Evaluation of Heavy Metals, Phytochemical Constituents and Median Lethal Dose of Two Anti-diabetic Plants (*Moringa oleifera* and *Carica papaya*)

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Abstract

Herbal medicine has been recognized as a major component of treatment for many diseases all over the world. Studies have shown that herbal specimens can be contaminated with heavy metals which can be detrimental to the health of the consumers if their concentrations exceed the allowable limits. The present study was aimed to determine the levels of heavy metals, phytoconstituents and median lethal dose of methanol extracts of two anti-diabetic plants; *Moringa oleifera* and *Carica papaya*. The heavy metals and phytoconstituents were evaluated using standard qualitative methods, while the median lethal dose was determined using Lorke's method. The results showed that zinc, arsenic, copper, nickel, cobalt and manganese were detected in the leaves of all the plants, while lead was only detected in the leaves of *C. papaya*. However, none of the plants was found to contain cadmium. The preliminary phytochemical analysis revealed the presence of saponins, carbohydrates, flavonoids, tannins, cardiac glycosides and steroids in both extracts, while alkaloids were only detected in the methanol extract of *C. papaya* leaves. The acute toxicity study showed that the median lethal dose (LD_{50}) of the two extracts was estimated to be above 5000 mg/kg body weight. The findings in this study showed that both extracts are practically non-toxic, and also, the levels of heavy metals detected didn't exceed the permissible limits approved by the World Health Organization (WHO), thus, the leaves of these anti-antidiabetic plants are safe for human consumption.

Keywords: Anti-diabetic, heavy metals, phytoconstituents, permissible limits, safe

Introduction

Diabetes mellitus is characterized by hyperglycemia (i.e high blood glucose) with alteration of carbohydrates, protein and fat metabolism, resulting from defects in insulin secretion or sensitivity of insulin to body cells or both (Hovens *et al*., 2005). Such alterations result in elevated blood glucose concentration which causes acute complications like hyperglycemia and hypoglycemia and long term complications in many organs. These complications may also lead to increase the risk of atherosclerosis, nerve damage, renal failure, blindness and coronary heart disease resulting in increasing disability (ADA, 2009).

Medicinal plants play a significant role in the management of diabetes mellitus, particularly in developing countries where many people do not have access to modern antidiabetic drugs (Acharya and Shrivastava, 2008), and the antidiabetic activity of some of those plants has been demonstrated using different diabetic models. Examples of such antidiabetic plants include *Moringa oleifera*, *Carica papaya*, *Acacia nilotica*, *Adonsonia digitata*, *Anacardium occidentalis*, *Azadirachta indica*, *Ficus sycomorus*, *Khaya senegalensis*, *Psidium guajava*, *Parkia biglobosa*, *Vernonia amygdalinia* among others (Oke, 1998; Mousa, 1994; Abo *et al*., 2008; Perez-Gutierrez and Domain-Guzman, 2012; Sabjan and Vinoji, 2012; Ibrahim and Islam, 2014; Mwangi *et al*., 2015; Abubakar *et al*., 2017).

Heavy metals pollution has continued to gain global attention; this is because of the toxicological risks posed by such metals to human and animal health (Ayodeji and Olorunsola, 2011). There are many health implications associated with higher levels of heavy metals in medicinal plants and herbal products. Higher level of heavy metals has been reported to cause cancer, asthma, cardiovascular disorders, allergic reactions, overproduction of red blood cells, anxiety, depression, premenstrual syndrome, respiratory problems, weakened immune systems, kidney and liver damage, inactivation of enzymes involved in Deoxyribo Nucleic Acid (DNA) synthesis and repair, nausea abdominal pain, severe diarrhea among others (Henok and Ariaya, 2013). In view of that, the World Health Organization (WHO) and other regulatory bodies have recommended that medicinal plants and other herbal formulations should be evaluated for the presence of heavy metals and their toxicity indexes determined; therefore, this study was aimed at determining the levels of heavy metals and the phytochemicals in the leaves of two known antidiabetic plants; *Moringa oleifera* and *Carica papaya*, as we as their median lethal dose (LD_{50}).

Materials and Methods

Experimental Animals

Adult Swiss albino mice of both sexes, weighing 20 to 35 g were obtained from the animal house of the Department of Pharmacology, Bayero University Kano, Nigeria and maintained under normal laboratory conditions of humidity, temperature and light for 7 days before the experiment and allowed free access to food and water. All experiments performed on the laboratory animals in this study were approved by the Local Ethical Committee for animal experimentation in the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Bayero University, Kano, Nigeria.

Collection, Identification and Preparation of Plant Materials

The plants were collected at the New Campus of Bayero University, Kano Nigeria, and then taken to the Herbarium of Ethnobotany and Multi-disciplinary Research Division of Bioresources Development Centre, Kano for authentication, and reference voucher numbers: BDCKN/EB/1623 for *Moringa oleifera* and BDCKN/EB/1605 for *Carica papaya* were deposited in the Herbarium. The leaves of *M. oleifera* and *C. papaya* were air dried and then ground into fine powder using mortar and pestle. The powdered plant materials (20 g each) were macerated separately with methanol (250 ml) for 48 hours, and each mixture was shaken occasionally. The filtrates obtained were evaporated to dryness at 40 ℃ using rotary evaporator and water bath.

Heavy Metals Analysis

This was conducted as described by Ali et al (2020). The powdered plant materials (200 mg each) were weighed separately and transferred into 90 ml microwave digestion vessel, 10 millilitres mixture of 15.9 N trace metal grade Nitric acid, Hydrogen peroxide and Perchloric acid (7:2:1) was added to the vessel. After standing for one hour, the samples were processed by microwave digestion system as follows: ramp temperature from ambient to 200 ℃ over 20 minutes, then hold at 200 ℃ for 20 minutes, after digestion, the sample was allowed cool to approximately 50 ℃ or lower before handling. The digests were transferred to 50 ml volumetric flask, the volume was adjusted to 50 ml with deionised water and filtered for heavy metals (zinc, arsenic, lead, copper, nickel, cadmium, cobalt and manganese) analysis using Agilent Micro Plasma Atomic Emission Spectrometer (MP-AES, 4200) available in the Centre for Dryland Agriculture, Bayero University, Kano, Nigeria.

Preliminary Phytochemical Screening

The preliminary phytochemical screening of the extracts was conducted using standard qualitative methods as described by Evans (1996), Harbone (1998), Sofowora (2008) and Prashant et al (2011).

Acute Toxicity Study

This was conducted in two phases using the method described by Lorke (1983). In the initial phase, mice were divided into 3 groups of three mice each. The first group received methanol extract of *M. oleifera* leaves (*i.p*) at a dose of 10 mg/kg body weight, followed by 100 mg/kg and 1000 mg/kg body weight to the second and third group respectively. This was also repeated on methanol extract of *C. papaya* leaves. The animals were then observed for 24 hours for signs and symptoms of toxicity and death. In the final phase, mice were divided into 3 groups of one mouse each, methanol extract *M. oleifera* leaves was administered to group 1, 2 and 3 at the doses of 1600, 2900 and 5000 mg/kg body weight respectively, and this was also repeated on methanol extract of *C. papaya* leaves. The median lethal dose (LD_{50}) was calculated from the results of the final phase as the square root of the product of the lowest lethal dose and the highest non-lethal dose.

Statistical Analysis

The heavy metals determinations were carried out in triplicates, and data was expressed as mean ± standard deviation (SD).

Results

Heavy Metals Analysis

The heavy metals analysis showed that zinc, arsenic, copper, nickel, cobalt and manganese were detected in the leaves of *M. oleifera* and *C. papaya*, while cadmium was not detected. However, lead was only detected in the leaves of *C. papaya* as presented in Table 1;

Table 1: Levels of Heavy Metals in the Leaves of *M. oleifera* and *C. papaya*

S/N	Heavy Metal	Concentration in mg/kg		WHO Permissible Limit in mg/kg
		M. oleifera	***C. papaya***	
1	Zinc	0.06±0.01	0.10±0.01	***
2	Cadmium	ND	ND	0.3
3	Copper	0.02±0.00	0.03±0.00	***
4	Nickel	0.01±0.00	0.01±0.00	***
5	Arsenic	0.12±0.17	0.04±0.01	10
6	Cobalt	0.02±0.00	0.02±0.00	***
7	Lead	ND	0.01±0.00	10
8	Manganese	0.02±0.00	0.31±0.00	***

ND = Not detected

*** = Not yet decided

Preliminary Phytochemical Screening

The preliminary phytochemical screening revealed the presence of saponins, carbohydrates, flavonoids, tannins, cardiac glycosides and steroids in both extracts, however, alkaloids were only detected in the methanol extract of *C. papaya* leaves as presented in Table 1 and 2;

Table 2: Phytochemical Constituents of Methanol Extracts *M. oleifera* and *C. papaya* Leaves

S/N	Phytochemicals	Methanol Extracts of;

		M. oleifera	*C. papaya*
1-	Tannins	+	+
2-	Anthraquinones	-	-
3-	Cardiac glycosides	+	+
4-	Saponins	+	+
5-	Flavonoids	+	+
6-	Alkaloids	-	+
7-	Steroids	+	+
8-	Carbohydrates	+	+

Key

+ = Present

- = Absent

Acute Toxicity Study

The acute toxicity study of methanol extracts of *M. oleifera* and *C. papaya* leaves resulted in no mortality and other behavioural changes as presented in Table 3, thus, the median lethal dose (LD_{50}) of the two extracts was estimated to be above 5,000 mg/kg body weight.

Table 3: Acute Toxicity Study of Methanol Extracts of *M. oleifera* and *C. papaya* Leaves in Mice

First Phase		Second Phase	
Dose (mg/kg)	**Mortality**	**Dose (mg/kg)**	**Mortality**
10	0/3	1600	0/1
100	0/3	2900	0/1
1000	0/3	5000	0/1

Discussion

The evaluation of heavy metals in the leaves of two anti-diabetic plants; *M. oleifera* and *C. papaya* revealed the presence of zinc, arsenic, copper, nickel, cobalt and manganese, while lead was detected in the leaves of *C. papaya*. The presence of arsenic, manganese, zinc, copper, nickel and lead agreed with the work of Edori et al (2019). The levels of arsenic detected in the leaves of these anti-diabetic plants didn't exceed the WHO 10 mg/kg permissible limit in medicinal plants and other herbal products (Gasser *et al*., 2009). Also, the level of lead detected in the leaves of *C. papaya* didn't exceed the WHO permissible limit (WHO, 2007). On the other hand, the WHO have not yet decided on the permissible limits of zinc, cobalt, manganese, copper and nickel in medicinal plants and herbal products; this is because, these metals are essential dietary micronutrients that are important for normal functioning of vital organs (Kosalec *et al*., 2009), however, the permissible limits for copper in some countries such as China and Singapore are 20 mg/kg and 150 mg/kg respectively (WHO, 2005).

The phytochemical analysis of the methanol extracts of *M. oleifera* and *C. papaya* revealed the presence of saponins, carbohydrates, flavonoids, tannins, cardiac glycosides and steroids, while, alkaloids were only detected in the methanol extract of *C. papaya*. The presence of flavonoids, saponins, cardiac glycosides and alkaloids could be responsible for the anti-diabetic activity of these plants (Singh *et al*., 2014).

Acute toxicity study is usually conducted to predict toxicity and provide guidelines for selecting safe doses for humans. It is the first step in toxicological investigation, which predicts the safety margin for any substance and hence the choice of doses for further studies (Aliyu *et al*., 2015; Lorke, 1983). The intraperitoneal median lethal dose of the methanol extracts of *M. oleifera* and *C. papaya* leaves in mice was estimated to be above 5000 mg/kg body weight. Substances with LD_{50} above 5000 mg/kg body weight are considered to be practically non-toxic, thus, both extracts could be considered as practically non-toxic (Loomis and Hayes, 1996).

Conclusion

The findings in this study showed that the methanol extracts of *M. oleifera* and *C. papaya* leaves are practically non-toxic, and also, the levels of heavy metals detected didn't exceed the permissible limits approved by the World Health Organization (WHO), thus, the leaves of these anti-diabetic plants are safe for human consumption.

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Conflict of Interest

The authors declare no conflict of interest.

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