ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: www.ijrps.com

The neuroprotective effects of the ethanolic extract of *Syzygium polyanthum* in benzene-induced rats

Andini Mandira, Chrismis Novalinda Ginting*, Linda Chiuman

Faculty of Medicine, Universitas Prima Indonesia, Medan, Sumatera Utara, 20117, Indonesia

Article History:

Received on: 22 Jun 2021
Revised on: 28 Jul 2021
Accepted on: 02 Aug 2021

Keywords:

Zyzygium polyanthum, Neuroprotective, Interferon-gamma, COX-2

ABSTRACT

The toxic and carcinogenic effects of benzene are caused by several factors such as the duration, mode and level of exposure, and individual susceptibility factors (age, gender, lifestyle, and pre-existing diseases). Syzygium polyanthum (Wight) Walp, known as the bay leaf, is a species of the Myrtaceae family. It is used as traditional medicine by various ethnic groups, especially in Southeast Asia, such as Malaysia and Indonesia. This study aimed to determine the neuroprotective effect of the ethanolic extract of Syzygium polyanthum using 44 rats. These experimental animals were divided into eleven groups, each consisting of 4 rats. The normal group was given only CMC (carboxymethyl cellulose), while negative 1 and 2 were given benzene 100 mg/kg BW every 6 and 3 days intraperitoneally. Furthermore, positive groups 1 and 2 were treated with vitamin c + benzene 100 mg/kgbw every 6 and 3 days, respectively. Group I-6 rats were given the extract at a dose of 400, 600, and 4800 mg/kgbw + benzene 100 mg/kgbw every 3 and 6 days during 21 days of the experiment. On day 22, the rats were injected with 1% ketamine and their blood samples were taken directly from the heart. This was followed by Interferon Gamma and COX-2. The result showed that the ethanolic extract of Syzygium polyanthum can reduce the biomarker of Interferon-gamma and COX-2 in benzene-induced rats.

*Corresponding Author

Name: Chrismis Novalinda Ginting
Phone:
Email: chrismis@unprimdn.ac.id

ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v13i1.13

Production and Hosted by

IJRPS | www.ijrps.com

INTRODUCTION

Benzene is one of the most common contaminants found in urban areas. It's made from gasoline vapours and automobile exhaust. In addition, the International Agency for Research on Cancer (IARC) classified it as a group 1 carcinogen in 1987. This is because there was enough evidence that inhaling this pollutant causes noncancerous and cancerous disorders in children, including acute myeloid leukaemia, myelodysplastic syndromes, and probably lymphoma and leukaemia. However, numerous studies have shown that benzene exposure can result in both acute and chronic illness, affecting a variety of human tissues and organs. Its effects can also have an impact on the central nervous system, reproductive and developmental systems, immune system, and respiratory system. This is due to the fact that metabolites play a role in its toxicity. Several factors, including the duration, manner, and level of exposure, as well as individual susceptibility factors, induce toxic and carcinogenic consequences (age, gender, lifestyle, and pre-existing diseases). As a result, risk should be assessed early on before irreversible impacts with severe social and health consequences show. Benzene is currently made mostly from petroleum, and due to its wide range

of applications, it ranks among the top 20 chemicals manufactured in the United States in terms of volume production. Many companies utilise it to manufacture other chemicals, including styrene (used in Styrofoam and other plastics), cumene (used in resins), and cyclohexane (for nylon and synthetic fibers). It's also used to make rubber, lubricants, dyes, detergents, medicines, and pesticides, among other things. Gas emissions from volcanoes and forest fires are natural sources that add to the amount of CO_2 in the environment. Additionally, benzene can be found in crude oil, gasoline, and cigarette smoke [1, 2].

At 500 parts per million, the hazardous substances data bank (HSDB) reported the negative consequences of benzene on life and health. Furthermore, an oral intake of 9-30 grams and an exposure to 20,000 ppm for 5-10 minutes in the air is anticipated to be deadly [3]. When benzene is released from huge locations, such as industrial sites, or containers, such as drums, cans, and bottles, it reaches the environment. The amount of dose, duration, age, gender, nutrition, family traits, lifestyle, and health issues, as well as the likelihood of concurrent exposure to other chemicals, all have a role in the harm and unfavourable impacts on health [4]. Furthermore, benzene exposure has been linked to oxidative DNA damage, resulting in multi-organ toxicity due to its harmful effects [5].

The safety and efficacy of herbal extracts or plant-derived chemicals used as monotherapy or adjuvant therapy in conjunction with conventional medications for hepatotoxicity have shown a positive reaction throughout the last five decades. Meanwhile, phytochemicals restore cellular defence and antioxidant systems, limit oxidative stress, and safeguard mitochondrial malfunction and inflammation, reducing necrotic cell death and preventing multi-organ damage. They also influence several cellular defence processes by regulating differential gene expression. Syzygium polyanthum (Wight) Walp, sometimes known as bay leaf, is a Myrtaceae species utilised in traditional medicine by a variety of ethnic groups. The secondary metabolite content of this plant is linked to its medicinal function. Furthermore, South East Asia's plant resources (PROSEA) are divided into spices. This group of plants includes candlenut (Aleurites moluccana (L.) Willd., galangal (Alpinia galanga (L.) Willd., and Curcuma longa) are all cooking spices. L. Plants used as cooking spices are typically utilised as colourants, scent enhancers, and flavour enhancers. They often exhibit antioxidant, anti-microbial, anti-diabetic, anti-digestive diseases, anti-hypertension, anti-cholesterol, and organoprotective properties (Etlingera elatior) [6].

The goal of this study was to see if an ethanolic extract of Syzygium polyanthum could protect rats against benzene poisoning. It was carried out by looking at the levels of Interferon Gamma and COX-2, both of which are inflammatory indicators [7].

METHODOLOGY

Reagents and chemicals

The materials used include Spectrophotometer capable of reading absorbance numbers at 450 nm, Piping device, Interval Timer, Cuvets and/or Test Tubes, Mixer (Vortex type), Constant temperature bath, or heating block set at 37°C or temperature-controlled cuvette, 10 cc pot, Elisa reader machine. Furthermore, the chemicals used include EESP, Benzene (Kairos, Yogyakarta), 0.9% NaCl (Widatra), 10% formalin, CMC-Na, chloroform (PT. Rudang jaya), Vitamin C (Ulvice-1000), calcium reagent, TCA, Calcium Carbonate (CaCO3), EDTA, liquid paraffin, toluene, and acetone (PT. Rudang jaya), Interferon Gamma Elisa kit (AB-Clonal-China), COX-2 Elisa kit (Abbkine-China).

Methods

The rat was healthy male Wistar rats weighing between 170 and 200 grammes. The experimental mice were given pellets and tap water ad libitum after being acclimatised for one week at room temperature (22-25°C) under a 12-hour light/dark cycle.

In addition, 500g of leaf powder was placed in reagent vials and macerated with 96 percent ethanol at a volume ratio of 1: 3 w/v. The mixture was agitated at 200-250 rpm for 48 hours before being filtered via filter paper. The maceration was carried out until a clear immersion was achieved. A rotary evaporator was also used to evaporate the ethanol extract solution at temperatures ranging from 45 to 50 degrees Celsius. The solution was then placed in a rotary evaporator and submerged in water to evaporate any remaining solvent.

The 44 rats in the experiment were divided into eleven groups, each with four rats. Negative 1 and 2 were administered benzene 100 mg/kgbw intraperitoneally every 6 and 3 days, respectively, whereas the normal group received only CMC (carboxymethyl celluloce). In addition, positive groups 1 and 2 were given 100 mg/kgbw vitamin C + benzene every 6 and 3 days, respectively. Every 3 and 6 days, Group I-6 rats were fed the extract at doses of 400, 600, and 4800 mg/kgbw + benzene 100 mg/kgbw. On day 22, the rats were given 1% ketamine and blood samples were collected directly from the heart, followed by Interferon Gamma and

COX-2 injections.

The one-way ANOVA (Analysis of Variance) test was used to analyse the data using SPSS version 21 (statistical application for social sciences). Significant and insignificant differences were defined as p-values less than or larger than 0.05 across groups, respectively.

RESULTS AND DISCUSSION

Table 1: Interferon gamma level

Groups	Interferon Gamma (ng/mL) Mean ± SD
Normal	12,2396 ± 2,53386913
Negative-1	75,1258 ± 4,09319503
Negative-2	59,1864 ± 5,31217006
Positive-1	17,2081 ± 2,97679183
Positive-2	14,1635 ± 3,80346493
Group-1	49,0091 ± 1,93512217
Group-2	34,2892 ± 3,4801458
Group-3	26,7403 ± 1,52683854
Group-4	21,2634 ± 0,92720217
Group-5	17,5557 ± 2,15642211
Group-6	15,5546 ± 1,294548

Table 2: COX-2 Level

Groups	COX-2 (ng/mL) Mean ± SD
Normal	0,98731 ± 0,03113144
Negative-1	2,78344 ± 0,08934113
Negative-2	2,57351 ± 0,06831548
Positive-1	0,98391 ± 0,07318176
Positive-2	0,89316 ± 0,05721487
Group-1	1,89631 ± 0,01863144
Group-2	1,78447 ± 0,04687137
Group-3	1,36734 ± 0,08631314
Group-4	1,29848 ± 0,07631467
Group-5	0,98561 ± 0,03714808
Group-6	0,98399 ± 0,04917310

From Table 1, the results showed that the benzene-induced groups 3 and 6 had the lowest interferon-gamma levels, with values of 75,1258 ± 4,09319503 and 59,1864 ± 5,31217006 ng/ml, respectively. Furthermore, benzene was used to induce groups 6 and 3, and an EESP dose of 800 mg/kgBW had no significant difference (p>0.05) when compared to the normal and positive controls. In contrast to the normal group, the negative 3 and 6 treated simply with benzene exhibited a significant difference (P<0.05).

From Table 2, the COX-2 levels in the benzene-induced groups 3 and 6 were highest, with values of 2,78344 ± 0,08934113 and 2,57351 ± 0,06831548 ng/ml, respectively. Furthermore, when compared to the normal and positive controls, groups 6 and 3 induced with benzene plus an EESP dose of 800 mg/kgBW exhibited no significant difference (p>0.05). When compared to the normal group, the negative 3 and 6 treated simply with benzene exhibited a significant difference (P<0.05).

Interferons (IFNs) are the main defence against pathogens due to their strong antiviral activity. IFN can be classified into three groups of type I, II, and III, according to their genetic, structural, and functional characteristics as well as their receptors on the cell surface. Type I IFNs are the largest group and include IFN-α, IFN-β, IFN-ε, IFN-ω, IFN-κ, IFN-δ, IFN-τ and IFN-ζ. The use of IFNs for the treatment of viral infectious diseases on their antiviral activity is an important therapeutic option, for example, IFN-α is well known for the successful treatment of hepatitis B and C virus infections. There is also a growing interest in the antiviral efficacy of other new classes of IFNs and their potential applications. Therefore, recent advances on the biological activity of all classes of type I IFNs and their potential applications in the treatment of infections with immunodeficiency, hepatitis, and influenza viruses were discussed. The type I IFN family in humans consists of 12 IFN-α subtypes encoded by 14 non-parallel genes. This includes one pseudogene and two genes encoding the same protein.

Meanwhile, one IFN-β is encoded by one IFNB gene despite the duplication. The various IFN-α subtypes share many points in common such as their similar clustering point on chromosome 9. They are composed of 165 to 166 amino acids with 80% sequence identity. IFN-βs consists of 166 amino acids and are N-glycosylated. In contrast, only IFN-α2 and IFN-α14 of the 13 subtypes of IFN- have glycosylation sites. IFN-α2 is O-glycosylated, while IFN-α14 is N-glycosylated [8].

The liver is highly reactive to benzene oxide, which causes bone marrow poisoning. After 18 minutes of incubation with liver microsomes, Lovern et al. (1997) found that this oxide makes up 7% of the benzene metabolites. Lindstrom et al. (1997) confirmed the presence in blood and calculated an 8-minute half-life. The susceptibility to toxicity is related to changes in the metabolic rate of benzene and the metabolites produced (Bayliss et al., 2002). In addition, benzene oxide produces 7-phenylguanine, which has an effect on other DNA. When compared to the reaction with thiols, it was said to have low reactiv-

ity. The enzyme epoxide hydrolase converts phenol to benzene dihydrodiol, which is then converted to catechol. It also goes through an iron-catalyzed ring-opening process to make trans, trans-muconaldehyde (MUC), which is then metabolised to produce trans, trans-muconic acid (t,t-MA). Another potential electrophilic metabolite is benzene dihydrodiol epoxide. The half-life was greater than 5 hours at near-physiological conditions (pH 7.6) and can be distributed to target tissues far from the site of initiation of generation. The CYP2E1 isoenzyme catalyses the conversion of phenol to hydroquinone, and the metabolic end product t, t-MA, is employed as a biomarker of benzene exposure [9]. Potential metabolic steps in the synthesis of t,t-MA have been found in vivo, in vitro, and animal studies (see Section 1.3.1). In addition, after incubation with benzene, Latriano et al. (1986) discovered muconaldehyde (t, t-MA dialdehyde) in rat liver microsomes. The ring-opening of benzene oxide or oxepin by cytochrome P450 (CYP) or oxygen radical-mediated processes are two possible ways of production. Muconaldehyde is a reactive electrophilic bifunctional aldehyde that combines with thiols and nucleic acids. Aldehydes can be reduced or oxidised in vitro to produce alcohol and aldehyde (6-hydroxy hexa-2,4-dienoic acid) or an acid and aldehyde (6-oxohexa-2,4-dienoic acid, muconic acid semialdehyde), which retains some of the electrophilicity of the parent dialdehyde but has a higher diffusion coefficient [9].

Reactive oxygen species (ROS) are extremely reactive molecules regulated by antioxidant defence systems, both enzymatic and non-enzymatic. ROS modulates cell proliferation, differentiation, and contraction-excitation multiplication in the heart, critical for cell homeostasis. Additionally, oxidative stress occurs when production surpasses the antioxidant defence system's buffering capability, resulting in cellular and molecular abnormalities that can contribute to heart failure [10]. Benzene should be broken down into several components that can build up in the bone marrow. They are subsequently activated to semiquinones and reactive quinones by myeloperoxidases and other hemeprotein peroxidases, resulting in the generation of reactive oxygen species (ROS). Superoxide radical anions, hydroperoxyl radicals, hydrogen peroxide (H_2O_2), and highly reactive hydroxyl radicals are examples. They are created by various physiological activities and can change the activity of specific protein kinases and transcription factors, hence influencing signal transduction cascades. Several radical scavenging enzymes keep ROS levels in cells under control for appropriate signalling. Increased generation of ROS, on the other hand, can result in excessive signalling to cells as well as direct damage to crucial components in the signalling pathway when ROS levels reach this threshold. When the balance between ROS creation and detoxifying levels is disrupted, oxidative stress ensues [11]. This could result in cellular dysfunction. Furthermore, ROS can cause irreversible damage to important macromolecular targets like DNA, proteins, and lipids, which can lead to cancer [12].

CONCLUSIONS

The ethanolic extract of Szygium polyanthum can reduce the biomarker of inflammation that are Interferon-gamma and COX-2 on benzene-induced rats. Further study should be conducted to develop this extract into additional therapy.

Funding Support

The authors declare that they have no funding support for this study.

Conflict of Interest

The authors declare that they have no conflict of interest.

REFERENCES

[1] B Liang, Y Zhong, K Chen, L Zeng, G Li, J Zheng, L Jiang, Z Xie, B Que, G Lai, B Wu, X Yang, J Wu, Y Xiao, W Chen, and Z Huang. Serum plasminogen as a potential biomarker for the effects of low-dose benzene exposure. *Toxicology*, 410:59–64, 2018.

[2] C Snyder. Experimental Benzene Toxicity. *Benzene carcinogenicity* , pages 23–32, 2017.

[3] S Kim, J Chen, T Cheng, A Gindulyte, J He, S He, and E E Bolton. PubChem 2019 update: improved access to chemical data. *Nucleic acids research*, 47(D1):1102–1109, 2019.

[4] N Barros, M Carvalho, C Silva, T Fontes, J C Prata, A Sousa, and M C Manso. Environmental and biological monitoring of benzene, toluene, ethylbenzene and xylene (BTEX) exposure in residents living near gas stations. *Journal of Toxicology and Environmental Health, Part A*, 82(9):550–563, 2019.

[5] N Pérez-Herrera, L D De León-Martínez, R Flores-Ramírez, O Barbier, M Ortega-Romero, F May-Euán, and F J Pérez-Vázquez. Evaluation of benzene exposure and early biomarkers of kidney damage in children exposed to solvents due to precarious work in Ticul, Yucatán, México. *Annals of global health*, 85(1):94, 2019.

[6] K L Silalahi, N Nainggolan, S Simanjuntak, and F A Ginting. The Effect of Brushed Salam Leaf on the Reduction of High Blood Pressure in Hypertension Patients. *Jurnal Penelitian Perawat Profesional*, 2(4):431–436, 2020.

[7] K R Cooper and R Snyder. Benzene metabolism (toxicokinetics and the molecular aspects of benzene toxicity). *Benzene carcinogenicity*, pages 33–58, 2017. ISBN: 9781351368919.

[8] A B Engin, E D Engin, K Golokhvast, D A Spandidos, and A M Tsatsakis. Glutamate-mediated effects of caffeine and interferon-γ on mercury-induced toxicity. *International Journal of Molecular Medicine*, 39(5):1215–1223, 2017.

[9] G Rindi, D S Klimstra, B Abedi-Ardekani, S L Asa, F T Bosman, E Brambilla, K J Busam, R R De Krijger, M Dietel, A K El-Naggar, L Fernandez-Cuesta, G Klöppel, W G Mccluggage, H Moch, H Ohgaki, E A Rakha, N S Reed, B A Rous, H Sasano, and I A Cree. A common classification framework for neuroendocrine neoplasms: an International Agency for Research on Cancer (IARC) and World Health Organization (WHO) expert consensus proposal. *Modern Pathology*, (12):1770–1786, 2018.

[10] R D'oria, R Schipani, A Leonardini, A Natalicchio, S Perrini, A Cignarelli, L Laviola, and F Giorgino. The Role of Oxidative Stress in Cardiac Disease: From Physiological Response to Injury Factor. *Oxidative Medicine and Cellular Longevity*, 2020:1–29, 2020.

[11] W Yoon, Y C Park, J Kim, Y S Chae, J H Byeon, S H Min, and B M Kim. Application of genetically engineered Salmonella typhimurium for interferon-gamma-induced therapy against melanoma. *European Journal of Cancer*, 70:48–61, 2017.

[12] W A I Khallaf, B A S Messiha, A M H Abo-Youssef, and N S El-Sayed. Protective effects of telmisartan and tempol on lipopolysaccharide-induced cognitive impairment, neuroinflammation, and amyloidogenesis: possible role of brain-derived neurotrophic factor. *Canadian Journal of Physiology and Pharmacology*, 95(7):850–860, 2017.