Research Article

BIOMEDICAL Journal of Scientific & Technical Research

ISSN: 2574 -1241

DOI: 10.26717/BJSTR.2024.56.008865

Traditional Chinese Herbal Drinks Improved Liver Injury by Increasing Antioxidant Capacity

Jian-Xin Wang[1], Yung Kai Lin[2], Yung Hsiang Lin[3] and Chi Fu Chiang[3]*

[1]*Shanxi Agricultural Valley BaoRenTang Food Co., Ltd. Taiwan*

[2]*Institute of Food Safety and Risk Management, National Taiwan Ocean University, Department of Food Science, National Taiwan Ocean University, Graduate Institute of Biomedical Engineering, National Chung Hsing University, Taiwan*

[3]*Research & Design Center, TCI CO., Ltd., Taiwan*

***Corresponding author:** Chi-Fu Chiang, Research & Design Center, TCI CO., Ltd., 11F, No.187, Gangqian Rd., Neihu Dist., Taipei City 114, Taiwan

ARTICLE INFO

Received: April 12, 2024

Published: May 08, 2024

Citation: Jian-Xin Wang, Yung Kai Lin, Yung Hsiang Lin and Chi Fu Chiang. Traditional Chinese Herbal Drinks Improved Liver Injury by Increasing Antioxidant Capacity. Biomed J Sci & Tech Res 56(3)-2024. BJSTR. MS.ID.008865.

ABSTRACT

The global prevalence of liver diseases remains persistently high, prompting the biopharmaceutical industry to actively focus on developing effective hepatoprotective agents or health supplements. Past research has indicated that traditional Chinese herbal medicine possesses hepatoprotective properties. This study primarily focuses on the development of a traditional Chinese herbal medicine drink with hepatoprotective functions, supported by scientific experiments to validate the efficacy of the product. This study investigates the antioxidant and hepatoprotective effects of Chinese herbal drinks, which include *Imperata cylindrica, Orange peel, Polygonati Rhizoma, Kudzu root, Myristica fragrans, Japanese raisin tree (Hovenia dulcis Thunb.)*, and Fructus mume. The results showed Chinese herbal drinks can increase superoxide dismutase (SOD), catalase (CAT), and decrease glutamyl pyrubic transaminase (GPT), ROS expression compared to H_2O_2 group *in vitro.* Chinese herbal drinks can prevent CCL4-induced acute liver injury in animal model. Chinese herbal drinks, with their demonstrated hepatoprotective effects, can indeed be considered as a traditional Chinese medicine for liver protection.

Keywords: Acute Liver Injury; Chinese Herbal; Oxidative Stress; Glutamyl Pyrubic Transaminase

Abbreviations: SOD: Superoxide Dismutase; CAT: Catalase; GPT: Glutamyl Pyrubic Transaminase; ROS: Reactive Oxygen Species; ELISA: Enzyme-Linked Immunosorbent Assay; DCF: ALT: Alanine Aminotransferase; GSH: Glutathione; GST: Glutathione–S–Transferase

Introduction

As one of the most vital organs in the human body, the liver plays various physiological roles, including metabolism, excretion, synthesis, and storage [1]. Acute liver injury refers to a rapid decline in liver function within a short period. It can be caused by various factors such as drug toxicity, viral infections, and alcohol abuse [2]. Oxidation is the process wherein molecules lose electrons, generating reactive oxygen species (ROS) with high reactivity that can damage cell structures and functions [3]. During acute liver injury, oxidative reactions are often accelerated, increasing intracellular oxidative stress [4]. The antioxidant defense system includes a series of enzymes and molecules that neutralize and scavenge reactive oxygen species and maintain the oxidative balance within cells [5]. In addition, oxidative stress may also activate inflammatory responses and aggravate the degree of liver damage [6]. Therefore, understanding the interrelationship between acute liver injury and oxidation is essential for developing preventive and therapeutic strategies. Some studies have shown that the extent of acute liver injury can be reduced by enhancing the activity of the antioxidant defense system [7]. Certain herbal extracts have shown potential effects in mitigating acute liver injury.

The homology of medicine and food refers to some Chinese herbal medicines with medicinal value closely related to their edible ingredients. Many foods considered medicinal value are also rich in antioxidants, such as vitamin C, E, polyphenols, flavonoids, etc [8]. These ingredients help neutralize free radicals, slow down the damage to the liver caused by oxidative stress, and play a liver-protective antioxidant role [9]. Some Chinese herbal medicines are used in traditional Chinese medicine to remove toxins from the body, regulate liver function, promote liver cell repair and regeneration, and also help strengthen the immune system, reduce chronic inflammation, and play a liver-protective role [10]. Chinese herbal medicines such as *Imperata cylindrica*, *Orange peel*, *Polygonati Rhizoma*, *Kudzu root*, *Myristica fragrans, Japanese raisin tree (Hovenia dulcis Thunb.)*, *Fructus mume*, are rich in natural antioxidants, such as polyphenols, flavonoids, vitamins, which can neutralize and eliminate antioxidants in the body. *Imperata cylindrica* [11], *Orange peel* [12], *Polygonati Rhizoma* [13], *Kudzu root* [14] can reduce oxidative damage. *Myristica fragrans* are thought to have anti-inflammatory and soothing effects on liver discomfort [15].

Fructus mume and Japanese raisin tree have a protective effect on the liver and can promote the repair and regeneration of liver cells [16]. *Imperata cylindrica* and *orange peel* help eliminate harmful substances from the body and are helpful for the liver's detoxification function [17]. *Kudzu root* is considered to have the effect of regulating blood lipids, helping to reduce the accumulation of fat in the liver. It may have specific benefits for fatty liver disease [14]. This study explores whether Chinese herbal drinks (including *Imperata cylindrica, Orange peel*, *Polygonati Rhizoma*, *Kudzu root*, *Myristica fragrans*, *Japanese raisin tree (Hovenia dulcis Thunb.)*, *Fructus mume* can improve liver injury. First, using H_2O_2-induced oxidative stress in HepG2 cells, Chinese herbal drinks were added, and antioxidant capacity was analyzed. Second, using carbon tetrachloride (CCL4) to induce liver injury in animal models, and using different doses of Chinese herbal drinks to treat liver injury.

Material and Methods

Cell Culture

Cell culture Human hepatoblastoma cell line HepG2 was cultured in Dulbecco's modified Eagle medium (DMEM; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) containing 10% fetal bovine serum (Sigma-Aldrich; Merck KGaA), 100 U/ml penicillin and 100 µg/ml streptomycin and placed in a cell culture chamber at 37°C and under a humidified atmosphere with 5% CO_2. After the cells adhered and were grown to 80% confluence, the culture medium was removed, washed with the appropriate amount of PBS, and digested with 0.25% trypsin (Sigma-Aldrich; Merck KGaA). Cells in the logarithmic phase of growth were selected for the following experiments.

Supplement Formulation

Chinese herbal drinks (Commercially name: BaoRenTang Raisin Tree Hydrosol) included *Imperata cylindrica, Orange peel*, *Polygonati Rhizoma*, *Kudzu root*, *Myristica fragrans*, *Japanese raisin tree*, *Fructus mume*, citric acid, water.

Quantification of Gene Expressions by Real-Time PCR

0.25% Chinese herbal drinks treated HepG2 cells for 24h, then cells were harvested, and total RNA was isolated from cells using an RNA purification kit (Geneaid, Taiwan). DNA-free total RNA was reversely transcribed to cDNA using a SuperScript™ Reverse Transcriptase kit (Invitrogen, Life Technologies Co., CA, USA). Quantitative real-time PCR was conducted using an ABI StepOnePlus™ Real-Time PCR System (Thermo Fisher Scientific, Inc., CA, USA) and the SYBR Green Master Mix (KAPA Biosystems, MA, USA) for transcript measurements. The gene-specific primers used in this study, human SOD1, forward 5'-ACTGGTGGTCCATGAAAAAGC-3' and reverse 5'-AACGACTTCCAGCGTTTCCT-3' human catalase, forward 5'-GCTCTTCTGGACAAGTACAATGCTG-3' and reverse 5'-TTACACGGATGAACGCTAAGCTTC-3'. The GAPDH gene was used as a normalization control.

Determination of Glutamate Pyruvate Transaminase

The GPT levels were determined using GPT enzyme-linked immunosorbent assay (ELISA) kits (Asan Pharmaceutical, Seoul, Korea) in accordance with the manufacturer's instructions.

Measurement of Intracellular ROS

The assay is based on the use of an established non-fluorescent (H2DCFDA)/fluorescent (DCF) system that measures ROS activity within the cell. HepG2 cells were seeded into 24-well plates (4×10^4 cells/well) and allowed to attach overnight. Cells were incubated in a serum-free medium in the presence of various concentrations of CF extract (100–250 µg/mL) after UVB radiation or CML treatment. The cells were washed with PBS, and the ROS detection reagent DCFDA was added to each well for 30 min. Images were captured using a fluorescence microscope (Leica DM IL LED, Leica Microsystems, Wetzlar, Germany), and the fluorescence (excitation/emission: 488 nm/520 nm) were measured using a microplate reader (Synergy HTX, BioTek Instruments, Winooski, VT, USA)

Animal Model

The procedures for this study were approved by PEKING UNIVERSITY BESCHOLOR. All animal experiments in this study were conducted by the ARRIVE guidelines for reporting experiments involving animals. All methods were carried out by relevant guidelines and regulations. The sixty Kunming mice (male n=30, female n=30), weighing 18-22 g, were purchased from the SPF(Beijing)BIOTECHNOLOGY Co., Ltd. All mice were housed under the controlled temperature (22–23 °C) and on a 12 h light and 12 h dark cycle with food and water ad libitum. All animals were adapted to their new housing conditions for one week before the experiments. The mice were randomly divided into 6 groups (n = 10 in each group) as follows: control group, CCL4-treated model group, Bicyclol-treated group (200mg/kg), high

(6g/kg), medium (3g/kg), and low (1.5g/kg) dose of Chinese herbal drinks co-treated group. The control group and CCL4-treated model group were administered corresponding volumes of distilled water. After the 6th day of administration, except for the control group, the mice in the other groups were intraperitoneally injected with 0.12% CCL4 corn oil solution at 0.1mL/10g, and the control group was given saline. Administration was carried out 15h later, and blood was collected from the orbit 1h later, centrifuged at 3000 rpm for 15 minutes, and the supernatant was taken to measure the serum ALT and AST.

Statistical Analysis

Data are expressed as mean ± standard deviation. Homogeneity of variance uses F-test, and comparison between groups uses t-test or ANOVA, as $p < 0.05$ was considered statistical significance

Results

Chinese Herbal Drinks Have Antioxidant Capabilities

First, explore whether Chinese herbal drinks have antioxidant capabilities, using H_2O_2-induced oxidative stress in HepG2 cells for 6h, then 0.25% Chinese herbal drinks to treat cells for 24h. The results showed that Chinese herbal drinks can significantly increase superoxide dismutase (SOD) by 1.1 times and catalase (CAT) by 2 times, and significantly decrease ROS expression compared to H_2O_2 group (Figures 1A & 1B). The glutamyl pyrubic transaminase (GPT), also known as, alanine aminotransferase (ALT), is a vital test item for health examination and an indicator of liver damage. Chinese herbal drinks can significantly decrease GPT compared to H_2O_2 group (Figure 1C). The above results showed that Chinese herbal drinks can increase antioxidant gene expression and reduce GPT.

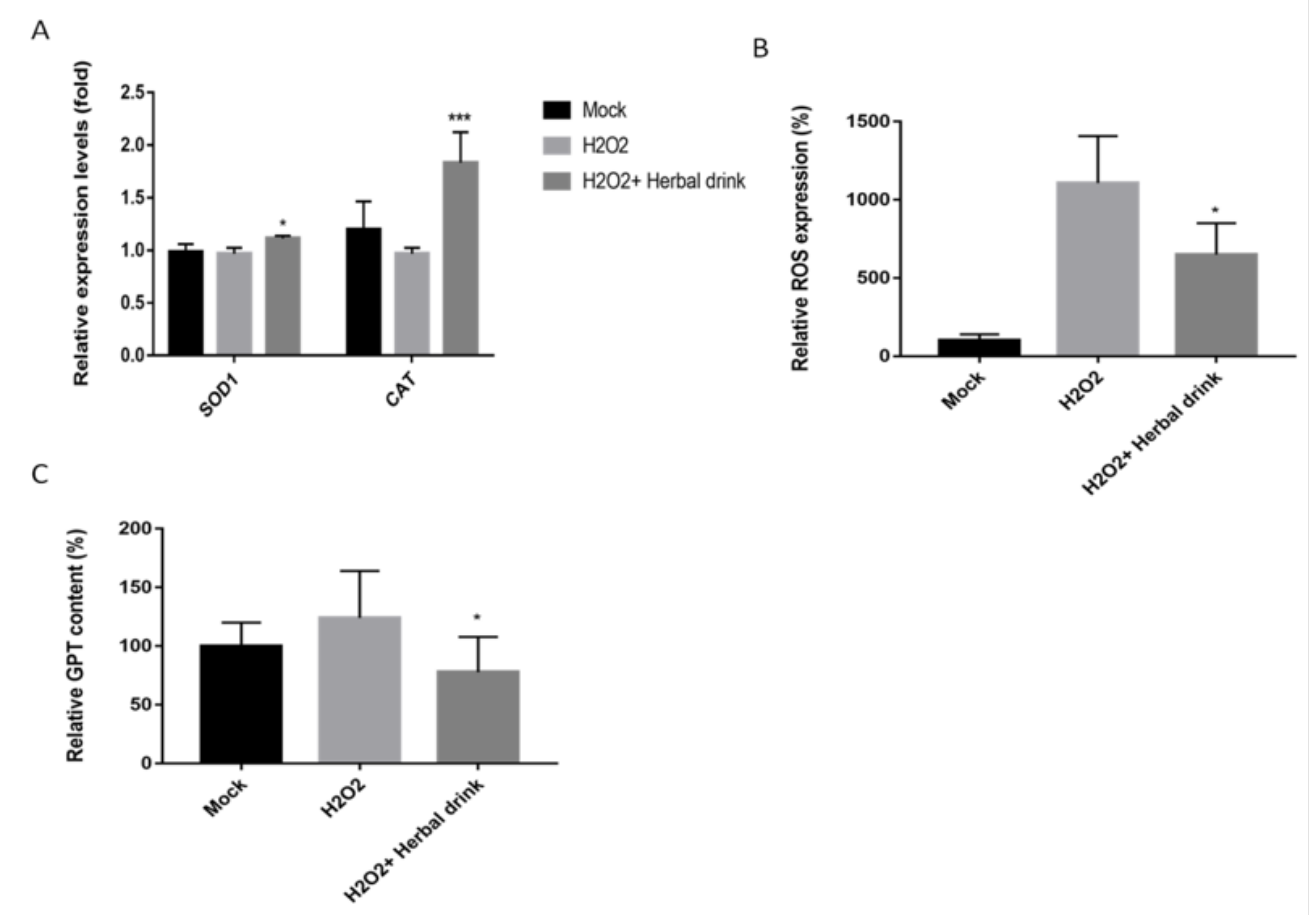

Figure 1: Chinese herbal drinks have antioxidant capabilities. Using H_2O_2-induced oxidative stress in HepG2 cells for 6h, then 0.25% Chinese herbal drinks to treat cells for 24h, and then examined (A) Superoxide dismutase (SOD), catalase (CAT) by real-time quantitative polymerase, (B) ROS level, (C) Glutamyl pyrubic transaminase (GPT). Significantly different from the H2O2 group : *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Chinese Herbal Drinks Prevent CCL4-Induced Acute Liver Injury in Mice

Table 1 showed that serum ALT/AST was significantly increased in the CCL4-treated model group compared with the control group. Serum ALT/AST was significantly decreased in the Bicyclol-treated group compared with the CCL4-treated model group. Serum ALT/AST was significantly decreased in the high (6g/kg), medium (3g/kg), and low (1.5g/kg) doses of Chinese herbal drinks groups compared to the CCL4-treated model group. The above results showed that Chinese herbal drinks can prevent CCL4-induced acute liver injury.

Table 1: Evaluation of Chinese herbal drinks as indicators of liver injury.

Group	AST(U/L)	ALT(U/L)
Control group	46.31±4.08	34.78±3.05
CCL4-treated model group	391.96±13.92**	539.28±29.81**
Bicyclol-treated group	68.88±3.30##	45.32±7.07##
High dose of Chinese herbal drinks	105.53±3.28##	56.30±10.64##
Medium dose of Chinese herbal drinks	123.79±2.50##	143.23±16.68##
Low dose of Chinese herbal drinks	327.35±29.12##	203.19±15.90##

Note: ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

Compared with control group, *P<0.05, **P<0.01.

Compared with CCL4-treated model group, #P<0.05, ##P<0.01.

Discussion

This study found that Chinese herbal drinks, including *Imperata cylindrica*, *Orange peel*, *Polygonati Rhizoma*, *Kudzu root*, *Myristica fragrans*, *Japanese raisin tree*, and *Fructus mume*, can increase antioxidant capacity *in vitro* and improve liver injury *in vivo*. Traditional Chinese herbs are typically composed of a combination of multiple compounds rather than a single chemical entity. This comprehensive effect allows Chinese herbs to impact the liver in various ways, including antioxidative, anti-inflammatory, and detoxifying effects, contributing to maintaining normal liver function [18]. Chinese herbs are generally considered safe and suitable for long-term use [19]. In comparison to certain Western medications that may entail side effects, Chinese herbs are often perceived as gentler on the body. Imperata cylindrica, belonging to the Gramineae family, is extensively distributed in southwestern Asia and is specifically indigenous to tropical and subtropical zones [20].

In traditional Chinese medicine, the dried rhizomes of Imperata cylindrica, known as "Bai mao gen," have been widely utilized [20]. Research indicates that Imperata cylindrica exhibits notable hydroxyl radical scavenging activity, with an IC50 of 0.0948 mg/mL, surpassing the efficacy of ascorbic acid (IC50: 0.1096 mg/mL) based on Fenton and potassium ferricyanide reduction methods [21]. Furthermore, studies have demonstrated that Imperata cylindrica extract enhances superoxide dismutase (SOD) activity in liver and brain tissues, inhibits hydroxyl free radicals, and reduces malondialdehyde levels [20]. Bioactive compounds such as protocatechuic acid, syringic acid, and catenarin contribute to its free radical scavenging properties. Orange peel, a primary byproduct in orange juice production, contains flavonoids associated with antioxidant activity [22]. Hesperidin and naringin glycosides play a vital role in the purported antioxidant effects of citrus peel extracts [23]. Rat studies indicate that hesperidin supplementation increases SOD, glutathione–S–transferase (GST), and glutathione (GSH) levels, offering protection against CCl4-induced liver injury [24].

Polygonati rhizoma (Huangjing) is a dual-purpose material in traditional Chinese medicine, containing various bioactive substances such as purine nucleosides, carbohydrates, bioflavonoids, alkaloids, saponins, lignins, amino acids, peptides, anthraquinones, cardiac glycosides, vitamins, and acids [25]. Polysaccharides in Polygonati Rhizoma contribute to its sweet taste and demonstrate metabolic regulation, immunomodulation, anti-aging, anti-inflammatory, and liver-protective effects [13]. Kudzu root (Gegen) from Pueraria lobata exhibits antioxidant and anti-inflammatory effects attributed to active ingredients like puerarin, baicalin, berberine, and glycyrrhizin [26]. Myristica fragrans Houtt (nutmeg) exhibits anti-inflammatory, antioxidant, and antimicrobial effects, regulating gut microbes and metabolites to attenuate non-alcoholic fatty liver disease in mice [27]. Hovenia dulcis, a traditional folk medicine, demonstrates antioxidant, immunostimulatory, anti-diabetic, and hepatic protective effects [28]. Fructus mume derived from Prunus mume Sieb. Et Zucc contains various components, including organic acids, exhibiting antioxidant and antibacterial properties [29]. The combined formula of Imperata cylindrica, Orange peel, Polygonati Rhizoma, Kudzu root, Myristica fragrans, Hovenia dulcis, and Fructus mume shows promising potential in preventing CCl4-induced acute liver injury. However, the specific mechanisms of this herbal combination require further research for clarification.

Limitations of this study include the fact that CCL4-induced toxicity depends on Cyp-induced CCL3 free radical formation. However, whether Chinese herbal drinks can regulate CCL3 requires more research to confirm. There is also a lack of genetic changes in cells treated with Chinese herbal drinks alone. The future applications of traditional Chinese herbal medicine in improving and safeguarding liver health may focus on clinical use and molecular mechanism research. Through further clinical trials, the actual effects of Chinese herbal medicine on human liver health, including the impact of supplements or formulations on liver function, enzyme levels, and tissue integrity, can be substantiated. Simultaneously, an in-depth exploration of the molecular mechanisms underlying the actions of Chinese herbal medicine can unveil its therapeutic mechanisms, providing concrete insights into potential targets and facilitating the development of more effective herbal treatment strategies.

References

1. Mitra V, Metcalf J (2009) Metabolic functions of the liver. Anaesthesia & Intensive Care Medicine 10(7): 334-335.
2. Yuan R, Tao X, Liang S, Pan Y, He L, et al. (2018) Protective effect of acidic polysaccharide from Schisandra chinensis on acute ethanol-induced liver injury through reducing CYP2E1-dependent oxidative stress. Biomed Pharmacother 99: 537-542.
3. Schieber M, Chandel NS (2014) ROS function in redox signaling and oxidative stress. Curr Biol 24(10): R453-R462.
4. Cichoz Lach H, Michalak A (2014) Oxidative stress as a crucial factor in liver diseases. World J Gastroenterol 20(25): 8082-8091.
5. Tudorachi NB, Totu EE, Fifere A, Ardeleanu V, Mocanu V, et al. (2021) The Implication of Reactive Oxygen Species and Antioxidants in Knee Osteoarthritis. Antioxidants (Basel) 10(6): 985.
6. Ambade A, Mandrekar P (2012) Oxidative stress and inflammation: essential partners in alcoholic liver disease. Int J Hepatol 2012: 853175.
7. Meng X, Tang GY, Liu PH, Zhao CJ, Liu Q, et al. (2020) Antioxidant activity and hepatoprotective effect of 10 medicinal herbs on CCl(4)-induced liver injury in mice. World J Gastroenterol 26(37): 5629-5645.
8. Rahaman MM, Hossain R, Herrera Bravo J, Islam MT, Atolani O, et al. (2023) Natural antioxidants from some fruits, seeds, foods, natural products, and associated health benefits: An update. Food Sci Nutr 11(4): 1657-1670.
9. Lobo V, Patil A, Phatak A, Chandra N (2010) Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev 4(8): 118-126.
10. Lu KH, Liu CT, Raghu R, Sheen LY (2012) Therapeutic potential of chinese herbal medicines in alcoholic liver disease. J Tradit Complement Med 2(2): 115-122.
11. Vidal C, Ruiz A, Ortiz J, Larama G, Perez R, et al. (2020) Antioxidant Responses of Phenolic Compounds and Immobilization of Copper in Imperata cylindrica, a Plant with Potential Use for Bioremediation of Cu Contaminated Environments 9(10): 1397.
12. Lv YX, Zhao SP, Zhang JY, Zhang H, Xie ZH, et al. (2012) Effect of orange peel essential oil on oxidative stress in AOM animals. Int J Biol Macromol 50(4): 1144-1150.
13. Wang S, He F, Wu H, Xiang F, Zheng H, et al. (2023) Health-Promoting Activities and Associated Mechanisms of Polygonati Rhizoma Polysaccharides. Molecules 28(3): 1350.
14. Chang BY, Lee DS, Lee JK, Kim YC, Cho HK, et al. (2016) Protective activity of kudzu (*Pueraria thunbergiana*) vine on chemically-induced hepatotoxicity: *in vitro* and *in vivo* studies. BMC Complement Altern Med 16: 39.
15. Zhao W, Song F, Hu D, Chen H, Zhai Q, et al. (2020) The Protective Effect of Myristica fragrans Houtt. Extracts Against Obesity and Inflammation by Regulating Free Fatty Acids Metabolism in Nonalcoholic Fatty Liver Disease. Nutrients 12(9): 2507.
16. Xu W, Chen S, Zhong G, Liu H, Xiu L, et al. (2020) Effects of a combination of Japanese Raisin Tree Seed and Flower of Lobed Kudzuvine against acute alcohol-induced liver injury in mice. Journal of Traditional Chinese Medical Sciences 7(1): 59-67.
17. Nayim P, Mbaveng AT, Ntyam AM, Kuete VA (2020) botanical from the antiproliferative Cameroonian spice, Imperata cylindrica is safe at lower doses, as demonstrated by oral acute and sub-chronic toxicity screenings. BMC Complement Med Ther 20(1): 273.
18. Lam P, Cheung F, Tan HY, Wang N, Yuen MF, et al. (2016) Feng, Y. Hepatoprotective Effects of Chinese Medicinal Herbs: A Focus on Anti-Inflammatory and Anti-Oxidative Activities. Int J Mol Sci 17(4): 465.
19. Liu SH, Chuang WC, Lam W, Jiang Z, Cheng YC (2015) Safety surveillance of traditional Chinese medicine: current and future. Drug Saf 38(2): 117-128.
20. Jung YK, Shin D (2021) Imperata cylindrica: A Review of Phytochemistry, Pharmacology, and Industrial Applications. Molecules 26(5): 1454.
21. Jung YK, Shin D (2021) Imperata cylindrica: A Review of Phytochemistry, Pharmacology, and Industrial Applications 26(5):1454.
22. Park JH, Lee M, Park E (2014) Antioxidant activity of orange flesh and peel extracted with various solvents. Prev Nutr Food Sci 19(4): 291-298.
23. Pyrzynska K (2022) Hesperidin: A Review on Extraction Methods, Stability and Biological Activities. Nutrients 14(12): 2387.
24. Tabeshpour J, Hosseinzadeh H, Hashemzaei M, Karimi G (2020) A review of the hepatoprotective effects of hesperidin, a flavanon glycoside in citrus fruits, against natural and chemical toxicities. Daru 28(1): 305-317.
25. Zhao L, Xu C, Zhou W, Li Y, Xie Y, et al. (2023) Polygonati Rhizoma with the homology of medicine and food: A review of ethnopharmacology, botany, phytochemistry, pharmacology and applications. Journal of Ethnopharmacology 309: 116296.
26. Wong KH, Li GQ, Li KM, Razmovski Naumovski V, Chan K (2011) Kudzu root: traditional uses and potential medicinal benefits in diabetes and cardiovascular diseases. J Ethnopharmacol 134(3): 584-607.
27. Zhao W, Guo M, Feng J, Gu Z, Zhao J, et al. (2022) Myristica fragrans Extract Regulates Gut Microbes and Metabolites to Attenuate Hepatic Inflammation and Lipid Metabolism Disorders via the AhR-FAS and NF-kappaB Signaling Pathways in Mice with Non-Alcoholic Fatty Liver Disease. Nutrients 14(9): 1699.
28. Meng X, Tang GY, Zhao CN, Liu Q, Xu XY, et al. (2020) Hepatoprotective effects of *Hovenia dulcis* seeds against alcoholic liver injury and related mechanisms investigated via network pharmacology. World J Gastroenterol 26(24): 3432-3446.
29. Chen ZY, Lin YS, Liu XM, Cheng JR, Yang CY (2017) Chemical Composition and Antioxidant Activities of Five Samples of Prunus mume Umezu from Different Factories in South and East China. Journal of Food Quality 2017: 4878926.

ISSN: 2574-1241

DOI: 10.26717/BJSTR.2024.56.008865

Chi-Fu Chiang. Biomed J Sci & Tech Res

Submission Link: https://biomedres.us/submit-manuscript.php