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Pharmacology

Histological evaluation and Physical characteristics behavior of Albino rats by (Rattus norvegicus) Moringa (Moringa oleifera), (Hibiscus sabdariffa), 'Ugwu' (Telfairia occidentalis) and Ginger (Zingiber officinale)

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ABSTRACT Moringa oleifera has gained popularity especially in recent times due to several publications reporting various nutritional and health benefits of the plant; though it is important to note that most investigations on this plant are basic and the reports would require proper trials to evaluate the exact benefits to human health. This investigation was an attempt to investigate the effects of ethanolic Moringa oleifera leaf extract on the histology of vital body tissues. The rationale is that histological observations would provide a more reliable and consistent picture of the effects produced by the interactions of the phytochemicals with the body cells and tissue. It may be helpful in observing the possible toxicological effects on body tissues or on the other hand, the positive effects on the body tissues. A total of twelve Wistar rats (n=12) were used for the investigation; divided in two groups of Control (A) and Treated (B). A daily dosage of 200mg/kg body weight of ethanolic moringa leaf extract was administered orally to the treated Group B for 28 days. All animals were fed ad libitum. Animals were sacrificed by cervical dislocation. The tissues were excised and processed using routine haematoxylin and Eosin staining techniques. Photomicrographs were taken with the aid of the Accuscope Photomicrographic at suitable magnifications. Analysis of each tissue's histo-morphology, general histo-architecture and cytological structures was critically done. The basis of analyses and inferences was clearly defined: whether Moringa oleifera leaf extract produced any observable deleterious effects on the tissue [toxicological evaluation]; or whether its effects would improve the tissue's histological architecture especially in manners that can produce improvement in physiological conditions of the individual tissue or general body health [medicinal and nutritional properties]. Extract produced positive effects in most tissues except in the testis and epididymis where the effects were anti-fertility. Various tissues 1enefited from the positive effects to various extents. The ameliorative efficacy of roselle, moringa, ginger, 'ugwu' and a mixture of the plant extracts on selected physical characteristics of rat exposed to cement dust was evaluated. Albino rats grouped into six of 18 rats each were exposed to cement dust at a cement factory in Sagamu, Ogun State, Nigeria. The test rats were administered 400 mg kg-1 ethanol extracts of roselle, moringa, ginger, 'ugwu' and a mixture of the plant extracts, respectively for 180 days. The control rats were fed with only distilled water. Significant ($p<0.05$) differences were observed between the test and control rats in all the tested health indices. The test rats weighed more than the control rats, and weight differences were also observed among the test rats. The births, number of newborn per litter and offspring survival rates of the test rats were higher than the control rats. Lower death rates were observed in the test rats compared with the control rats. These findings highlight the roles food plants containing phytonutrients and phytochemicals may play in maintaining good health in polluted environments.

KEYWORDS : Cement dust, food plants, phytonutrients, phytochemicals, and body weight.

INTRODUCTION

Environmental pollution and control became a global issue during the industrial revolution in the 18th century (Doyle, 2003). The industrial revolution meant that more goods could be produced for human consumption. However, it also meant that more natural resources would be exploited, and more pollutants would be discharged into the environments (Clive, 1991). Presently, environmental pollution is among the problems the world is facing, increasing in threat with every passing year. All industries generate harmful waste substances, causing contamination of water, air and soil with harmful effects on both humans and environments. The main environmental pollutants include particulate matters, aluminium, dioxins, heavy metals, sulphur dioxide, benzene, carbon monoxide, nitrogen dioxide, industrial effluents and agricultural runoff (European Public Health Alliance, 2009; Yahaya and Okpuzor, 2011). Pollutants can cause both acute and chronic health problems in animals and humans, involving several organs and systems. Short- and long-term exposures to pollutants have also been associated with high mortality and shortened life expectancy (Marilena and Elias, 2008). Despite these health consequences, industries keep expanding because they make life easier. Hence, the need to control and ameliorate health effects of pollutants from various industries.

Pollution prevention and control strategies have not been successful, especially in developing nations due to lack of funds, ignorance, strategy technicalities and weak environmental protection laws (Yahaya et al., 2012). Even in developed countries, pollution control has not been 100 % successful with large proportions of annual national budgets allocated to pollution control. According to a researcher from Kansas State University, United States spend more than $ 4.3 billion annually on freshwater pollution mainly from agricultural runoff. The country also spends well over $ 50 billion annually on pollution from automobiles and more than $ 234 billion yearly on industrial pollution (KSU, 2008). Since phytomedicine is one of the strategies employed in health care delivery, it is necessary to assess the potentials of some food plants in ameliorating effects of pollutants. This study evaluates the ameliorative efficacy of some selected food plants on some physical characteristics of albino rats exposed to cement dust. There are several publications, reporting the various benefits of Moringa oleifera leaf and other parts of the plant. It is however important to note that there is the need for more specific reports, especially considering the scope of the research activities leading to the presented results. While the plant shows huge potentials to alleviate hunger and provide herbal and plant-derived medicinal products, especially for the developing nations; it is important to establish the primary effects of the leaf phytochemicals' activities and interactions with the body tissues cum organs. In other words, toxicological research efforts would do well to first establish the safety of consumption of the plant products and to the various extents; as this will be vital to the establishment of the use of the plant's products as standard nutritional supplements and natural or bio-medicinal products. To this end, we employed histological methods to evaluate the effects of moringa leaf extracts on vital body tissue-organs.

The rationale is that histological methods of observations would provide a more reliable and consistent picture of the effects produced by the interactions of the phytochemicals with the body cells and tissue better than in vitro tests and analysis of the highly dynamic biochemical activities as contained in extracted tissue fluids. Also, the use of Histological methods of assessment of moringa leaf extract effects on body tissues is important because literatures are comparatively scarce on such methods of investigation of the plant's extracts' effects.

Few publications have reported some of the specific effects of Moringa oleifera leaf extract on the some body tissues or organs. The

cerebroprotective effect of moringa oleifera against focal ischemic stroke has been reported (Woranan et al., 2013). Chatchada et al., (2013) reported that moringa leaf extract was neuroprotectant when administered to animal Models of Age-Related Dementia when their hippocampus was observed. It was also reported that Lyophilized hydroalcoholic extract of M. oleifera showed myocardial preservative effect in isoproterenol (ISP)-induced model of myocardial infarction (Nandave, 2009). The report of Ouédraogo (2013) in their efforts to evaluate the protective effect of Moringa oleifera leaf extract against gentamicin-induced nephrotoxicity in rabbits stated that histological preparations of the kidney of intoxicated animals treated with moringa leaf extract exhibited reparative tendencies. Moringa oleifera ethanolic leaf extract reportedly have hepatoprotective abilities in various induced conditions such as using diclofenac (Hamza, 2007); acetaminophen (Fakurazi et al., 2008) antitubercular drug (Pari and Kumar, 2002) and carbon tetrachloride (Selvakumar and Natarajan, 2008) . Awodele et al., (2012) estimated the LD(50) for aqueous extract of moringa leaf and tested various dosages of extract on the sperm, haematological and biochemical parameters as well as histopathological preparations; and they concluded that orally administered moringa leaf extract at their estimated sub-lethal dosages were relatively safe for tested body organs. The aim of this particular investigation was to observe the effects of Moringa oleifera leaf extract on the histological architecture on a dozen vital tissues of the body.

MATERIALS

Animal Care

One hundred and fifty albino rats weighing between 185 and 200 g were sourced from the Department of Biochemistry, University of Ibadan in August 2009. The rats were made to acclimatize to the ambient environment before commencing the experiment. Pellet feeds from the F. A Feeds industry, Agege-Lagos and water were given to the rats ad libitum.

Elemental Analysis of the Cement Dust.

The elemental analysis of the cement dust was carried out by Atomic Absorption Spectroscopy (AAS). Spectrophotometer (UNICAM model 969) was used for the analysis in the Department of Chemistry, University of Lagos.

Source of the Plant Materials

The plant materials- roselle, moringa, ginger and 'ugwu' were purchased from Ketu in Lagos metropolis, Nigeria. They were identified by a curator, Mr. Odewo T. Kolawole, in the Department of Botany, University of Lagos. The voucher numbers of the authenticated samples are LUH 4394, LUH 4558, LUH 4396 and LUH 4395 for roselle, moringa, ginger, and 'ugwu', respectively.

Preparation of the Plant Materials

Fresh leaves of the individual plant materials were washed gently to remove impurities and air-dried under shade for one week. The dried leaves were milled into a powder using laboratory mill, Norris Limited, Poole, England at the Department of Pharmacognosy, University of Lagos. A mixture of the individual plant materials was also obtained by mixing the four parts each of the ground plant materials in the ratio 1:1:1:1. The ground plant materials were then stored in desiccators before use.

Preparation of the Plant Extracts

The bio-active compounds were extracted from the plant materials using the method of Okigbo and Ogbonnaya (2006). Fifty grams (50 g) powder of each plant material and the mixture were put in 500 ml 95% cold ethanol for 72 hours. The extracts thus obtained were filtered with muslin cloth and evaporated to dryness at a temperature of 40±2oC. The resulting dried extracts of each plant material yielded 6.6 g, 6.5 g, 6.2 g, 5.9 g, and 6.1 g of roselle, moringa, ginger, 'ugwu' and mixture, respectively. These dry extracts were reconstituted in water and were the decoctions used for the experiment.

Phytoconstituents Screening of the Plant Extracts

The phytochemicals in the plant extracts were identified using standard procedures as described by Harbone (1973) and Sofowora (1993). The phytonutrients were screened using thin layer chromatography (TLC) method as described by Meloan (1999).

Acute Toxicity Test

The acute toxicity of the crude extracts of the plants was measured using the 'Classical LD50' method described by Gabriel et al. (2008). Albino rats (36) of both sexes weighing between 183 and 205 g were used for the studies. The rats were randomly distributed into six groups of 6 rats each and were denied food and water 12 hours before commencing the study. The rats in the test groups were orally administered doses of 200, 400, 500, 700, 1500, and 2000 mg kg-1 of the crude extracts. The control rats received only distilled water. The general symptoms of toxicity were monitored and recorded for each group within 24 hours.

Dosage Administered to the Rats

The acute toxicity test showed the plant extracts were nontoxic to the rats even at a dose of 2000 mg kg-1. However, 400 mg kg-1 dose was chosen because a previous study by Adedapo et al. (2009) showed some of the understudy plants work best in rats at the dose.

Twelve male Wistar rats (n=12) were used for the investigation. They were divided in two groups of Control (A) and Treated (B). A daily dosage of 200mg/kg body weight of ethanolic moringa leaf extract was administered orally to the treated Group B for 28 days. All animals were fed ad libitum. Animals were sacrificed by cervical dislocation. The tissues were excised and processed using routine haematoxylin and eosin staining techniques. Analysis of tissues was done using qualitative methods with emphasis laid on histo-morphology, general histo-architecture and cytological structures features of the prepared specimen.

METHODS

Study Design

The rats were placed into six groups of 18 rats each. Group one was the control, and groups two through six formed the test rats. The entire rats were exposed to cement dust at 200 km from a cement factory in Shagamu, Nigeria. The body weights of the rats were measured before commencing the experiment. The test rats were thereafter treated with 400 mg kg-1 ethanol extracts of roselle, moringa, ginger, 'ugwu' and a mixture of the plant extracts, respectively. The control rats received only distilled water. The body weight, percentage death, average number of newborn per birth, and percentage offspring survival rates were monitored for 180 days.

Relative Growth Rate (RGR) of the Rats

The relative growth rate of rats across the groups was calculated using the formulae below:

$$RGR\,(\%) = \frac{WF - WI}{T}\ X\ 100$$

Where W^F =final weight;

W_i= initial weight; and T = period of exposure (Winder et al., 1990)

Percentage Death, Average Number of Newborn per Birth, and Percentage Offspring Survival.

The percentage death, average number of newborn per birth, and percentage offspring survival of the rats were calculated from the formula below:

$$Death\ (\%) = \frac{Number\ of\ deaths\ recorded}{Number\ of\ rats}\ x\ 100$$

$$\text{Average Number of Newborn per Birth} = \frac{Number\ of\ Newborns}{Number\ of\ Delivery}$$

$$Offspring\ survival\,(\%) = \frac{Nuumber\ of\ newborns\ that\ survived}{Number\ of\ newborns}\ x\ 100$$

RESULTS AND DISCUSSION

Bone Marrow

The bone marrow tissue of the Group A animals [Control] is being illustrated as photomicrographs A1 and A2 the cellular elements. Photomicrographs B1 and B2 are photomicrographs of the Group B animals administered moringa oleifera extract-the cellular elements are also observable (BMC). In both Groups (A and B), the cells appear relatively normal, and there are no sign to suggest anomalies especially in the untreated Group. In both sets of photomicrographs, the extracellular materials are abundant and well distributed. There is not enough evidence to suggest that moringa leaf extract has improved the physiological condition of the bone marrow from the photomicrographs; a deducible fact however, is that the administration of Moringa oleifera leaf extract has not produced any observable

deleterious effects on the bone marrow tissue in the models employed in this investigation. This strongly suggests that the use of the extract would not compromise vital functions of the active bone marrow which primarily would include haematopoiesis. The report of Adedapo et al., (2009) showed dose pended responses of haematological parameters to moringa extract ingestion; however at 400 mg/kg-bw significant increase in packed cell volume (PCV) was recorded; this could be an indication of a positive effect.

Brain Cerebrum

The cerebral cortices of the experimental animals are illustrated at various magnifications. The control Group A cortex is illustrated at various magnifications in A1, A2 and A3. A1 illustrates the cross section of the cerebral cortex in an attempt to observe the general organization and arrangement of neurons and other supportive structures across the cortical layers [though this could only be effectively done at such low magnification]. A2 and A3 are larger illustrations of the cerebral cortex of the Group A models-deeper and superficial layers respectively. Neurons are clearly observable as well as the glia in their various peculiar forms. While glia could be differentiated using their basic forms, neurons as well could be seen as they assume various shapes and forms across the various layers of the cerebral cortex. The neuropil is also normal in appearance. These observations altogether show that the Control Group A cerebral cortex is normal and could serve as a suitable reference. B1 provides a cross-sectional observation of the brain cortex of the treated Group B at the lowest suitable magnification. The neurons appear well distributed across the layers. At the larger magnifications- B2 and B3; the neurons and the glia are also well defined and observable in their various forms are shapes. There are no abnormal observations that could suggest a damaging or deleterious effect of the administered substance on the tissue as illustrated on the photomicrographs. In other words, moringa leaf extract administration did not produce any histologically observable disrupting or damaging effects on the tissue of the cerebral cortex in this investigation. Ganguly et al., (2005) reported positive effects of moringa extracts on the cerebral cortex and suggested it could give protection against devastating disease like Alzheimer's; it has also been shown to have neuroprotective effects in focal cerebral ischemia (Kirisattayakul). Moringa also has positive anti-lead ameliorative effects (Owolabi et al., 2014). There are however very scarce literatures on the specific effects of the extract on tissue morphology and histo-architecture.

Brain Cerebellum

The brain cerebellum of the control Group A animals models is histologically illustrated in the photomicrographs labelled A1, A2 and A3; the basic layers or regions of the cerebellum are clearly illustrated, so also the primary elements of the layers or regions A2 and A3. The cells of the molecular layer [MC], Purkinje Cells [PC] and the granular layer cells [GC] are clearly defined. The layers are also indentified and labelled- molecular layer [ML], most peripheral, the middle layer of Purkinje cells {PCL] as well as the inner granular cells layer (GCL) together with the white matter [WM] core. These are obviously normal features of a healthy cerebellum and as such the control is considered a suitable reference for the study. All the aforementioned features are also well defined and clearly observable in the treated Group B model cerebellum as illustrated in the photomicrographs labelled B1, B2 and B3. All evidences point to a conclusion that the extract did not produce any histologically observable deleterious effect on the cerebellum of the treated Group B animal models. Moringa's anti-toxicity effect in the cerebellum has been previously reported (Owolabi et al., 2014). The brain tissue remains a tissue on which more specific reports about the results of moringa phytochemicals' int-eractions with the tissue should be properly documented

Brain Hippocampus

The hippocampus of the control Group A animal is represented in photomicrographs. Photomicrographs A1, A2 and A3 are illustrations of the control Group animals (dentate gyrus); the various regions or zones of the dentate gyrus are observable- the molecular granular and polyform layers are all observable. The neurons and glia are also present- all are morphologically normal. The treated Group B hippocampus tissue is illustrated in B1, B2 and B3. Tissue has no sign of disruption or damage. The granular cells are clearly defined and compact in organisation, suggesting they are also normal. The dentate gyrus granular layer of cells appears relatively thin; this is however not enough to infer a compromising consequence. It could rather be an observation to be investigated furthermore. Moringa leaf extract as administered in this investigation did not produce any histologically compromising effects on the hippocampus of the treated animals. Not many reports are available on the particular effects of Moringa oleifera on the hippocampus; our previous report however showed that it could produce an anti-lead toxicity in the hippocampus (Owolabi et al., 2014).

Epididymis

The epididymis of the control Group A animals is being illustrated in A1, A2 and A3 at various suitable magnifications. The epithelium of the epididymis could be observed (EE) as well as the lumen of the tubular structure (L). The observations could be correctly used to adjudge the epididymis in this group to the normal. B1, B2 and B3 are histological representation of the epididymis of the treated Group B animals at three various magnifications. B1, B2 and B3 show the epididymis of the treated Group B models obviously with certain anomalies: the epithelium for the entire structure is grossly disrupted and there is an abnormal accumulation of tissues- tissue debris (TD)- in the lumen. The loss of the epithelium is no doubt an indication that the functions of this organ as a store and nurture chamber for produced spermatozoa until copulation and ejaculation is seriously compromised. Worst still, the lumen appears to contain more than the usual and normal germ cells or spermatozoa. These could possibly be an aggregate of deformed spermatozoa and in addition, the lost epithelial cells. Even if spermatozoa are produced normally in the testis and stored in the epididymis, there are indications that their forms and functions or viability could be seriously compromised. How effective the damaged tissue can respond to reparative stimuli cannot however be measured; but the signs suggest that in cases of continuous administration, the epididymis might have its form and functions disrupted by the effects of moringa extract phytochemicals. This supports pre-existing reports that moringa plant parts could produce anti-fertility effects when ingested. Lilibeth and Glorina (2010) had earlier reported moringa extract producing unusual effects of the mice epididymis including unusual thickening of the wall and epidermis inactivity; this shares similarities with the current investigation.

Cardiac Muscle

The photomicrographs in A1 and A2 illustrate the cardiac muscle tissue of the control Group A animals. The cardiac muscle cells or myocytes (CMC) nuclei as well as the fibrils (CMF) are observable at both magnifications. The organisation of the cells into tissue also appears normal for a healthy heart muscle. B1 and B2 are histological illustrations of the heart muscle of the Group B animals treated with moringa leaf extract; the basic elements are also observable as labelled and they are all normal in form, organisation and distribution. Moringa leaf extract therefore did not produce any histologically observable deleterious effects on the heart muscle of the treated animals. While it may be ambiguous to suggest from observations in this context that the extract effects could improve the structure of the heart muscle; it may however be adjudged to be safe for the heart tissue as used in the investigation. Positive effects of moringa extract against isoproterenol - induced myocardial damage in rats has been reported (Nandave, 2009). Davis (2010) also reported moringa to have affected heart muscle contractility in a way that could produce positive effects to counter anomalies such as hypertension while Gunjal et al., (2010) reported that the stem back extract could have protective effects on myocardial tissue.

Kidney

The renal tissues of the experimental animals are being illustrated histologically in the photomicrographs presented, illustrates the cortical structures especially the glomerulus (G) and the neighbouring renal tubules (RT). The renal corpuscle that contains the glomerulus as a whole is quite well defined and the constituting elements are clearly observable.

There is the Bowman's capsule; the glomerulus with its capillaries and their constituting elements (endothelial cells); the terminal ends of the afferent arteriole and the beginning of the efferent arteriole; the mesangium partly within the glomerulus partially and extending outside the glomerulus. The constituent cells of the elements are not distinguished by the staining mechanism of the dye employed, understandably a dye for general and proper demonstration of tissues' histo-architecture; the morphology of the cells however suggest that they are normal and properly organised as they should be in a functional kidney cortex. The podocytes are cells that wrap their processes round the capillary tufts to achieve effective ultrafiltration in

the process of urine formation-they are prominent in glomerular presentations. The endothelial cells also form primarily the walls of the capillaries; and there is the mesangial cell. The thin epithelium- simple squamous that forms the Bowman's capsule is equally observable.

B1 is a demonstration of the kidney cortex of the Group B animals treated with the moringa leaf extract. All the aforementioned features of the renal corpuscle (for the control) are present and appear normal. In addition, the glomerulus appear quite well defined, relatively better compactly organised with the constitute cells being more relatively prominent. While there are no usual disadvantages reported in association with better corpuscular element organisation, disorganisations or disruptions could easily be linked with renal malfunctioning; therefore improved organisation of this structure as observed could most likely be of an additional physiological advantage for the kidney, especially with respect to ultra filtration cum urine production- the primary activities that take place within the cortex. In both groups, the photomicrographs portray healthy renal tubules- both proximal and distal convoluted. Renal tubules are normal in the treated animals' photomicrographs; like the glomerulus relative to the control, they also appear better defined.

Paliwal et al., (2011) reported the anti-nephrotoxic effect of Moringa oleifera Lam; Ezejindu et al., (2014) reported that Moringa oleifera leaf extract would not produce any deleterious effects on the kidney of experimental animals even in cases of chronic administration. Awodele et al., (2012) reported moringa leaf consumption to be relatively safe at sub-lethal doses especially with respect to its effects on the kidney and liver tissues. Oyagbemi et al., (2013) however suggested that chronic use could predispose animals to hepatic and kidney damage. The current finding however shows that at moderate doses, moringa leaf extract ingestion is safe for the renal tissues.

Liver

The liver tissue of the Control Group A is being illustrated at various suitable magnifications in photomicrographs A1, A2 and A3. The lowest magnification shows a normally organised liver tissue with the plates of hepatocytes being separated by sinusoids. The central vein is also observable. At the higher magnifications- A2 and A3; the hepatocytes are observable (H), arranged in plates as well as the sinusoids (S) separating them. A few Kupfer cells (KC) are also observable. The portal triad- artery, vein and bile duct branches are also observable (PT). The liver tissue as a whole is healthy and normal. Group B photomicrographs (B1, B2 and B3) are illustrations of the Group B animals' liver tissues. All the basic features of a normal liver tissue as found in the control are present. The hepatocytes are however quite prominent and they appear better defined. While these observations show that the administered moringa leaf extract would not produce deleterious effects on the liver tissues; it could also suggest that it could stimulate better state of health and functional status of the hepatocytes; and consequently, improve the functions. It should be noted that hepatocytes acute states of health depend greatly on their functional response to systemic bio- and chemo-assaults. Moringa could therefore have produced synergistic anti-toxicity [such as anti-oxidant actions]or effects to either complement the liver's similar functions, provide prophylactic effects against the consequences of cellular (hepatocytes) activities or help the liver cells improve their state of health. It is important to note that a number of murine model investigations have reported the potency Moringa oleifera leaf extracts in protecting the liver from chemical toxicity and damage (Pari and Kumar, 2002; Ndong et al., 2007; Fakurazi et al., 2008; Buraimoh, 2011).

Lung

The lung tissue is being illustrated in A1, A2 and A3 are photomicrographs of the control Group of experimental animals. The alveolar sacs (AS) and the alveolar ducts (AD) leading to them are labelled. The supportive tissues are also of adequate structural integrity. The lung tissue of the control group is therefore normal and well defined. The treated Group B lung tissue is also normal and all the basic features as mentioned for the Group A are also observable and they appear normal. There is therefore histological evidence that moringa extract treatment does not produce any form of deleterious effect on the lung tissue of the treated animals; there is however not adequate evidence to suggest a structural or functional improvement of the Group B lung tissue over the Group A- control. Only a few publications have reported the effects of moringa leaf extracts on its own on the lung tissue; however, the antiproliferative effects of moringa leaf extract against alveolar epithelial cell cancer was reported by Tiloke et al., (2013). Dany et al., (2012) and Jung (2014) also suggested that the leaf contains specific anticancer (active for the lung) agent; Owolabi et al., (2012) reported the anti-lead toxicity effects of then leaf on the lung tissue while Yahya et al., (2014) reported that the antioxidant properties of moringa could make it ameliorate the effects of cement dust exposure.

Skeletal Muscle

A1, A2 and A3 are photomicrographs illustrating the skeletal muscle of the control Group A animals. The general histological architecture is normal in cross sections and the fibre bundles or fascicle [MF] are observable even at the lowest magnification employed. The perimysium [P] bundling the fibres are also observable especially at the medium, magnification. In B3, the myocytes nuclei [MCN] are observable as well as the endomysium [E] surrounding the syncytium-fibre. The treated Group B skeletal muscles have all the basic histological features of a normal skeletal muscle tissue. An important observation worth of attention is the prominence and the relative abundance of myocytes nuclei. This observation suggests hyperplasia- a condition that could build muscle tissues via stimulated or increased cell division, hence producing more cells and subsequently, more fibres. It could be surmised therefore that moringa extract treatment improved muscle volume by inducing hyperplasia in the skeletal muscle; this is normal of a physiological implication. Bhattacharya (2014) reported moringa effect as muscle relaxant; general body growth effects in poultry- including muscles-has also been reported by Teteh et al., (2013)

Spleen

The spleen tissue of the control animals is illustrated in A1 and A2; the white pulps (WP) and the red pulps (RP) are clearly defined at both magnifications used. This spleen tissue is normal in histological presentation and could serve the purpose of a suitable reference. In B1 and B2; the photomicrographs illustrate the spleen issue of the Group B animals administered moringa leaf extract. The pulps are also well defined and the entire tissues structural integrity is normal. The white pulps appear relatively quite prominent. This structure is quite important for the immune roles of the spleen. Observations therefore strongly suggest that moringa leaf extract treatment did not produce any deleterious effect on the spleen tissue structure; there however could have been structural improvement that could translate into improved physiological functions of the tissue. Moringa has been reported to produce positive effects on the body's immunity (Gupta, 2010); while Ogunlade et al., (2013) observed that moringa leaf extract could ameliorate the toxicity of aniline on the spleen, thus preventing splenomegaly and splenic hyperplasia.

Testis

The testicular tissues of the experimental animals are histologically illustrated in photomicrographs.

A1, A2 and A3 are photomicrographs of the tissue of the control Group A animals. The seminiferous tubules (ST) are well defined and the interstitial tissues are also defined. The serminferous tubule epithelium (STE) is well defined and has a normal histo-architecture; so also the central lumen. The control Group testicular tissue is therefore normal. B1, B2 and B3 are the histological representations of the Group B animals' testis. Extensive disruption of the histo-architecture is observable at all magnifications. The seminiferous tubules have grossly disrupted epithelium and consequently, large lumens. At the higher magnifications, it is evident that the disruptions cut across the various zones or layers of germinal cells which arrangement also correspond with the stages of development. Consequently, spermatogenesis would be generally compromised irrespective of the stage of the maturation of the cells. Only a few isolated and deformed cells are left of the epithelia. A few basal cells in the epithelium appear to remain [B3]; this could be an indication for possible regeneration upon withdrawal of extract administration or introduction of therapeutic agents. The interstitial structures are also damaged by the effects of the administered substance. All observations as stated point to compromises in the process of producing spermatozoa-spermatogenesis. This is evidence that moringa leaf extract has anti-fertility effects in the male. Paul and Didia (2012) had earlier reported the deleterious effects of the root extract on the testis. Traditional and early experimental reports have implicated this plant product has having fertility influencing effects such as being abortifascient (Nath et al., 1992; Tarafder 1983), and as a birth control (Shukla et al.,1988a, b, c, 1989; Faizi et al., 1988). While moringa leaf extract would produce deleterious effects on the testis; it appears to ameliorate certain induced deleterious effects on the same organ by certain other agents such as diabetes induced testicular damage(Ebong, 2014); lead

induced testicular damage (Owolabi et al., 2012); mercury (Asomugha, 2014) and alcohol-induced testicular toxicities (Bassey et al., 2013).

The elemental analysis of the cement dust shows it contains 57 % calcium, 23 % silicon, 10.5 % aluminium, 8.5 % chromium and 8.0 % lead.

The phytochemicals found in the roselle extract include alkaloids, tannins, glycosides and reducing sugars, and moringa contains all the tested phytochemicals except flavonoids and phlobatanins. Ginger extract has glycosides, reducing sugars, saponins, and flavonoids, and 'ugwu' extract has all the phytochemicals except reducing sugars and phlobatanins. The mixture extract has all the tested bio-active compounds. The phytonutrients analysis of the extracts shows roselle contains calcium, iron, zinc, magnesium, vitamins A, and vitamin C, and ginger extract has zinc, magnesium, vitamin A and vitamin C. Moringa, 'ugwu' and mixture extracts have all the tested nutrients.

The results of the acute toxicity test showed the plant extracts were nontoxic to the rats even at a dose of 2000 mg kg-1. The general observations showed no mortality occurred 24 hours after administering the plant extracts. The rats that received roselle extract displayed a readiness to take more and were licking the cannula used to administer the extract. The rats that received ginger, moringa, 'ugwu', and mixture extracts did not show any signs of illness.

Table 1 shows both the control and test rats gained considerable weights during the period of exposure. However, a significant difference ($p< 0.05$) in weight was observed between the control and test rats. The control rats had a weight increase of 14.29 mg kg-1, whereas the test rats fed with roselle, moringa, ginger, 'ugwu', and mixture extracts had 25.24, 29.61, 20.56, 28.94 and 34.89 mg kg-1, respectively. Significant differences ($p< 0.05$) were also observed in the test rats. The mixture of the plant extracts had the highest body weight increase of 34.89 mg kg-1, followed by moringa, 29.61> 'ugwu', 28.84> roselle, 25.24 > ginger, 20.56.

In Fig. 1 the relative growth rates of the exposed rats per day, where a significant difference ($p<0.05$) was observed between the control and test rats. The control rats had the relative growth rate of 15.1 %. The rats that received extracts of roselle, moringa, ginger, 'ugwu', and the mixture had relative growth rates of 26.1, 30.9, 23.0, 30.7 and 36.5 %, respectively.

Table 1: Effect of plant extracts on the body weight (g) of the exposed albino rats

Day	0	30	60	90	120	150	180	Min. Wgt.	Max. Wgt.	RGR	Wgt. Increase
Extract											
Control	190.42^a ±11.2	193.67^a ±9.6	198.07^a ±10.3	202.30^a ±9.39	207.83^a ±8.88	212.35^a ±10.23	217.60^a ±10.61	176.9	228.9	15.10 ±3.4	27.2 ±7.8
Roselle	185.82^a ±9.75	190.77^a ±8.12	199.42^a ±6.26	209.17^b ±6.76	215.15^b ±6.47	222.82^a ±5.91	232.73^b ± 7.78	176.8	243.0	26.10 ±4.1	46.9 ±6.98
Moringa	187.93^a ± 9.57	196.60^b ± 7.83	206.88^a ±9.71	217.48^b ±6.11	226.48^a ± 8.21	234.00^a ±6.96	243.48^b ±7.78	175.9	249.8	30.90 ±4.6	55.6 ±6.90
Ginger	200.88^a ± 7.87	204.92^a ±5.82	213.55^b ±6.13	219.62^a ±6.31	227.35^b ±5.91	233.00^a ±7. 38	242.22^b ±7.98	192.3	249.9	23.00 ±3.2	41.3 ±5.87
'Ugwu'	191.08^a ±9.11	200.30^a ±8.75	207.42^a ±9.06	213.20^a ±10.5	222.98^a ±8.14	236.22^b ±7.56	246.35^a ±8.51	173.5	255.8	30.70 ±5.1	55.3 ±6.45
Mixture	188.60^a 11.91	199.20^b ±12.01	209.92^a ±13.18	221.13^b ±15.8	231.58^a ±12.9	242.02^b ±13.45	254.37^a ±12.01	167.2	267.3	36.50 ±5.07	65.8 ±6.34

Data are expressed as MEAN±SE
Mean values in the same row with different superscripts 'a' and 'b' were significantly different at $p<0.05$
RGR = Relative Growth Rate

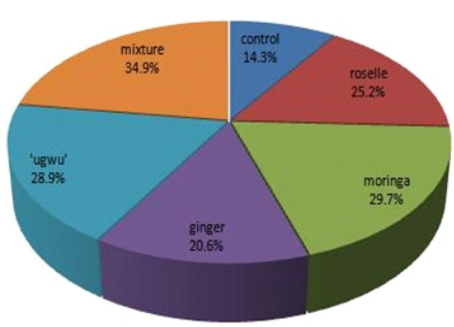

Figure 1: Relative growth rates of the exposed albino rats fed with different plant c extracts for 180 days.

Table 2 describes the effects of the plant extracts on some physical characteristics of the rats. The physical characteristics of the control and test rats showed a significant difference ($p<0.05$), which was also observed among the test rats. Roselle had the highest death of 33.3 %, and the mixture of the plant extracts had the lowest death of 16.7 %. Moreover, the mixture of the plant extracts produced the highest average number of newborn of 14.0/litter, and ginger had the lowest average number of newborn of 8.0/litter. The highest offspring survival of 76.4 % occurred in the mixture rats, and roselle fed rats had the lowest offspring survival of 50.0 %.

The rats that were administered with extracts of mixture, moringa, ginger and 'ugwu' were active, however, the rats that received mixture of plants extracts rats being the most active. The rats that were fed with roselle extract were not as active, and the control rats moved sluggishly.

Table 2: Efficacy of the different plant extracts on some physical characteristics of the exposed albino rats

Physical characteristics	Death (%)	Ave. Number of Newborn per birth	Offspring survival (%)	Physical condition
Extract				
Control	58.3	6.0^a ±1.00	50.0	Sluggish
Roselle	33.3	8.33^b ±0.58	52.0	Partially active
Moringa	25.0	12.00^b ±1.00	75.8	Active
Ginger	41.7	8.0^a ±1.00	58.3	Active
'Ugwu'	25.0	11.0^b ±1.00	73.3	Active
Mixture	16.7	14.00^b ±1.00	76.4	Very active

Mean values with different superscripts 'a' and 'b' from the control are significantly different at $P < 0.05$

DISCUSSION

Several studies including the present study have shown that cement dust may contain cytotoxic elements such as heavy metals, dioxins and particulate matters. However, some plants have been reported to prevent or ameliorate the cell and tissue damage processes of these elements. The weight increase noted in the test rats compared with control rats could be attributed to the antioxidant and cell rebuilding activities of some chemicals and nutrients in the plant extracts. Phytochemical analysis of the plant extracts showed the plants contained glycoside and saponin, both of which could have increased the body weight of the rats. George et al. (2002) reported that glycosides and saponins increased the feed intake and development of experimental animals. Okwu (2005) also reported that glycoside indirectly increases the levels of calcium in animals, which specializes in blood and bone formation leading to body weight increase. The food plants also contain essential nutrients such as calcium, magnesium, iron, sodium, potassium and zinc, all of which could have contributed to the body weight of the exposed rats. Adedapo et al. (2009) observed an increase in the weight of mice administered with the extracts of moringa. Moreover, increase in weights of rats and birds fed with 'ugwu' diets have been reported by Fasuyi and Nonyeren (2007), and Iweala and Obidoa (2009).

The low birth rates of the control rats may be due to the destruction of their reproductive systems by the toxic elements in the cement dust. Several studies on rats and other rodents indicated that blood lead concentrations above 30-40 mg dl-1 were associated with impairment of spermatogenesis and reduced concentrations of androgens (Apostoli, 1998). Studies have also shown that most of the infertility, birth defects and aborted pregnancies that happened in the United States in the 90s were due to exposure to heavy metal (ATSDR, 1999). The high fertility and birth rates of the test rats may be attributed to the scavenging activities of antioxidants in the plant extracts. The extracts contain flavonoids and tannins; all which could have prevented or mopped free-radicals in the test rats. It could also be attributed to the replenishing activities of the phlobatanins and alkaloids found in the plant extracts. Moringa has been reported by Adedapo et al. (2009) and Cajuday and Pocsido (2009) to improve sexual activity in rats by promoting testosterone production. Salman et al. (2008) also reported an improvement in sperm count and quality in rats following treatment with 'ugwu' extract. Alkaloids and phlobatanins detected in the plant extracts have been reported by George et al. (2002) and Okwu (2004) to increase sexual activity in rats. Alkaloids are aphrodisiac (Harisaranraj et al., 2009) and phlobatanins can synthesize sex hormones (Okwu, 2001; Edeoga et al., 2005).

The low death and high offspring survival rates of the rats fed with the plant extracts may be linked with the prophylactic action of the phytoconstituents. The phytochemicals in the plant extracts may have triggered the mopping of the free-radicals generated by the toxic elements in cement dust. This could have worked in the wellness of the test rats. Antioxidants protect cells, tissues and organs from oxidative damage. Therefore, the antioxidants in the test plants could have contributed to the observed decrease in death rate, and high offspring survival rate. Also, the essential nutrients in the plant extracts were actively involved in cell rebuilding processes, resulting in improved health condition of the exposed rats. Phytochemicals have been pointed in the reduced mortality rates observed in people consuming high levels of plant-based foods (Arts and Hollman, 2005). Alkaloids are strong antioxidants which can improve physiological activities of animals resulting in improved health status and low death rate (Harisaranraj et al., 2009).

CONCLUSION

The food plants used in this study have been shown to be bio-protective and can be used to ameliorate negative effects of pollutants. It is also suggested that since the plants have high nutritive values, they would be of immense benefit in healthcare delivery and animal husbandry. Moringa oleifera leaf extract did not produce histologically observable deleterious effects on the brain- cerebrum, cerebellum and hippocampus, kidney, liver, bone marrow elements; however, it produced extensive histological disruptions of the reproductive organs- testis and epididymis, thus acting as an anti-fertility agent. Certain tissues assumed improved Histomorphology due to the effects of moringa extract treatment; these include the kidney, spleen and liver. In conclusion, Moringa oleifera extract as used is not toxic; it is rather anti-fertility.

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