RESEARCH PAPER

MEDIAN LETHAL DOSE (LD$_{50}$) EVALUATION OF SOME POLYHERBAL FORMULATIONS MARKETED IN NORTHERN NIGERIA

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ABSTRACT

The polyherbal preparations reported here are traditionally used in Northern Nigeria for the treatment of wide range of illnesses. The aim of this study was to evaluate the acute toxicity potential of 70% ethanol extracts of forty polyherbal products by determining their median lethal dose (LD$_{50}$) estimates intraperitoneally and orally using the Lorke's method in mice. Overall 90% of the extracts indicated values that were either less toxic or slightly toxic intraperitoneally, while 10 % had values that were practically non toxic using the same route. Oral administration of the extracts showed that 25% had values that were only slightly toxic while 75 % of the herbal products had median lethal dose values that were practically non toxic. From our results this could imply that most of the extracts tested may be safe for oral use and this could explain the continuous use of the polyherbal preparations by the local people in traditional management of various ailments in the Northern part of Nigeria.

Keywords: Herbal products, AcuteToxicity, Lorke's method, Northern Nigeria
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INTRODUCTION

Folklore or herbal medicine is gaining greater recognition in the developed countries and often represents the popular therapeutic system to which people are referred for their primary health care in the developing countries. Medicinal plants are the oldest known health-care products because people have been utilizing herbal medicine for the treatment, control and management of a variety of diseases since ancient times (Kong *et al.,* 2003,). It is generally presumed that herbal medicines are more effective and because of their natural source are free from undesirable side effect (Mbaka and Owolabi, 2011). This belief has led to serious abuse such as prolonged administration without appropriate dose monitoring thereby undermining the greater potential for adverse effect.

The extensive traditional use of plants as medicines has enabled those medicines with acute and obvious signs of toxicity to be well recognised and their use avoided. However, the premise that traditional use of a plant for perhaps many hundreds of years establishes its safety does not necessarily hold true (De Smet, 2004). The increase in the number of users as opposed to the scarcity of scientific evidence on the safety of medicinal plants has raised concerns regarding toxicity and detrimental effects of these remedies. In rural communities, the exclusive use of herbal drugs prepared and dispensed by herbalists without formal training in the drug formulation and preparation for disease treatment is still very common, thus there is the need for screening methods be established to ascertain the safety and efficacy of these herbal products (Ogbonnia *et al.*, 2008).

Majority of the traditional herbal medicines used in Africa are provided by practitioners who live within the communities. They have been trusted over time and are often willing to assist the patients with their knowledge and skills, sometimes at minimal costs to the patients. Most of these herbal medicines are procured in their crude forms although some pharmaceutical prepackaged forms also exist and are available over the counter. Interestingly a genuine interest in various traditional practices now exists among practitioners of modern medicine and growing number of practitioners of traditional, indigenous or alternative systems are beginning to accept and use some of the modern technology. This will help foster teamwork among all categories of health workers within the framework of primary health care.

In the determination of safety of drugs and herbal products for human consumption, toxicological evaluation is carried out in various experimental animals to predict toxicity and to provide guidelines for selecting a 'safe' dose for humans. The proof of safety of these medicines in animals should take precedence over establishing efficacy. Hence, this study sought to investigate the acute toxicity profiles of Forty (40) polyherbal preparations marketed by members of Kano State branch of National Association of Nigerian Traditional Medicine Practitioners (NANTMP) through determination of the median lethal doses (LD_{50}) in mice.

MATERIALS AND METHODS

Study Area: Kano State located in the northwest political zone of Nigeria (11^0 30'N, 8^0 30'E), has a total area of $20,131$ km^2 and population of about 13 million people is divided into 44 local government areas (LGAs). A large number of traditional medicine practitioners practice in the state, some of which practice independently while others practice under the umbrella of a union. The National Association of Nigerian Traditional Medicine Practitioners (NANTMP) is one of such associations that have been recognized by the Government of Nigeria.

Collection of Test Materials: Herbal products were collected from various local government areas across the state under the umbrella of the National Association of Nigerian Traditional Medicine Practitioners. Some of the products had their compositions made available. All products were powdered materials.

Preparation of Extracts: For each product tested, 50 g of the powdered material was weighed out and submerged in 250 ml 70% ethanol and left to macerate for 72 h with occasional shaking. After maceration the resultant mixture were filtered using Whatman filter paper (No.1) and the filtrates evaporated to complete dryness using water bath at 50^0C (Aliyu *et al.,* 2014). The resulting dry extracts were then weighed to determine the percentage yield for each product. Aliquot portions of the extracts were weighed and dissolved in distilled water for use in the study.

Experimental Animals: Adult Swiss albino mice (weight: 20 to 33 g) of both sexes were used for the experiments. The animals 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 prior to 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 Clinical Sciences, Bayero University, Kano, Nigeria.

Acute Toxicity Study (Median Lethal Dose (LD_{50}) Determination): 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 and treated at doses of 10, 100 and 1000 mg/kg, respectively of each extract, intraperitoneally (*i.p.*). These were then observed for 24 h for signs of toxicity, including death. In the final phase, mice were divided into either 3 or 4 groups of one mouse each depending on the result of the first phase and treated with each extract at the doses suggested by Lorke, respectively (Table 1). The 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, i.e., the geometric mean of the consecutive doses with 0 and 100% survival, respectively. The same procedure was used for the oral route of administration *(p.o)*.

RESULTS

Percentage Yield: Twelve point five percent (5) of the herbal products had percentage yield between 1- 9, while 25 % (10) had yields in the range of 10 – 19 %. A percentage yield of 20 – 35 was recorded for 62.5 % (25) of the test products (Table 2).

Median Lethal Dose (LD$_{50}$): The intraperitoneal LD$_{50}$ values for the test products were within the range of 178 to > 5,000 mg/kg body weight. Forty percent (16) had values ranging from 100 to 1,000 mg/kg body weight, while LD$_{50}$ values in the range of 1,001 to 5,000 mg/kg body weight was recorded for 50 % (20) of the herbal preparations. Median lethal dose (LD$_{50}$) values for 10 % (4) of the products were greater than 5,000 mg/kg body weight *i.p* (Table 2). The oral LD$_{50}$ values were between 1,256 to > 5,000 mg/kg body weight. Twenty five percent (10) of the herbal products had values up to 5,000 mg/kg body weight, while 75 % (30) had values greater than 5,000 mg/kg body weight *p.o* (Table 2).

Table 1: Suggested Doses in the Two Phases Using Lorke's Method.

Doses in mg/kg body weight Result of the initial investigation			Doses chosen for the second test body weight)			(mg/kg
10	100	1000				
0/3*	0/3	0/3		1,600	2,900	5,000
0/3	0/3	1/3	600	1,000	1,600	2,900
0/3	0/3	2/3	200	400	800	1,600
0/3	0/3	3/3	140	225	370	600
0/3	1/3	3/3	50	100	200	400
0/3	2/3	3/3	20	40	80	160
0/3	3/3	3/3	15	25	40	60
1/3	3/3	3/3	5	10	20	40
2/3	0/3	0/3	2	4	8	16
3/3	3/3	3/3	1	2	4	8

* Number of Animals which died/ number of animals used

Table 2: LD$_{50}$ Values of Forty (40) Polyherbal Preparations Marketed in Northern Nigeria

SN	Product Name	Product Composition	% Yield	LD$_{50}$ mg/kg (*i.p*)	LD$_{50}$ mg/kg (*p.o*)
1	Urinary Disorder Formula	*Equsetum arvense, Chondrus crispus, Arctostaphylus uvaursi*	22.0	1,131	> 5000
2	Reharb (1000) Capsules		24.5	570	3,800
3	Asthmatic Herbal Powder		12.1	471	3,800
4	Habbuk Capsules		17.1	775	> 5000
5	Bawa Energetic Capsules		21.5	775	> 5000
6	Measles Formula	*Vitex cienkowski,Cuiera genkowski, Parkia leaf*	10.7	1,265	> 5000
7	Hemorrhoids Formula	*Vitex cienkowski, Centaura calcitraps, Lanea acide*	23.1	178	> 5000
8	Rheumatic Tea Formula	*Salix alba, Eucalyptus glubolus, Albizia chive*	12.0	> 5000	> 5000
9	Breast Cancer Formula	*Fiscus platyphylla, Ximenia Americana, Sarcocephalus russegen*	27.5	471	> 5000
10	Smar Capsules		20.7	1,200	> 5000
11	Girfa Powder		24.0	3,800	> 5000
12	Hypertension Formula	*Verbena offcinalis, Foeniculum vulgare, Hibiscus abelmoschus, Viscum album*	31.0	> 5000	> 5000
13	Hepatitis Formula	*Berberis Spp, Tarxacum Spp, Verbena officinalis, Vitex ciekowski*	27.0	> 5000	> 5000

#	Name	Composition			
14	Tuberculosis Tea	*Anogeissus leocarpus, Glycyrrza Spp, Allium cepa, Equisetum arvense*	32.0	3,800	> 5000
15	Diabetes Tea Formula	*Artemisia absithium, Trigonella foenum graecum, Viscum album, Momodica balsamina*	25.0	2,154	> 5000
16	Taipoid Formula	*Rheum palmatum, Inula helenium, Parkia filicoidea*	19.0	2,154	> 5000
17	Skin Cancer Formula	*Fiscus platyphyda, Parkia pilpcoidea, Carica papaya, Securida longependumculata*	18.0	289	> 5000
18	Ulcer Tea	*Myric cerifera, Tusilago furfara, Glycyrrhiza glabra, Achillea millefilum*	26.0	1,265	3,808
19	Impotence Formula	*Carthamus tinctorius, Turnera aphrodisaca, Similax officinalis*	7.6	2,154	> 5000
20	Asthma Tea	*Ephedra sinensis, Euphorbia hirta, Anogeissus lerocarpus*	7.6	3,808	> 5000
21	Stomach Cancer		22.7	775	3,808
22	Immunozin Capsules	*Gulera senegalensis, Allium sativum, Azadiracta indica, Balanites aegyptiaca*	28.0	1,131	> 5000
23	Diarrhea & Vomiting Formula		23.0	775	3,808
24	B$_6$ Formula	*Carthamus tinctorius, Turnera aphrodisaca, Similax officinalis*	23.8	471	> 5000
25	Pile- Hemorrhoids Formula	*Achilea millefolium, Quercus alba, Plantago major*	35.0	471	> 5000
26	Ajingawa Stomach Formula		28.0	1,265	3,808
27	Sha'a Formula		24.0	283	3,808
28	Sherif Uba Formula		9.3	2,154	> 5000
29	Joje Powder		21.0	775	3,808
30	Champion Capsules	*Nauclea latifolia, Ageratum conyzoides, Cassia alata, Morida lucida*	30.2	2,154	> 5000
31	Greba Powder		24.0	3,808	> 5000
32	Fortrezin Capsules	*Zingiber officinallis, Moringa oleifera, Momordica charantia, Boswella Odorata*	30.0	2,154	> 5000
33	Gadaudine Formula		24.0	471	1,265
34	Sikla Formula		14.0	1,265	> 5000
35	Takadari Powder		16.4	3,808	> 5000
36	Madubiya Formula		18.3	566	2,154
37	Markazu Shifan		8.0	1,131	> 5000
38	Shajaratul- Mukarrama		11.5	775	> 5000
39	Shukura Herbal Tea		9.3	2,154	> 5000
40	Munazzil Powder		22.7	> 5000	> 5000

DISCUSSION

Acute systemic toxicity measures relative toxicological response of an experimental organism to single or brief exposure to a test substance (OECD, 2001).The assessment of acute toxicity of an unknown substance is the first step in toxicological investigation (Lorke, 1983). The most frequently used acute toxicity test involves the

determination of median lethal dose (LD_{50}). The LD_{50} is a statistically derived expression of a single dose of a material that can kill 50 percent of animals. The value represents the amount of toxicant per unit weight of the animal, which will kill 50 percent of a particular population of the animal species exposed to the chemical within a specific time, usually 24 to 72 hours. The routes of administration and animal species used affect the median lethal dose.

The LD_{50} determines the safety margin for any substance or chemical and hence the choice of dose for the study of the substance or chemical for any work. The smaller the LD50 value, the more toxic is the chemical. The opposite is also true: the larger the LD50 value, the lower the toxicity. A scale proposed by Lorke, (1983) roughly classifies substances according to their LD_{50} as follows: very toxic ($LD_{50} < 1.0$ mg/kg), toxic (LD_{50} up to 10 mg/kg), less toxic (LD_{50} up to 100 mg/kg), only slightly toxic (up to 1000mg/kg). Substances with LD_{50} values greater than 5,000 mg/kg are practically non toxic.

The culture of the indigenous population, though compounded at times by poor accessibility to healthcare facilities, largely influences the people's healthcare-seeking behavior. Traditional healers and herbalists are an integral part of the culture around parts of Africa (Sorsdahl *et al*., 2013). Despite the growing market demand for herbal medicines, there are still concerns associated with not only their use, but their safety. For majority of these products in use, very little is known about their active and/or toxic constituents. Toxicity testing can reveal some of the risks that may be associated with use of herbs, therefore avoiding potential harmful effects when used as medicine.

Herbal preparations could be contaminated with microbiological and foreign materials such as heavy metals, pesticides, residues or even aflatoxins. Contaminants when present in herbal preparation may produce prominent health defects underscoring the claimed safety. An increase in the morbidity and mortality associated with the use of herbal or traditional medicines has raised universal attention in the last few years (Bondaranayake, 2006). The substitution of toxic Aristolochia species in traditional Chinese medicines (TCM) has resulted in cases of serious renal toxicity and renal cancer in Europe, China and America (EMEA, 2005). The emergence of interactions between *Hypericum perforatum* (St John's wort) and certain prescription medicines has necessitated regulatory action world-wide. Serious cases of liver damage including a number of fatalities associated with the use of *Piper methysticum* (Kava) have led to restrictions on its use in many countries (O'Sullivan and Lum, 2005).

The need to evaluate the toxicity profile of some herbal preparation extracts was prompted by their widespread use in the management of various disorders in Northern Nigeria. This study has shown the diversity in the toxicity of the herbal products tested. The intraperitoneal median lethal doses of the tested extracts indicated that 40% were less toxic while 50 % were only slightly toxic. Ten percent (10 %) of the extracts were practically non toxic by this route.

The oral LD_{50} of the herbal products showed that 25 % were only slightly toxic while 75 % had median lethal doses that were practically non toxic. Thus, our results has shown that the use of most of these herbal formula extracts may be relatively safe on acute exposure especially when administered orally which is the main route employed by the practitioners. Most of the extracts are therefore not relatively harmful after single oral administration and this could explain the continuous use of the preparations by the local people in traditional management of various ailments in Northern part of Nigeria.

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AUTHOR'S CONTRIBUTION

This work was carried out in collaboration between all authors. Author AM designed the study, performed the experiments, wrote the first draft of the manuscript and did all the corrections mentioned by the reviewers. Author YAH wrote the protocol and performed the experiments. Author CBAZ managed the literature searches and contributed to manuscript review. Author SAI contributed to manuscript review. All authors read and approved the final manuscript.

We however state that the limitations of this study include the fact that the samples used for the study were voluntarily submitted by the practitioners and may not be consistent always. Also, the determining of the efficacy and veracity of the constituents as claimed by the practitioners is beyond the scope of this work. Above all, the authors declare that no competing interests exist.