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Characterisation of Freeze-Dried Powdered Aqueous Slurry of *Pennisetum Glaucum (Poaceae)* Grains and its Galactogogue Properties in an Animal Model

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A – Research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: Pearl millets (*Pennisetum glaucum*) are important but underutilized crops found in tropical and semi-arid regions of the world, including Northern Nigeria. A survey report of women in Kaduna, Northern Nigeria, revealed that milk obtained from grains of Pearl millet have been utilized to enhance lactation, even in cases of adoptive nursing.

Objectives: The aim of this study is to prepare aqueous slurry of Pearl millet grains, freeze-dry into powders, characterize and then evaluate the powders for galactogogue activity in female Wistar rats, in comparison to domperidone, a dopamine antagonist used as a lactogenic agent.

Methods: The aqueous slurry of millets was freeze-dried and characterized for morphology, crystallinity (Fourier Transform Infra-red spectroscopy, FTIR and X-ray diffraction analysis, XRD), viscosity, flow and compaction properties. Animal studies were carried out to evaluate the galactogogue effect and histopathological examination was done on the 15th day of parturition. Toxicological testing to determine the LD_{50} was performed using Lorke's method.

Results: The morphology, FTIR and XRD spectra confirmed the disruption of the granules when freeze-dried. Freeze-drying improved flow and showed good compactibility, indicating potential for formulating the powders into tablets. Weight gain and histopathological examination revealed improved milk secretion and milk emptying in rats. The LD_{50} showed that the highest dose of 6000µg/kg gave no mortality.

Conclusion: The freeze-dried powders of Pearl millet slurry were free flowing, capable of compaction and produced increased secretion and emptying of milk in Wistar rats, showing potential as a galactogogue for use in humans and the dairy industry.

Keywords: *Pennisetum glaucum* grains, Compaction properties, Freeze-dried powdered extracts, Galactagogue effect

INTRODUCTION

Lactation can be defined as the secretion of milk from the mammary glands and as the process of producing and releasing milk, a process that can occur in all female mammals who have gone through pregnancy (Capuco and Akers, 2009). Lactation is very important to infants as it provides specific nourishments which aid growth and development, serves as protection against diseases, as well as boosts their immunity (Dewey and Brown, 2003, Berry and Gribble, 2008). However, lactation insufficiency has been a major cause of concern, especially among breastfeeding women and has resulted in early discontinuation of breastfeeding and the introduction of infant formula feeds.

Galactogogues are medicinal substances that have been used to assist in the synthesis of breast milk, even

in women that are not necessarily nursing mothers (Forinash *et al*, 2012). Common indications for galactagogues are adoptive nursing (induction of lactation in a woman who did not carry the pregnancy of the baby being nursed), re-lactation (re-establishing milk supply after weaning) and increasing a faltering milk supply because of maternal or infant illness or separation (Forinash *et al*, 2012). Plants such as Fenugreek (*Trigonellafoenum graecum*), Shatavari (*Asparagus racemosus*), Fennel (*Foeniculum vulgare*) and Blessed thistle (*Cnicus benedictus*) have been reported to have been used as galactogogues (Albert - Puleo, 1980; Hilchcock, 1987; McGuffin *et al*, 1997). These plants exhibit their galactogogue activity through interactions with dopamine receptors by influencing the adreno-hypothalamo-hypophyseal gonadal region which enhances prolactin concentration and hence improve milk production (Swafford and Berens, 2000; Reeder *et al*, 2011; Gbadamosi and Okolosi, 2013). Phytochemical screening of many of these plant extracts either with organic or aqueous solvents has revealed the presence of numerous active principle including flavone, curcumin, vanillic acid, ferulic acid, saponins, glycosides, essential oils, isoflavones, racemosol, α-pinene, asparagamine, zingiberene, bisabolene, cadina-1,4-diene, isopelletierine, anaferine, andrograpolide, pregnane derivatives etc. which contribute to galactapoetic effect (Bharti *et al*, 2002).

Millets are important but underutilized crops which are found in tropical and semi-arid regions of the world, including Northern Nigeria. Nigeria has for many decades, accounted for more than half of the Pearl millet production in West Africa (ICRISAT, 2001). The crops are valued due to their greater resistance to pests and diseases, good adaption to a wide range of environment, good production yield, ability to withstand significant levels of salinity, short growing season, and resistance to water logging (Seghal and Kawatra, 2006; Tadele, 2016). The galactogogue effect of Pearl millet may be attributed to its high levels of calcium, iron, lipids, high quality proteins and high energy value (Amer and Mustafa, 2010). The phenolic properties found in millets also compromise phenolic acids, flavonoids, and tannins (Hassan *et al*, 2021). An unpublished survey of women in Kaduna, Northern Nigeria reveals that milk obtained from grains of Pearl millet have been utilized to enhance lactation, even in cases of adoptive nursing (Jideani *et al*, 2021).

Despite popular and historical use, it appears from literature search that there has been little or no research work conducted to determine the galactogogue activity of millet grains. Hence, studies are required to assess the efficacy and safety of this grains that are commonly used during breastfeeding. Formulation of the aqueous slurry of Pearl millet grains into powder dosage form by the process of freeze-drying is expected to produce light, free flowing powders which retain most of the nutritional quality of the millet, will not require refrigeration and hence prevent microbial growth and chemical changes that could lead to deterioration of the product. In addition to providing an extended shelf-life, successful freeze-drying should yield the product that has a short reconstitution time with acceptable potency levels (Searles *et al*, 2001). Furthermore, the freeze-drying avoids the denaturation caused by the heating procedures used in conventional drying methods and the powders formed allow portability of large amounts and cheaper transportation cost (Nail and Garlin, 1992). Hence, in this study, aqueous slurry of Pearl millet grains were prepared and freeze-dried into powders, the powders were characterized using physicochemical and compaction properties and then evaluated for galactogogue activity in female Wistar rats, in comparison with domperidone, a standard lactogenic agent.

METHODOLOGY

Materials and Methods

Materials

Millet grains were procured from a local market in Kaduna State. Domperidone (Motilium®) 10 mg

tablets were obtained from Janssen, Belgium; NAFDAC REG number: 04-1293) while Xylene was from BDH, England. All other chemicals used were of analytical grade.

Ethical approval for animal studies

The experimental protocol was approved by the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC) with assigned number UI-ACUREC/18/0050.

The Laboratory animals used in this study were fifty five (55) virgin female Wistar rats (150-180g) and ten (10) male Wistar rats (180 - 200g). They were obtained from the Animal Housing Unit of the Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria. The animals were housed in clean cages, placed in well-ventilated housing conditions. They were fed with special rat pellets and fresh water ad libitum. The cages were cleaned once in two days. The female animals were acclimatized to laboratory condition for one month before the commencement of the experiments.

All institutional and national guidelines for the care and use of laboratory animals were followed.

Methods

Preparation of aqueous slurry of Pearl millet grains

The slurry was prepared according to the traditional method used by the Northern women in Nigeria as described below:

Five hundred grams of whole millet grains were soaked in 2 L of distilled water for 12h, followed by blending of the soaked grains. The grinded millet was sieved through a muslin cloth to obtain the slurry.

Preparation of dispersions and freeze-drying of milky extracts of Pearl millet grains

The slurry was divided into three (3) batches: uncooked slurry, cooked slurry without additives and cooked slurry with an additive, ginger (based on the local recipe). The three batches of slurry were freeze-dried into powder (Freeze-Drier, Lyotrapplus model®, Great Britain). The freeze-dried powders were then prepared as 20%w/v dispersions in water. The fourth batch consisted of the slurry of Pearl millet grains that simulated the indigenous method of preparation by cooking only without freeze drying.

Characterization of freeze-dried powders

Organoleptic properties

The samples were observed for appearance and odor.

Determination of particle size and morphology

The particle sizes and morphology of the sample powders were determined by optical microscopy (Digital Microscope, VJ 2005 DNMODEL BIO-MICROSCOPE®, China.) on 100 particles. The mean particle size for each sample was determined (TS View CX Image® Software, File version 6.2.4.3, China and Motic Image 2000, China).

Fourier Transform Infra-red (FTIR) analysis

The samples were analyzed using Fourier Transform Infra-red Spectroscopy (FT-IR Spectrum BXII Perkin Elmer, Waltham, MA, USA) in transmission mode. The transmission spectra range was 4000-400cm^{-1} using 64 scans with resolution of 8cm^{-1}.

X-ray Diffractometry

The X-ray diffraction pattern was recorded using an X-ray diffractometer (ARL X'TRA Thermo Scientific, The Netherlands) with copper- cobalt radiation. The samples were exposed to the X-ray beam at 25 ^{o}C. The scanning region of the diffraction angle (2^{θ}) was from 5 to 70^{o} at a scan rate of 12 ^{o}/min. The integration time was 0.150 s and step was 0.030 ^{o}.

Determination of swelling index

A 5 g quantity of the powdered millet was weighed into a 100-mL measuring cylinder. The tapped volume (Vx) occupied by the powders was determined and recorded. A dispersion of the powder was made with about 80 mL of distilled water. The dispersion was made up to the 100 ml volume with water and allowed to settle. The dispersion was allowed to stand for 24 h and the sedimentation volume (Vv) measured. The swelling capacity was calculated using the relation:

$$Swelling\ index\ = {}^{Vv}/_{Vx} \quad (1)$$

Density measurements

A 5 g quantity of powdered millet sample was weighed and carefully transferred into a 100-mL measuring cylinder. The initial volume occupied by the powder in the cylinder was noted as the Loose Bulk Density (LBD).The powder in the cylinder was tapped until no further change in volume occurred. Tapped bulk density (TBD) and LBD were calculated using the formulae of equations 2 and 3 below:

$$\text{LBD} = \frac{weight\ of\ powder}{volume\ of\ the\ packing} \quad (2)$$

$$TBD = \frac{weight\ of\ powder}{tapped\ volume\ of\ packing} \quad (3)$$

Particle density values were determined for all samples using the pycnometer method with xylene as the displacement fluid:

An empty 50- mL pycnometer bottle was weighed (W) and then filled with xylene, and excess xylene cleaned off the bottle. The bottle plus the xylene was weighed (W1). The difference between the weights (i.e.W1–W) was calculated (W2). Two grams of the powder was weighed (W3) and quantitatively transferred into the pycnometer bottle. The excess xylene was again wiped off and the bottle weighed again (W4). The particle density (ℓs) was calculated from equation 4:

$$\frac{W2W3}{50(W3 - W4 + W2 + W)} gcm^{-3} \quad (4)$$

Flow properties

The flowability of the powders was evaluated using the Carr's index and Hausner's ratio. The Carr's compressibility index and Hausner's ratio were respectively calculated using Equations 5 and 6:

$$Carr's\ index = \frac{tapped\ density - bulk\ density}{tapped\ density} x\ 100 \quad (5)$$

$$Hausner's\ ratio = \frac{Tapped\ density}{Bulk\ density} \quad (6)$$

The angle of repose was determined as follows: Ten grams of starch was weighed and poured through a funnel, into an opened cylinder placed on a wooden base of similar diameter. The starch was allowed to cascade into a heap. The angle of repose was then calculated, using:

$$Tan\ \theta = \frac{height}{radius} \quad (7)$$

Compaction properties

The powder sample (10 g) was allowed to flow freely through a funnel into a glass measuring cylinder. The volume occupied by the starch powder was noted. One hundred taps were applied to the starch powder and the volume occupied after each set of 10 taps was determined. The volume reduction of the powder due to tapping was evaluated using the Kawakita equation stated in Equation 8:

$$N / C = N / a + 1 / ab \quad (8)$$

where N is the number of taps and both 'a' and 'b' are constants. Constant 'a ' describes the compressibility while the reciprocal of the constant 'b' (i.e. 1/b) describes cohesiveness of powders or the time of onset of final packing. Term 'C' describes volume reduction during tapping and can be calculated from Equation 9 (Kawakita and Ludde, 1971):

$$C = (Vo - Vn)) / Vo \quad (9)$$

where Vo is the loose volume of the powder before tapping and Vn is the volume of the powder after a certain number of taps.

The data obtained was used to access the consolidation behaviour of the excipients using the method described by Neumann *et al.* (1967) to study the relative decrease in powder volume and density as a function of applied load according to the equation below:

$$Log\ (PT - PB)/PT = K\ Log\ N + C \quad (10)$$

where P_T and P_B are the tapped and bulk densities respectively. N is the number of taps. C is the consolidation index. K is the rate of consolidation.

Characterization of dispersions of freeze-dried millet powders

The freeze-dried powders of Pearl millet slurries were prepared as 20%w/v aqueous dispersions and evaluated with the slurry of Pearl millet grains prepared using the indigenous method of without freeze drying:

Organoleptic properties of the dispersions

The color and odor of the dispersion samples were determined by visual observation.

pH determination

The pH of each sample was determined in triplicate using the pH meter (Model 720 A, Thermo Electron Corporation, MA, and USA).

Viscosity

The viscosity of each sample was measured with a Brookfield rheometer (DV-III + model, Brookfield Engineering, Middleborough, MA, USA) at 29.6 0C using spindle no. 3.

Flow rate

The flow rate was obtained by determining the time it took 5 mL of each sample to pass through the orifice of a 5-ml pipette.

Redispersibility

The redispersibility was evaluated qualitatively. The test consisted of pouring 10 mL of each sample into four calibrated tubes, and were stored at room

temperature. At the end of one week storage period, each tube was inverted at ninety degree and no of inversions to redisperse the sedimented suspension was noted (Bashir et al, 2014).

Animal studies

Adult Wistar rats (150 – 200 g body wt.) were housed in standard conditions of temperature (27 ± 2°C) and relative humidity (55 ± 5%). The rats were kept on wood shavings in plastic boxes with wire covers and the lighting was adjusted with 14 h of lightness (06.00 a.m. - 08.00 p.m.) and 10 h of darkness (08.00 p.m. - 06.00 a.m.) in a day with standard commercial feed and water *ad libitum*.

Oral administration of millet dispersions to evaluate their effect on milk production

The virgin female Wistar rats were exposed to male rats and separated once mating was confirmed (through vagina plug analysis) within 48 hours, after which the rats were allowed to deliver their litters (a gestational period of 19 to 22 days). The first day of parturition was designated as Day 1 of lactation. The lactating rats were randomly divided into seven groups of five rats each (n=5) (Himanshu *et al*, 2016). Each litter size was adjusted to six per dam within 48 hrs. Group I was treated as control and were administered distilled water. Group II was treated with Domperidone (0.7 mg/kg body wt.) administered as the standard control. Group III, which consisted of the Dams and litters were treated using the slurry of millet grains that was not freeze-dried. Group IV consisting of virgin female Wistar rats, were treated with the slurry of millet grains. Group V - VII was treated using dispersions of the freeze-dried powders: Freeze-dried uncooked millet slurry (FDUM); Freeze-dried cooked millet slurry (FDCM) and Freeze-dried cooked millet slurry with additive (FDCM+A), respectively. The suspension was orally administered at 750mcg/kg body wt. The administration to lactating rats was done daily using animal feeding tube at 08.00 a.m., starting from Day 5 to the 14th day of lactation. The average weights of the litters before and after administration of the last dose were obtained to estimate milk yield. The duration of administration was 10 days. The weight gain by litters as well as Dams during the experimental period were weighed and compared between the treatment groups and the control groups 1 and II (Himanshu *et al*, 2016). All weights were determined with accuracy of 0.01 g using an electronic balance.

Histopathological Examinations

On the 15th day of parturition, the lactating mother rats were euthanized through anesthesia with ketamine (100 mg/kg IM), then the whole mammary glands were excised. The mammary tissues were fixed in 10% buffered formalin (pH 7.2) and dehydrated through series of acid-alcohol solutions, embedded in paraffin and routinely processed for histological analysis using H&E staining protocol.

Histopathological assessment and photomicrography of the prepared slides was done by a Histopathologist. Photomicrographs were taken at x40, x100 and x400 magnifications with a Digital Microscope, VJ-2005 DN MODEL BIO-MICROSCOPE®. The morphometrical analyses was done using TS View CX Image® Software,File version 6.2.4.3 and Motic Image 2000 (China).

Toxicological Testing; Determination of LD_{50}

The Lorke's method, a trial for the investigation of the acute toxicity in animals, was used to estimate the lethal dose i.e. the drug concentration that causes mortality of 50% of the test animals (LD_{50}). The method adapted for determination of the LD50 of the preparations in Wistar rats is as described below:

Nine rats (176.33 ± 17.14 g body weight) were randomly divided into three (n=3). A separate group of three rats was used as unexposed control. Each group was kept in plastic containers with water and fed ad libitum and dosed orally with a single daily concentration. The first group was administered with 1500 mcg/kg/day, the second group with 3000 mcg/kg/day while the third got 6000 mcg/kg/day. A daily observation for mortality was done for 48 h (Lorke, 1983).

Data presentation and statistical analysis

The data for physicochemical, compaction and dispersion properties were expressed as mean ± standard deviation.
For the animal studies, Graph-pad prism version 5 was used for statistical analysis. The one-way analysis of variance (ANOVA) in addition to the Tukey Kramer's multiple comparison tests were used to compare means across groups. The probability limit for all experiments was set at $p < 0.05$

RESULTS AND DISCUSSION

Characterization of powdered millet extracts

Organoleptic properties

The Freeze-dried uncooked millet slurry (FDUM) appeared light brown in colour, with a pleasant aromatic odour. The Freeze-dried cooked millet slurry (FDCM) had a dark brown colour and was odourless while the Freeze-dried cooked millet slurry with additive (FDCM+A) was brown with a yellow tint and ginger-like odour. Like FDUM, the cooked millet slurry was light brown in colour but odourless.

Morphology

The photomicrographs of the powders of freeze-dried uncooked millet slurry (FDUM), freeze-dried cooked millet slurry (FDCM) and freeze-dried cooked millet slurry with additive (FDCM+A) are shown in Fig. 1. The particle shape of FDUM was ovoid with smooth edges and an average diameter of 14.68µm. The cooked millet slurry (FDCM) had larger, less spherical particles with diameter of 46.74µm and rough edges with tiny pores on their surfaces. The freeze-dried cooked slurry with additive were in clusters forming elongated structure with rough edges and produced the largest diameter of 188.00 µm. The ranking of particle diameter observed was in the order: FDCM+A > FDCM > FDUM. The increase in size observed with the cooked samples could be as a result of increase in particle enlargement owing to deformation of the internal structure by heat during its processing.

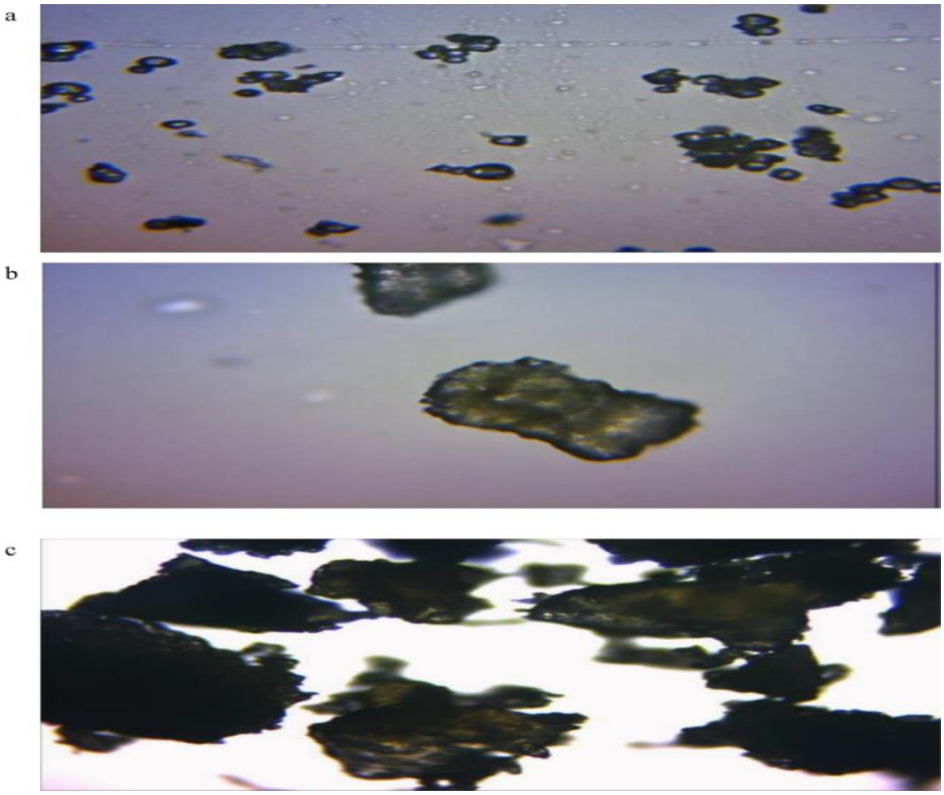


Fig. 1: Photomicrographs of powders of (a) Freeze-dried uncooked millet slurry, FDUM (b) Freeze-dried cooked millet slurry, FDCM and (c) Freeze-dried cooked millet slurry with additive, FDCM+A (mg x400)

Fourier Transform Infra-red (FTIR) analysis

The Fourier Transform Infra-Red Spectroscopy (FTIR) spectra of the samples are shown in Fig. 2. The FTIR spectra of the freeze-dried powders of the cooked slurries revealed a decrease in the ordered structure that was initially observed for the uncooked millet slurry powder. This was characterized by reduction in the band intensity at various wavelengths which could be attributed to hydrogen-bonded hydroxyl groups on the cooked molecules.

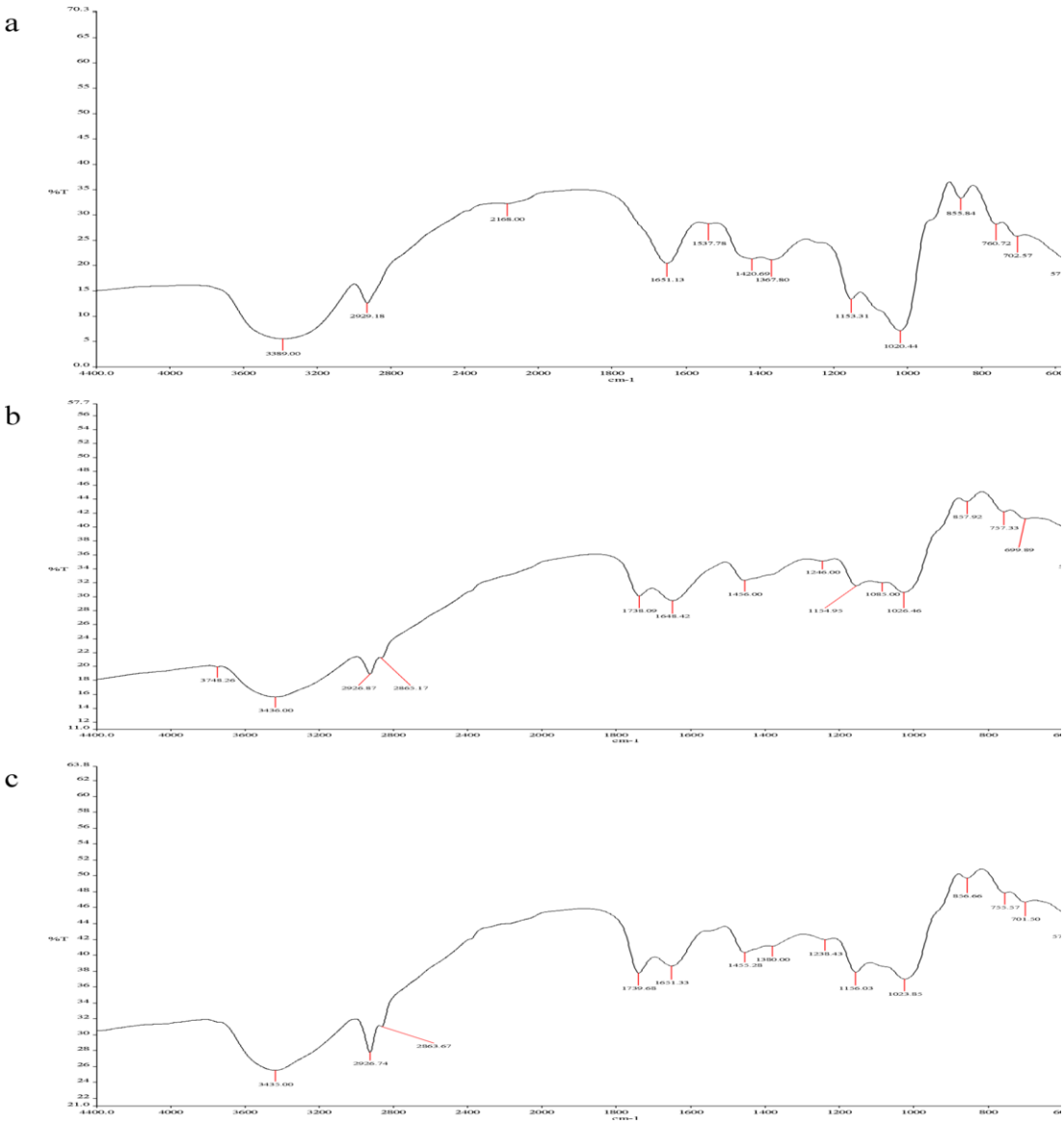
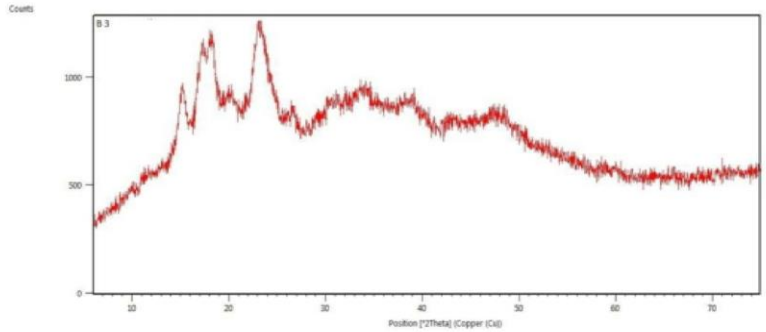


Fig. 2: FTIR spectra of (a) Freeze-dried uncooked millet slurry, FDUM (b) Freeze-dried cooked millet slurry, FDCM and (c) Freeze-dried cooked millet slurry with additive, FDCM+A

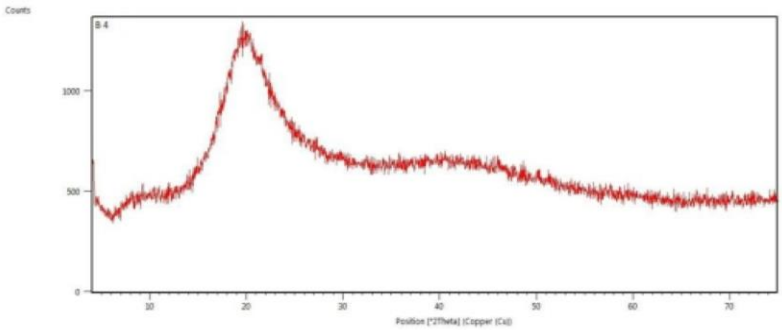
X-ray diffraction (XRD) spectra

The XRD spectra of the samples are shown in Fig. 3. The XRD spectra of the cooked samples also confirmed the disruption of the internal order of the uncooked millet slurry powder. Hence the semi-crystalline nature of the uncooked millet slurry appeared to have become more amorphous when cooked.

a



b



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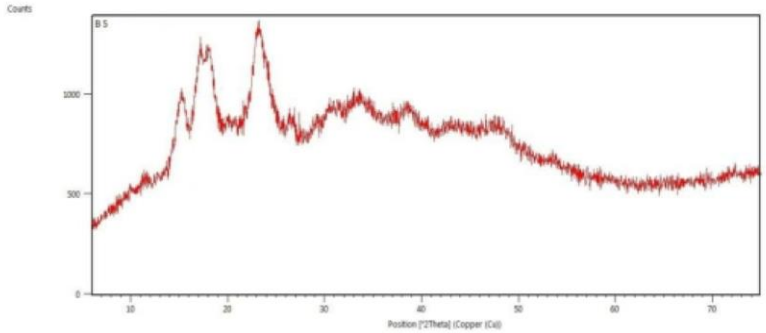

Fig. 3: XRD spectra of (a) Freeze-dried uncooked millet slurry, FDUM (b) Freeze-dried cooked millet slurry, FDCM and (c) Freeze-dried cooked millet slurry with additive, FDCM+A

Swelling index

The results of swelling index for all samples are presented in Table 1. Swelling index of freeze-dried millet slurry samples was in the rank order: FDCM+A > FDCM > FDUM. The result showed that cooking of the slurry enhanced swelling of the powders owing to the disrupted and loose structure of the cooked extracts.

Table 1: Physicochemical and material properties of freeze-dried powders of Pearl millet slurries (mean ± sd)

Sample	Particle diameter µm	Swelling index	Particle density g/cm3	Bulk density g/cm3	Tapped density g/cm3	Hausner's ratio	Carr's index %	Angle of repose °
FDUM	14.68±5.66	1.54±0.16	0.48±0.22	0.75±0.10	0.93±0.58	1.24±0.13	19.35±3.10	36.39±2.78
FDCM	46.74±17.27	1.90±0.04	0.41±0.02	0.86±0.06	1.20±0.21	1.40 ±1.23	28.33±.2.79	36.84±2.37
FDCM+A	188.00±98.18	1.92±0.10	0.37±0.24	0.69±0.04	1.21±0.75	1.75±0.78	42.98±2.30	41.38±1.74

Key: FDUM - freeze-dried uncooked millet slurry; FDCM- freeze-dried cooked millet slurry; FDCM+A freeze-dried cooked millet slurry with additive

Flow properties

The ranking of particle, bulk and tapped densities were similar to that of particle diameter and were in the order of: FDCM+A > FDCM > FDUM. From the bulk and tapped density values, the Carr's index and Hausner's ratio were obtained. The Carr's index (percentage compressibility) and Hausner's ratio measure the compressibility and flowability of a material, respectively. The values also give an indication of the likely compaction behaviour of powders when subjected to compression forces to form a compact mass like tablet. The higher the Hausner's ratio, the greater is the propensity to form a compact mass (Olayemi et al, 2011). On the other hand, the smaller the Carr's Index, the better the flow properties. Hausner's ratios less than 1.25 indicate good flow; Carr's index below 15 % indicates good flowability while values above 35 % indicate cohesiveness (Carr, 1965; Bakre and Jaiyeoba, 2009). The ranking of the values for Carr's Index was FDCM+A > FDCM > FDUM. The rank order for Hausner's ratio was similar to that obtained for Carr's index and indicated good flow of the uncooked millet powder, fair flow for the cooked powder (FDCM) while the presence of additives reduced flow in FDCM+A.

The angle of repose (θ) is the angle of elevation to the horizontal plane, at which a powder commences to slide upon itself. It is also another important criterion used for predicting the flow characteristics of a powder and it ranges from 0 – 90°. Particle size and the frictional force present between the particles of a loose powder are important determinants of its flowability. Angle of 30^{o} or below usually indicate that the powder is free flowing; values of 40^{o} or above is an indication that the flow is uneven, eventually ceasing altogether at angles of ≥ 60 (Geldart and Wong, 1984). The angles of repose of all the powders confirmed flow.

Compaction properties

The freeze-dried powders of the millet slurries exhibited good compressibility and this warranted the evaluation of their compaction properties in order to assess their potential for compression into tablet dosage forms. Tablets have been reported to be one of the most acceptable and convenient dosage forms for oral administration (Aulton, 2017); thus, formulating the free flowing powders into compact tablets offers another convenient alternative for the oral delivery of the processed millet grains.

Compressibility is the ability of a material to reduce in mass or volume by the application of pressure. A highly compressible powder enables particles to get closer to each other and interact, thereby facilitating inter-particulate bonding and the formation of stronger compaction. The degree of volume reduction of powders undergoing densification under externally applied pressure were analyzed by Kawakita's equation (Kawakita and Ludde, 1971). The equation relates the degree of volume reduction to the approved powder under tapping. The constant 'a' is equal to the minimum porosity of the material before compression while the constant 'b' is related to the plasticity of the material after compression. The reciprocal of 'b' gives a pressure term P which is the pressure required to reduce the powder bed by 50% (Podezeck and Sharma, 1996). A low value of 'a' indicates that the powder system was well packed densely on initial pouring into the cylinder indicating that the powder is well packed before tapping, since tapping did not considerably improve their flow[17]. On the other hand, the value of 'b' is an inverse measure of cohesiveness. The lower the value, the more cohesive the material. The basis for the Kawakita's equation for powder compression is that particles subjected to a compressive load in a confined space are viewed as a system in equilibrium at all stages of compression, so that the product of the pressure term and volume term are constant (Lin and Cham, 1995; Celik, 1992). The Kawakita plots of N/C versus N for the freeze-dried powder extracts are shown in Fig. 4. The constants 'a' and 'b' of the Kawakita equation were observed from the slope and intercept, respectively. The values of the constants are presented in Table 2. The ranking for 'a' was in the order: FDCM+A > FDCM > FDUM while that of 'b' was FDCM+A < FDUM > FDCM. These results suggested that processing of the millet slurry by cooking produced powders with good compressibility, with potential for tableting in to a solid compact mass (Shivanand and Sprockel, 1992; Ogunjimi and Alebiowu, 2013). As expected, the addition of additives acted as solid barriers that reduced their attractive forces and hence the cohesiveness of the powders containing ginger was reduced.

The values of bulk and tapped density values were used to plot relative change in density with applied load versus number of taps; from which the slope K, the rate of consolidation, and intercept C, the consolidation index, were determined (Ogunjimi and Alebiowu, 2013). The value of K is a measure of the rate of packing of powder. The higher the consolidation index, C, the higher is the rate of packing of the powder. The plots of relative change in density with applied load versus number of taps are shown in Fig. 5 while the values of C and K obtained from the plots are also presented in Table 2. Ranking for consolidation index (C) was FDCM+A > FDCM > FDUM while that of consolidation rate (K) was FDCM > FDCM+A > FDUM. The results showed that the processing of the millet slurry by cooking resulted

in increase in consolidation index and consolidation rate implying higher rate of packing.

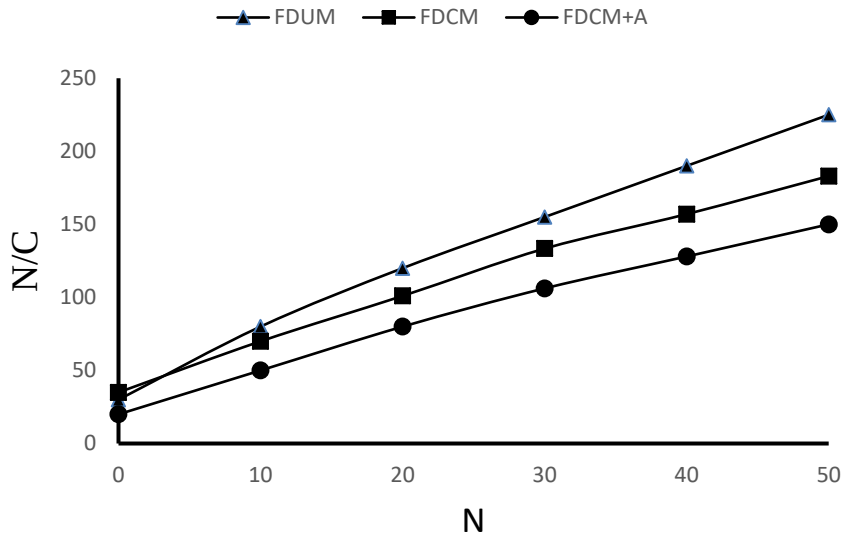

Fig. 4: Kawakita plots of N/C versus N for Freeze-dried uncooked millet slurry (FDUM), Freeze-dried cooked millet slurry (FDCM) and Freeze-dried cooked millet slurry with additive (FDCM+A)

Table 2: Compaction properties of freeze-dried millet powders

Sample	Kawakita		Neuman	
	a	b	C	K
FDUM	0.2662	0.0974	0.0757	0.0192
FDCM	0.3462	0.0721	0.1847	0.0547
FDCM+A	0.3950	0.1023	0.2153	0.0379

FDUM - freeze-dried uncooked millet slurry; FDCM- freeze-dried cooked millet slurry; FDCM+A freeze-dried cooked millet slurry with additive

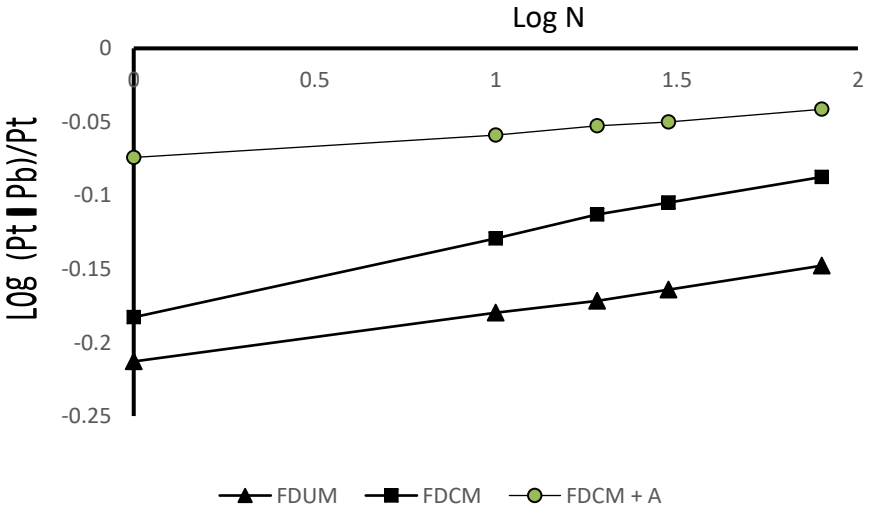

Fig. 5: Plots of Log Pt – Pb / Pt vs Log N

Characterization of dispersions of powdered samples of Pearl millet slurry

pH

The pH of the dispersion samples including the cooked millet slurry prepared using the traditional method are presented in Table 3. The ranking of pH followed the order: FDUM = cooked millet slurry > FDCM+A > FDCM. All the samples were acidic. pH plays an important role in food processing as constant pH measurement compensates for intangible process variations and assures reliable product quality (Troiler and Taylor, 2014)

Flow rate

The values of the flow rate are presented in Table 3 and these were ranked as follows: FDCM > FDCM+A > FDCM >cooked millet slurry. The cooked millet slurry had the least flow rate owing to the irregularly-shaped and large particles that retarded flow.

Table 3: Characterization of Pearl millet dispersions

Sample	pH	Flow rate /s	Viscosity cP	Rate of redispersibility (cycles)
FDUM	4.55	0.13 ± 0.04	3.50 ± 0.70	12.00± 2.00
FDCM	2.45	0.25 ± 0.07	1.50 ± 0.00	7.00± 1.00
FDCM+A	3.55	0.20 ± 0.01	4.50 ± 0.00	8.00± 1.00
Slurry of millet (not freeze-dried)	4.55	0.10 ± 0.01	10.50 ± 2.12	**NA**

NA - test not applicable to the slurry

Viscosity

The viscosity values was in the rank order: cooked millet slurry > FDCM+A > FDUM > FDCM. The viscosity of the cooked millet slurry was significantly higher than those of the freeze-dried millet slurries formulated into dispersions. When it comes to the freeze- -dried extracts, low viscosity of the system may be obtained due to retrogradation at cooling, and then gel freezing prior to freeze-drying (Blaszczak *et al*, 2001). The high viscosity of the cooked millet slurry accounted for its low flow rate.

Redispersibility

The samples all had good redispersibilty and high sedimentation volumes with larger percentage ease of redispersibility observed for those dispersions of the cooked millet slurries possible due to their larger, less cohesive particles that permitted separation and redispersion (Elkheshen *et al*, 1997).

Animal studies

In this study, virgin female Wistar rats were exposed to male rats and separated once mating was confirmed after which the rats were allowed to deliver their litters (a gestational period of 19 to 22 days). The lactating rats were randomly divided into seven groups of five rats each (n=5) as shown in Table 4.

Table 4: Groups of Wistar rats and formulations administered

Group	Formulation administered
I	Water (control)
II	Domperidone (standard.)
III	Conventional cooked slurry
IV	Conventional slurry of cooked millet administered to virgin rats
V	Freeze-dried uncooked millet slurry
VI	Freeze-dried cooked millet slurry
VII	Freeze-dried cooked millet slurry with additive

The weight gain of the dams and litters of the Wistar rats treated with the samples and domperidone as standard are presented in Table 5. The weight gain by the rats may not be an absolute determinant of improved lactation through dosage administration, but it may serve as a pointer especially when the animals are subjected to the same feeding and environmental

condition, in particular with the weight gained by litters (Cambraia *et al,* 1997).

The ranking of the groups in terms of weight gain was: Group treated with the standard domperidone > Group treated with FDCM > Group treated with FDCM+A > Group treated with cooked millet slurry > Group treated with FDUM > Group of virgin rats administered with cooked millet slurry > Group treated with water as control. From these results, it appears that the millet slurries had some influence on the weight gain of both the Dams and the litters.

Using the Turkey's multiple comparison, the statistics of weights of dams revealed there was no significant differences between the change in weight of virgin rats, those treated with cooked millet slurry, those treated with FDUM, those treated with FDCM and those treated with FDCM+A. However, the change in weight of the group treated with domperidone and those treated with water were significant.

Most food products require drying to remove excess water in order to prolong shelf life and prevent microbial degradation. Often, product quality degrades during the process of drying due to thermal degradation and denaturation. A low-temperature drying process such as freeze-drying will preserve characteristics such as taste, colour/appearance as well as minimize the degradation of thermolabile compounds, many of which are responsible for the nutritional value of the crops (Karam *et al*, 2016). Processing of the millet grains by freeze-drying maintained the nutritional quality of the grains as observed in the results above in which there was no significant difference between the change in weight of rats treated with cooked millet slurry prepared the traditional way and those treated with the freeze-dried samples.

Table 5: Weight gain of Wistar rats administered with millet preparations and domperidone (mean ± sd)

Sample	Wistar rats	Initial weight of Dams g	Final weight of Dams g	Change in weight	Number of Litters (n)	Initial weight of litters g	Final weight of litters g	Change in weight of litters
Slurry	VWR	146.46±24.74	160.96±26.06	14.7±6.55	-	-	-	-
Slurry	WR	165.36±14.35	187.12±12.80	21.76±11.63	7.00±3.16	73.90±15.45	94.90±14.48	20.26±16.37
FDUM	WR	173.60±16.11	174.42±17.49	1.22±20.61	6.40±1.94	36.50±7.22	76.96±14.22	40.45±13.82
FDCM	WR	180.82±25.79	205.70±26.85	24.88±8.94	5.40±1.14	96.34±34.18	117.52±36.49	21.18±5.19
FDCM+A	WR	173.70±14.80	195.84±16.68	22.14±5.68	5.20±1.48	77.40±32.99	94.74±28.94	22.30±15.08
Distilled water	WR	170.17±8.37	169.42±12.33	0.75±6.11	8.00±2.73	95.85±09.65	107.00±13.45	31.53±15.43
Domperidone	WR	80±1557.62	203.00±6.29	47.16±5.44	7.40±2.30	135.76±17.61	184.8±31.13	49.04±14.87

Histological assessment of the mammary glands of the female Wistar rats.

The photomicrographs explaining the results of the histopathological assessment are shown in Fig. 6 and Fig. 7. The acini of the mammary glands containing secretory cells are indicated by the green arrow, the lumen that housed the milk production is shown by the black arrow while the epithelium cells are indicated by the red arrow. The group tested with FDUM (A) showed well-developed glandular structures, surrounded by adipose tissue. Few of the lobules were distended with compressed glandular epithelium, showing heightened secretory activity. The heightened secretion revealed an increased emptying to the dam (Reeder *et al*, 2011). The group tested with water (B) showed glandular structures with only few lobules showing secretory activity. A lot of interlobular connective tissue is also seen, the visible interlobular connective tissue showed that the dam underwent lactogenesis but emptying of milk to the litters was reduced. The group treated with cooked millet slurry prepared in the traditional way (C) showed well-developed glandular structures. Most of the acini were greatly distended with compressed glandular epithelium, showing heightened secretory activity. Secretory materials are also seen prominently in the lumen. The heightened secretion and prominence could also be attributed to increase in milk emptying to litters. The virgin group treated with the slurry of cooked extract (D) showed a mammary gland ducts, with surrounding hypodermal and interlobular connective tissue observed to be very prominent. No glandular secretory acini were present. This could further be inferred that the millet extracts did not, on its own, induce lactogenesis but rather enhanced the volume secretions and emptying to litters. The group treated with Domperidone (E) showed a well-

developed glandular structure, the glandular units were well developed and highly distended, showing a very high secretory activity (Bharti *et al,* 2002). This could be due to the activity of domperidone as a standard galactogogue. Though it also did not induce lactogenesis, it had a very high emptying rate and improved secretion. The group treated with FDUM (F) also showed a well-developed glandular structure, most of the glandular units were well developed and highly distended, indicating a very high secretory activity. Pink staining secretory materials were found in the lumina of the acini. The glandular epithelia were also compressed by the secretory material. The virgin group treated with FDCM (G) showed glandular structures but the acini did not show considerable secretory activity, with the lumen of the acini reduced. It could be inferred that no lactogenesis occurred in the virgin rats. The group tested with FDCM+A (H) showed glandular structures with few of the lobules showing high secretory activity. Most of the secretory units appeared quiescent. In summary, Figures A (group of virgin rats treated with FDUM), E (group treated with domperidone) and F (group of dams with litters treated with FDUM) showed the highest depth of secretory activity while the other groups except Figure D (group of virgin rats administered the cooked millet extract) appeared to show moderate activity. Figure D showed no secretory activity.

The oral administration of both processed and traditionally-prepared millet slurries showed some effects on milk secretions and milk emptying in the groups of dams. However, lactogenesis was not induced in virgin rats that were administered both the freeze-dried and cooked millet slurry prepared by the traditional method. Hence, the folklore use of cooked millet slurry in adoptive nursing by women who have never been pregnant or nursing mothers, could not be justified by the study.

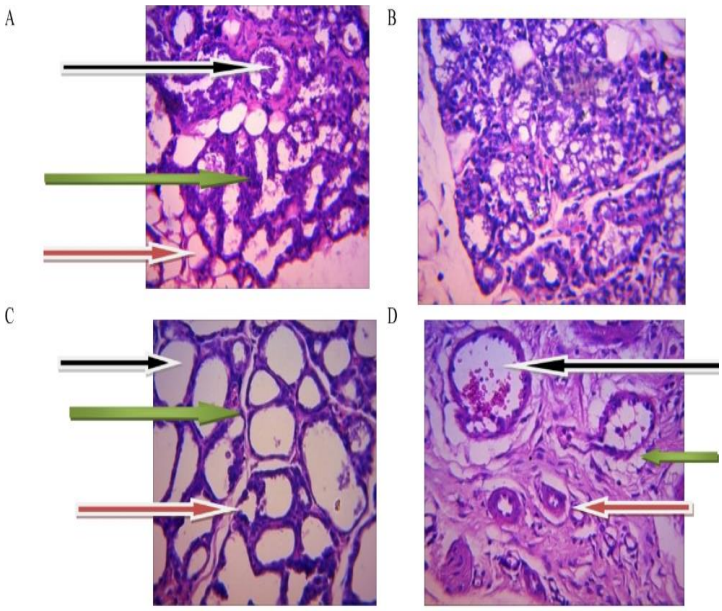

Fig. 6: Photomicrographs showing the mammary gland acini of groups administered with: A, Freeze-dried uncooked millet slurry; B, water; C, slurry of cooked millet grains; D, Slurry of cooked millet grains (Virgin Wistar rats) (mg x400)

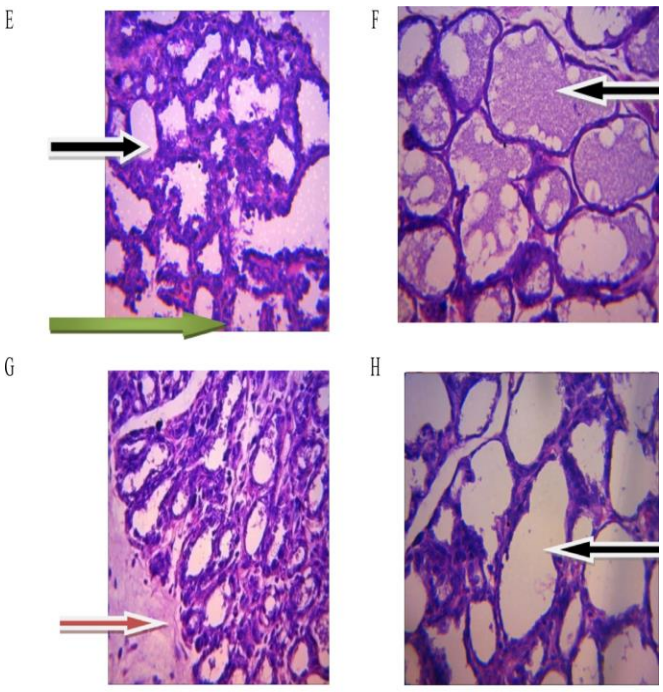

Fig. 7: Photo micrographs showing the mammary gland acini of group administered with: E, Domperidone; F, Freeze-dried uncooked millet slurry t; G, Freeze-dried cooked millet slurry; H, Freeze-dried cooked millet slurry with additive (mg x400)

Toxicological testing: determination of LD_{50}

The Lorke's method is a trial for the investigation of the acute toxicity in animals, in order to estimate the Lethal Dose which is the drug concentration that causes mortality of 50% of the test animals (LD_{50}). A daily observation for mortality was made for two days (Lorke, 1983). There was no mortality reported even after administration of the highest dose of the millet slurries (6000 µg/kg).

CONCLUSION

Slurries of Pearl millet, *Pennisetum glaucum* (Poaceae), were freeze-dried for convenience of handling, extension of shelf life and improved flow. Evaluation of physicochemical. properties revealed larger particles with improved swelling and adequate compressibility, showing the potential of the freeze-dried powders of millet slurry for compression into tablet dosage form, if required. The histopathological examination in rats revealed that millet extract improved milk secretion and emptying comparable to domperidone. There was no mortality after the administration of the highest dose of the extract. Pearl millet grains extract showed potential as a galactogogue which may be applied not only in humans but can be further studied for its potential application in improving milk production in the dairy industry.

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