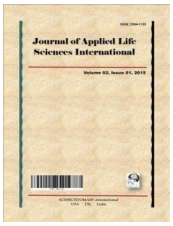

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Chemo-therapeutic Potential of *Dialium guineense* (wild) Leaf Extract on Dumpsite Leachate Induced Hepatoxicity of Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author ACI designed the study, performed the statistical analysis, and wrote the first draft of the manuscript. Author SOAE wrote the protocol and managed the analyses of the study. Author JEO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To investigate the chemo-therapeutic potential of *Dailum guineense* leaf extract on dumpsite leachate induced hepatoxicity of Wistar rats.
Study Design: A Cross-sectional study.
Place and Duration of Study: Department of Animal and Environmental Biology (Animal Unit) and Department of Life Science, University of Benin, Benin City, Edo state, Nigeria, between January 2016 and July 2017.
Methodology: A total of 20 wistar rats were acclimatized for two weeks and randomly distributed into four groups A to E, and were administered 2ml each different treatment protocol once every 48 hours for 30days.After the exposure period, the surviving rats were examined and sacrificed. Blood and organs were collected for analysis. After which the clinical biochemistry, tissue histology and the expression of some hepatic pro-inflammatory genes were examined.

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Results: The concentration of heavy metals and anions in the test samples were above standard permissible limits. Phytochemical analysis showed the presence of alkaloids, tannin, saponin, phlorotannins, steroids, and flavonoid except for cardiac glycoside was absent in both leaf extract. The concentration of heavy metals and anions in the test samples were above standard permissible limits. Biochemical analysis showed that leachate administration in Wistar rats caused an increase in glucose 24.15%, albumin 32.73% with a decrease in alkaline phosphatase 63.91% and glucose 13.96%. Histopathological investigations indicated that the leachate provoked alterations in the liver tissue; which include mild infiltration vascular congestion, patchy vascular ulceration and a mild periportal filtrates of inflammatory cells. An increased expression of CCL11 mRNA, TGF-α mRNA, and IL-4 in hepatic tissues as a result of leachate administration was observed. However, the administration of *Dialium guineense* leaf extract with the leachate prevented tissue damage in the Wistar rat to varying degree

Conclusion: The findings of the present study have shown the potentials of Ikhueniro dumpsite leachate to induce tissue and genetic dysfunction probably via direct and/or indirect chemical disruption of the blood. However, *Dialium guineense* protected the liver against dumpsite leachate by reducing the effect of the leachate on the liver.

Keywords: Chemo-therapeutic; Dialium guineense; dumpsite leachate; hepatoxicity; wistar rats.

1. INTRODUCTION

The liver is a vital organ which regulates many important metabolic functions and is responsible for maintaining homeostasis of the body. It has an immense task of detoxification of xenobiotics, environmental pollutants, and chemotherapeutic agents. Hence, this organ is subjected to a variety of diseases and disorders [1]. Hepatotoxicity is one of very common ailment resulting in serious debilities, ranging from severe metabolic disorders to even mortality [2].

Most heavy metals are toxic and are contained in dumpsite leachate, and are said to induce hepatotoxicity in wistar rats [3]. Dumpsite leachate forms when waste deposited in a dumpsite decomposes. The generation of waste, however, is inevitable and waste disposal has become a common way of managing waste worldwide. In an attempt to guide against the release of hazardous chemicals capable of polluting the environment and harming biotic communities, different methods of waste management (incineration, landfilling, recycling, composting, disposal into sea and water ways, burning along major roads and surface dumping) are utilized worldwide. Among these, landfilling and open dumping are the most common methods of managing these wastes [4-6]. This is because they are the cheapest and most convenient way of disposing of municipal solid wastes (MSW); however, open dumping is even cheaper. Furthermore, dumpsites in most developing nations, for instance, Nigeria, and some developed nations are unsanitary without liner, covers and leachate collecting systems. They are also not designated for specific waste type and are located in public places surrounded by residential quarters and in wetland or other places with seasonally high-water tables. Unlined dumpsite or landfill have been reported to release large amounts of hazardous and deleterious chemicals to nearby ground water and to the air, via leachate and landfill gas respectively [7-9], which can induce liver damage. Most hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and oxidative stress in liver, and can further lead to liver disease.

A number of chemical agents and drugs which are used on a routine basis produce cellular as well as metabolic liver damage [10]. Certain natural antioxidants overdose in both animals and man has been shown to produce hepatic necrosis [11]. Administrations of the proper dosage of these natural antioxidants play a major role in the management of various liver disorders. A number of plants possess hepato-protective properties [12]. Howbeit, further research on various natural antioxidants that can be used against leachate induced toxicity is ongoing. *Dalium guineense* however, is a medicinal plant that possesses anti-mutagenic, antiulcer, anti-neuralgic, anti-inflammatory and anti-microbial activities [13-15] is gaining consideration recently. The leaf extract of *Dalium guineense* contains phytochemicals such as flavonoids, alkaloids, tannin, and saponin, which are responsible for their antioxidant properties. Study to evaluate the chemo-therapeutic potential of *Dalium guineense* leaf extract on

dumpsite leachate induced hepatotoxicity in wistar rats is therefore important.

2. MATERIALS AND METHODS

2.1 Collection of Dumpsite Leachate

Raw leachate was collected from five different leachate collection point around the dumpsite and thoroughly mixed to provide a homogenous representative sample for each sampling site. This was transferred to the laboratory in pre-cleaned 4-liter plastic containers, filtered with a muslin cloth to remove suspended particles and stored at 4^{o}C until use. This was considered as the stock sample (100%) and was labeled Ikhueniro dumpsite leachate. The sample was analyzed for a number of standard physical and chemical properties according to procedures outlined in the Standard Methods for the Examination of Water and Wastewater [16,17].

2.2 Collection, Identification and Classification of Plants

Fresh leaves of *Dialium guineenses* (black velvet) was collected from Upper Sakponba, Benin city; The taxonomic identity of the plant was confirmed at the Department of Plant Biology and Biotechnology, Faculty of Life Science, University of Benin, Benin City, Edo State, Nigeria. Phytochemical analysis (such as carbohydrate, alkaloids, tannin, saponin, cardiac glycoside, anthraquinone, phlorotannins, steroids and flavonoid) of the crude powder of the leaves collected was determined according to the standard procedures to identify the constituents [18-21].

2.3 Preparation of Abatements

The abatement was the leaf extract of black velvet tree (*Dialium guineenses*). The leaves collected were air-dried to crispiness in the laboratory (prevailing room temperature of 30 ± 2°C) for two weeks. The dried materials were reduced to coarse form using a pestle and mortar and further pulverized to very fine particles using Viking Exclusive Joncod pulverizing machine (Model: YLH2M2 - 4). 28g of the powdered leaves was subjected to infusion extraction and exhaustively extracted with 0.5L of warm water for Four hours. The extracts were filtered and stored at a temperature of about 4°C in a clean container prior to use.

2.4 Collection and Acclimatization of Experimental Rats

Thirty (30) male and female wistar rats (6-7 weeks old) weighing within the range of 100g to 150g were obtained from the Anatomy Department, University of Benin, Nigeria; and housed in wooden cages with wire mess covers. The rats were acclimatized for 2 weeks until they were 8-9 weeks and their weights were taken. The animals were fed with standard rodent chow (Bendel Livestock Feeds Limited, Ewu, Edo State, Nigeria) and given distilled water ad labium.

2.5 Experimental Setup

The rats were distributed randomly into five groups of six animals (3 males and 3 females) each. The rats were administered different treatment protocol as stated below.

Group A – Control (C)
Group B - Leachate (L)
Group C - Leachate + Abatement A (LA)

This was allowed for 30 days. Half of the rats were sacrificed while the remaining rats in group B and C were given the protocol below for another 30 days.

Group B_N – Clean water with no leachate (NL)
Group C_N – Abatement A with no leachate (NLA)

The rats were maintained in laboratory conditions; and had access to drinking water and standard rodent chow (Bendel Livestock Feeds, Ewu, Edo State, Nigeria®) *ad libitum.* Each animal in a group was gavaged 2ml of the different protocol as described above for 30 consecutive days (once every 48 hours). At the end of the exposure period, survivors were fasted overnight, weighed (using Acculab® USA, Model-Vic-303 electronic analytical weighing balance) and sacrificed under slight Anesthesia. Blood sample and the liver were collected.

2.6 Organ Weight Measurement, Collection, and Preparation of Samples

Blood was collected from the inferior vena cava of the rats with a plain 5ml sterilized syringe into a vial containing heparin (lithium and ammonium), fluoride oxalate (sodium fluoride and potassium oxalate) and plain bottles for

biochemical analysis under a light anesthesia. The blood in the bottles containing anticoagulants was directly centrifuged at 3000 rpm for 10 minutes to separate the plasma (supernatant); while the blood in the plain bottle was allowed to clot, centrifuge at 3000 rpm for 10 minutes to separate the serum (supernatant). The blood plasma and serum were stored at -80^{o}C prior to biochemical analysis. The liver was surgically removed, rinsed with ice-cold physiological saline and blotted dry. While slices of the liver from exposed and control animals were fixed in 10% neutral buffered formalin before being processed.

2.7 Laboratory Analysis

2.7.1 Serum biochemical analysis

Serum biochemical markers such as total protein, albumin, and alkaline phosphate were measured as a functional marker for hepatotoxicity These biomarkers were determined colorimetrically by employing the standard ready-to-use kits and methods of Human. Alkaline phosphate was determined using TECO diagnostic assay kits (TECO Diagnostics, CA); total protein, albumin, and glucose were determined using RANDOX assay kits (RANDOX laboratories Limited, UK). The manufacturer's instructions for each biochemical parameter were strictly followed in the course of the investigations. The absorbance of the tests was measured spectrophotometrically using OPTIMA, SP-300 (Japan).

2.7.2 Histopathological analysis of the liver

Slices of the liver from exposed and control animals were fixed in 10% neutral buffered formalin before being processed. All organs tissue was processed with the standard protocol using Leica automated tissue processor (Model - TP1020). The organ was cut into bits and put into a tissue cassette. The cassette was label accordingly and fixed in 10% formalin solution for two hours. After fixation, the organ was dehydrated in ascending grades of alcohol (starting from 70%, 90%, 96% and absolute) for two hours each. The dehydrated organ was cleared in xylene for two hours and impregnated in using molten wax for two hours.

Processed tissue was then embedded using Thermo scientific Histo star embedding machine (Model - E312010). The impregnated tissue was set on a mold and paced in the hot section of the machine to dissolve the paraffin wax. Then the organ was properly set in the mold and transferred to the cold section to solidify. Sectioning was carried out using a Leica micro tomb (Model - RM 2235). The section was transferred to a Thermo scientific digital section flotation bath (Model - A82000101) to all the section spread out properly. The section was picked from the water bath using the slides and transferred to a thermal scientific slime hotplate (Model- A82100116) for the slides to dry.

The organ section was dewaxed in xylene and hydrated using ascending grades of alcohol (Absolute alcohol, 96% alcohol, 90% alcohol and 70% alcohol) to water for 3minutes in each solution. The hydrated tissue section was stained with Gill 2 hematoxylin for 10 minutes and then rinsed in water. The section was differentiated briefly with 10% acid alcohol and blue in running water for 10 minutes to develop the color of the hematoxylin. The slide was counter stained with Shandon Eosin and rinsed in water afterward. The stained slides were again dehydrated using ascending grades of alcohol (starting from 70%, 90%, 96% and absolute); and cleared in xylene for 5 minutes. The section was mounted using shandom's mount (Distrene Dibutyl Phathalate xylene), covered with a cover slip and allowed to dry. The slides were examined Leica CME light microscope (Model – 1349522X).

2.7.3 Expression of hepatic pro-inflammatory genes

The levels of expression of certain hepatic pro-inflammatory genes were assessed using semi-quantitative reverse transcriptase polymerase chain reaction (RT-PCR) techniques. In brief, RNA from the liver samples was extracted using the spin column kit obtained from Aidlab's EASYspin PlusVR (Aidlab Biotechnologies Co., Ltd, Beijing, China) according to the instructions of the manufacturer. The RT-PCR was carried out with 500 ng RNA template using the Transgenic EasyScriptVR one-step RT-PCR supermix (Beijing TransGen Biotech Co., Ltd, Beijing, China) according to the instructions of the manufacturer. Samples were subjected to an initial incubation at 45ºC for 30 min for cDNA synthesis, followed by PCR amplification, using gene-specific primers (GSP) (Table 1), 94°C for 5 min followed by 50 cycles of 94°C for the 30s, 5 min at the annealing temperature of GSP, and 1 min at 72°C. All amplifications were carried out in C1000 Touch^{TM} Thermal Cycler (BioRad, Hercules, CA). The intensity of the amplicon

bands on 1.5% agarose was analyzed using Image J software [22]. Results were presented as the relative expression of the gene in comparison with the level of expression of B-Actin gene.

2.8 Data Analysis

All statistical analyses were conducted with Statistical Package for Social Scientists (SPSS) and Microsoft Excel computer software. Data are presents as mean ± Standard Error (SE). One-way Analysis of Variance (ANOVA) was used to determine the differences among various groups.

3. RESULTS AND DISCUSSION

The results of the biochemical analysis showed that leachate administration in wistar rat caused an increase in serum glucose (118.25 ± 26.75 mg/dl), alkaline phosphatase (ALP) (47.71 ± 5.86 mg/dl) and total protein (TP) (8.61 ± 0.63 mg/dl) and a reduction in albumin (2.65 ± 0.23 mg/dl) (Table 2). Similar finding has earlier been reported by Farombi et al. [23]; stating that Olushosun municipal landfill leachate from Ojota in Lagos State of Nigeria increased ALP in wistar after 14 days exposure. Alimba et al. [3] also reported an increase in ALP and a decrease in Albumin and total protein in wistar rats as a result of leachate from Olusosun and Aba-Eku landfills in South-western Nigeria.

The co-administration of leachate and abatement caused a reversal in the trend observed in the leachate administered group of wistar rats (Table 2). Similar trend was also reported in groups of rats where leachate administration was stopped. The reversal in the negative trends noted when leachate was co-administered with the leave extract of *Dalium guineense* may be attributed to the therapeutic properties of the plant. Okemo *et al*. [24] noted that some plant parts have been used as antimicrobial agents, especially their extract as decoctions, infusions, or oral administration. Osuagwu and Eme [25] also reported that the leave extract of *Dalium guineense* extracts inhibited the growth of these pathogens.

Furthermore, the administration of *Dialium guineense* abatement caused a significant increase in glucose in rats where it was administered with leacahte (181.75 ± 51.75 mg/dl) and without leachate (153.25 ± 9.75 mg/dl). This significant increase may be associated with the high carbohydrate concentration in the leave extract. The pulp of *Dalium guineense* fruit has been reported to contain 75% carbohydrate [26].

The relative expression of the hepatic pro-inflammatory genes of Wistar rats administered different protocol is shown in Fig. 1. It was noted that the administration of only leachate (0.56) caused an increase in the relative expression of hepatic Eotaxin (CCL12) Interleukin 4 (IL-4) and Transforming growth Factor (TGF) gene in Wister rats, when compared to the control (0.48). It was noted that the co-administration of Ikhueniro dumpsite leachate with leaf extracts of *Dalium guineenses* in Wistar rats caused a further increase in the relative expression of the hepatic CCL_{12} gene (0.56 and 0.91 respectively)

Table 1. Sequences of gene-specific primers

S/N	Gene	Sequence (50–30)
1.	B-Actin	Forward: GTCAGGTCATCACTATCGGCAAT
		Reverse: AGAGGTCTTTACGGATGTCAACGT
2.	TGFβ	Forward: GCTGAACCAAGGAGACGGAA
		Reverse: CCACGTAGTAGACGATGGGC
3.	CCL11	Forward: CACCCAGGTTCCATCCCAAC
		Reverse: GGGGATGGGTGCCGATATTC
4.	IL1α	Forward: CCATCCAACCCAGATCAGCA
		Reverse: TCTCCTCCCGATGAGTAGGC

Table 2. Some liver function parameters of Wistar rats administered Ikhueniro dumpsite leachate and *Dialium guineense* leaf extract

	C	L	LA	NL	NLA
Glucose (mg/dl)	95.25±4.75	118.25±26.75	181.75±51.75	104.00±0.50	153.25±9.75
Albumin (mg/dl)	2.75±0.05	2.65±0.23	3.38±0.18	3.98±0.02	3.28±0.35
Alkaline phosphatase (mg/dl)	40.92±8.85	47.71±5.86	21.10±4.40	41.25±0.69	40.98±4.30
Total protein (mg/dl)	7.18±0.33	8.61±0.63	7.05±0.20	6.90±0.40	7.51±0.40

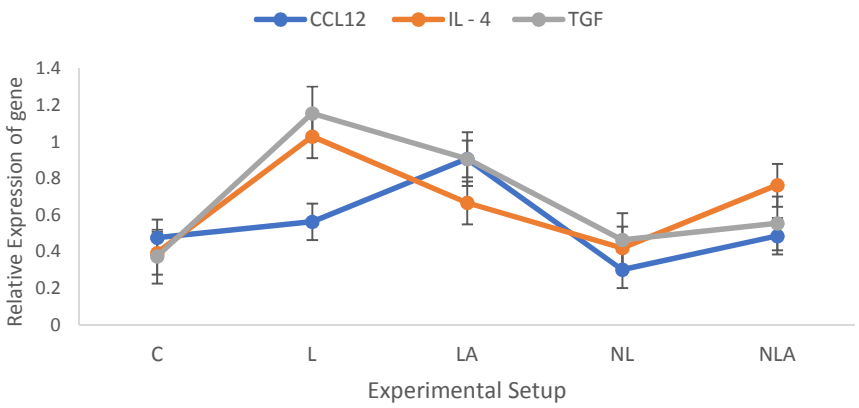

Fig. 1. Relative expression of hepatic pro-inflammatory gene of Wistar rats administered Ikhueniro dumpsite leachate and *Dialium guineense* leaf extract

when compared to the control (0.48); while the expression of Interleukin 4 (IL-4) and Transforming growth Factor (TGF) gene was reduced when compoared to the leachate administered group. Similar trend has been reported by Soliman et al. [27] who reported that Curcumin administration decreased the expression of IL-1 and IL-8 expression in the liver of wistar rats previously increased by paracetamol administration. However, when leachate administration was stopped, the relative expression of the hepatic CCL_{12} gene in Wistar rats decreased when compared to the leachate administered group.

Plates 1 to 4 showed the histological findings in the hepatic tissues of the Wistar rats exposed to the different protocol. Haemato-xylene and Eosin stained hepatic tissue micrographs revealed that leachate administration in wistar rats caused a moderate periportal infiltrates of inflammatory cells (Plate 2). Which was also noticed mildly after leachate administration was stopped after 30days (Plate 4). The histological alteration may be associated with corrosive nature of Ikhueniro dumpsite leachate. Similarly, a mild to severe multifocal degenerative and necrotizing hepatitis which is shown by the presence of a diffused hydropic degeneration of hepatocytes and multiple foci of periportal zones; shrunken hepatocytes and diffused hepatic necrosis with cellular infiltration by macrophages and lymphocytes has been reported by Alimba et al. [28] reported as a result of Olusosun and Aba-Eku landfills in South-western Nigeria.

Furthermore, the administration of the leachate along with abatement, and the administration of abatement after leachate administration have been discontinued, however, caused a mild activation of the kupffer cell (Plates 3 and 5). Kupffer cells (KC) reside within the lumen of the liver sinusoids, and are intimately involved in the liver's response to infection, toxins, ischemia, resection and other stresses [29]. Ajiboye *et al*. [30] reported that *Phyllanthus muellarianus leaves* prevented necrotic damage caused by Acetaminophen by activation of Kupffer cells in the liver of mice.

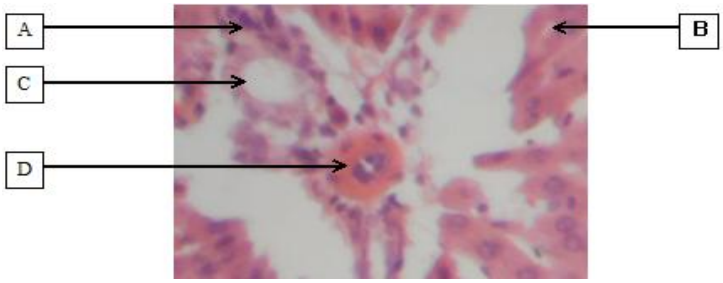

Plate 1. Photomicrograph of Wistar rat liver in the control group (A, hepatocytes, B, sinusoids, C, bile ducts and D, hepatic artery) (H&E x 100)

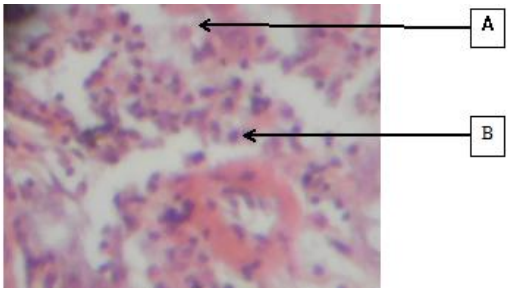

Plate 2. Photomicrograph of Wistar rat liver exposed to leachate only (A, mild vascular congestion and B, moderate periportal infiltrates of inflammatory cells (portal hepatitis)) (H&E x 100)

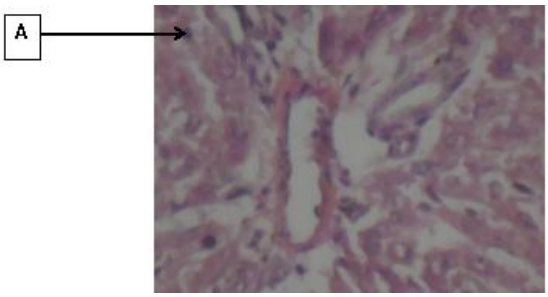

Plate 3. Photomicrograph of Wistar rat liver exposed to leachate +*D. Guinness* (A, mild kupffer cell activation) (H&E x 100)

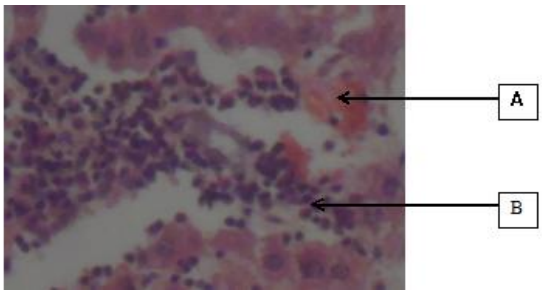

Plate 4. Photomicrograph of Wistar rat liver with leachate exposure discontinued (A, mild vascular congestion, and B, moderate periportal infiltrates of inflammatory cells) (periportal hepatitis) (H&E x 100)

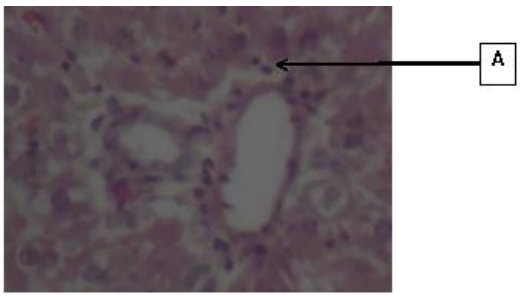

Plate 5. Photomicrograph of Wistar rat liver with leachate exposure discontinued but *D. guinensis* exposure continued (A, mild kupffer cell activation) (H&E x 100)

4. CONCLUSION

The findings of the present study have shown the potentials of Ikhueniro dumpsite leachate to induce tissue and genetic dysfunction probably via direct and/or indirect chemical disruption of the blood. However, *Dialium guineense* protected the liver against dumpsite leachate by reducing the effect of the leachate on the liver.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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