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# Alteration of the Hepatic Antioxidative System and Cytochrome P450 Enzymes by Pineapple Soup in Rats with MCAO-Induced Ischemic Stroke

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ABSTRACT

Oxidative stress is a consequence of ischemic stroke caused by an imbalance between free radicals and the body's antioxidant defense system. This imbalance primarily affects the liver, which is highly susceptible to oxidative damage. This study investigated the effects of pineapple soup and bromelain on the hepatic antioxidant system in rats subjected to ischemic stroke induced by middle cerebral artery occlusion (MCAO). Adult male Wistar rats were orally administered pineapple soup (500, 1,000, and 1,500 mg/kg/day) or bromelain (250 mg/kg/day) once daily for two weeks. Ischemic stroke was subsequently induced using the MCAO method. Induction of ischemic stroke increased hepatic levels of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and lipid peroxidation while decreasing the ratio of reduced glutathione to oxidized glutathione (GSH/GSSG = 0.40±0.09). Activities of hepatic CYP1A2, CYP2B1, and CYP3A4 were also significantly reduced in MCAO rats. Pineapple soup normalized CYP1A2 and CYP2B1 activities, while CYP3A4 activity was markedly higher than in the sham-control. Both pineapple soup and bromelain significantly reduced SOD, CAT, GPX, and lipid peroxidation levels while increasing the GSH/GSSG ratio (0.43±0.06 to 0.56±0.02) and normalizing CYP activities in the liver of rats with ischemic stroke. The effects of pineapple soup were dose-dependent. Therefore, pineapple soup and bromelain show potential for mitigating acute oxidative stress in the liver of rats with MCAO-induced ischemic stroke.

***Keywords:*** Bromelain, Cytochrome P450, Ischemic Stroke, Lipid Peroxidation, Oxidative Stress, Pineapple soup.

## Introduction

Stroke is the second leading cause of mortality and disability globally, posing a significant public health challenge. Ischemic strokes, accounting for approximately 87% of all stroke cases, have increased notably between 1990 and 2016.[1] Most ischemic strokes occur in the middle cerebral artery territory,[2] making middle right cerebral artery occlusion (MCAO) a standard model for stroke research in animals.[3] Ischemic strokes result in neuronal injury due to inadequate blood supply, leading to the overproduction of reactive oxygen species (ROS) and oxidative stress throughout the body.[4]Cytochrome P450 (CYP) is a crucial enzyme in the initial metabolism of drugs and foreign substances (phase 1). It is widely distributed across various cell types, with the highest abundance in the liver. CYP activity is significantly affected by health conditions, medications, and dietary factors. Concurrent consumption of specific foods and drugs may increase the likelihood of interactions, potentially impacting drug effectiveness and altering their availability for therapeutic purposes.[5]

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There is a slight change in hepatic CYP enzyme activity during the acute phase of stroke. Therefore, the expression of CYP, which is highly influenced by disease conditions, might also be altered under stroke conditions.[6] Oxidative stress arises from an imbalance between free radicals and antioxidants, leading to tissue damage, including DNA and cell membrane impairment. This can have potential implications for various diseases, such as cardiovascular, neurodegenerative (e.g., Parkinson's, Alzheimer's), and organ degeneration.[7,8] Ischemic stroke-induced oxidative stress can trigger alterations in gene expression, particularly in genes controlling antioxidant enzyme production. Organs like the liver, kidneys, and heart are particularly susceptible to oxidative stress-induced free radical imbalances, intensifying the need for antioxidant defense mechanisms.[9,10] Pineapple (*Ananas cosmosus*) has been widely used as an edible fruit for a long period. Additionally, it has been used for the treatment of various health problems in traditional medicine.[11] The notable therapeutic properties of pineapple may be due to the complex natural mixture of proteolytic enzymes called bromelain. The well-known properties of bromelain include anti-inflammatory, anti-cancer, antithrombotic, and fibrinolytic effects, which could improve the circulatory system.[12] In recent years, pineapple has gained acceptability due to its long history of safe use and it has been used as an active ingredient in nutritional supplements. Nonetheless, bromelain and pineapple extract have been shown to inhibit CYP2C9 activity.[13] As the concept of "food is medicine" continues to gain popularity, food-based interventions are being adopted by people worldwide.[14] However, there is limited information on how bromelain and pineapple affect other CYP P450 enzymes, which could potentially lead to food-drug interactions.

This study focused on the influence of bromelain and pineapple soup on hepatic CYP profiles and oxidative stress in stroke conditions. The findings from this study could enhance our understanding of the complex nature of food-drug interactions and their

potential impact on oxidative stress for future stroke treatment. Furthermore, this study highlights the novel potential of bromelain and pineapple in mitigating acute oxidative stress in the liver of rats subjected to MCAO-induced ischemic stroke.

## Materials and Methods

*Study area*
The study was conducted at PANPB Research Group and HHP&HP Research Institute of Khon Kaen University, Thailand, from January 2021 to March 2023.

*Preparation of the pineapple soup*
Pineapple (*Ananas comosus* L., cultivar Sriracha) was planted in Nakhon Phanom Province, Thailand in July 2019 and the fruits were harvested in March 2021. The plant specimen (Voucher No. PANPB-AC2021-004) was deposited at the Research Group for Pharmaceutical Activities of Natural Products using Pharmaceutical Biotechnology (PANPB), Faculty of Pharmaceutical Sciences, Khon Kaen University. The pineapple soup was prepared by stir-frying all ingredients, including fresh pineapple (50%), oyster mushrooms (9%), shallots (3%), parsley (2%), garlic (2%), milk (15%), brown sugar (3%), salt (0.2%), pepper (0.15%), and soybean protein (1%). Drinking water (15%) was added to the mixture and stirred until it boiled. The pineapple soup was then ground using an electric grinder and dried in an oven at 50°C. The resulting powder was dissolved in distilled water before being fed to the rats.

*Animal treatment*
Male Wistar rats, aged 8-10 weeks and weighing 300-350 grams, were procured from Nomura Siam International Co., Ltd. (Thailand) and housed at NELAC, Khon Kaen University. The experimental protocol was approved by the IACUC of Khon Kaen University (Approval No. IACUC-KKU-95/63), following US-NIH Guidelines for the Care and Use of Laboratory Animals and the Declaration of Helsinki. The rats were kept in polycarbonate cages with sterile wood shaving bedding, supplied with commercial animal pellets, and maintained on a 12/12 light/dark cycle at a controlled temperature of 23±2°C and humidity ranging from 30 to 60% RH. This housing condition was maintained for 7 days before the commencement of the treatment. The rats were divided into 7 groups, with the number of rats ranging from 6 to 9 (n = 6-9 per group). Over the initial 2 weeks, the rats received oral administration of either distilled water (control), bromelain at a dosage of 250 mg/kg/day (B250), vitamin C at a dosage of 250 mg/kg/day (C250), or pineapple soup at doses of 500, 1,000, or 1,500 mg/kg/day (P500, P1000, or P1500). Each group received a maximum of 1.0 mL of the respective treatments orally at the same time every day for 14 days (D1-D14).

*Induction of right middle cerebral artery occlusion (MCAO)*
On D15 (day 15), the ischemia/reperfusion technique was used to induce focal cerebral ischemia. After administering anesthesia (thiopental 60 mg/kg), a nylon filament was inserted through the right common carotid artery into the internal carotid artery. Following a 90-minute period of occlusion, the filament was removed to provide reperfusion, and the incision was sealed with sutures.[3]

*Determination of protein content*
Liver homogenization was performed using a manual homogenizer in 0.01 M phosphate-buffered saline (PBS) at a ratio of 1 mg tissue to 3 mL buffer. The protein content in the liver homogenate was assessed using the Bradford assay. Protein quantification was conducted at a wavelength of 595 nm using an EnSight® Multimode plate reader (Shelton, CT, United States) and the concentration was determined using standard bovine serum albumin (BSA) ranging from 12.5 to 150 µg/mL.[15]

*Determination of catalase (CAT) activity*
After a 1-minute incubation of $H_2O_2$ and liver homogenate at 37°C, the reaction was halted by introducing ammonium molybdate. The absorbance was measured at 405 nm using an EnSight® Multimode plate reader (Shelton, CT, United States) to determine CAT activity compared with the standard CAT (2.5 units/µL).[15]

*Determination of superoxide dismutase (SOD) activity*
After treatment with chloroform and ethanol, the liver homogenate was centrifuged at 14,000×g using a Benchtop Thermo Scientific centrifuge (Waltham, Massachusetts, United States) for 30 minutes at 4°C. The supernatant was mixed with a reaction solution comprising 1.1 mM xanthine, 0.1 mM EDTA, 0.6 mM nitroblue tetrazolium, 56 mM $Na_2CO_3$, and 70 µg/mL BSA. This mixture was then supplemented with 0.1 mM $CuCl_2$ and allowed to stand at room temperature for 20 minutes. The absorbance was measured at 550 nm using an EnSight® Multimode plate reader (Shelton, CT, United States) to determine SOD activity using standard SOD (0.04 units/µL).[15]

*Determination of glutathione peroxidase (GPX)*
The liver homogenate was mixed with assay buffer (PBS, EDTA, sodium azide) and incubated at 30°C for 10 minutes. The enzymatic reaction was initiated by adding 0.7 mM reduced glutathione (GSH) and 1.2 mM $H_2O_2$. The reaction was subsequently terminated using sulfosalicylic acid and treated with 4-vinyl pyridine (4-VP) for 1 hour, following the addition of GPX standard, GSH, 6 units/mL glutathione reductase, 1.5 mg/mL 5,5'-dithiobis-2-nitrobenzoic acid, and 0.16 mg/mL NADPH. GPX activity was determined as the amount of enzyme required to generate 1 µmol of oxidized glutathione (GSSG) per minute.[15]

*Determination of malondialdehyde (MDA)*
The liver homogenate was mixed with 10% trichloroacetic acid (TCA; 1:1) and centrifuged at 3,000×*g* at 4°C for 10 minutes. The supernatant was added with 0.8% thiobarbituric acid (1:1) and heated to 100°C for 15 minutes. The reaction was stopped by rapidly cooling in an ice bath. The fluorescence intensity was measured at excitation/emission wavelengths of 520/590 nm using an EnSight® Multimode plate reader (Shelton, CT, United States) to determine MDA formation using a standard curve ranging from 5 to 40 µM.[15]

*Determination of total glutathione content*
Liver homogenates were subjected to mixing with sulfosalicylic acid before centrifugation. The supernatant was then used, complying with the previously described methodology, for the GPX assessment. The absorbance was measured at a wavelength of 405 nm. A slope of the standard GSH (6.25-50 mg/mL) was constructed to evaluate total glutathione content. The samples were incubated with 4-VP at room temperature for one hour, and the GSSG content was assessed similarly to the total glutathione content using a slope of standard GSSG (5-30 mg/mL). The GSH content was calculated by deducting the GSSG control from the total glutathione content.[15]

*Preparation of hepatic microsomes*
The liver homogenate was centrifuged at 10,000×g for 10 minutes at 4°C in 1.15% potassium chloride to obtain the microsomal fraction. Subsequently, the supernatant required ultracentrifugation at 104,000×*g* at 4°C for 60 minutes )Beckman Coulter XE ultracentrifuge, California, United States(. The microsomal protein content was determined using standard BSA.[16]

*Determination of hepatic alkoxyresorufin O-dealkylase activity*
The activity of hepatic alkoxyresorufin *O*-dealkylase was determined using the dealkylation of ethoxyresorufin, methoxyresorufin, and benzyloxyresorufin (EROD, MROD, and BROD, respectively). A 5 mg aliquot of hepatic microsomes was added to a mixture of Tris–HCl, NADPH, and either EROD, MROD, or BROD. Following incubation at 37°C, the generation of resorufin was promptly measured using a spectrofluorometer with excitation/emission wavelengths of 520/590 nm using an EnSight® Multimode plate reader (Shelton, CT, United States), compared to a resorufin standard.[16]

*Determination of aniline hydroxylase (ANH) activity*
ANH activity was measured using a mixture of 5 mM MgCl2, 1 mM NADPH, 15 mM aniline hydrochloride, 10 nM nicotinamide, 40 mM Tris–HCl (pH 7.8), and 10 mg hepatic microsomes. The reaction was allowed to proceed at 37°C for 20 minutes and was stopped by adding 10% TCA, followed by centrifugation at 1,000×g for 15 minutes. The supernatant was added to a mixture of 20% $Na_2CO_3$ and 4% phenol in 0.2 M NaOH. The absorbance was measured at 630 nm after a 30-minute incubation and compared to a p-aminophenol standard.[16]

*Determination of erythromycin N-demethylase (ENDM) activity*
ENDM activity was assessed by measuring the amount of formaldehyde generated in the Hantzsch reaction. The reaction mixture consisted of 1 mM NADPH, 15 mM $MgCl_2$, 1 mM erythromycin, 70 mM PBS (pH 7.4), and 15 mg of hepatic microsomes. The reaction was initiated by adding NADPH and incubating at 37°C for 20 minutes before termination with 12.5% TCA, followed by centrifugation at 1,000×g for 15 minutes. An aliquot of the supernatant was incubated with Nash reagent at 60°C for 15 minutes. The absorbance was measured at 405 nm compared to a formaldehyde standard.[16]

*Statistical analysis*
The results are expressed as mean ± standard deviation (SD) with n = 6-9 per group and were analyzed using one-way analysis of variance (ANOVA) coupled with Tukey's honest significance test using IBM SPSS software (version 23, Chicago, IL, USA). A *p*-value less than 0.05 was considered statistically significant.

## Results and Discussion

*The effects of pineapple soup on hepatic antioxidant enzymes*
Ischemic stroke is characterized by an imbalance between free radicals and the antioxidant system, resulting in oxidative stress due to the excessive generation of ROS, which surpasses the body's elimination capacity.[17] Oxidative stress can be evaluated by examining the genetic regulation of antioxidant enzymes in vital organs such as the liver, kidneys, and heart. The liver is particularly vulnerable to the consequences of oxidative stress.[9] This study employed MCAO induction to investigate the effects of pineapple soup and bromelain on the antioxidant system in rat livers under acute oxidative stress caused by ischemic stroke. In the MCAO rats, there were significant increases in CAT, SOD, and GPX levels (Fig. 1A-C). Bromelain (B250) and pineapple soup (P500-P1500) notably restored CAT activity to a level similar to the sham group ($p<0.05$ vs MCAO-control), while vitamin C (C250) did not achieve the same effect (Fig. 1A). For SOD activity, B250 and P500 normalized SOD levels, whereas C250 led to a slight reduction in SOD activity compared to the MCAO-control group ($p<0.05$). Interestingly, high doses of pineapple soup (P1000-P1500) significantly decreased SOD activity compared to the control group (Fig. 1B, $p<0.05$). All treatments significantly suppressed GPX activity compared to the sham or MCAO-control groups (Fig. 1C, $p<0.05$). Pineapple and bromelain were observed to enhance the activities of SOD, CAT, and GPX enzymes to levels adequate for scavenging free radicals generated after ischemic stroke induction. Consequently, a decrease in enzyme activities was noted, as elucidated in the research of Jebur et al.[18] where the administration of bromelain 250 mg/kg/day restored SOD, CAT, and GPX enzyme activities in the testes of Wistar rats subjected to oxidative stress induced by aluminum. This finding is consistent with earlier research by Mohamad et al.[19] which demonstrated that administering pineapple vinegar (2 mL/kg/day) ameliorated liver conditions in male mice exposed to paracetamol-induced toxicity, leading to a significant increase in SOD enzyme activity.

*The effects of pineapple soup on MDA level and glutathione profile*
Elevated hepatic glutathione synthesis has the potential to mitigate inflammation and oxidative stress following an acute ischemic stroke.[20] Oxidative stress, characterized by an increased generation of oxygen-free radicals, results in the production of lipid peroxides such as MDA. A significant increase in plasma MDA has been observed in stroke and transient ischemic attack patients.[21] Hepatic MDA levels were significantly increased in ischemic stroke-induced rats (MCAO-control) compared to the control (sham, $p<0.05$). Bromelain (250 mg/kg) returned lipid peroxidation to a level comparable to the control. Vitamin C (250 mg/kg) considerably lowered lipid peroxidation, compared to the control and the MCAO-control ($p<0.05$).

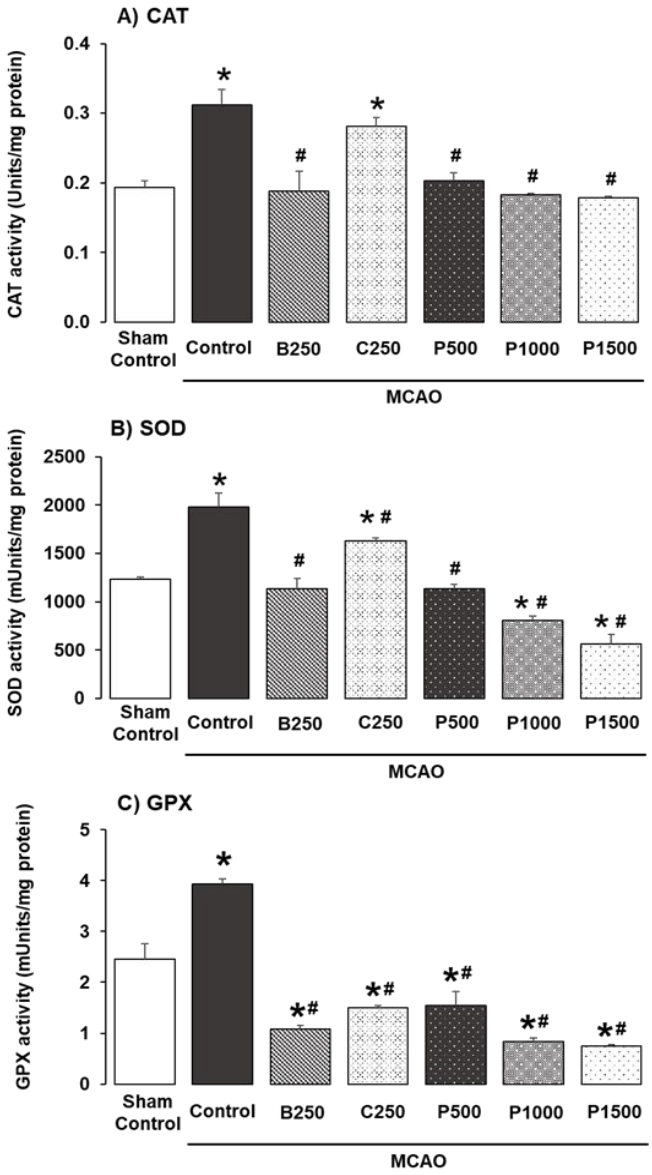


**Figure 1:** The effects of pineapple soup and bromelain on hepatic antioxidant enzymes. A) catalase (CAT); B) superoxide dismutase (SOD); C) Glutathione peroxidase (GPX).
The data are presented as mean ± SD (n=6-9). B250, bromelain 250 mg/kg/day; C250, vitamin C 250 mg/kg/day; P500, P1000, and P1500, pineapple soup 500, 1,000, and 1,500 mg/kg/day, respectively. A significant difference was determined by one-way ANOVA followed by Tukey's *post hoc* test. *$p<0.05$ vs Sham-Control; $^{\#}p<0.05$ vs MCAO-Control.

Pineapple soup (500 to 1,500 mg/kg) significantly reduced lipid peroxidation, compared to the MCAO-control ($p<0.05$); the highest dose markedly reduced lipid peroxidation to a level below the MCAO-control (Table 1). Total glutathione, reduced glutathione (GSH) and oxidized glutathione (GSSG) contents were all significantly increased in ischemic stroke-induced rats (MCAO-control) compared to the sham control. In MCAO rats receiving bromelain (250 mg/kg), total glutathione content was greater than the sham control ($p<0.05$), but lower than the MCAO-control ($p<0.05$, Table 1).

**Table 1:** The effect of pineapple soup and bromelain on malondialdehyde (MDA) level and glutathione profiles in the rat livers.

| | MDA | Glutathione (µmol/mg protein) | | | |
|---|---|---|---|---|---|
| | (nmol/mg protein) | Total GSH | Reduced GSH | Oxidized GSSG | GSH/GSSG ratio |
| **Sham-Control** | 0.126 ± 0.01 | 86.65 ± 6.51 | 24.41 ± 0.29 | 59.95 ± 0.92 | 0.53 ± 0.08 |
| **MCAO** | | | | | |
| - **Control** | 0.159 ± 0.04* | 135.76 ± 8.09* | 43.57 ± 0.75* | 91.75 ± 1.83* | 0.40 ± 0.09* |
| - **Bromelain 250** | 0.116 ± 0.02 | 101.93 ± 4.39*,# | 32.10 ± 0.30*,# | 71.87 ± 1.30*,# | 0.45 ± 0.04 |
| - **Vitamin C 250** | 0.102 ± 0.07*,# | 136.24 ± 6.15* | 32.11 ± 0.44*,# | 103.05 ± 6.40*,# | 0.43 ± 0.06 |
| - **Pineapple soup 500** | 0.131 ± 0.03# | 91.73 ± 3.92# | 30.55 ± 0.46# | 63.11 ± 3.54*,# | 0.45 ± 0.11 |
| - **Pineapple soup 1000** | 0.112 ± 0.01# | 82.36 ± 4.06# | 26.08 ± 0.26# | 56.28 ± 2.03*,# | 0.46 ± 0.04 |
| - **Pineapple soup 1500** | 0.106 ± 0.08*,# | 82.11 ± 3.74# | 29.99 ± 0.20# | 53.58 ± 1.28*,# | 0.56 ± 0.02 |

***Note.*** The data are presented as mean±SD (n=6). Bromelain 250, bromelain 250 mg/kg/day; Vitamin C 250, vitamin C 250 mg/kg/day; Pineapple soup 500, 1000, and 1500, pineapple soup 500, 1,000, and 1,500 mg/kg/day, respectively. A significant difference was determined by one-way ANOVA followed by Tukey's *post hoc* test. *$p$<0.05 vs Sham-Control; #$p$<0.05 vs MCAO-Control.

In contrast, vitamin C (250 mg/kg) did not reduce total glutathione content. For MCAO rats receiving pineapple soup at all doses, there was a marked decrease in total glutathione content compared to the MCAO-control. For reduced glutathione, MCAO rats receiving pineapple soup had markedly lower GSH contents compared to the MCAO-control, whereas the bromelain and vitamin C treated MCAO rats did not (Table 1). For oxidized glutathione, MCAO rats receiving bromelain and all doses of pineapple soup had considerably lower GSSG contents than the MCAO-control ($p$<0.05), even though the GSSG content remained higher than the sham control ($p$<0.05). In contrast, vitamin C did not reduce GSSG content in MCAO rats.

The MCAO-control rats displayed a lower GSH/GSSG ratio than the control ($p$<0.05). In contrast, rats receiving bromelain and pineapple soups exhibited a higher GSH/GSSG ratio compared to the MCAO-control, while those receiving vitamin C did not. Notably, the highest dose of pineapple soup (1,500 mg/kg) slightly elevated the GSH/GSSG ratio compared to the MCAO-control. These findings are consistent with a previous report by Seenak et al.[22] demonstrating that pineapple (100 to 200 mg/kg/day) reduced lipid peroxidation in the hearts of male Sprague-Dawley rats fed high-cholesterol diets. Similarly, pineapple vinegar (2 mL/kg/day) reduced lipid peroxidation and restored the hepatic glutathione profile in rats exposed to paracetamol.[19]

*The effects of pineapple soup on hepatic CYP activity*

The group of hepatic monooxygenase enzymes known as CYPs, including CYP1A, CYP2B, CYP2E1, and CYP3A4, plays an essential role in the oxidation of over 50% of pharmaceutical drugs.[23] ROS are involved in various diseases, including ischemic stroke, and can be generated by CYPs or biotransformation pathways during various physiological processes. Metabolizing enzymes are pivotal in upholding the redox balance and preventing the onset of oxidative stress.[24,25]A shown in Table 2, MROD, BROD, and ENDM activities were significantly suppressed in the MCAO-control group ($p$<0.05). EROD and ANH activities exhibited only marginal changes. Bromelain (250 mg/kg) restored ENDM to normal levels, though other CYP enzyme activities were not comparably affected. In contrast, vitamin C (250 mg/kg) restored all CYP enzyme activities to the levels observed in the sham-control. Pineapple soup at all doses normalized MROD and BROD activities, but significantly increased ENDM activity compared to the sham-control ($p$<0.05). Pineapple has been shown to enhance EROD and ENDM activities in hepatic mice CYPs ($p$<0.05), while concurrently lowering MROD activities.[26] As a result, the potential for drug interactions involving pineapple soup through ENDM induction should be considered.

## Conclusion

Pineapple soup (500 to 1,500 mg/kg) and bromelain (250 mg/kg) significantly reduced SOD, CAT, GPX, and lipid peroxidation levels while improving the GSH/GSSG ratio and restoring the activities of hepatic CYP enzymes in rats with MCAO-induced ischemic stroke. Notably, the activities of hepatic CYP1A2, CYP2B1, and CYP3A4 were markedly reduced in MCAO rats. Pineapple soup normalized CYP1A2 and CYP2B1 activities to the baseline levels, yet exhibited a noticeable increase in CYP3A4 activity, compared to the control. Therefore, pineapple soup is a potential candidate for inclusion in targeted health supplements for stroke patients to mitigate hepatic oxidative stress. Nevertheless, caution is advised due to the potential of pineapple soup to induce CYP3A4, which could lead to drug interactions with concomitant medications. Since consuming large amounts of pineapple soup and bromelain may also cause undesirable effects in some individuals, the acute and chronic toxicity profiles should be considered. Clinical trials to assess the efficacy and safety of pineapple soup and bromelain in stroke patients and their impact on CYP3A4 activity would help guide safe usage, benefit broader liver health applications, and reveal underlying protective mechanisms.

**Table 2:** The effect of pineapple soup and bromelain on cytochrome P450 activities in the rat livers

| | Cytochrome P450 activity (pmol/min/mg protein) | | | | |
|---|---|---|---|---|---|
| | **CYP1A1 (EROD)** | **CYP1A2 (MROD)** | **CYP2B1 (BROD)** | **CYP2E1 (ANH)** | **CYP3A4 (ENDM)** |
| **Sham Control** | 12.90 ± 2.37 | 22.04 ± 0.80 | 23.72 ± 2.61 | 650.00 ± 62.50 | 331.37 ± 30.22 |
| **MCAO** | | | | | |
| - **Control** | 8.60 ± 0.13 | 10.44 ± 2.94* | 10.76 ± 2.25* | 572.92 ± 30.90 | 262.75 ± 28.11 |
| - **Bromelain 250** | 9.32 ±1.71 | 11.06 ± 1.50* | 13.35 ± 2.62* | 614.58 ± 55.90 | 311.76 ± 33.25# |
| - **Vitamin C 250** | 12.76 ±0.88# | 15.46 ± 3.09 | 21.50 ± 3.78# | 606.25 ± 60.02 | 313.92 ± 27.20# |
| - **Pineapple soup 500** | 10.05 ± 1.19 | 15.72 ± 0.45 | 16.39 ± 4.30* | 597.92 ± 20.83 | 73.86 ± 39.62*# |
| - **Pineapple soup 1000** | 11.27 ± 3.78 | 17.58 ± 4.48 | 19.52 ± 3.18# | 631.25 ± 47.96 | 388.24 ± 18.86*# |
| - **Pineapple soup 1500** | 14.09 ± 1.29# | 19.14 ± 3.30# | 21.34 ± 3.73# | 652.08 ± 78.78 | 390.20 ± 52.80*# |

***Note.*** The data are presented as mean±SD (n=6). EROD, ethoxyresorufin *O*-deethylation; MROD, methoxyresorufin *O*-demethylation; BROD, benzyloxyresorufin *O*-dealkylation; ANH, aniline hydroxylation; ENDM, erythromycin *N*-demethylation. Bromelain 250, bromelain 250 mg/kg/day; Vitamin C 250, vitamin C 250 mg/kg/day; Pineapple soup 500, 1000, and 1500, pineapple soup 500, 1,000, and 1,500 mg/kg/day, respectively. A significant difference was determined by one-way ANOVA followed by Tukey's *post hoc* test. *$p$<0.05 vs Sham-Control; #$p$<0.05 vs MCAO-Control.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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## References

1. Kuriakose D, Xiao Z. Pathophysiology and treatment of stroke: present status and future perspectives. Int J Mol Sci. 2020; 21:7609. DOI: 10.3390/ijms21207609
2. Abdu H, Tadese F, Seyoum G. Comparison of ischemic and hemorrhagic stroke in the medical ward of Dessie Referral Hospital, Northeast Ethiopia: a retrospective study. Neurol Res Int. 2021; 2021:1-9. DOI: 10.1155/2021/9996958
3. Palachai N, Wattanathorn J, Muchimapura S, Thukhammee W. Phytosome loading the combined extract of mulberry fruit and ginger protects against cerebral ischemia in metabolic syndrome rats. Oxid Med Cell Longev. 2020; 2020:1-15. DOI: 10.1155/2020/5305437
4. Orellana-Urzúa S, Rojas I, Líbano L, Rodrigo R. Pathophysiology of ischemic stroke: role of oxidative stress. Curr Pharm Des. 2020; 26:4246-4260. DOI: 10.2174/1381612826666200708133912
5. Zhao M, Ma J, Li M, Zhang Y, Jiang B, Zhao X, Huai C, Shen L, Zhang N, He L, Qin S. Cytochrome P450 enzymes and drug metabolism in humans. Int J Mol Sci. 2021; 22:12808. DOI: 10.3390/ijms222312808
6. Muscari A, Collini A, Fabbri E, Giovagnoli M, Napoli C, Rossi V, Vizioli L, Bonfiglioli A, Magalotti D, Puddu GM, Zoli M. Changes of liver enzymes and bilirubin during ischemic stroke: mechanisms and possible significance. BMC Neurol. 2014; 14:1-8. DOI: 10.1186/1471-2377-14-122
7. Dorszewska J, Kowalska M, Prendecki M, Piekut T, Kozłowska J, Kozubski W. Oxidative stress factors in Parkinson's disease. Neural Regen Res. 2021; 16:1383-1391. DOI: 10.4103/1673-5374.300980
8. Teleanu DM, Niculescu A-G, Lungu II, Radu CI, Vladâcenco O, Roza E, Costachescu B, Grumezescu AM, Teleanu RI. An overview of oxidative stress, neuroinflammation, and neurodegenerative diseases. Int J Mol Sci. 2022; 23:5938. DOI: 10.3390/ijms23115938
9. Maritim A, Dene BA, Sanders RA, Watkins III JB. Effects of $\beta$-carotene on oxidative stress in normal and diabetic rats. J Biochem Mol Toxicol. 2002; 16:203-208. DOI: 10.1002/jbt.10038
10. Su XT, Wang L, Ma SM, Cao Y, Yang NN, Lin LL, FisherM, Yang JW, Liu CZ. Mechanisms of acupuncture in the regulation of oxidative stress in treating ischemic stroke. Oxid Med Cell Longev. 2020; 2020:7875396. DOI: 10.1155/2020/7875396
11. Debnath B, Singh WS, Manna K. A phytopharmacological review on Ananas comosus. Adv Tradit Med. 2023; 23:291-298. DOI:10.1007/s13596-021-00563-w
12. Hikisz P, Bernasinska-Slomczewska J. Beneficial properties of bromelain. Nutrients. 2021;13:4313. DOI: 10.3390/nu13124313
13. Muneaki H, Masashi N, Yohei K, Hiroshi S, Hirofumi KAI, Keishi Y, Manabu O. Inhibitory effects of fruit juices on cytochrome P450 2C9 activity in vitro. Biosci Biotechnol Biochem. 2008; 72:406-411. DOI: 10.1271/bbb.70511
14. Downer S, Berkowitz SA, Harlan TS, Olstad DL,

Mozaffarian D. Food is medicine: actions to integrate food 10.1136/bmj.m2482
15. Sriset Y, Sukkasem N, Chatuphonprasert W, Jarukamjorn K. Nephroprotective effects of hesperidin and myricetin against high-fat diet plus ethanol-induced renal oxidative damage in mice. Rev Bras Farmacogn. 2022; 32:555-562. DOI: 10.1007/s43450-022-00275-5
16. Nopwinyoowong N, Chatuphonprasert W, Tatiya-Aphiradee N, Jarukamjorn K. *Garcinia mangostana* and α-mangostinrRevive ulcerative colitis-modified hepatic cytochrome P450 profiles in mice. Pak J Biol Sci. 2022; 25:843-851. DOI: 10.3923/pjbs.2022.843.851
17. Chiang T, Messing RO, Chou WH. Mouse model of middle cerebral artery occlusion. JVis Exp. 2011; e2761. DOI: 10.3791/2761
18. Jebur AB, El-Demerdash FM, Kang W. Bromelain from *Ananas comosus* stem attenuates oxidative toxicity and testicular dysfunction caused by aluminum in rats. J Trace Elem Med Biol. 2020; 62:126631. DOI: 10.1016/j.jtemb.2020.126631
19. Mohamad NE, Yeap SK, Lim KL, Yusof HM, Beh BK, Tan SW, Ho WY, Sharifuddin SA, Jamaluddin A, Long K, Rahman NMAN, Alitheen NB. Antioxidant effects of pineapple vinegar in reversing of paracetamol-induced liver damage in mice. Chin Med. 2015; 10:1-10. DOI: 10.1186/s13020-015-0030-4
20. Inderhees J, Schwaninger M. Liver metabolism in ischemic stroke. Neurosci. 2024; 550:62-68. DOI: and nutrition into healthcare. BMJ. 2020; 369. DOI: 10.1016/j.neuroscience.2023.12.013
21. Kaur J, Arora S, Singh B, Thakur LC, Gambhir J, Prabhu KM. Role of oxidative stress in pathophysiology of transient ischemic attack and stroke. Int J Biol Med Res. 2011; 2:611-615.
22. Seenak P, Kumphune S, Malakul W, Chotima R, Nernpermpisooth N. Pineapple consumption reduced cardiac oxidative stress and inflammation in high cholesterol diet-fed rats. Nutr Metab (Lond). 2021; 18:1-10. DOI: 10.1186/s12986-021-00566-z
23. Zanger UM, Schwab M. Cytochrome P450 enzymes in drug metabolism: Regulation of gene expression, enzyme activities, and impact of genetic variation. Pharmacol Ther. 2013;138:103-141. DOI: 10.1016/j.pharmthera.2012.12.007
24. Orhan H, Karakuş F, Ergüç A. Mitochondrial biotransformation of drugs and other xenobiotics. Curr Drug Metab. 2021;22:657-669. DOI: 10.2174/1389200222666210628125020
25. Veith A, Moorthy B. Role of cytochrome P450s in the generation and metabolism of reactive oxygen species. Curr Opin Toxicol. 2018;7:44-51. DOI: 10.1016/j.cotox.2017.10.003
26. Chatuphonprasert W, Jarukamjorn K. Impact of six fruits-banana, guava, mangosteen, pineapple, ripe mango and ripe papaya-on murine hepatic cytochrome P450 activities. J Appl Toxicol. 2012;32:994-1001. DOI: 10.1002/jat.2740