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The Effect of the Administration of Salam Leaf Ethanol Extract on IL-6 and IL-4 on Benzene-Induced Brain Networks of Rats

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ABSTRACT

Benzene is poisonous to the human body and has been linked to neurotoxic and neurodegenerative illnesses. Bay leaf is extremely safe to consume because it has been shown in experimental animals to have no toxic, teratogenic, or genotoxic effects and to have a strong antioxidant effect. The purpose of this study is to assess the neuroprotective impact of an ethanolic extract of bay leaf on rats exposed to benzene. The study was divided into 11 groups: group 1 was assigned to a control condition, groups 2 and 3 were assigned to negative controls, groups 4 and 5 were assigned to positive controls, and groups 6-11 were assigned to receive an ethanol extract of bay leaf. For 21 days, benzene was injected intraperitoneally every three and six days and the extract was delivered orally. On day 22, rats were sedated and their brains were collected and examined for IL-4 and IL-6 levels using an Elisa kit and a 450 nm microplate reader. The results indicated that the levels of IL-4 and IL-6 increased statistically substantially in rats given just benzene (p0.05), whereas they decreased in animals given the highest extract, 800 mg/kg BW. The levels of IL-4 and IL-6 were not statistically significantly different from those in the normal group (p>0.05). Thus, it can be inferred that the ethanolic extract of bay leaves has a neuroprotective effect due to the presence of flavonoids that contribute to the prevention of inflammation caused by benzene exposure.

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INTRODUCTION

Ambient air pollution and particulate matter (PM) has been linked to a number of detrimental health impacts, including respiratory disease, cardiovascular disease, and lung cancer [1]. Water and soil pollution, such as drinking contaminated water, can result in a variety of diseases, including typhoid fever, diarrhea, vomiting, cancer, kidney, and liver damage [2], and experimental and clinical neurotoxicology lists over 350 synthetic and natural compounds that are known to cause functional or structural damage to the nervous system [3]. Each year, over seven million people die as a result of air pollution. According to WHO (World Health Organization) data, nine out of ten people breathe air that exceeds the WHO guideline limit for high levels of pollutants, with low- and middle-income nations bearing the brunt of the burden [4]. According to the WHO, ambient air pollution caused 3.7 million deaths worldwide in 2012, accounting for 6.7% of total fatalities, and was responsible for 16% of lung cancer deaths and 11% of deaths from chronic obstructive pulmonary disease. Disease-related deaths, 29% of heart disease and stroke deaths, and approximately 13% of respiratory infec-

tion deaths [1].

The exposure limit for benzene, a common element pollutant, is extremely high in humans. This is because benzene is frequently employed in industry as a solvent, as a chemical intermediary, and as a component of gasoline, among other things. Although inhalation is the most common route of exposure to benzene, oral and cutaneous routes should also be addressed. The toxicokinetics of benzene has been examined in humans and experimental animal species. Benzene is rapidly absorbed and transported throughout the body of test animals and humans. The parent chemical is preferably stored in fat, and the percentage absorption appears to be depending on the level of blood circulation in the tissue. The metabolism of benzene is essential for the manifestation of benzene toxicity [5].

Benzene is a volatile pollutant found of high concentrations in toxic substances such as tobacco smoke and black smoke from combustion that contaminates animals and humans during pregnancy. If exposed chronically, it can cause neurological effects such as euphoria, headache, vertigo, ataxia, narcosis, confusion, neuropathy, and EEG abnormalities, while its derivatives can increase the risk of neurobehavioral changes. And if acute benzene exposure in pregnant rats results in long-term behavioral abnormalities in their offspring, specifically a reduction in motor activity and cognitive function [6].

Bay leaf, scientifically known as Syzygium polyanthum (Wight) Walp, is a member of the Myrtaceae family. It is a well-known plant that is frequently used as a kitchen spice or cooking spice due to its characteristic aroma. Additionally, bay leaf (Syzygium polyanthum (Wight) Walp.) is frequently utilized by the population for alternative medicine due to its widespread availability and ease of acquisition. In Java, Madura, and Sunda, salam leaves are referred to as-salam, kastolam leaves are referred to as kastolam leaves, manting leaves are referred to as manting leaves, and meselengan leaves are referred to as meselengan leaves [7].

The nutritional content of bay leaves has been shown to improve hemoglobin levels. Additionally, the secondary metabolite content of bay leaf is quite beneficial for decreasing cholesterol, treating hypertension, diarrhea, gastritis, and diabetes mellitus. Bay leaf filtrate has been shown to lower uric acid levels. Salam includes essential oils such as citral and eugenol, as well as tannins and flavonoids. The bay leaf's thick extract contains tannins and flavonoids, the most abundant of which are fluorethin and quercitrin [8]. Flavonoids are also antioxidants that act as neuroprotectors against neurotoxins, suppress neuroinflammation, inhibit apoptosis, and induce angiogenesis, neurogenesis, and beneficial changes in neuron morphology, whereas tannins are a class of phenolic metabolites that have free radical scavenging activity and the ability to inhibit lipid peroxidase and lipoxygenase. Tannins may also act as antioxidants by scavenging free radicals and activating antioxidant enzymes. Tannins can also stimulate glucose absorption via insulin signaling pathway mediators such as PI3K activation, P38 MAPK activation, and GLUT-4 translocation. The capacity of tannins to trap ROS is believed to result in tannins being able to inhibit neuronal apoptosis and brain cell atrophy [9].

Due to the dangers of benzene and its association with neurotoxic and neurodegenerative diseases, as well as the high nutritional and antioxidant content of bay leaf (*Syzygium polyanthum* (Wight) Walp.), the researchers sought to determine the effectiveness of bay leaf as a preventative and treatment in male Wistar rats induced with benzene neurotoxicity, by analyzing (1) screening for immunization. (2) determined the anti-inflammatory cytokine Interleukin-4's activity. (3) histologically examine brain tissue.

METHODOLOGY

Reagents and chemicals

The materials used include UV spectrophotometer (Microlet 3000), rotary evaporator, centrifugation, microtube, test tube, animal scale (Presica), Elisa Rider (Thermo), Spectrophotometer capable of reading absorbance numbers at 340 nm, Accurate piping device, Timer Interval, 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, bay leaf ethanol extract, 0.9% NaCl, Vitamin C (Ulvice-1000), calcium reagent, TCA, Calcium Carbonate (CaCO3), EDTA, liquid paraffin, toluene, and acetone, IL-6 and IL-4 reagents Rat-Elisa kit (AB-Clonal-China). IL-6 and IL-4 reagents Rat Elisa kit (ABclonal-China).

Methods

Male Wistar rats weighing 150–200 g were used in this study. Prior to the start of the research, the test animals were acclimated for one week at room temperature (22-25°C), on a 12-hour light/dark cycle, and fed pellets and free access to tap water. There were 11 test groups, each of which contained four male Wistar rats, for a total of 44 rats.

Extract Preparation

500 g of bay leaf powder was placed in a reagent container and macerated with 96 percent ethanol

at a volume ratio of 1:3 w/v powder to solvent. This combination was shaken for 48 hours at a speed of 200-250 rpm using a shaker. The solution was then filtered with filter paper. The maceration process was repeated until a clear immersion was achieved. Following the maceration procedure, the bay leaf ethanol extract solution was evaporated using a rotary evaporator at temperatures ranging from 45 to 50°C; the solution was then placed in a water bath to evaporate any leftover solvent [10].

Experimental Design

44 rats were divided into 11 groups: group 1 served as a control, groups 2 and 3 were given benzene 1,940 mg/kg BW/intraperitoneal for 3 and 6 days, respectively, groups 4 and 5 (positive control) were given benzene 1,940 mg/kg BW/intraperitoneal for 3 and 6 days plus vitamin C 1.62 mg/oral, and groups 6 and 7 were given benzene 1,940 mg/kg BW/intraperitoneal day 21, each group of rats was randomly selected for up to three rats, anesthetized with chloroform, and rat brains were harvested for IL-4 and IL-6 analysis.

Data Analysis

The SPSS version 22 application was used to analyze the research data. The Shapiro-Wilk test was used to confirm normality (P > 0.05), and a homogeneity test was also done. Then, using the One Way ANOVA approach, calculate the mean difference between groups. If there is a difference (P 0.05), the Post Hoc Tukey HSD test is used to determine the true difference between treatments. If the data, on the other hand, are not normally distributed, the Kruskal-Wallis test is utilized.

RESULTS AND DISCUSSION

IL-4 Level

The results showed that the highest levels of Interleukin-4 were found in the group induced by benzene per 3 days with a value of 534.9399 ± 45.40621326 pg/mL and induced by benzene 6, namely 389.2613 ± 58.34844844. Statistically, the group that was given benzene 6 and benzene 3 plus EESP at a dose of 800 mg/kg BW was not significantly different p>0.05 with the normal group and the positive control group, while the negative group that was only given benzene 3 and 6 had a significant difference. P<0.05 in the normal group. (Table 1)

IL-6 Level

Interleukin-6 is a proinflammatory cytokine with pleiotropic effects that acts as a regulator of the acute phase response to inflammation. The involvement of IL-6 in biological processes such as immune response control, inflammation, and hematopoiesis. Interleukin-6 has a critical function in the etiology of neurotoxicity. When a trigger for this illness occurs, IL-6 levels rise and antibody production is excessive, resulting in an immunological response. Thus, in this study, benzene promotes inflammation in the brain, resulting in a rise in IL-6 levels.

The results showed that the highest levels of Interleukin-6 were found in the group induced by benzene per 3 days with a value of 3220.9592 ± 119.6722076 pg/ml and induced by benzene per 6 days, namely 2180.4749 ± 353.8480554. Statistically, the group that was given benzene per 6 days and benzene per 3 days plus ethanol extract of the bay leaf at a dose of 800 mg/kg BW was not significantly different p>0.05 with the normal group and the positive control group, while the negative group that was only given benzene per 3 days and per 6 days have a significant difference. P<0.05 in the normal group. (Table 2)

Additionally to benzene metabolism via enzymatic and non-enzymatic processes ultimately result in the formation of 1,2,4-benzentriol and oxidative stress that causes damage to cerebrovascular tissues. Cerebrovascular injury and brain function can be mediated through biological processes associated with the brain's autonomic function. Release of pro-oxidative and pro-inflammatory mediators from the lungs into the systemic circulation as a result of particle inhalation, as well as translocation of ultrafine particles and their soluble contents into the systemic circulation. The mechanism by which particle pollution adversely affects the central nervous system, thereby increasing the risk of paralysis and death linked with particulate pollution exposure [11].

Human exposure to benzene occurs in the chemical and petroleum industries, as well as in the environment, via automobile exhaust, gasoline, and cigarette smoke [12]. Cytochrome P450 2E1 oxidizes benzene in the liver to generate benzene oxide. The oxide can then be transformed either non-enzymatically to phenol or enzymatically to catechol (through epoxide hydrolase and subsequently dihydrodiol dehydrogenase). Further hydroxylation reactions on the phenol result in the formation of catechol or hydroquinone; each of these products can be hydroxylated to form 1,2,4-benzenetriol.

Additionally, ring-open metabolites can be generated, the most prevalent of which are trans-trans-muconic acid (t,t-MA) and 6-hydroxy,t-2, 4-hexadecenoic acid in the urine. While each of these metabolites may cause toxicity on its own, mixtures of metabolites can have synergistic effects

Table 1: IL-4 levels of rat brain tissue in each group

No.	Group	IL-4 (pg/mL) Mean ± SD
1.	Normal	115,7571 ± 5,016050235
2.	Negative-1	534,9399 ± 45,40621326
3.	Negative-2	389,2613 ± 58,34844844
4.	Positive-1	134,2826 ± 15,21296096
5.	Positive-2	117,4425 ± 5,598576772
6.	Group 1 (400/3D)	438,9869 ± 39,58614152
7.	Group 2 (400/6D)	352,9873 ± 33,10648005
8.	Group 3 (600/3D)	268,9846 ± 13,11968518
9.	Group 4 (600/6D)	203,5772 ± 6,487385728
10.	Group 5 (800/3D)	138,8755 ± 15,85305402
11.	Group 6 (800/6D)	119,5768 ± 9,555040175

Table 2: IL-6 levels of rat brain tissue in each group

No.	Groups	IL-6 (pg/mL) Mean ± SD
1.	Normal	894,0539 ± 53,33906436
2.	Negative-1	3220,9592 ± 119,6722076
3.	Negative-2	2180,4749 ± 353,8480554
4.	Positive-1	915,0278 ± 42,19581073
5.	Positive-2	893,2517 ± 61,0544254
6.	Group 1 (400/3H)	1191,1997 ± 13,5234949
7.	Group 2 (400/6H)	1086,1378 ± 138,5887527
8.	Group 3 (600/3H)	908,4563 ± 32,20282146
9.	Group 4 (600/6H)	946,4331 ± 13,46860357
10.	Group 5 (800/3H)	784,7578 ± 162,5153177
11.	Group 6 (800/6H)	700,7828 ± 50,30281168

on several cellular targets, resulting in greater toxicity. Benzene toxicity is most likely caused by benzene's oxidative conversion to reactive compounds. Bioactive substances are activated in the presence of reactive intermediates that can result in an increase in reactive oxygen species (ROS). The peroxidation of arachidonic acid and the activation of oxygen to superoxide radicals occurred as a result of the peroxidation of benzene, phenol, and hydroquinone metabolites, respectively. Silva et al. (2003) [13] discovered that hydroquinone can be converted to possibly hematotoxicity, genotoxic, and carcinogenic benzoquinone, which can also increase the creation of radical species, hence predisposing cells to oxidative stress. Chronic benzene exposure can result in DNA degradation in bone marrow cells and peripheral blood lymphocytes, as well as impaired antioxidant enzyme activity. Oxidative stress, caused by increased free radical generation and deficiencies in antioxidant defenses, plays a role in the etiology of a number of disorders [11].

Neurotoxicity appears to be a common mechanical link between numerous environmental agents, including pesticides, heavy metals, and organic pollutants; and the inflammatory process appears to be a common mechanical link between this devastation. Indeed, toxins have been shown repeatedly to promote the production of pro-oxidant and pro-inflammatory substances from immunocompetent microglia, resulting in the destruction and death of midbrain dopamine (DA) neurons. Proinflammatory cytokines such as tumor necrosis factor- and interferon-, which are produced locally in the brain by microglia, have been implicated in the loss of DA neurons in toxin-based models of Parkinson's disease, and an increasing body of evidence points to a role for the inflammatory enzyme cyclooxygenase-2. Similarly, immune-activating bacteria and viruses have been shown to have neurodegenerative effects

on their own and to exacerbate the detrimental effect of chemical toxins on AD neurons [14, 15]

Bay leaf is an excellent source of antioxidants; it has been shown to scavenge free radicals, which are known to contribute to the development of cancer and atherosclerosis by producing oxidative damage to lipids, proteins, and nucleic acids. Numerous studies on the antioxidant properties of S. *polyanthum* have been conducted on various sections of the plant, including the leaves, ripe and unripe fruits, and bark. The most frequently used method is the diphenyl-1-picrylhydrazyl (DPPH) radical scavenging test, 27-29, followed by others such as the iron-reducing assay (FRAP), 10,26,27 beta bleaching carotene assay, 7,12,25, and 2,2-azino-bis (3-ethylbenzothio-zoline-6-sulphonic acid) cation radical scavenging assay (ABTS) [16–18]

CONCLUSION

The research findings indicate that the ethanol extract of bay leaves includes chemicals that inhibit neurotoxicity caused by benzene and that, based on phytochemical analysis, bay leaf ethanol extract inhibits IL-4 and IL-6 production in benzene-induced rats brain tissue. Bay leaf ethanolic extract exhibits a neuroprotective effect against benzene-induced rat brain tissue at all dosages.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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