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HISTOPATHOLOGY ASSESSMENT OF THE EFFECTS OF *LEDEBOURIA OVATIFOLIA* ON EXPERIMENTALLY INDUCED REFLUX ESOPHAGITIS

Ndebia EJ*, Zweni L, Seipone ID, Mathulo S, Gondwe M.

Department of Human Biology, Faculty of Health Sciences, Walter Sisulu University, 27 Sisson St, Fort Gale, Mthatha, 5100 South Africa

***Author for Correspondence:** endebia@wsu.ac.za

ABSTRACT

Ledebouria ovatifolia is a plant known in *isiXhosa* as *"Icubudwana"* and is generally used by the Xhosa tribe in South Africa for medicinal purposes, including stomach-ache, diarrhoea, influenza, gargle and skin irritation. This study aimed at evaluating the healing effect of *L. ovatifolia* on experimentally induced reflux esophagitis. Reflux esophagitis was induced on rats by ligating the pylorus and the fore stomach. Animals were then autopsied 4 hours after the double ligation to examine the protective and deleterious effect of drugs. Prior to induction of esophagitis, different groups of rats were pre-treated orally with 100mg/kg *L. ovatifolia,* 200 mg/kg *L. ovatifolia*, 20mg/kg omeprazole and distilled water**.** Induction of reflux esophagitis caused marked increase of gross esophageal lesions, which corresponded with histopathological changes. Microscopic evaluation of ulcerated esophagus of *L. ovatifolia* pre-treated groups showed a reduced disruption of the surface epithelium at the lower dose. Higher dose of the plant extract showed an eroded esophageal mucosa with no recovery. The results suggest that *L. ovatifolia* could reduce the severity of reflux esophagitis and prevent the esophageal mucosal damage. This may confirm its therapeutic use in esophageal reflux disease.

Keywords: Ledebouria ovatifolia, esophagitis, Reflux

INTRODUCTION

Reflux esophagitis is a common chronic upper gastrointestinal disease associated with damage to the lining of the upper digestive tract and is increasingly recognized as significant health problem (Kwon et al. 2015). It is the inflammation or burning of the oesophagus that occurs during gastroesophageal reflux disease (GERD) as a result of repetitive exposure to vomited acidic stomach contents over prolonged periods of time (Takeuchi and Nagahama, 2014; Mahattanadul et al. 2011). The existing therapeutic strategy for GERD is primarily acid suppression such as using antacids, H_2 receptor antagonists, and proton pump inhibitors (Katz et al. 2013). Despite their well-known effectiveness, several patients have experienced relapse, incomplete mucosal healing, and the development of severe complications like Barrett's oesophagus (Kwon et al. 2015). There are medicines that irritate the oesophagus and worsen GERD on patients. These medicines include non-steroidal anti-inflammatory drugs, such as aspirin, ibuprofen, or naproxen (Ruszniesski et al. 2008 and Cryer et al. 2000). Age also has an effect as older people take medications known to decrease the sphincter tone more regularly, which may promote reflux (Kwon et al. 2015).

Numerous medicinal plants have been shown to be effective and safe with better tolerance by patients. They provide traditional therapies that are considered safe and effective compared to the synthetic chemicals (Dubey et

al. 2004). The use of medicinal plants for the treatment of many diseases is associated with folk medicinal use from different parts of the world for thousands of years, and is the main source of structurally important chemical substances that lead to the development of innovative drugs (Dubey et al. 2004). *Ledebouria* is genetically an African bulbous perennial herb in the *Asparagus* family, *Asparagaceae*, subfamily S*cilloideae*. Most members were previously part of the genus *Scilla* (Arnold and De Wet, 1993). The majority of species are grown by cacti and succulent enthusiasts for their patterned leaves. Most of the species are inhabitant to Africa or Madagascar, but a few are from India, Sri Lanka, or the Arabian Peninsula. The genus is reputed to be poisonous in Africa, although it is reported that Bushmen eat the bulbs of *L. apertiflora* and *L. Revolute*. *L. ovatifolia* is generally used in the Eastern Cape for medicinal purposes, such as the relief of back and stomach aches, dysentery or diarrhoea, influenza, skin rashes, wounds, as well as relief of sore throat with gargle (Germishuizen and Meyer, 2003)**.** This study aimed to investigate the potential preventive and therapeutic ability of *L. ovatifolia* against experimentally induced reflux esophagitis.

MATERIALS AND METHODS

Plant Material

L. ovatifolia was collected in the Eastern Cape, South Africa on its natural form, and processed as previously described and detailed in our prior published work with the same specimen (Ndebia et al. 2018). Briefly, after taxonomically identifying the collected plant material (voucher herbarium specimen, Ref: NDE003), 100g of the bulbs were pulverized to powder, which was then concentrated to dryness. The 9g brownish end-product obtained after processing was utilized for experiments within fourteen days of its production.

Animals

Twenty-five wistar rats weighing 200 - 250 g each were used. They were procured by the South African Vaccine Producers PTY (Ltd) in Johannesburg, South Africa, and were transported to Mthatha by air and kept in the animal holding facility at Walter Sisulu University, Department of Human Biology. Rats were kept in grouped cages and subjected to a 12 h light/dark cycle at room temperature. Animals were maintained on standard rodent chow with *ad libitum* access to food and clean tap water. They were allowed 2 weeks acclimatization in the new environment before the start of the experiment. All animals received humane care and experiments were performed according to the Society for the Prevention of Cruelty to Animals (SPCA) and Animal Welfare Organisation principles and recommendations. The study was approved by the ethical review committee of Walter Sisulu University under the reference 063/2016.

Treatment

Eighteen hours before the experiments the rats were deprived food but were allowed *ad libitum* access to tap water. These rats were divided into five groups with five animals per group. Group 1: Reflux esophagitis not induced. Group 2: Control (treated with distilled water), reflux esophagitis induced. Group 3: Treated with *L. ovatifolia (100 mg/kg),* reflux esophagitis induced. Group 4: Treated with *L. ovatifolia (200 mg/kg)*, reflux esophagitis induced. Group 5: Treated with omeprazole (20 mg/kg), reflux esophagitis induced. All treatments were given orally prior to induction of esophagitis.

Reflux esophagitis induction

Induction of reflux esophagitis was carried out using the rat model of acid-reflux esophagitis described by Takeuchi et al. 2014. In summary, the rats were put under deep sleep using anesthesia. Their abdomen was incised along the middle, and then both the pylorus and junction between the forestomach and corpus were ligated. Following ligation of the pylorus and forestomach, severe hemorrhagic damage developed in the proximal 3cm of the esophagus in a time - dependent manner due to the flow back of acid in the esophagus. Animals were autopsied 4 hours after the double ligation to examine the protective and deleterious effect of drugs (Takeuchi et al., 2014).

Histomorphology studies of ulcerated oesophagus

Oesophagus obtained from each rat were

fixed in 10% buffered formalin and were routinely processed for histology using a TP102 automatic processor. The oesophagus glandular portions were then embedded in paraffin wax after trimming. Five micrometre thick tissue sections were cut from the same area on the different esophagi from each group of animals and stained with Haematoxylin and Eosin (Sigma-Aldrich, St Louis Missouri, USA) for evaluation. The sections were analysed using a DMD 108 Imaginer microscope at x40 magnification (Comanescus et al. 2012).

RESULTS

The histology study of group 1 comprised of rats that were not induced showed normal oesophagus epithelium while the ulcerated untreated oesophagus of the rats in group 2 showed a disrupted mucous membrane in the surface epithelium and detachment of the mucous barrier (Figure 1A). The oesophagus of rats in group 3 pre-treated with a dose of 100 mg/kg of *L. ovatifolia* extract showed less disruption and a level of recovery of the mucosa with a developing mucous membrane (Figure 1B) while group 4 rats treated with 200 mg/kg of the plant extract showed no recovery with a tilted mucus barrier (Figure 1C). Oesophagus of rats pre-treated with omeprazole (group 5) showed a level of recovery with a well-organized epithelium and well-developed mucus barrier (Figure 1D).

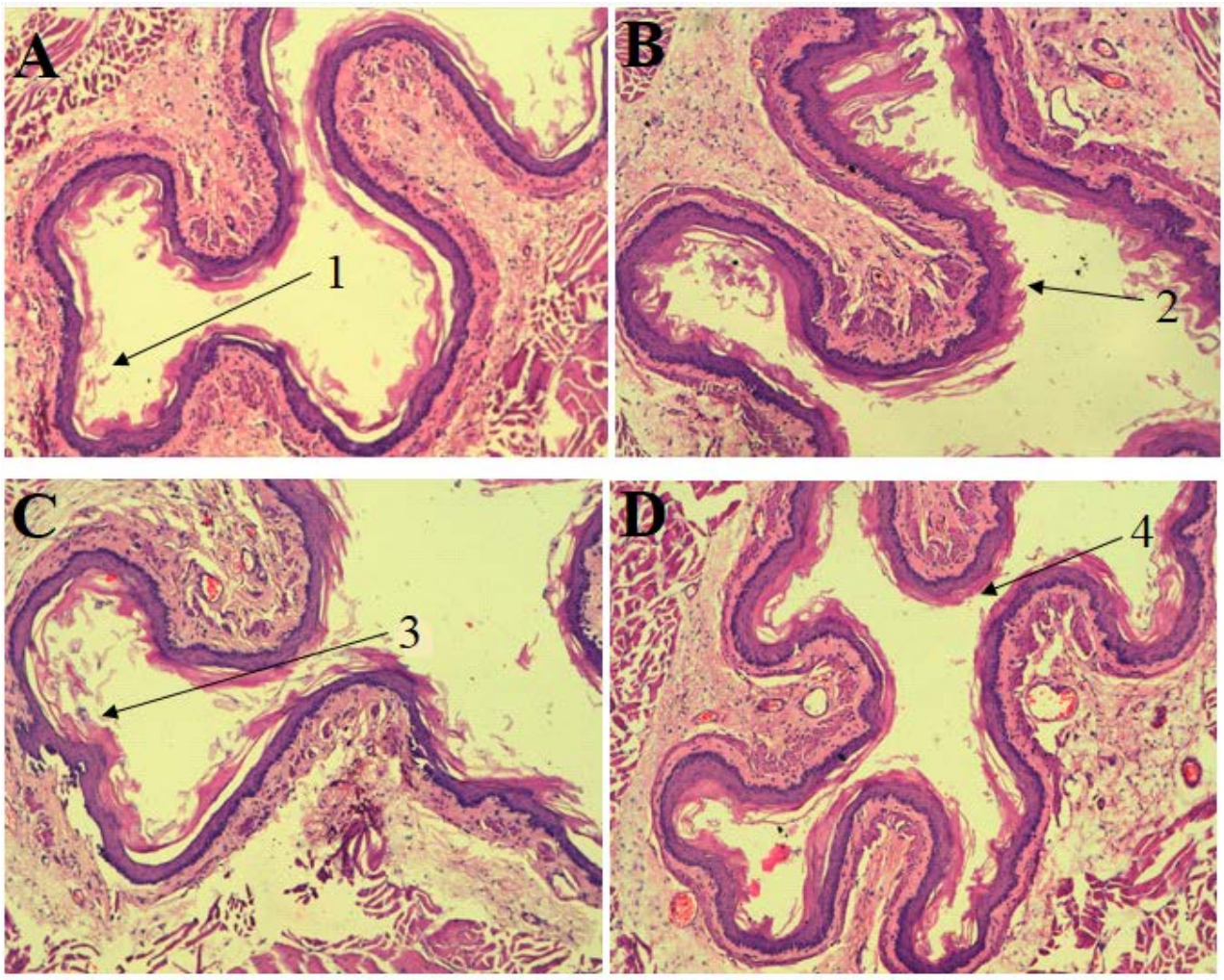

Figure 1. *Photomicrographs of esophagus mucosae in experimentally induced reflux esophagitis.* A: The untreated group (Group 2) showing eroded mucus membrane as indicated with 1A, B: 100mg/kg of *Ledebouria ovatifolia* (Group 3) showing recovering of the surface epithelium marked 2B, C: 200mg/kg of *Ledebouria ovatifolia* (Group 4) show destructed mucus barrier as indicated in 3C, D: Omeprazole (Group 5) show recovering surface epithelium with mucus barrier marked 4D. Magnification 40X.

DISCUSSION

Ulceration of the esophagus due to gastric acid exposure could be managed by diminishing the aggressive factors and by a natural substance enhancing the protective factors of the upper gastrointestinal tract (Ndebia et al. 2013). *L. ovatifolia* is the plant generally used in the Eastern Cape for medicinal purposes, including pregnancy, diarrhoea, stomachache, influenza, backache, skin irritations, wounds and relief of sore throat with gargle (Germishuizen and Meyer, 2003). This is the first study evaluating the effect of this plant on reflux oesophagitis. Acid secreted from gastric parietal cells is a potentially harmful factor in the esophageal lumen, it increases the oxidative stress which plays a significant role in the depletion of the adherent gastric mucus layer and damages the esophageal mucosa (Orlando, 2010). Reflux of caustic gastric contents, reactive oxygen species such as superoxide radical and hydroxyl radical, and release of lysosomal enzymes, is known to directly or indirectly cause symptoms such as heartburn and nausea (Ku et al. 2009). It is well known that the prolonged contact of the esophageal mucosal membrane with acid and pepsin due to the impaired anti-reflux barrier can lead to detrimental morphological changes as observed in our study (Figure 1A).

Our study showed that the group pre-treated with *L. ovatifolia at 100 mg/kg* (Figure 1B), has a level of recovery of the esophageal mucosa with a well-developed recovered surface epithelium as indicated with 2 (Figure 1B), meaning that the acid may be inhibited by the plant extract because of its well-known anti-inflammatory activity (Sparg et al. 2002). This is also supported by a previous study showing that the plant has a therapeutical effect against ulcer induced by anti-inflammatory drugs, cold, stress and alcohol (Ndebia et al. 2018). The therapeutic effect on reflux esophagitis may be due to the presence of flavonoid which is the major phytochemical component of the *L. Ovatifolia* (Waller et al. 2013). Literature has shown that flavonoid compounds exhibit anti-inflammatory properties by augmenting the function of cyclooxygenase (COX) and Prostaglandin E_2 (PGE2) (Waller et al. 2013) *L. ovatifolia* at the 200 mg/kg dose was not effective in healing reflux esophagitis as indicated with label 3 in both the oesophagus (Figure 1C). This might mean that the plant may have no therapeutic effect at a higher dose, and this may be explained by the fact that the plant may have reach its therapeutic saturation at dosage higher than 100 mg/kg.

However, the group pre-treated with omeprazole showed a good level of recovery of the mucus barrier and well developed esophageal epithelium (Figure 1D). Omeprazole is the standard drug used to manage GERD; it acts as a proton pump inhibitor. Proton pump inhibitors are the most effective class of agents used in the treatment of GERD. It acts by covalently binding to and inactivate the H^+/K^+- adenosine triphosphate which is the enzyme located on the apical membrane of the gastric parietal cell, there by blocking the final common pathway for gastric acid secretion (Shin and Kim, 2013). As a result, there would be less acid secretion, therefore no acid backflow (Lowe, 2006).

CONCLUSION

From this study it can be concluded that *L. ovatifolia* at the dose of 100mg/kg might be effective in preventing reflux esophagitis induced by pylorus and fore stomach ligation through its anti-inflammatory activity. This accounts for its use in the traditional settings for ailment such as sore throat. Further studies are however needed to elucidate which mechanism is responsible for the anti-reflux activity.

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