

Tiger nut and vocal folds healing education: Epithelization and histological investigations

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Abstract

This study investigated lead (Pb) metal intoxication and the ameliorating effects of tiger nuts and vitamin C (Vit C) on the vocal folds as a means of vocal education for theater performers. Lead is a component of paints, batteries, water pipelines, and lubricants for agricultural equipment and automobiles. It enters the body through plant-based edibles, lead-contaminated air, and water. Its versatility and ability to withstand corrosion, its residential and industrial applications, ignite public health concerns to humans and animals as it bioaccumulates. The antioxidant and radical-scavenging properties of Tiger nuts informed this study. Thirty-six (36) male Wistar rats, 150-200 g body weight, were categorized into six groups (n=6) for the study. Control (Distilled water only), Lead (40 mg/kg only), Low Tiger Nut + Lead (TN 5 mL/kg) + Pb (40 mg/kg), Medium Tiger Nut + Lead (TN10 mL/kg) + Pb (40 mg/kg), High Tiger Nut + Lead (TN 20 mL/kg) + Pb (40 mg/kg), vit C (200 mg/kg) + Pb (40 mg/kg). All administrations were done through oral gavage for 14 days. Thereafter, the rats were weighed, sacrificed, and vocal folds dissected out for histological analysis. In comparison to the control and other treatment groups, the Lead group (Pb) had squamous epithelial metaplasia, hyperplasia, ulceration, and mucosal inflammation of the vocal fold. Epithelization in the Tiger Nut-treated group increased with dose, while the vitamin C group (vit C) had considerable amelioration. In conclusion, tiger nut and vitamin C have beneficial effects on vocal fold healing following lead intoxication.

Keywords: Epithelization, Healing, Tiger nut, Vocal folds

1. Introduction

Theatre performers are generally educated on vocal hygiene as a means to meet the vocal demands expected of them. Actors are confronted with meeting optimal vocal production with less regard to components around them that are hazardous to the vocal apparatus. The actor works vigorously to increase volume, and pitch variations and extend the frequency range beyond typical conversation (Pinczower & Oates, 2005). The demand is through their career (Walzak et al., 2008). One important factor in the vocal fold's creation process

is voice. The breath from the lungs causes the vocal folds of the larynx to vibrate (Zen & Senior, 2014). The wedge-shaped vocal fold divides the larynx into three parts: superior to the fold is the laryngeal vestibule (supraglottic cavity), and inferior to it is the infraglottic cavity and a small middle chamber in between. Apart from its phonation ability, which should be a concern for theatre performers (Goulart & Vilanova 2011), vocal folds are also inspiratory sphincters. In regular breathing, the vocal folds and rima glottides, a small aperture between two vocal folds, allow air to pass through the laryngeal cavity into the lower respiratory tract during its opening and relaxation.

However, lead intoxication is the most prevalent metal poisoning that affects people and other animals because of their susceptibility and indiscriminate feeding practices and habits (Makokha et al., 2008; Majiya et al., 2015). Lead is an integral component of paints, batteries, lubricants, and oils for agricultural equipment, cars, and other automobiles. Artisans also use and come in contact with lead in their mining processes (Majiya et al., 2015). Its numerous residential and industrial applications are a reflection of its special chemical characteristics and its health dangers to both humans and animals.

2. Literature review

Aside from paint and exposure to petroleum products, which have been extensively documented in the bulk of the literature, the most usual ways that individuals are exposed to lead more frequently and daily are through edibles, agro-based contacts, and the soil and also through air (Gyasi et al., 2021), with children being more particularly at risk (Rees & Richard, 2020). The individual performers must be conscious of their food consumption and how it affects their mental health (Robb, 2018). For both young and old, one of the main ways that lead exposure occurs is through food, by consuming game meat or lead-based ammunition (Sullivan & Green, 2020), consuming plants grown on soil with lead traces or high densities, especially people close to lead mining sites and other associated businesses, or consuming synthetic fertilizers and vehicle emissions as occupational risks which as well endangers family members (Rinsky et al., 2018). These factors can raise the blood lead levels of victims. People who breathe in coal dust may experience long-term health issues and vocal fold damage (Steffan et al., 2018; Jin et al., 2020). This can develop into dangerous compounds that can bioaccumulate in tissues with low excretion. If this heavy metal buildup is not controlled, it can lead to vocal fold dysfunction, which manifests as a variety of symptoms, including fatigue, throat clearing, globus sensation, tightness or soreness in the chest, dysphagia, sporadic aphonia, and air hunger. All these symptoms have the potential to cause anxiety, panic, and fear, all of which can exacerbate respiratory symptoms (Hoyte, 2013). Many studies have described patients with Vocal fold Dysfunction with concomitant cough (Andrianopoulos et al., 2000; Lerner et al., 2013; D'haeseleer et al., 2017). Lead toxicity, however, makes all bodily systems vulnerable, such as the liver, urogenital, embryologic, and neurologic organs (Firat et al., 2009). Lead toxicity works by causing oxidative stress, a damaging tissue reaction that impacts metabolism and cells through the generation of reactive Oxygen Species (Bamishaiye and Bamishaiye, 2011).

Accordingly, it has been demonstrated that the small tuberous rhizome of the tiger nut, which contains significant amounts of water-soluble flavonoids, alkaloids, glycosides, polyphenols, natural acids, glycerol, vitamins, and other active substances, serves as a source of good nutrition as much as it is for medication (Nina et al., 2019; Vega-Morales et al., 2019). Scientifically called *Cyperus esculentus*, tiger nut is highly prized by theatre performers after fatigue on the vocal folds (Leyns et al., 2022) for their health benefits and nutritional value (Oguwike et al., 2017), black, yellow, and brown colored species. Tiger nut extract is contained in a number of culinary and industrial products, such as beverages, milk, flour, beer, liqueur, chocolate, candies, and non-edibles like soaps (Asante et al., 2014). According to Oladele and Aina (2007), *Cyperus esculentus* can be consumed freshly or dried with or without any form of processing or fortification,

or be made into a cool, refreshing beverage called "Kunu." Tiger nuts are also an excellent source of edible non-saturated fatty acids and oils with nutritional value similar to olive oil (Rosello-Soto et al., 2018).

Although it has a relatively reduced protein composition, it has been shown to aid persons with digestive issues and diabetics after consumption, as well as ameliorate cardiac-related diseases (Ogunlade et al., 2015). The tiger nut oil's antioxidant activity makes it more resistant to oxidation than other plant-based oils, among other things, rich in vitamins, lipids, zinc, alkaloids, and flavonol (Nina et al., 2020). According to certain research, daily administration of tiger nut powder to rats prevented abnormality in the functioning of the rats' testicles by arresting inflammation and apoptosis (Adelakun et al., 2020), improved male arousal (Allouh et al., 2015), reduced diarrheal symptoms in Wistar rats (Shorinwa & Dambani, 2020), and reduced inflammation with atherosclerosis and oxidative stress in the liver (Achoribo & Ong, 2017). It also inhibits α -amylase, α -glucosidase, and lipid peroxidation activities, and it eliminates the activities of free radicals (Ademosun & Oboh, 2015), which is why it was chosen as an option for lead toxicity in the vocal fold in this study.

3. Research method

3.1. Chemicals

Twenty grams (20 g) of Lead acetate ($\text{Pb}(\text{CH}_3\text{COO})_2$) was obtained from the Laboratory of the Department of Pharmacology and Therapeutics, University of Ibadan, Ibadan, Nigeria.

3.2. Plant authentication and extract preparation

Fresh *Cyperus esculentus* nuts were purchased from Bodija market, Ibadan, and authenticated at the Department of Botany, University of Ibadan. The nuts were rinsed in clean water and blended using an electric blender. The mixture was separated, and the filtrate was stored in a container, labelled appropriately, and kept in a refrigerator until it was needed for use (Nina et al., 2019).

3.3. Animals

Thirty-six (36) male Wistar rats 150-200 g body weight, were selected for the investigation. The rats were maintained in adequately ventilated rat cages in the Central animal house of the College of Medicine, University of Ibadan, Nigeria in an environment (22 ± 2 °C on a 12 hour light and 12 hour darkness) with unlimited access to food and water. They were divided up into six groups of six rats each after being acclimatized for seven days. Every animal was treated humanely.

3.4. Experimental Design

Group 1: (control) water only

Group 2: Pb (40 mg/kg) only

Group 3: Low TN (5 mL/kg) + Pb (40 mg/kg)

Group 4: Med. TN (10 mL/kg) + Pb (40 mg/kg)

Group 5: High TN (20 mL/kg) + Pb (40 mg/kg)

Group 6: Vit. C + Pb (40 mg/kg)

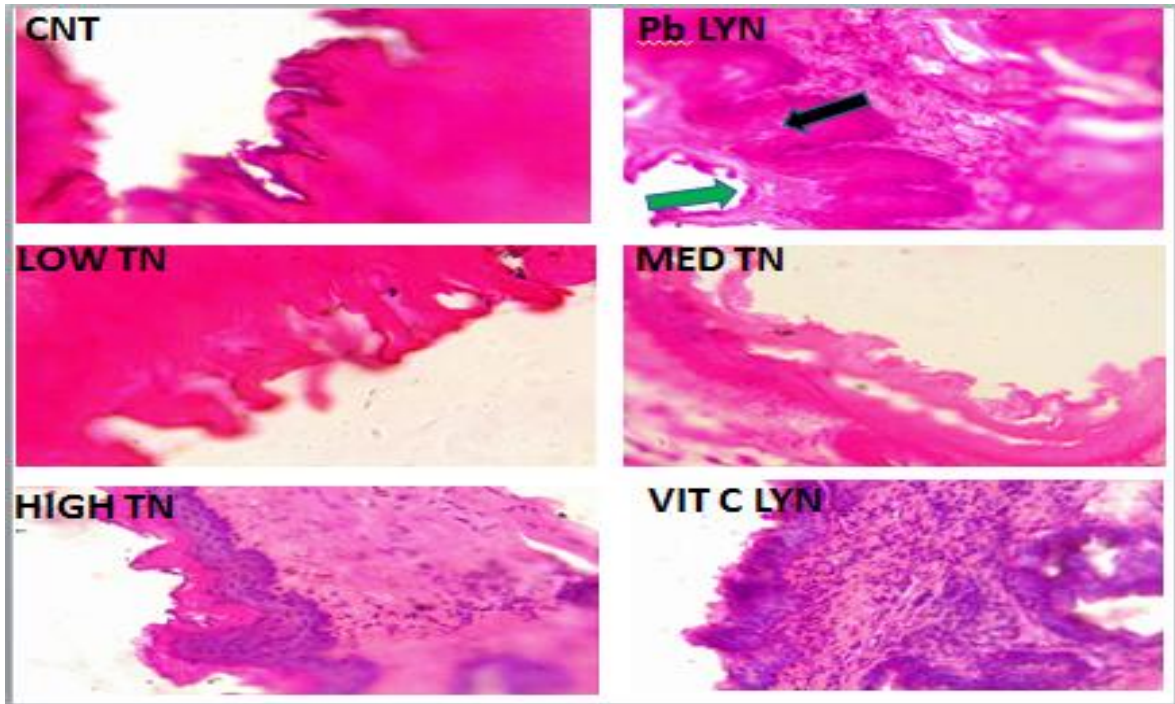
All administrations were done through oral gavage for 14 days. Thereafter, the rats were weighed and sacrificed. The vocal folds were dissected out for histological analysis.

4. Data analysis

4.1. General observation

There was a general body weakness of the rats in the lead-treated group compared to other groups. This was shown by the drastic reduction in the exploratory ability of the lead group. There was also a serious weight loss in the lead group, which could have been a result of reduced appetite seen from their poor feeding habit following lead intoxication.

Figure 1: A photomicrograph of the vocal fold of control and experimental adult wistar rats H&E x100



In comparison to the control and other treated groups, the Lead group in the larynx (**Pb Lny**) exhibited notable squamous metaplasia, epithelial hyperplasia (black arrow), ulceration (green arrow), and mucosal inflammation in the vocal fold's epithelial lining. The group that received tiger nut treatment showed a dose-dependent increase in epithelization, with high tiger nut (**HIGH TN**) exhibiting the highest amount. Vitamin C group in the larynx (**Vit C Lny**) likewise demonstrates considerable improvement when compared with the Lead group.

5. Findings and discussion

The larynx's vocal folds are extremely sensitive and specific structures. The vocal folds resonate due to complementary rhythmic air pulses in the glottis caused by the adjacent vocal folds' synergy and similar microstructure. This brings about oscillation for the creation of voice for communication and singing, which are its major functions. The tone of the sound generated is determined by the vocal folds' length and tension.

While the ventricular folds and trachea next to the vocal folds are made up of a ciliated columnar epithelium, the vocal fold epithelium is made up of a stratified, non-keratinized squamous epithelium (Stiblar-Martincic, 1997). The vocal fold's mucosa is subject to nearly continuous harm from a variety of causes, including systemic or environmental irritants like pollution and acidic reflux, as well as physical harm brought on by phonation (Levendoski et al., 2014).

From this study, lead was seen to elicit negative histological alterations in the mucosal lining of the vocal folds, with significant squamous metaplasia and epithelial hyperplasia, ulceration, and mucosal inflammation. Thus, it is believed that this mucosal lining serves as a shield to the underlying connective tissues from a

variety of external and kinetic dangers. Hence, this demonstrates the connection between diseased states of the vocal fold and the structural disturbance and deficit of the mucosa (Levendoski et al., 2014).

It is generally accepted that the mouse larynx's squamous metaplasia is an adaptive reaction to lead exposure. According to this study, lead poisoning caused reversible mucosal alterations and suppressed mucosal immunity. But in each instance, giving Tiger nut plus vitamin C sped up the epithelization of the lead toxicity-induced damage of the rat vocal folds.

Ximenes et al. (2003) showed that as people age, their mucosal cell density significantly decreases. They argued and maintained that glandular mucus production is essential to the vocal fold's ability to operate maximally. In addition to providing immune-specific protection, these secretions maintain the structural integrity of the epithelium and shield the underlying tissues from air pressure contacts and other shear and wearing forces (Masanobu, 2017). Toxicants, including lead, have the potential to decrease the quantity and integrity of mucous secretions.

In individuals over 70, shrinking-related alterations in the ventricular folds' mucous glands have been reported. Age-related shrinkage of the vestibular glands' secretory units occurs. And the loss of parenchymal tissue and the buildup of fatty tissues both inside and outside the glands are characteristics of these alterations. Following the glandular deterioration, a reduction in the amount and the quality of secretions may alter the vocal fold's viscoelasticity and lessen defense against external and kinetic threats. Two notable effects of this include squamous metaplasia and epithelial hyperplasia (Masanobu, 2017).

Scar formation was also linked to the disruption of these delicate multilayered structures and resulted in notable alterations in tissue biomechanics, which in turn led to a voice issue. There are numerous reasons for vocal fold scarring, such as iatrogenic damage, inflammation, or irritation from toxins. Scar development is the principal cause of dysphonia, defined by difficulty in making sounds, typically resulting in changes in pitch, volume, or quality of the voice. The basement membrane of the Reinke's space of the vocal fold is disturbed by scarring.

Changes to the normal basement membrane in the scarred condition include reduced elastin, hyaluronic acid, and the entire fiber aggregation, as well as increased precursor protein, mature protein, and major protein component of blood plasma. Treatments must target both the cellular and basement membrane components in order to restore vocal fold histology.

Using plant-based interventions has become possible as regenerative medicine has thrived and flourished in recent years. For instance, Johnson et al. (2010) used a rat model to inject bone marrow-derived multipotent stromal cells and synthetic basement membrane into surgically injured vocal folds and found that the vocal fold had higher levels of precursor protein, fibronectin, and transforming growth factor- β . Kim et al. (2014) used a rabbit model and adipose-derived multipotent stromal cells on surgically inflicted vocal fold scars and found improvements in the viscoelastic properties of the vocal fold along with increased synthesis of collagen and growth factors.

6. Implications of the study

Heavy metal toxicity, like lead, has been proven to be a source of diverse health risks in excess amounts as it accumulates in the body and food chain with long-term effects. Almost all human body parts are affected by lead toxicity (Aliyu & Musa, 2021). Lead is highly persistent in the environment and because of its continuous use, its levels rise in both developed and developing countries and particularly Nigeria where it occupies unique physical and chemical properties that make it suitable for a large number of applications occurring at various levels, following environmental factors and occupational exposures (Sridevi & Umamaheswari, 2020). About 193,000 deaths occur each year worldwide as a result of poisoning by chemical

agents (WHO, 2016). Exposure to lead, like other chemical substances, causes hoarseness, loss of voice, sore throat and inflammation, secretion, and burning in the throat, dry throat and mouth, lump in the throat associated with difficulty in swallowing and articulating words in verbal expression (Carina & Márcia, 2018). These could either be due to a possible irritation caused by chemical agents or due to diseases developed during or after the exposure (Carina & Márcia, 2018). However, most of the above-stated voice defects may be experienced by theatre performers due to the vocal demands of their profession. So, apart from the natural course of intoxication of lead via various routes with health implications having impacts on the vocal folds, occupational engagements of theatre performers may exacerbate the symptoms and may even raise complications. It becomes pertinent that natural interventions be applied to ameliorate the impacts. Tiger nut has good antioxidant properties that can neutralize the oxidative stress exposed to the vocal folds by these complications. It has abundant phytochemical components such as flavonoids, quercetin, myricetin, minerals, vitamins (Vega-Morales et al., 2019), and many more. And these informed the choice of this study.

7. Recommendation

Further studies should be carried out to fractionate the Tiger nut to get the active component most responsible for the intervention in this study.

8. Conclusion

Theatre performers, as singing and speaking voice users, need to consider the benefits of tiger nut as a beverage to improve their vocal folds from hazards. Tiger nut and vitamin C have positive effects on the repair of vocal folds after lead intoxication, since they hasten the epithelization of the damaged rat vocal folds. Tiger nut and vitamin C are inexpensive, have a low profile of adverse effects, and may help avoid scarring, according to the study.

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