

Proinflammatory effects of *Launaea taraxacifolia* aqueous leaves extract in benzene induced haematotoxicity in albino male wistar rats

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ABSTRACT

Background: Haematotoxicity induced by benzene exposure has been associated with altered gene expressions of proinflammatory cytokines, such as Interleukin-6 (IL-6) and Tumor Necrosis Factor-alpha (TNF- α). *Launaea taraxacifolia*, a plant known for its traditional medicinal uses, has emerged as a subject of interest due to its reported anti-inflammatory properties. Therefore, the aim of this study was to determine the proinflammatory effects of aqueous leaf extracts of *Launaea taraxacifolia* in benzene induced haematotoxicity in albino Wistar rats.

Methods: A total of sixty (60) adult male albino Wistar rats were divided into six (6) groups namely groups A, B, C, D, E and F, representing control, benzene group, cyclophosphamide group, benzene + 100mg/kg *Launaea taraxacifolia*, benzene + 200mg/kg *Launaea taraxacifolia* and benzene + 400mg/kg *Launaea taraxacifolia* respectively. mRNA expressions of IL-6 and TNF- α were determined using polymerase chain reaction (PCR). Data obtained was analysed by the Statistical Package for Social Science (SPSS) software.

Results: mRNA expression of IL-6 in group B was significantly higher when compared to group A ($p < 0.05$). The mRNA expression of IL-6 was significantly higher in groups E and F when compared to group A ($p < 0.05$). There was a significant increase in the mRNA expression of TNF- α in group B when compared to group A ($p < 0.05$).

Conclusion: This study concludes that benzene exposure induced an increase in the mRNA expression of IL-6 and TNF- α . Co-administration of benzene and varying *Launaea taraxacifolia* concentrations subsequently reduced TNF- α while further increase was noted in IL-6 expression after administration of 200mg/kg with benzene.

Keywords: Albino Wistar Rats, Benzene, Interleukin-6, *Launaea taraxacifolia*, Tumor Necrosis Factor-alpha

1. INTRODUCTION

The use of medicinal plants as a source of medicine is an ancient practice that has been utilized for millennia by diverse cultures all over the world. Many traditional medical systems have depended significantly on medicinal plants to cure a variety of diseases [1]. Medicinal plants contain a wide array of bioactive compounds which can include alkaloids, flavonoids, terpenes, and phenols, among others [2]. These bioactive compounds can have diverse pharmacological properties such as anti-inflammatory, antimicrobial, analgesic, antioxidant, and anticancer activities [3]. *Launaea taraxacifolia* is a plant that belongs to the Asteraceae or Compositae family. It is considered to be one of the largest and most significant families of flowering plants (angiosperms) in terms of its size and utilization. *Launaea taraxacifolia* is native to Africa, particularly West Africa, where it can be found in Nigeria, Senegal, Guinea, and Sierra Leone. The plant may thrive in a variety of environments, including savannahs, grasslands, and disturbed regions [4]. In traditional African medicine, *Launaea taraxacifolia* has been used for its medicinal properties [5]. Inflammation is a natural immune reaction triggered when the body detects adverse stimuli such as infections or tissue damage [6]. Immune cells emit signaling chemicals (cytokines) that attract other immune cells to the site of injury or infection during the inflammatory phase [7]. Cytokines are tiny proteins that cells employ for intercellular communication and to regulate the conditions in which they function. They are produced by cell types that have important roles in the immune response, inflammation, haemopoiesis, healing and systemic response to injury [8]. Cytokines may be pro-inflammatory or anti-inflammatory. Pro-inflammatory cytokines or simply inflammatory cytokines are special types of signaling molecules that promote inflammation. The most prominent among them are interleukins (IL-1, IL-6) and tumor necrosis factor (TNF- α and TNF- β) [9]. Benzene is a well-known aromatic

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hydrocarbon that is commonly found in industrial processes and products, including gasoline, solvents, and plastics [10]. It is considered a hazardous chemical and a known human carcinogen, as prolonged exposure to benzene has been linked to the development of various cancers, including leukemia and lymphoma. Benzene metabolism in the body produces reactive oxygen species (ROS) and other toxic intermediates, leading to cellular damage and oxidative stress [11]. This cellular damage triggers an inflammatory response as the body attempts to repair the affected tissues. This response involves the activation of immune cells, including macrophages, which produce pro-inflammatory cytokines like IL-6 and TNF- α [12]. Excessive production of IL-6 and TNF- α due to benzene exposure can lead to chronic inflammation, disrupting the balance of the immune system and potentially contributing to the development of various health issues. Additionally, chronic inflammation has been associated with an increased risk of cancer development, including hematopoietic cancers like leukemia [13]. Investigating the impact of *Launaea taraxacifolia* on these pro-inflammatory biomarkers in respect to benzene induced haematotoxicity, will aid in elucidating the extract's inflammatory and immune-modulatory properties, which can then be applied to other inflammatory disorders. Hence this study which aims to determine the proinflammatory effect of *Launaea taraxacifolia* leaf extracts in benzene-induced haematotoxicity in albino male Wistar rats.

2. MATERIALS AND METHODS

2.1. Materials

Eppendorf Containers, Sorvall biofuge, Germany eppendor mastercycler, Germany Labnet, Electrophoresis system, USA micro pipettes, Hisense Microwave, UV-visible, Trizol, Chloroform, Zymo DNA extraction kit, Loading dye, EZ-Vision, TBE buffer, Nuclease Free Water, Agarose.

2.2. Methods

2.2.1. Study Population

In this study, animal (rats) model was used. A total of sixty (60) adult male albino Wistar strain were purchased from the animal holdings of the Department of Anatomy, University of Benin, Benin City, Nigeria. The rats were housed at the animal housing wing of the Department of Anatomy, University of Benin [14].

2.2.2. Identification of the *Launaea taraxacifolia* leaves

Launaea taraxacifolia leaves were harvested from Faculty of Agriculture (in the month of February, 2023) in the University of Benin, Nigeria. The leaves were then identified and authenticated by Dr. A. O Akinnibosun of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City [15].

2.2.3. Preparation of Plant Extract

About 3.65kg leaves were pulverized (after drying) by a commercial blender and (665g of powder obtained), soaked in distilled water using 1g of powder to 5ml of distilled water and allowed to stand at room temperature for 72 hours at room temperature. The extract was filtered using Whatman's (Nitro cellulose 45; 0.45 μ m pore size) filter paper and the filtrates were concentrated to dryness at 100°C in a water bath. Thereafter, it was put in an airtight container and refrigerated until use [15].

2.2.4. Animal Care

Animals were housed in a cross ventilated room in the animal holdings of the department of anatomy, University of Benin, Benin City. Animals were exposed to 12 hours dark and light cycles with access to feed and water *ad libitum*. The rats were acclimatized for a period of two (2) weeks before commencement of the experiment [15].

2.2.5. Ethical Consideration

Ethical approval was obtained from Research Ethics Committee on animal subjects from Edo State Ministry of Health, Benin City (Ref Number: HA/737/23/B/200600143 issued on 30th June, 2023).

2.2.6. Preparation of Benzene and Cyclophosphamide Drug Solution

2.2.6.1. Benzene Solution

Benzene solution was made by mixing benzene (Manufactured by LOBA Chemie LABORATORY REAGENTS and FINE CHEMICALS LTD, Batch Number: L246641711), distilled water v/v and 2-propanol in the ratio of 1:5:5. That is, 1 part of benzene was mixed with 5 part of distilled water v/v and 5 parts of 2-propanol. 0.2ml of this benzene

solution was administered to each animal in the various test groups with an average weight of 150g every 48 hours for 28 days [14].

2.2.6.2. Cyclophosphamide Drug Solution

Cyclophosphamide drug solution was made by mixing 500mg of the powdered drug in 25ml of distilled water. 0.3ml of this drug solution was administered orally to each animal in group C of an average weight of 150g every 48 hours for 28 days [16].

2.2.7. Research Design

Grouping of Animals: Sixty (60) Mature Wistar rats weighing 150-200g were randomly selected and divided into six groups (n = 10 per group). The Groups were the Group A, Group B, Group C, Group D, Group E and Group F.

Group A: This was the control group. Animals in this group received only standardized feed (Manufactured by KARMA AGRIC FEEDS AND FOOD LIMITED, Oyo State) and clean water *ad libitum*.

Group B: This group received only benzene intraperitoneally.

Group C: Animals in this group were administered benzene solution and treated with the standard drug solution (cyclophosphamide) intraperitoneally.

Group D: Animals in this group were administered benzene solution intraperitoneally and treated with low dose of *Launaea taraxacifolia* leaves extract orally.

Group E: Animals in this group were administered benzene solution intraperitoneally and treated with a higher dose of *Launaea taraxacifolia* leaves extract orally.

Group F: Animals in this group were administered benzene solution intraperitoneally and treated with the highest dose of *Launaea taraxacifolia* leaves extract orally [14].

2.2.8. Administered Doses of *Launaea taraxacifolia* Extract

Group A (control) received only standardized feed and clean water *ad libitum*. Group B (benzene treated group) were administered 0.2ml of benzene solution intraperitoneally every 48 hours for 28 days. Group C (cyclophosphamide drug solution treated group) were administered 0.2ml of benzene solution intraperitoneally every 48 hours for 28 days and treated with 0.3ml of 6mg/ml of cyclophosphamide 48 hourly for 28 days. Group D were administered with 0.2ml of benzene solution intraperitoneally every 48 hours for 28 days and treated with 0.15ml of 100mg/kg body weight of *Launaea taraxacifolia* leaves extract orally using a gavage tube every 24 hours for 28 days. Group E were administered with 0.2ml of benzene solution intraperitoneally every 48 hours for 28 days and treated with 0.3ml of 200mg/kg body weight of *Launaea taraxacifolia* leaves extract orally using a gavage tube every 24 hours for 28 days. Group F were administered with 0.2ml of benzene solution intraperitoneally every 48 hours for 28 days and treated with 0.6ml of 400mg/kg body weight of *Launaea taraxacifolia* leaves extract orally using a gavage tube every 24 hours for 28 days [14].

2.2.9. Sacrifice of Animals and Collection of Samples

At the end of the experimental period, the animals were grossly observed for general physical characteristics. A midline incision was made through the ventral wall of the rats after anaesthetizing (using chloroform) and cervical dislocation. Five milliliters (5ml) of blood were collected from each rat using a sterile syringe and placed in an Ethylene Diamine Tetra-acetic Acid (EDTA) container for full blood count analysis and peripheral blood film preparation. Bone marrow samples were also obtained from the rats by opening the femur longitudinally and exposing the marrow cavity. Sterile forceps were used to obtain the bone marrow from the cavity and placed in an Eppendorf container containing Trizol for molecular analysis [14].

2.2.10. Interleukin 6 (IL-6) and Tumor Necrosis Factor Alpha (TNF- α) mRNA Assay

2.2.10.1. Isolation of Total RNA

Total RNA was isolated from tissue samples with Quick-RNA MiniPrep™ Kit (Zymo Research). The DNA contaminant was removed following DNase I (NEB, Cat: M0303S) treatment. The RNA was quantified at 260 nm and the purity confirmed at 260 nm and 280 nm using A&E Spectrophotometer (A&E Lab. UK).

2.2.10.2. cDNA Conversion

One (1) μ g of DNA-free RNA was converted to cDNA by reverse transcriptase reaction with the aid of cDNA synthesis kit based on ProtoScript II first-strand technology (New England BioLabs) in a condition of 3-step reaction: 65 °C for 5 min, 42 °C for 1 h, and 80 °C for 5 min [17].

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2.2.10.3. PCR Amplification and Agarose Gel Electrophoresis

Polymerase chain reaction (PCR) for the amplification of IL-6 and (TNF- α) gene was carried out with OneTaqR2X Master Mix (NEB) using the following primers (Inqaba Biotec, Hatfield, South Africa). PCR amplification was performed in a total of 25 μ l volume reaction mixture containing cDNA, primer (forward and reverse SEE BELOW) and Ready Mix Taq PCR master mix. Under the following condition: Initial denaturation at 95°C for 5 min, followed by 30 cycles of amplification (denaturation at 95°C for 30 seconds, annealing for 30 seconds and extension at 72°C for 60 seconds) and ending with final extension at 72°C for 10 min. The amplicons were resolved on 1.0% agarose gel. The Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) gene was used to normalize the relative level of expression of each gene, and quantification of band intensity was done using “image J” software [17].

2.2.10.4. Primer sequences

IL-6

Forward: TCTCTCCGCAAGAGACTTCCA

Reverse: ATACTGGTCTGTTGTGGGTGG

TNF α

Forward: ACCACGCTCTTCTGTCTACTG

Reverse: CTTGGTGGTTTGCTACGAC

GAPDH

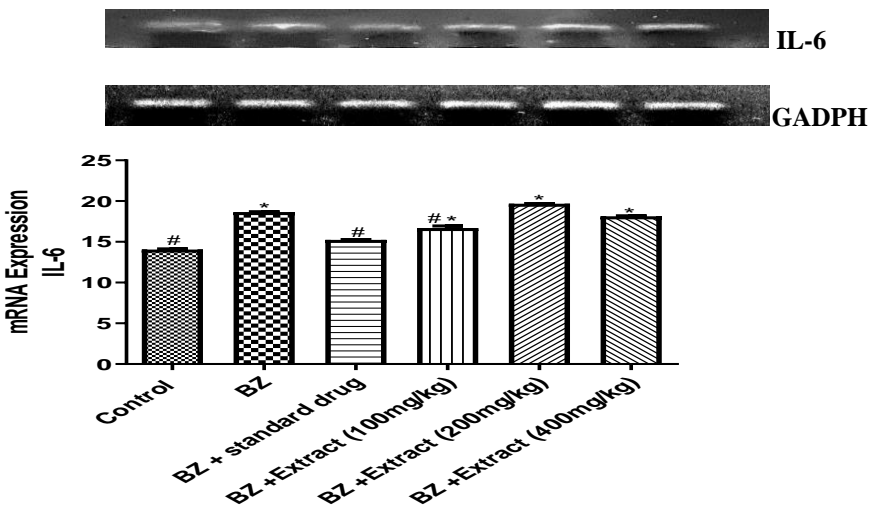
CTCCCTGGAGAAGAGCTATGA

AGGAAGGAAGGCTGGAAGA

2.3. Statistical Analysis

Data obtained from this research was presented and analyzed using statistical package for social sciences (SPSS) version 21.0 (IBM Inc. USA). Bar charts were used to represent the mRNA gene expression patterns. A p value of ≤ 0.05 was considered statistically significant.

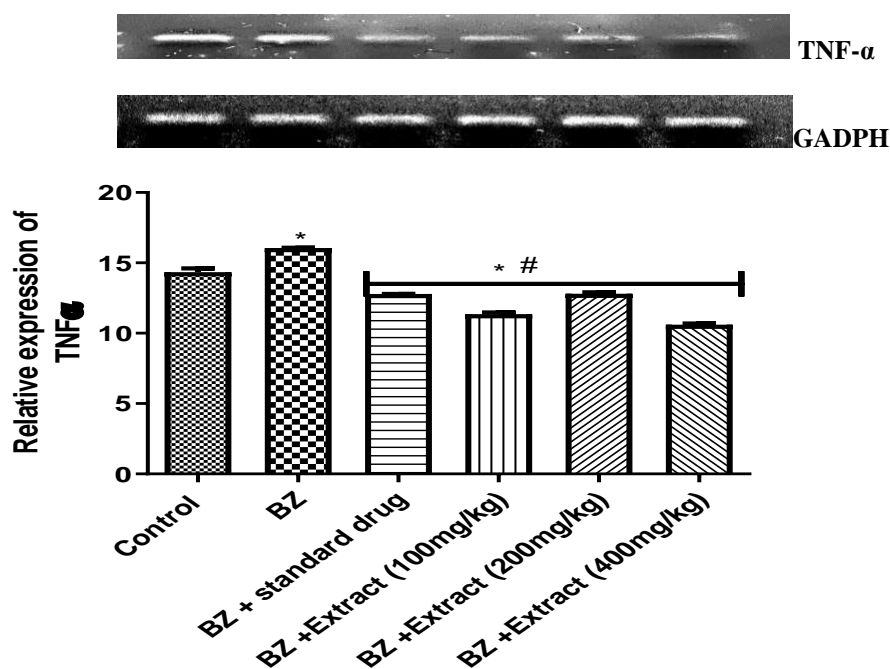
3. RESULTS



* Represents statistical difference to control. # Represents statistical difference to benzene induced group at $p < 0.05$.

Key: BZ=Benzene, GAPDH=Glyceraldehyde-3-Phosphate Dehydrogenase

Figure 1: mRNA Expression of IL-6 of the Studied Groups.



* Represents statistical difference to control. # Represents statistical difference to benzene induced group at $p < 0.05$.
 Key: BZ=Benzene, GADPH=Glyceraldehyde-3-Phosphate Dehydrogenase

Figure 2: mRNA Expression of TNF- α of the Studied Groups.

4. DISCUSSION

In recent years, there has been a significant surge of interest in the usage of plants for their medicinal purposes, driven largely by the exploration of their bioactive constituents. Bioactive compounds are naturally occurring chemicals found in plants that have the potential to exert physiological effects on the human body [18]. Benzene, an environmental pollutant, has been linked to detrimental effects on human health, particularly haematological toxicity [19]. Haematotoxicity induced by benzene exposure has been associated with altered gene expressions of key inflammatory cytokines, such as Interleukin-6 (IL-6) and Tumor Necrosis Factor-alpha (TNF- α), both of which play crucial roles in the regulation of immune responses and inflammatory processes [20]. *Launaea taraxacifolia*, a plant known for its traditional medicinal uses, has emerged as a subject of interest due to its reported anti-inflammatory and antioxidant properties. Interleukin-6 (IL-6) is a pro-inflammatory cytokine that plays a central role in orchestrating immune responses and inflammation. In this study, it was observed that benzene administration caused a significant increase in the expression of IL-6 gene. This finding aligns with the known pro-inflammatory effects of benzene exposure [21]. This result suggests that benzene, as a toxic environmental pollutant, has triggered an immune response characterized by the up regulation of pro-inflammatory cytokines like IL-6. This type of response is the body's attempt to address potential damage and initiate protective mechanisms. TNF- α is another pro-inflammatory cytokine produced primarily by immune cells, including macrophages and T cells. In this study, it was also observed that benzene administration caused a significant increase in the expression of TNF- α gene. TNF- α is known to play a pivotal role in initiating and amplifying inflammatory responses. It is produced by immune cells, particularly macrophages and certain T cells, in response to various stressors, including toxins, infections, and tissue damage. The release of TNF- α triggers a cascade of events that recruit immune cells to the site of inflammation and promote various inflammatory processes [22]. The activation of these pro-inflammatory cytokines may be attributed to the fact that benzene is metabolized in the body primarily by cytochrome P₄₅₀ enzymes in the liver [23], and one of its key metabolites which is benzene oxide can react with cellular components and form reactive intermediates. These intermediates can damage cellular structures and trigger inflammatory responses as a result of oxidative stress [24]. The inflammatory responses may be in the form of release of these proinflammatory cytokines. Also, benzene and its metabolites can activate transcription factors such as nuclear factor-kappa B (NF-B) and activator protein-1 (AP-1). These transcription factors which are key regulators of immunological and inflammatory responses, following

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activation can lead to an increase expression of proinflammatory genes, including cytokines like interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- α) [25]. The subsequent administration of different doses of *Launaea taraxacifolia* with benzene demonstrated differential effects on the expression of these genes. While the extract reduced TNF- α expression following benzene exposure, it further elevated IL-6 expression at 200mg/kg concentration of the extract. This contrasting response warrants careful consideration. The reduction in TNF- α expression suggests a potential anti-inflammatory effect of *Launaea taraxacifolia* which aligns with the findings of previous studies [26]. However, the further increase IL-6 expression after administration 200mg/kg of the extract with benzene raises questions about the extract's dose-dependent effects and its potential to modulate immune responses.

5. CONCLUSION

This study concludes that benzene administration led to an increase in the expression of the IL-6 gene. However, subsequent administration of 200mg/kg of *Launaea taraxacifolia* led to further increase in IL-6 expression. Similarly, benzene administration also led to an increase in the expression of the TNF- α gene. However, subsequent administration of different doses of *Launaea taraxacifolia* led to significant decrease in TNF- α expression.

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Conflict of Interest

There is no conflict of interest as declared by the Authors.

Contribution of the Authors

Progress A. Obazelu: Corresponding Author, conceptualization and manuscript writing. Favour E. Wasa: Analysis.

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