine the percentage of yield. Using high-performance liquid chromatography, a preliminary phytochemical analysis was conducted. Using previously reported techniques (16,17), the biologically active substances, such as anthocyanins, phenolics, and flavonoids, were filtered out.

Animal treatments

Under the direction of the Animal Ethics Committee for Use and Care (Approval No. AEKSU 92/2559), fifty male ICR mice at seven weeks of age were provided by the National Laboratory Animal Center (Mahidol University, Nakhonpathom, Thailand) and housed at Kalasin University, Thailand. Unless otherwise specified, the mice were housed in cages with water, wood chip bedding, and a commercial mouse diet supplied *ad libitum*. The mice's quarters featured air conditioning set at 23 ± 2 ℃ and a 12/12-h light/dark cycle.

Fifty mice were assigned to two categories, control and APAP (18-21). Each category was divided into 5 groups (five mice each) as follows: the control study included (the treatment was administered by oral gavage once a day for 14 days) group 1 received 0.1 mL/mouse of deionized water (ColT-DW); groups 2 to 4 were administered 512 mg/kg/day of HM (ColT-HM) (22), HNg (ColT-HNg) (23), and HN (ColT-HN) bran rice extract (11); and 150 mg/kg/day of NAC (ColT-NAC) (6), respectively. In contrast, the mice of all five groups of the APAP treatment trial (administered 60 mg/kg/day orally once a day for 14 days) received the same portions of treatment as those in the control study. For studying the effects of the three rice, groups 2-5, HM, HNg, HN, and NAC (APAP-HM, APAP-Hng, APAP-HN, and APAP-NAC) were administered orally to mice of the 14 days-paracetamol-treated groups, respectively (Fig. 1). On day 15th, the mice were sacrificed. For hematological and biochemical analyses, heart blood was collected. Internal organs were excised, frozen, and kept at -80 °C for later analysis.

Biochemical parameter analysis

The plasma samples were analyzed using an automatic chemistry analyzer (Konelab 20i, Thermo Fisher Scientific) at the Veterinary Laboratory Diagnostic Service, Faculty of Veterinary Medicine, Khon Kaen University, Thailand. Biochemical parameters including alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were evaluated (24-29).

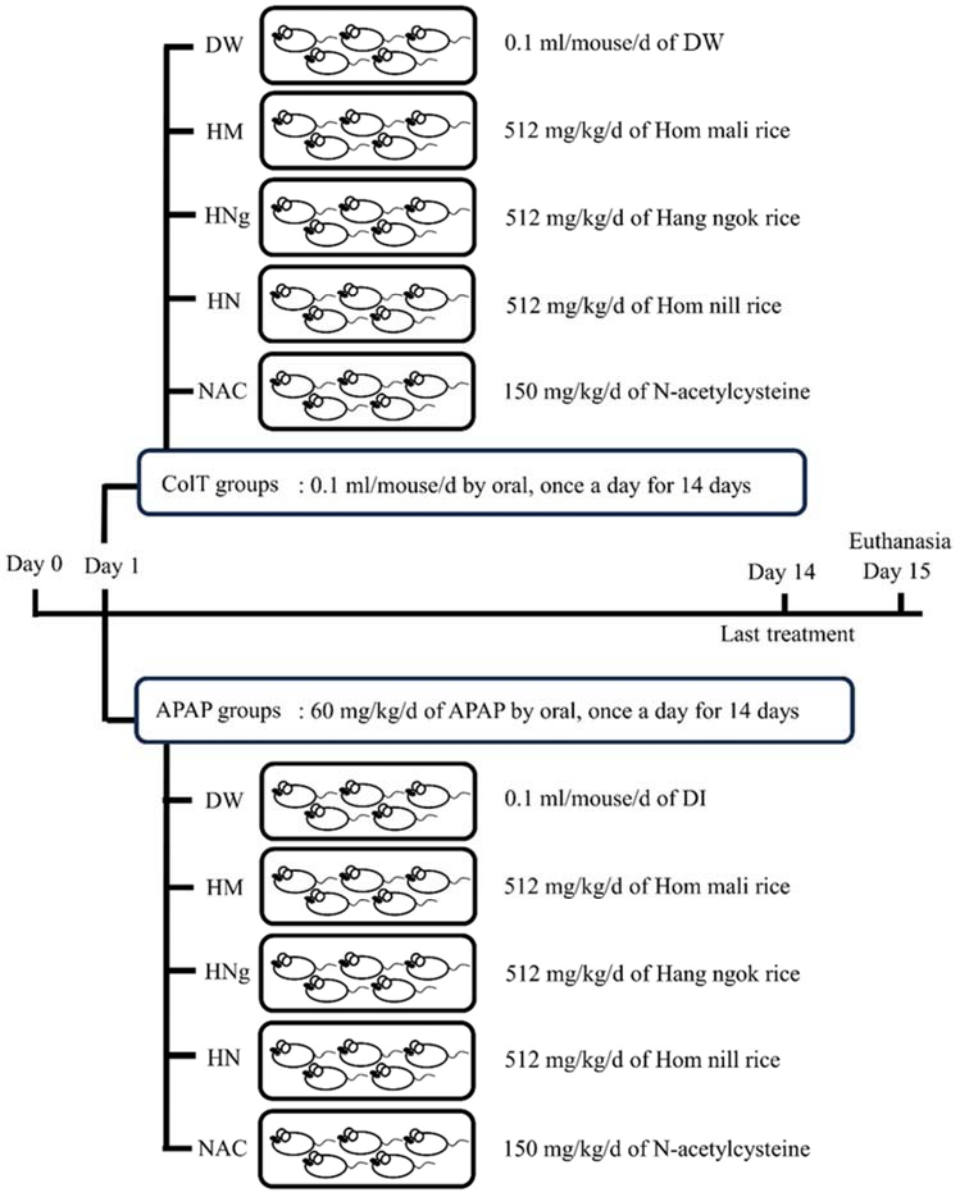

Fig. 1. Experimental treatments of mice. ICR mice at 7 weeks of age (n = 50) were divided into two treatment categories including control (ColT) and APAP-induced hepatotoxicity group (APAP). Each category was treated with DW, HM, HNg, HN, and NAC. Each experimental treatment was performed on five mice. APAP, N-acetyl-ρ-aminophen; DW, deionized water; HM, Hom mali rice; HNg, Hang-Ngok rice; HN, Hom Nil rice; NAC, N-acetylcysteine.

Determination of antioxidant activity

The amount of SOD activity was evaluated by the degree of inhibition of formazan production. An aliquot of homogenate was removed using a solution of ethanol and chloroform. The SOD assay was conducted using the supernatant. The reagent mixture, consisting of xanthine, ethyl-enediaminetetraacetic acid (EDTA), nitrotetrazolium blue chloride, Na_2CO_3, BSA, and xanthine oxidase, was mixed with the supernatant and the bovine SOD standards. After incubating the mixture for 20 min at 25 °C, the reaction was terminated using $CuCl_2$. At the wavelength of 550 nm, formazan's absorbance was measured. A comparison was made between the formazan inhibition% and the SOD standard.

The homogenate sample was incubated for 1 min at 37 °C in an H_2O_2 substrate before ammonium molybdate was added to cease the reaction and measure the level of CAT activity. A wavelength of 405 nm was used to measure the yellow complex. The hepatic bovine CAT standard was used to compare the percentage of yellow complex inhibition.

The sample homogenate, sodium azide, EDTA, and sodium phosphate buffer (pH 7.4) were all included in the reaction mixture. GSH was then added. Subsequently, the mixture was incubated at 30 °C for 10 min. H_2O_2 was added to begin the reaction, and 5-sulfosalicylic acid (SSA) was used for terminating it. The glutathione peroxidase (GPx) activity was calculated using the GSSG concentration of the supernatant, which was obtained after centrifuging the reaction mixture for 15 min at 350 *g*. Based on a GPx unit of mmol(s) of GSSG formed/min at pH 7.4 and 30 °C (30), the protein results were expressed as a unit/mg.

Measurement of GSSG, GSH, and glutathione content

The homogenate samples were deproteinized using SSA and then centrifuged at 10,000 *g* for 10 min at 4 °C after being stored for 10 min at 2-8 °C. The reaction mixture, which further included glutathione reductase, EDTA, NADPH, DTNB, and potassium phosphate buffer (pH 7.0), was combined with the supernatants in order to measure the total GSH. Over the course of 5 min, the absorbance of the thiol anions at 405 nm (A405) was measured every 60 s. To determine the total GSH levels, the sample's A405/min (slope) was compared to the glutathione standard series. The contents of total GSH were subtracted from the amounts of GSSG to determine the GSH contents. 4-Vinylpyridine was incubated for 60 min at room temperature with an aliquot of the sample supernatant and the GSSG standard in order to determine the GSSG concentration. The process for determining the contents of GSSG was similar to that of GSH (31).

Determination of lipid peroxidation

The amount of lipid peroxidation was measured using the TBA assay. The homogenate sample and MDA standard were incubated for 1 h at 37 °C before the reaction mixtures (trichloroacetic acid, TBA, and acetic acid) were added. After that, the samples were heated to a 15-min boil. The thiobarbituric acid-reactive species was measured with a spectrofluorometer that was calibrated to emit at 551 nm and excite at 528 nm (32).

Cytokine analysis

Samples of serum were warmed to room temperature. Using commercial kits from Invitrogen, Thermo Fisher Scientific, USA, the inflammatory cytokines (tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6)) were quantified by enzyme-linked immunosorbent assay (ELISA), adhering to the manufacturer's protocols. The lower limit of detection was 4.4 pg/mL. The intra- and inter-assay coefficients of variation for TNF-α were 5.2% and 9.2%, respectively. While the minimum detection of IL-6 was estimated to be 2 pg/mL, the intra- and inter-assay coefficients of variation for IL-6 were 9.2% and 8.4%, respectively.

Assessment of antioxidant activity and total phenolic compounds

DPPH radical scavenging activity

The 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) scavenging activity of the extracts was evaluated with various modifications (16). One-tenth mL of the sample extract was mixed with 1.9 mL of 0.1 mM DPPH in ethanol. The mixture was vortexed for 1 min, and after being at room temperature in the dark for 30 min, the absorbance of this solution at 517 nm was determined. The % inhibitory activity was calculated using the equation below:

$$\text{Activity inhibition (\%)} = \frac{\text{Ao} - \text{Ae}}{\text{Ao}} \times 100 \quad (1)$$

where Ao stands for absorbance without extract and Ae for absorbance with extract.

Ferric reducing/antioxidant power assay

Fresh ferric reducing/antioxidant power (FRAP) reagent was made by combining 12 mL of distilled water at 37 °C with 100 mL of acetate buffer (300 mM, pH 3.6), 10 mL of 2,3,5-triphenyl-tetrazolium chloride solution (10 mM 2,3,5-triphenyl-tetrazolium chloride in 40 mM/HCl), and 10 mL $FeCl_3 \cdot 6H_2O$ (20 nM)

in a 10:1:1 ratio. To conduct the assay, identical test tubes, 180 µL of Milli-Q water, 1.8 mL of FRAP reagent, and 60 µL of sample, standard, or blank were added. The absorbance of the tubes was measured at 593 nm following 4 min of incubation at 37 °C, using the FRAP working solution as a blank. If the relative absorbance reading is not between 0 and 2.0, the sample must be diluted. To calculate the sample's FRAP values in the FRAP assay, the antioxidant potential of the sample was determined using a standard curve plotted using the $FeSO_4 \cdot 7H_2O$ linear regression equation (16).

Determination of total phenolic content

The Folin-Ciocalteu reagent was used to measure the total phenolic content (TPC). After the extract was left at room temperature for 5 min, 2.25 mL of diluted Folin-Ciocalteu reagent (diluted ten times) and distilled water were combined with 300 µL of the extract. Then, 2.25 mL of a 60 g/L sodium carbonate solution was added. A spectrophotometer was used to measure the absorbance at 725 nm following 90 min at room temperature. The results are expressed as milligrams of gallic acid equivalents to each gram of dried sample (mg GAE/g) (16).

Determination of total flavonoid content

The total flavonoid content (TFC) was calculated using the colorimetric technique. In a test tube, 0.5 mL of the extract and 2.25 mL of distilled water were combined with 0.15 mL of a 5% $NaNO_2$ solution. Following a six-minute stand time, 0.3 mL of a 10% $AlCl_3 \cdot 6H_2O$ solution and 1.0 mL of 1 M NaOH were added 5 min apart. The mixture was mixed using a vortex mixer. At 510 nm, the absorbance was promptly determined with a spectrophotometer. According to the findings, 1 g of the dried sample (16) included mg of rutin equivalent (mg RE/g) as well as mg of quercetin equivalent (and mg QE/g).

Determination of total anthocyanin content

With a minor modification, the colorimetric approach was used to determine the colored rice's total anthocyanin content. Using 10 mL of distilled water, three extractions totaling 10 mg of rice brans were produced. The mixture was centrifuged at 10,000 *g* for 10 min, and the supernatants were collected (17).

Evaluation of in vitro anti-inflammation activity

The BSA assay was utilized to determine the crude extract's anti-inflammatory properties, with few modifications (33). Phosphate buffered saline (PBS, pH 6.4), 0.2 mL of 1% bovine albumin, and 0.02 mL of extract made up the reaction mixture (5 mL). The mixture was then allowed to incubate for 15 min at 37 °C in a water bath. For 5 min, the mixture was heated to 70 °C. The turbidity at 660 nm was measured using a UV-visible spectrometer after the mixture cooled. Phosphate buffer solution was used as a control. The percentage inhibition of BSA denaturation was calculated as follows:

$$Antidenaturation\ activity\ (\%) = \frac{Absorbance\ of\ control - absorbance\ of\ sample}{Absorbance\ of\ control} \times 100 \quad (2)$$

Statistical analysis

The obtained data, represented as mean ± SD. were analyzed using; SPSS software (version 17, Chicago, IL, USA). One-way analysis of variance (ANOVA) followed by Tukey's post-hoc tests was applied to compare the groups. The *P*-values < 0.05 were considered statistically significant.

RESULTS

Phytochemical contents of rice bran extracts

Using the FRAP and DPPH radical scavenging activity (% inhibition) assays, the antioxidant capacity of Thai rice bran was evaluated. Table 1 illustrates the results of these examinations. HN rice bran had the highest value of scavenging activity and FRAP (87.83% and 16.73 mM $FeSO_4$/g), followed by HNg rice bran (35.03% and 2.52 mM $FeSO_4$/g) and the lowest activity was found in HM rice bran (24.97% and 1.20 mM $FeSO_4$/g). In comparison to other rice brans, HN rice bran demonstrated a substantially greater capacity for free radical scavenging and reduction.

Table 1. Antioxidant activity and total phenolic compounds of Thai rice bran extracts. The data are presented as mean ± SD (n = 5). Different lower-case letters indicate a significant difference among different precipitation at the level of *P*-values < 0.05.

Rice bran	Hom Mali	Hang-Ngok	Hom Nil
1,1-Diphenyl-2-picrylhydrazyl radical (% inhibition)	24.97 ± 0.33^{c}	35.03 ± 0.62^{b}	87.83 ± 0.10^{a}
Ferric reducing/antioxidant power (mM $FeSO_4$/ g)	1.20 ± 0.02^{c}	2.52 ± 0.02^{b}	16.73 ± 0.70^{a}
Total phenolic content (mg GAE/g)	14.11 ± 0.09^{c}	57.23 ± 0.18^{b}	316.29 ± 2.08^{a}
Total flavonoid content (mg RE/g)	2.32 ± 0.08^{c}	6.37 ± 0.13^{b}	27.45 ± 0.23^{a}
Total flavonoid content (mg QE/g)	9.73 ± 1.04^{c}	20.10 ± 0.14^{b}	183.40 ± 4.72^{a}
Total anthocyanin content (mg cya3glu/100 g)	Not detected	Not detected	9.63 ± 0.06

GAE/g, Gallic acid equivalents for each gram of dried sample; RE/g rutin equivalents for each gram of dried sample; QE, quercetin equivalents for each gram of dried sample.

Table 1 also shows the TPC, TFC, and total anthocyanin content of Thai rice bran. The TPC values were significantly different amongst different varieties. Thai rice bran's TPC varied from 14 to 316 mg GAE/g. HN rice bran had the highest TPC with a concentration of 316.29 mg GAE/g, followed by HNg rice bran (57.23 mg GAE/g), while HM rice bran had the lowest TPC (14.11 mg GAE/g). There was a significant difference in flavonoids amongst varieties of Thai rice brans in this study. HN rice bran had higher levels of TFC than HM and HNg rice bran, which was consistent with the findings of antioxidant activity and TPC (Table 1). HN rice bran had the highest TFC with a concentration of 27.45 mg RE/g and 183.40 mg QE/g, followed by HNg rice bran (6.37 mg RE/g and 20.10 mg QE/g), while HM rice bran had the lowest TFC (2.32 mg RE/g and 9.73 mg QE/g). Total phenolic and flavonoid levels and antioxidant activity were shown to be strongly correlated in the data. Measuring 9.63 mg of cya3glu per 100 g, anthocyanin was exclusively detected in pigmented rice bran (HN). It was not detected in non-pigmented rice bran (HM and HNg).

In vitro anti-inflammation assays of rice bran extracts

Protein denaturation was inhibited by ethanolic extracts of Thai rice bran in a manner that depended on the variety of rice used. Figure 2 illustrates the inhibitory effect of various Thai rice brans on protein denaturation. These Thai rice brans had an inhibition percentage of protein denaturation that ranged from 21.74% to 68.12%. HN rice bran showed the highest level of inhibition, whereas HM rice bran showed the lowest levels of inhibition.

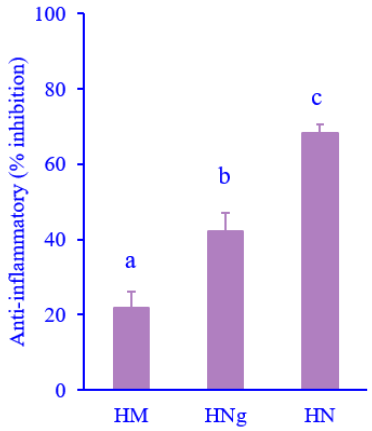

Fig. 2. The percentage inhibition of anti-inflammation of various Thai rice bran cultivars. Data are expressed as mean ± SD, n = 5. Different letters (a-c) indicate significant differences amongst the groups at the level of *P*-values < 0.05. HM, Hom mali rice; HNg, Hang-Ngok rice; HN, Hom Nil rice.

Effect of Thai rice cultivars on biochemical parameters

Serum AST and ALT levels (IU/L) were measured to evaluate the liver's function following intoxication. The levels of AST and ALT were rather constant in all groups without APAP. However, liver function increased after APAP intoxication. HM, HNg, HN, and NAC treatment decreased the levels of AST and ALT, although those levels in the groups treated with HN were rather close to those of NAC. Changes in the levels of AST and ALT followed the same trends; however, the levels of AST in both CoIT and APAP were much higher than those of ALT (Fig. 3A and B).

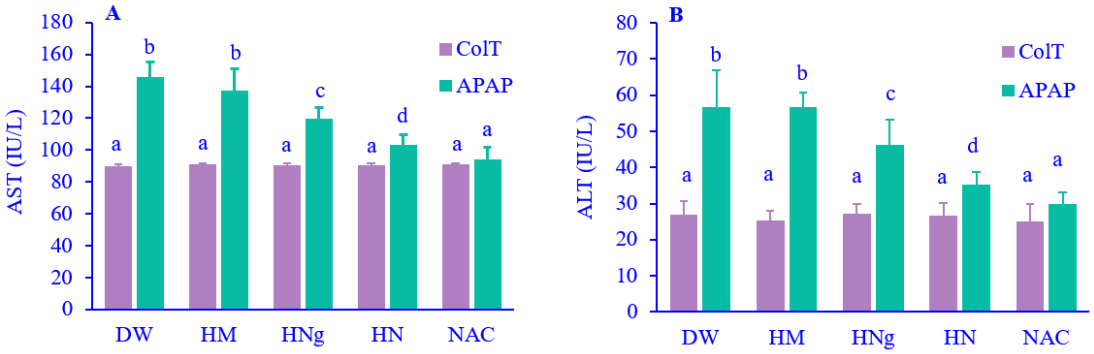

Fig. 3. Evaluation of liver function by measuring (A) AST and (B) ALT levels in APAP-induced hepatotoxicity mice. Data are expressed as mean ± SD, n = 5. Different letters (a-c) indicate significant differences amongst the groups at the level of *P*-values < 0.05. ColT, Control category not receiving APAP; APAP, N-acetyl-ρ-aminophen; DW, deionized water; HM, Hom mali rice; HNg, Hang-Ngok rice; HN, Hom Nil rice; NAC, N-acetylcysteine.

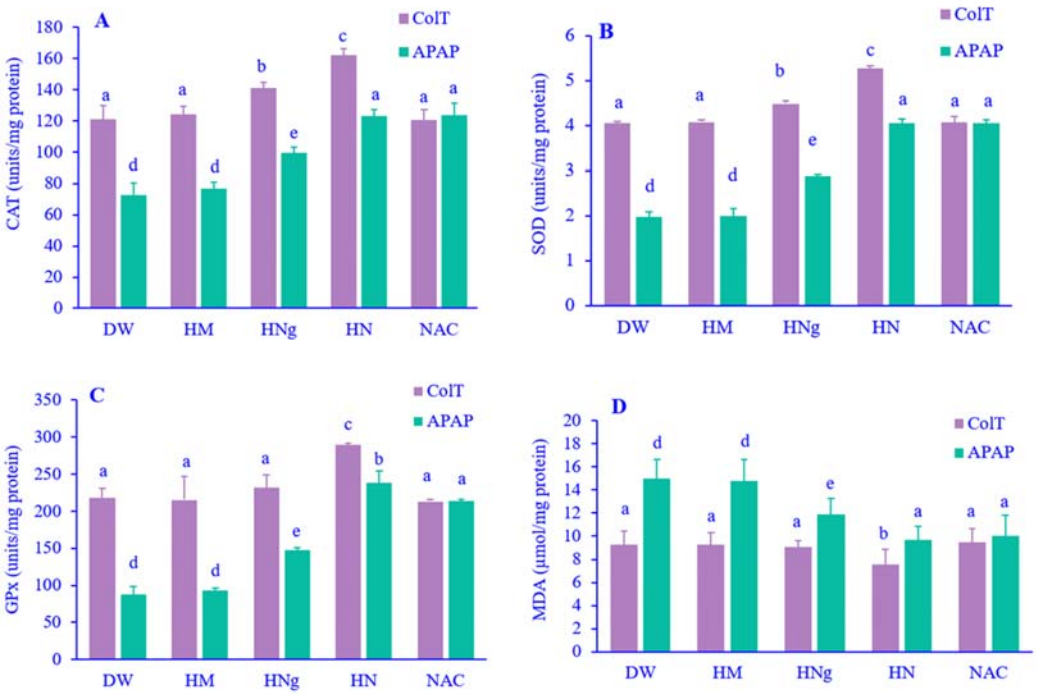

Fig. 4. Evaluation of lipid peroxidation and antioxidant enzyme activities by measuring (A) CAT, (B) SOD, (C) GPx, and (D) MDA levels in APAP-induced hepatotoxicity mice. Data are expressed as mean ± SD, n = 5. Different letters (a-e) indicate significant differences amongst the groups at the level of *P*-values < 0.05. ColT, Control category not receiving APAP; APAP, N-acetyl-ρ-aminophen; DW, deionized water; HM, Hom mali rice; HNg, Hang-Ngok rice; HN, Hom Nil rice; NAC, N-acetylcysteine; CAT, catalase; SOD, superoxide dismutase; GPx, glutathione peroxidase; MDA, malondialdehyde.

The effect of Thai rice cultivars on antioxidant activities

In the ColT groups of mice treated with HNg and HN, the antioxidant activity of CAT and SOD, also GPx activity following treatment with HN markedly increased (compared to the DW group which was the same as HM and NAC). On the other hand, after APAP intoxication, the enzyme activities were the lowest in the APAP-DW group. The antioxidant enzyme activity levels significantly increased in APAP groups treated with HNg, HN, and NAC. Surprisingly, after administration of HN, the activity of the enzymes CAT, SOD, and GPx gradually increased which was not different from that in the NAC group (Fig. 4A-C).

The lipid peroxidation level in APAP mice was unusually high during the intoxication

period as showed by the high concentration of MDA (Fig. 4D). MDA levels substantially decreased after treatment with HNg, HN, and NAC (MDA level was similar in HM and DW groups). Intriguingly, the HN rice-treated population showed a significant reduction in lipid peroxidation, similar to that of the NAC group. The levels of MDAs were similar in ColT but only the lowest in HN.

The effect of Thai rice cultivars on glutathione and the GSH/GSSG ratio

No significant differences in the level of total glutathione, GSH, GSSG, and GSH/GSSG were observed in all ColT groups. While in APAP mice, a lower total glutathione level was presented. When mice were given HN and NAC, the level of total glutathione significantly increased. Not only total glutathione value was raised through treatment with the extract of HN rice but also all of the glutathione profiles including GSH, GSSG, and the Ratio of GSH/GSSG were improved (Table 2).

Effect of Thai rice cultivars on the serum inflammatory cytokines

The levels of TNF-α and IL-6 increased in APAP-intoxicated mice treated with DW, HM, or HNg. The TNF-α level in the HN-treated group is similar to that of the NAC-treated group which was much lower than that of the other APAP groups. The trends followed by both inflammatory factors, but the levels of IL-6 were almost higher in comparison with TNF-α, as shown in Fig. 5A and B. It was also observed that there was no statistically significant difference between the ColT groups' serum inflammatory profile levels.'

Table 2. The total glutathione level and ratio of GSH/GSSG in APAP-induced hepatotoxicity mice. The two treatments including the control and APAP-induced hepatotoxicity group and each treatment were treated with DW, HM, HNg, and HN, and NAC. The data are presented as the mean ± SD (n = 5). Different lower-case letters indicate a significant difference among different precipitation at the level of *P*-values < 0.05.

Groups		Total glutathione	GSH	GSSG	GSH/GSSG
ColT	DW	7.30 ± 0.21^{a}	5.10 ± 0.11^{a}	2.31 ± 0.17^{a}	2.245^{a}
	HM	7.27 ± 0.27^{a}	5.12 ± 0.15^{a}	2.24 ± 0.09^{a}	2.234^{a}
	HNg	7.48 ± 0.43^{a}	5.19 ± 0.30^{a}	2.48 ± 0.24^{a}	2.265^{a}
	HN	7.92 ± 0.17^{a}	5.47 ± 0.27^{a}	2.51 ± 0.08^{a}	2.259^{a}
	NAC	7.49 ± 0.02^{a}	5.26 ± 0.43^{a}	2.29± 0.44^{a}	2.188^{a}
APAP	DW	3.18 ± 0.17^{b}	1.73 ± 0.10^{b}	1.47 ± 0.07^{b}	1.152^{b}
	HM	3.04 ± 0.42^{b}	1.69 ± 0.12^{b}	1.34 ± 0.02^{b}	1.123^{b}
	HNg	4.61 ± 0.13^{ab}	2.42 ± 0.06^{ab}	2.13 ± 0.45^{ab}	1.141^{ab}
	HN	7.11 ± 0.28^{a}	4.92 ± 0.09^{a}	2.37 ± 0.27^{a}	2.125^{a}
	NAC	7.31 ± 0.32^{a}	5.31 ± 0.23^{a}	2.20 ± 0.47^{a}	2.525^{a}

GSH, Reduced glutathione; GSSG, oxidized glutathione; APAP, N-acetyl-ρ-aminophen; ColT, control; DW, deionized water; DW, deionized water; HM, Hom mali rice; HNg, Hang-Ngok rice; HN, Hom Nil rice; NAC, N-acetylcysteine;

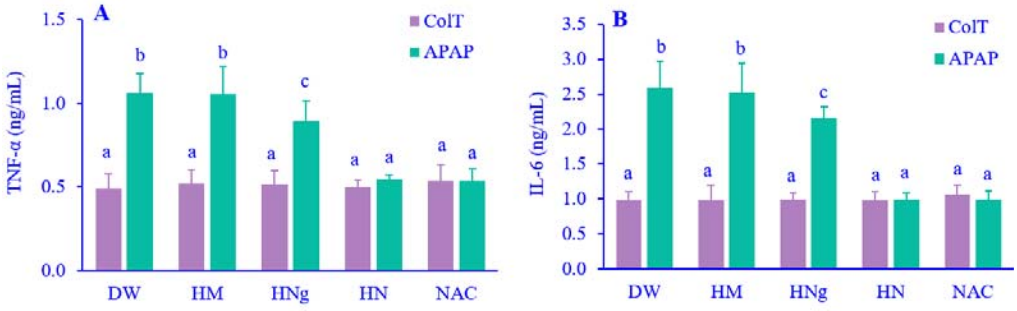

Fig. 5. Evaluation of serum inflammatory profile by measuring (A) TNF-α and (B) IL-6 levels in APAP-induced hepatotoxicity mice. Different letters (a, b, and c) indicate significant differences amongst the groups at the level of *P*-values < 0.05. ColT, Control category not receiving APAP; APAP, N-acetyl-ρ-aminophen; DW, deionized water; HM, Hom mali rice; HNg, Hang-Ngok rice; HN, Hom Nil rice; NAC, N-acetylcysteine; TNF-α, tumor necrosis factor-alpha; IL-6, interleukin-6.

DISCUSSION

Similar to other rice-consuming nations, Asia in particular, Thailand cultivates and consumes a lot of rice. There are about 5000 recognized types of rice in the nation; one of the natural plants that are grown is Thai-colored rice (*Oryza sativa L. indica*). Black rice contains more protein, vitamins, minerals, and bioactive substances than white rice, according to many studies comparing the two types of rice (11). It has been noted to have health-promoting compounds and to be a potent antioxidant source (34). Rice contains essential bioactive components, including γ-oryzanol, flavonoids, phenolic acids, anthocyanins, sterols, tocopherols, and tocotrienols, which have been shown to manage and alleviate human health disorders, including oxidative diseases (11,12). In this study, the finding of phytochemicals of black rice for nutraceutical development was presented in the total anthocyanin, phenolic, and flavonoid contents in HN rice bran which was one of the black rice cultivated in Thailand.

Anthocyanin is one of the main antioxidants that protect cells in all animals, including humans against ROS. Research has indicated that it contains antiviral, anti-inflammatory, and anti-aging properties. In addition, it lowers the chance of developing serious conditions like cancer and obesity (35-37). The HN extract of black rice cultivars had all of the phenolic and flavonoid components.

This outcome is consistent with the research conducted by Settapramote *et al*. (34) which discovered that rice that has been pigmented (black, red, brown, dark purple, and rice berry rice) has flavones, anthocyanins, proanthocyanidins, tannins, phenolics, γ-oryzanols, tocopherols, phytosterols, and essential oils, all of which are shown to be beneficial to human health. Natural compounds that protect the liver often have a range of properties, such as antiviral, immunomodulatory, anti-inflammatory, and antioxidant effects (38,39). Because these substances have the function of decreasing liver damage caused by APAP, they can be further developed as hepatoprotective agents or antioxidants. APAP is one of the most often used medications due to its antipyretic and analgesic qualities. The recommended doses are safe and effective. On the other hand, hepatotoxicity and acute liver failure could result from an overdose (40).

The etiology of APAP-induced hepatotoxicity is mostly attributed to oxidative stress and mitochondrial dysfunction (38). ROS are produced when the thiol groups in proteins are oxidized by the high amount of APAP, which also depletes the glutathione reservoir. Damage to mitochondrial DNA is caused by both NAPQI and ROS. The subsequent binding of NAPQI to cellular macromolecules, such as proteins, lipids, and nucleic acids, causes hepatocyte death and centrilobular liver damage (38,41,42). The results, corroborating with those of others, demonstrate that the higher dose of APAP leads to liver damage with the increase of liver enzyme function (ALT and AST). Following the research conducted by Hosack *et al*. (26) and Kuna *et al.* (27) for acute or chronic drug-induced hepatotoxicity or liver injury, drug-induced liver injury can be diagnosed by liver biopsy histology, hepatotoxicity mechanism, hepatocellular, cholestatic, or mixed clinical presentation. It is the leading cause of acute liver failure in the US and elsewhere, although its prevalence is unknown. Hepatotoxicity is caused by dose-dependent intrinsic and unexpected idiosyncratic processes. Most drug-induced liver injury patients have asymptomatic jaundice. Hepatocellular and cholestatic injuries elevate ALP and aminotransferases in lab tests. Although not necessary for diagnosis, a liver biopsy may rule out other liver disease causes (28,29).

According to the findings of the study by Mrakic-Sposta *et al*. (43) and Sun *et al*. (44), correctly determining the prevalence of liver injury in humans is difficult, but it is the main cause of acute liver failure. Drug-induced liver injury including acute or chronic liver damage might be caused by the use of natural or synthetic drugs (45-46). The clinical symptoms, cellular and molecular mechanism, or liver biopsy histology can classify drug-induced liver injury. APAP-induced liver damage certainly contributes to acute liver failure (46). NAPQI is produced abundantly in liver cells by APAP. Increased NAPQI lowers glutathione

levels in the cytosol and mitochondria, causing oxidative stress. Lipid oxidation and ROS generation increase. High liver enzyme levels including ALT and AST correspond to the increase of ROS and lipid peroxidation in the liver damage and necrotize hepatocytes. This study shows that drug-induced liver damage (44-46) requires ROS-targeted treatments.

The serum inflammatory cytokines of the APAP-intoxicated animals showed elevated levels of TNF-α and IL-6, which indicated liver damage. A high dose of APAP can result in redox imbalance in addition to liver injury (6,47).

Due to their considerable increase over those in mice without APAP loading, the MDA levels can be used as an indicator of oxidative stress caused by APAP. Considering these findings, the APAP animals had a markedly higher antioxidant state as evidenced by the relative activity of antioxidant enzymes (SOD, CAT, and GPx). Thus, this behavior suggests that oxidative stress is a greater threat to APAP mice. The first line of defense against oxidant processes is antioxidant enzymes. For biological systems to repair the damage induced by oxidative stress in diverse tissues, such as the hematopoietic and reproductive organs, or to scavenge oxidative metabolites, an equilibrium between oxidants like MDA and antioxidants like CAT, SOD, and GPX is crucial (41).

During the APAP hepatotoxicity damage process, NAPQI primarily targets mitochondrial proteins. Additionally, this substance disrupts the electron transport chain complex in the mitochondria, allowing electron leakages from the electron transport chain to oxygen and forming superoxide radicals (48). After superoxide radicals are created, SOD converts them to molecular oxygen (O_2) and hydrogen peroxide (H_2O_2), or they can combine with endogenous nitric oxide (NO) to make peroxynitrite ($ONOO^-$). Subsequently, H_2O_2 is either directly detoxified by GSH or scavenged by various antioxidant enzymes in hepatocytes, including CAT and GPx. Additionally, $ONOO^-$ formed in mitochondria may react with GSH to facilitate detoxification (38,39). As a result of these excessive free radicals, GSH is depleted, which leads to the accumulation of $ONOO^-$, which damages mitochondrial DNA and forms nitrotyrosine protein adducts (48).

Clinically, NAC is utilized as the conventional antidote for APAP poisoning, mostly by replenishing GSH to improve NAPQI detoxification. NAC also provides protective effects when taken during the oxidative damage phase, when the mitochondria produce ROS and hepatocellular GSH is reduced (48,50,51). However, there are some negative aspects to this medicine, including a limited therapeutic window and initial infusion-related adverse effects such as nausea, vomiting, and anaphylactoid responses (51,52). Therefore, due to their anti-inflammatory and antioxidant qualities, Thai rice cultivars have been explored for the treatment of APAP at high doses (11,34).

A crucial aspect of APAP intoxication in this investigation is the elevation of liver enzyme function, serum inflammatory cytokine, and MDA levels. Blood interacts with all organs and tissues creating reactive species (43,53-55). Blood also includes oxidizable substrates and compounds like thiobarbituric acid-reactive species that indicate oxidative stress. The blood levels of these indicators reflect tissue changes. Mrakic-Sposta *et al.* also evaluated a human ROS production profile (43). We found that measuring ROS production in capillary blood can detect free radical-induced alterations in skeletal muscle, heart, and liver.

The findings demonstrated a decrease in these parameters after the administration of HN rice bran. In addition, mice fed 512 mg/kg HN rice bran had lower MDA levels than those of the mice in the APAP group. These findings support previous research showing that HN rice provides better overall antioxidant capacity protection against oxidative damage. Additionally, the studies using HN rice have demonstrated that flavonoids, by increasing SOD, CAT, and GPx activity and decreasing MDA levels, can enhance antioxidant capacity, promote nonspecific immunity, and minimize oxidative stress. The primary explanation for the impact is that anthocyanin, phenolic, and flavonoid compounds have the capacity to function as hydrogen donors and reducing agents, neutralizing ROS, and removing superoxide and hydrogen peroxide ions. Numerous studies have found that HN rice bran

has two main functions in lowering oxidative stress through (1) direct interaction with ROS and (2) augmentation of antioxidant enzyme activity (56-60).

The pro-inflammatory cytokines TNF-α and IL-6 play a crucial role in mediating inflammation activation and pathogenic progression in APAP-induced hepatotoxicity (6,61). The present investigation showed that the HN rice and NAC-treated groups exhibited reduced levels of TNF-α and IL-6, potentially signifying a decrease in hepatic cell death. Similarly, it has been indicated that eating HN rice reduces pro-inflammatory components, (11,36,60,62,63).

CONCLUSION

In summary, this study demonstrated how Thai rice bran cultivars' anti-inflammatory and antioxidant properties shielded mice against APAP-induced hepatotoxicity. Based on the findings, Thai black rice bran, or rice cultivars known as Hom Nil, could be an effective natural source of antioxidant and anti-inflammatory agents that can be used as an active natural pharmaceutical ingredient in functional foods and nutraceuticals, especially ones that promote liver protection.

Conflict of interest statement

All authors declared no conflict of interest in this study.

Authors' contributions

C. So-In made a substantial contribution to the concept and design of the article; P. Wanyo potentially interpreted the data and revised the article critically. The finalized article was approved by all authors.

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