http://dx.doi.org/10.4314/jpb.v13i1.9
Vol. 13 no. 1, pp. 58-65 (March 2016)
http://ajol.info/index.php/jpb

Journal of
PHARMACY AND BIORESOURCES

Acute toxicity studies and characterisation of local dietary salts in Nigeria

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Received 12th February 2015; Accepted 30th March 2016

Abstract
Kanwa and *Shem* are food additives and also local dietary salts which are commonly used in Nigeria for various purposes both in rural and urban communities. *Kanwa* and *Shem* were subjected to acute toxicological studies using the modified Lorke method while the elemental, qualitative analysis and pH were determined by employing standard methods. The FT-IR of the samples was determined in order to characterise the samples. The LD_{50} for both salts was 2,820mg/kg while the elemental and qualitative analysis revealed the presence of potassium in high concentration both in *Kanwa* and *Shem* (24.5 ± 9.60mg/L and 29.4 ± 10.4mg/L) respectively followed by calcium, magnesium and sodium. The qualitative analysis revealed the presence of trioxocarbonates (IV) (CO_3^{2-}), hydrogen trioxocarbonates (IV) (HCO_3^-) and tetraoxosulphates (VI) (SO_4^{2-}) while trioxonitrates (NO_3^-) and chlorides (Cl^-) are absent in both salts. The characterization of the salts through infrared spectroscopy showed that both salts had carbonyl groups in the finger print region inferring that the chemical structure may likely be similar. The suggested chemical formula for both salts is a complex inorganic salt which is a combination of carbonates and sulphates. *Kanwa* and *Shem* salts showed similarities in structure. They therefore are likely to have similar properties.

Keywords: *Kanwa*; *Shem;* Acute toxicity, Atomic Absorption Spectroscopy, Infrared Spectroscopy

INTRODUCTION

"A food additive is any substance not normally consumed as a food in itself and not normally used as a characteristic ingredient of food whether or not it has nutritive value, the intentional addition of which to food for a technological purpose in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food results, or may be reasonably expected to result, in it or its by-products becoming directly or indirectly a component of such foods" (Council Directive 89/107/EEC). Food additives and their metabolites are subjected to rigorous toxicological analysis prior to their approval for use in the industry. The lowest level of additive producing no toxicological effects is termed the no-effect level (NOEL). The NOEL is generally divided by 100 to determine a maximum acceptable daily intake (ADI) (Singh, 2014). The use of food additives is however not without its negative effects. These include behavioural problems (hyperactivity) in

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ISSN 0189-8442

children, allergies, asthma, headaches and seizures (Gonen, 2008).

Local dietary salts in various forms are used in Nigeria as food seasoning and food additives. Potash is commonly consumed in Nigeria especially in the northern parts where two forms are commonly available: *kanwa* and *shem. Kanwa*, also known as natron, a sesquicarbonate or hydrated carbonate of sodium (Alawa *et al.*, 2012). *Kanwa* is also known scientifically as trona (Omajali *et al.*, 2010). Okehie-Offoha in 1996 however described *kanwa* as a potassium salt in combination with other salts. *Kanwa* is a base with a pH of 8.9. It contains 10% sodium as bicarbonates, 70% potassium, 0.33% calcium and 8% phosphorous (Yakasai *et al.*, 2004). *Kanwa* is a mould growing out of the soil in rainy season. It is scrapped off, dried in the sun and sold without further treatment. In Nigeria *kanwa* occurs as a natural deposit in saline rocks. The deposit is covered with shallow water, less than two feet deep. It occurs mostly in northern Nigeria, particularly in Kano and Maiduguri, extending to border countries like Chad and Niger. *Kanwa* is the second most commonly used salt in Nigeria after table salt (Omajali *et al*., 2010). *Shem, shim or tokan tsanyi* is extracted from wood ash. No known studies have been reported on it. In Plateau State, North-Central Nigeria, these salts find common use as antacids, antibacterial in the treatment of sore throats and mouth ulcers. *Kanwa* is used to soften food and reduce cooking time when boiling beans and tough meat. *Kanwa* is also an essential ingredient in making special porridges such as *kunun kanwa* literally translated from the Hausa language as 'saltpetre porridge' (Okehie-Offoha, 1996). In the Hausa-Fulani tribe of northern Nigeria, *kanwa* is used in postnatal care of the puerperium as nursing mothers consume large quantities of natron (about 40 g equivalent to 450 M of sodium) daily in a pap of guinea corn as part of the forty-day postpartum practice in the belief that it increases the quantity and quality of breast milk (Sanderson *et al.*, 1979). Consequently, this has been implicated in the incidence of peripartum cardiac failure (PPCF) among nursing mothers in this region (Davidson *et al.*, 1974). Similarly, Alawa *et al.* (2012) studied the pharmacological effects of Natron (*Kanwa*) varieties on murine virgin uterine contractility. They reported that a variety known as *Ja Kanwa* elicited a dose-dependent transient contractile response followed by relaxation of the isolated rat uterus, which confirmed the efficacy of *Ja kanwa* in childbirth and further supported the rationale of its folkloric use as an abortifacient.

Omajali *et al.* (2010) investigated the effect of *kanwa* on rat gastrointestinal phosphatases. The activity of Alkaline Phosphatase (ALP) fluctuated in the small intestine. There was an increase in activity of ALP in the stomach throughout the period of *kanwa* administration. It was concluded that *kanwa* may not be toxic if consumed in moderate levels in homes. *kanwa* and *shem* both have been shown to have blood pressure lowering effects (Obasaju, 2006).

This study was aimed at determining the toxicological effects of the salts, determining the level of potassium, magnesium, calcium and sodium present in *kanwa* and *shem* salts located in Pankshin Local Government area of Plateau State, North Central Nigeria and to characterise the salts.

EXPERIMENTAL

Animal studies. The acute toxicity test was carried out on thirteen (13) Wistar rats of either sex weighing between 120 –150 g. All animals were obtained from the Animal House Unit of the Department of Pharmacology, University of Jos, Nigeria. Ethical approval on the handling of animals was obtained from the Faculty of

Pharmaceutical Sciences, University of Jos Nigeria, which was in line with the 'Principles of Laboratory Animal Care' from the NIH publication No. 85-23. The animals were maintained in standard environmental conditions (25^{o}C, 12:12h dark light cycle, frequent air change); the animals were fed with standard feeds daily (Vital feed, Nigeria) and allowed water *ad libitum* all through the research. The animals were allowed to acclimatize for 5 days before commencement of the research.

Collection and identification of salts. *Kanwa* and *shem* were both bought in a local market in Pankshin town, Pankshin Local Government Area of Plateau State, North Central Nigeria. They were identified and authenticated by Mr. Daniel, Chief Technologist, Chemistry Department, University of Lagos. The samples were then carefully ground into powdered forms and stored at room temperature.

Acute toxicity studies. Acute toxicity test of the samples was conducted using the modified method of Lorke (1983). The method was divided into two phases. In the initial phase, 3 groups of three rats each were treated with the samples at doses of 10, 100 and 1,000 mg/kg body weight intraperitonealy and observed for signs of toxicity (restlessness, hair sprang up, red coloration of eyes, shivering then reduced activity) and death for 24 hours. In the second phase, 4 groups of one rat each was injected with four more specific doses of the samples (1,600, 2,400, 2,750 and 2,900 mg/kg) based on the result of the first phase. The LD_{50} value was determined by calculating the geometric mean of the lowest dose that caused death and the highest dose for which the animal survived (Magaji *et al.,* 2008; Iyiola *et al.,* 2011).

Sample preparation. Samples were digested by the wet digestion method. Ten milliliter (10mL) of nitric acid was added to 5 g of accurately weighed dried sample in a 250 ml conical flask and was heated on a hot plate at 95°C for 15 min. The digest was cooled and 5 ml of concentrated nitric acid was added and heated until the brown fumes present turned white. The sample was removed and allowed to cool. 10 ml of distilled water was added to the sample after cooling and filtered through a Whatman No.1 filter paper into a 50ml standard volumetric flask and made up to the mark with distilled water.

Sample analysis. Digested samples were analyzed for Ca, Mg, K and Na using flame atomic absorption spectrophotometer (A-Analyst 200 Perkin Elmer). The 1000 ppm standard solutions of elements were diluted in three different concentrations {2, 4, and 6 parts per million (ppm)} to obtain calibration curve for quantitative analysis. All the experiments were run in duplicate for the samples and standard solutions. The values are expressed as Mean ± Standard Error of Mean (SEM) as described in Ibukun *et al*. (2010).

IR spectra. The crushed powdered samples were mulled in nujol (spectroscopic grade) and smeared on the cell and covered. Spectra were recorded in absorbance mode from 500 to 4000 cm^{-1} on an IR spectrophotometer (M500 IR spectrophotometer, Buck Scientific, USA).

Qualitative inorganic analysis. This analysis was carried out on the salts for the presence of anions such as carbonates (CO_3^{2-}), sulphates (SO_4^{2-}), nitrates (NO_3^{-}) and chlorides (Cl^{-}) by employing the standard methods described in Sevhla (1979).

Determination of pH. The pH of the salts solutions was determined by using PHS-3C pH meter (Shanghai, China).

RESULTS

Physical characteristics of the salts. The white variety of *kanwa* is made up of whitish-grey crystal aggregates with blackish-grey

stains. *Shem* is a light brown crystalline powder, extracted from wood ash as seen in Figs. 1 and 2.

Acute toxicity test. Acute toxicity test showed a LD_{50} = 2,820 mg/kg through ip route as seen in the Table 1.

Qualitative inorganic analysis. The following anions are present in the *Kanwa*: trioxocarbonates (IV) (CO_3^{2-}), hydrogen trioxocarbonates (IV) (HCO_3^-) while *Shem* contains the following anions: trioxocarbonates (IV) ion (CO_3^{2-}), hydrogen trioxocarbonates (IV) ion (HCO_3^-) and tetraoxosulphates (VI) ion (SO_4^{2-}). The two salts did not show the presence of trioxonitrates (V) ion (NO_3^-) and chloride ion (Cl^-).

pH determination. The pH of *kanwa* solution is 13.20 while *shem* is 7.95. *Kanwa* solution is very alkaline in nature while *shem* is neutral.

Infrared (IR) spectroscopy. The IR spectra of *kanwa* and *shem* are shown in Figs. 3 & 4.

DISCUSSION

Acute toxicity test showed that the LD_{50} through ip route is 2820 mg/kg for both *kanwa* and *shem* as seen in Table 1. According to OECD (2001), chemicals with LD_{50} values between 2,000 and 5,000 mg/kg are considered to be of relatively low acute toxicity hazard (category 5). This may explain why it is used in most homes in Nigeria without significant toxic effects.

Fig. 1. White variety of *kanwa*

Fig. 2. Light brown crystalline *shem* powder

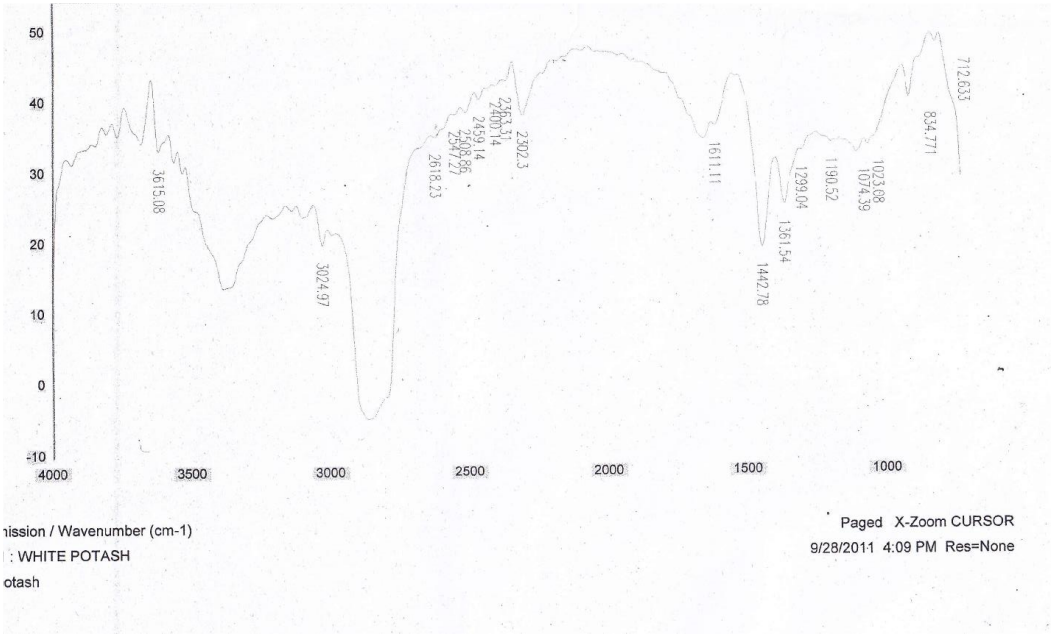

Fig 3. Infrared (IR) spectrum of *kanwa*

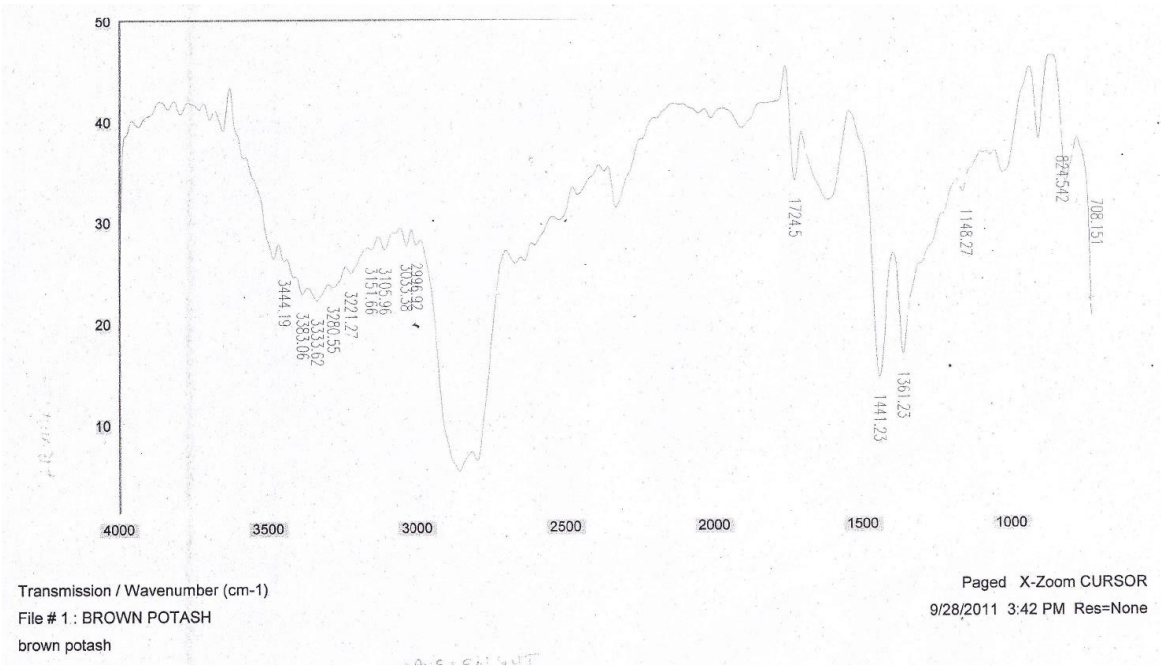

Fig 3. Infrared (IR) spectrum of *shem*

Table 1. LD_{50} for *Kanwa* and *Shem*

Phase	Dose (mg/kg)	Number of Animals	Number of Deaths
Phase I	10	3	-
	100	3	-
	1,000	3	-
Phase II	1,600	1	-
	2,400	1	-
	2,750	1	-
	2,900	1	1

Table 2. Elemental Analysis of *Kanwa* and *Shem*

Sample	Mean ± SEM (mg/ml)			
	Mg	Ca	K	Na
Kanwa	8.76 ± 0.52	13.08 ± 4.73	24.52 ± 9.61	6.36 ± 3.05
Shem	8.80 ± 1.43	10.61 ± 3.38	29.37 ± 10.42	5.91 ± 2.42

The concentrations of the elements presented in table 2 reveal that both *Kanwa* and *shem* contained high levels of potassium (24.52 mg/l and 29.37 mg/l respectively), followed by calcium (13.08 mg/l and 10.60 mg/l for *kanwa* and *shem* respectively) then magnesium (8.75 mg/l and 8.79 mg/l respectively). Sodium had the lowest value in both *kanwa* and *shem* (6.36 mg/l and 5.91 mg/l respectively).

Kanwa showed high levels of potassium (24.52 mg/l) as was similarly obtained in other studies (Okehie-Offoha, 1996; Yakasai *et al.,* 2004). The high potassium level especially in *kanwa* probably confirms one of its commonly used names as potash, and its ability to lower blood pressure (Obasaju, 2006). Sodium was however the dominant element in *Kanwa* in other studies (Ekanem, 1977; Ankrah and Dovlo, 1978; Alawa *et al*, 2000 and Alawa *et al*, 2012). This verifies the claim that different varieties of *kanwa* vary in composition depending on their location (Ikwuegbu *et al.*, 1985; Ekanem and Harrison, 1997). *Shem (tokan tsanyi)* had higher potassium levels (29.37 mg/ml) than *kanwa* (24.5 mg/l). Potassium is the major intracellular cation in the body. The potassium ion in the Extra Cellular Fluid (ECF) is filtered freely at the glomerulus of the kidney, reabsorbed in the proximal tubule, and secreted into the distal segments of the nephron. The kidneys however have a limited ability to conserve potassium (Schwartz and Garrison, 2009).

The infrared spectroscopy showed the presence of carbonyl and carboxylate groups, suggestive of the presence of carbonates and hydrogencarbonates of potassium, calcium, sodium, magnesium and probably other elements; although potassium ion is the dominant element in both salts. Increased potassium intake has been found to be associated with decreased blood pressure (Cappuccio and MacGregor 1991; Whelton *et al.,* 1997, Obasaju, 2006). Omajali *et al.*, (2010) however described *kanwa* as hydrated sodium carbonate ($Na_2CO_3.NaHCO_3.2H_2O$) which also corroborated the analysis of *kanwa* in this study. Two signals which can be seen clearly in this area is the carbonyl group, which is a very strong peak around 1700 cm^{-1}, and the C-O bond can be one or two strong peaks around 1200 cm^{-1} (a peak of 1361cm^{-1} was obtained for both salts). This complex lower region is also known as the "fingerprint region" because almost every organic compound produces a unique pattern in this area. Therefore, identity can often be confirmed by comparison of this region to a known spectrum (Merlic *et al.,* 2000; Reusch, 2013). *Kanwa* and *shem* both absorbed at 1361cm^{-1}, which is the fingerprint region; inferring that they likely have the similar molecular structure. The fingerprint region is 1500 to 910 cm^{-1}. Absorptions in this region include the contributions from complex interacting vibrations, giving rise to the generally unique fingerprint for each compound. A good match between the IR spectra of two compounds in all frequency ranges, particularly in the fingerprint region, strongly indicates that they have the same molecular structures (Hsu, 2000).

Conclusion. The acute toxicity test for *kanwa and shem* showed that the salts were of relatively low acute toxicity hazard. The elemental analysis revealed that both salts contain required elements for metabolic processes of the body and qualitative analysis revealed the presence of trioxocarbonates, hydrogen trioxocarbonates and sulphates ions.

The characterization showed that *kanwa* is a complex inorganic salt that contains a combination of the carbonates and hydrogen carbonates of potassium, sodium, calcium and magnesium while *shem* is a complex inorganic salt that contains a combination of carbonates, hydrogen carbonates and sulphates of potassium, sodium, calcium and magnesium. This study shows that *kanwa* and *shem* have similarity in their chemical formula and they are safe for consumption.

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