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RESEARCH ARTICLE

IMPACT OF *KALANCHOE PINNATA* LEAF EXTRACT ON ALCOHOL-INDUCED GASTRIC INJURY IN ADULT WISTAR RATSDeborah Calmday-Ombo^{ab*}, Endurance O. Imafidon^a, Seun Bidemi Olukayode^{ac}^a Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin City, Nigeria,^b Department of Anatomy, Faculty of Basic Medical Sciences, Federal University Otuoke, Nigeria,^c Department of Pathology, Federal Medical Centre, PMB 1033, Asaba, Delta State*Corresponding Author Email: calmday-ombodd@fuotuoke.edu.ng

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ABSTRACT

Kalanchoe pinnata, also known as *Bryophyllum pinnatum*, is a succulent plant native to Madagascar that has been extensively utilized in traditional medicine for its curative effects. This study looks at the gastroprotective properties of an aqueous extract of *Kalanchoe pinnata* (AKP) against ethanol-induced stomach ulcers in Wistar rats. The plant's phytochemicals, including flavonoids, saponins, tannins, and alkaloids, are known for their antibacterial, anti-inflammatory, and wound-healing properties. Thirty Wistar rats were separated into six groups and given AKP, omeprazole, or no therapy after ethanol-induced stomach injury. Histological investigations revealed that the control group had normal gastric architecture, but the ethanol-only group suffered severe stomach injuries. Treatment with 0.5 ml AKP resulted in limited protection, while 1 ml AKP had strong gastroprotective effects comparable to omeprazole. The untreated group showed persistent severe damage. These findings support the historic usage of *Kalanchoe pinnata* to as treatment agent in traditional medicine while also highlighting its potential for modern therapeutic applications. The study recommends more investigation into the biochemical principles and clinical efficacy of *Kalanchoe pinnata* to fully grasp its therapeutic potential.

KEYWORDS

Kalanchoe pinnata, Ethanol-induced gastric damage, Aqueous extract, Malondehyde, Traditional medicine, Gastroprotective effects.

1. INTRODUCTION

Kalanchoe pinnata, also known as *Bryophyllum*, the air plant, cathedral bells, life plant, miracle leaf, and Goethe plant, is a succulent native to Madagascar (Wagner and Lorence, 2020). It is commonly naturalized in tropical and subtropical regions and is notable for the miniature plantlets forming on its leaf margins (Wagner and Lorence, 2020). Widely used in folkloric medicine across tropical Africa, America, India, China, and Australia, *Kalanchoe pinnata* is renowned for its medicinal properties, particularly in wound healing (Wagner and Lorence, 2020). The plant is rich in phytochemicals, including flavonoids, saponins, tannins, and alkaloids, as well as vitamins such as ascorbic acid, riboflavin, thiamine, niacin, and minerals like calcium, magnesium, potassium, phosphorus, sodium, Micro elements: iron, zinc, vitamins, ascorbic acid (Kendeson et al., 2021; Fernandes et al., 2019; Nagpal and Sharma, 2020).

The leaves of *Kalanchoe pinnata* exhibit numerous medicinal properties, including antimicrobial, antifungal, antihypertensive, antidiabetic, analgesic, anti-inflammatory and wound healing effects (Wagner and Lorence, 2020; Tajudin and Ismail, 2022; Tom et al., 2021; Agüero-Hernández et al., 2020; Matthew et al., 2013; Anandan and Shanmugam, 2024). It is also reported to have anti-cancer, sedative, and muscle relaxant properties (Stefanowicz-Hajduk et al., 2022; Mora-Pérez and Hernández-Medel, 2016). Additionally, the plant may be effective in treating leishmaniasis (Oliveira et al., 2018). In Nigeria and other parts of Africa, herbal practitioners use the aqueous extract of the leaves to treat coughs and as a prophylactic for asthma and lung injury treatment (Singh et al., 2021; Ehi-Omosun and Etunim, 2023). Research indicate that *Kalanchoe*

pinnata can hasten wound healing processes, suggesting potential anti-ulcer activity (Nayak et al., 2010). The widespread use of *Kalanchoe pinnata* in traditional medicine, combined with its diverse phytochemical composition, emphasize its potential for further research and application in contemporary medicinal practices.

Alcohol, particularly ethanol, is as known central nervous system depressant (Pervin and Stephen, 2021). Alcohol, according to World Health Organization (2022), and National Agency for Food and Drug Administration and Control (NAFDAC) (2019) is a regulated substance with known health risks that necessitates responsible consumption and adherence to legal guidelines. Initially, it causes relaxation and euphoria, but as use grows, it impairs judgment, coordination, and motor skills (Pervin and Stephen, 2021). It has been proven from research studies to be hepatotoxic, cardiotoxic and nephrotoxic (Subramaniyan et al., 2021; Fernández-Solà, 2020; Eteng et al., 2020). Chronic alcohol usage can cause a variety of health problems, addiction, and an increased chance of developing some forms of cancer (National Institute on Alcohol Abuse and Alcoholism, 2021; National Cancer Institute, 2021). Furthermore, excessive alcohol intake promotes weight gain and can lead to illnesses such as alcoholic liver disease, which is caused by the liver's metabolic processing of ethanol (Schwartz, 2022; Subramaniyan et al., 2021). Despite its widespread recreational use, alcohol's long-term health consequences highlight the significance of careful usage and compliance with the restrictions governing its production, sale, and consumption.

The stomach, located in the upper abdomen, has a J-shaped structure that includes the cardiac, fundus, body, and pyloric regions (Chaudhry et al.,

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2022). It produces gastric juice containing hydrochloric acid and enzymes such as pepsin, which aids digestion by breaking down proteins into smaller molecules (Hsu and Forshing Lui, 2018). The wall layers are mucosa, submucosa, muscularis externa, and serosa (Chaudhry et al., 2022). It is supplied by the celiac artery and innervated by the vagus and sympathetic nerves, which are necessary for digestion and nutrient absorption (Chaudhry et al., 2022). Embryologically, it originates from the foregut, developing stratified squamous and simple columnar epithelia (Chaudhry et al., 2022). Despite its known therapeutic properties, *K. pinnata*'s aqueous extract ability to ameliorate or reduce gastric ulcers caused by excessive alcohol consumption has not been previously investigated hence this study.

2. MATERIALS AND METHODS

2.1 Plant procurement and extraction of Aqueous extract of *Kalanchoe pinnata* (AKP)

Fresh leaves of *Kalanchoe pinnata* were obtained from Uselu Market in Egor Local Government area, Edo State, Nigeria, and identified in the herbarium of the Department of Plant Biology and Biotechnology, University of Benin. Aqueous extraction of the leaves of the plant was done by cold water Maceration. *K. pinnata* leaves were washed with running tap water, air dried and grinded to powder. The powder obtained was weighed to be 180g, and soaked in distilled water for a period of 24 hours in a separating funnel with occasional shaking. The solution was filtered. The filtrate was allowed to settle and then decanted. The decant was placed in an evaporating dish and then placed in a water bath at 60°C for excess water to evaporate gently after which the dried residue was scraped out, yielding 45.6g of extract (26.5% yield). The extract was stored in a refrigerator at the Department of Anatomy, University of Benin, and 10g of it was dissolved in 100ml of distilled water for use in the experiment.

$$\text{Yield \%} = \frac{\text{Final weight of AKAP (g)}}{\text{Weight of powdered } K. pinnata \text{ leaves (g)}} \times 100$$

2.2 Alcohol administration

1ml of absolute ethanol (Manufactured by EMPARTA® ACS with CAS Number: 64-17-5) was administered to 24 hours-starved experimental rats to induce gastric ulcerations.

2.3 Mechanism of Action/ Administration of Omeprazole Drug

Omeprazole is a proton pump inhibitor. It inhibits the parietal cell H⁺ / K⁺ ATP pump, the final step of acid production. In turn, omeprazole suppresses gastric basal and stimulated acid secretion (Shah and Srivastava, 2023). 20mg/kg of omeprazole tablets (manufactured by Fredum Pharmaceutical. Ltd with batch number CA0050) were dissolved in 100ml of distilled water. 2ml of omeprazole solution was administered orally to the standard treatment group using a gavage to compare effectiveness of the plant extract to that of the drug.

2.4 Experimental animals

A total of thirty (30) Wistar rats weighing 180-250g were used for this study. The animals were bred and housed in the animal House unit, Department of anatomy, University of Benin. The animals were maintained in adequate and conducive temperature, Grower's mash and water were provided ad libitum. They were grouped into six groups of five rats per group. Rats were made to acclimatize for 14 days (two weeks), and administered daily treatment of extract and Omeprazole where applicable for twenty-eight (28) days after 24-hour starvation across all group except for the control group. One group out of the six groups administered alcohol was euthanized to confirm gastric ulcerations after starvation. Treatment was continued for the other groups.

GROUP A: Control group (Water and feed)

GROUP B: 1ml C₂H₅OH (100%) + 0.5ml AKP

GROUP C: 1ml C₂H₅OH (100%) + 1ml AKP

GROUP D: 1ml C₂H₅OH (100%) + 2ml Omeprazole solution

GROUP E: 1ml C₂H₅OH (100%) + No treatment

GROUP F: Euthanization following 1ml C₂H₅OH (100%) administration

2.5 Ethical Clearance

In the course of this research work, ethical clearance was applied for and

approved by ethics committee of the College of Medical sciences, University of Benin, Benin city, Nigeria; Resignation number: **CMS/REC/2023/340**. The determination of gastric malondialdehyde (MDA), a marker of lipid peroxidation, was conducted using the thiobarbituric acid reacting substances (TBARS) assay. Gastric tissue homogenates were mixed with a reagent containing TCA, TBA, and HCl, then boiled to form a pink MDA-TBA adduct. After cooling and centrifugation, the supernatant's absorbance was measured at 535 nm. MDA concentration was calculated using the extinction coefficient $1.56 \times 10^{-5} \text{ M}^{-1} \text{ cm}^{-1}$.

2.6 Histological examination

On the 28th day of administration, rats were euthanized under chloroform, and the stomach was removed and fixed in 10% buffered formalin for 72 hours before being processed using Drury and Wallington's (1980) hematoxylin and eosin staining procedure. The processed tissue slides were examined with a Leica DM750 research microscope paired with a digital camera (Leica ICC50). Tissue sections were imaged digitally at 100x magnification.

3. STATISTICAL ANALYSIS

Data were subjected to statistical analysis using the IBM SPSS statistics software (Statistical Package for Social Science) (Version 25) and relevant statistical values were obtained. One-way analysis of variance (ANOVA) was carried out and data were presented as mean \pm SEM. LSD post-hoc test was used. Values of P<0.05 were considered significant. The statistical values obtained were converted into graphical representations in the form of bar charts.

3.1 Effects of AKP Treatment on Body Weight Changes in Experimental Animals

Table 1: There were no statistically significant differences (P>0.05) between initial and final body weights across all groups. Alcohol administration and treatment with *Kalanchoe pinnata* (AKP) or Omeprazole did not significantly impact the overall body weight of the rats. This suggests that the interventions were not detrimental to general physiological growth or health during the study period.

3.2 Effects of AKP Treatment on Oxidative Stress (Malondialdehyde, MDA Levels) in Experimental Animals

Figure 1 Significant decreases (P<0.05) in MDA levels were noted in groups treated with AKP (0.5 ml, 1 ml) and Omeprazole (2 ml) compared to the control group.

Lower MDA levels indicate reduced lipid peroxidation, suggesting that AKP and Omeprazole have antioxidant properties that mitigate oxidative stress induced by alcohol.

3.3 Effects of AKP Treatment on the Gastric Histology of Experimental Animals

Control Group (Plate 1): Histological examination in Control group showed normal mucosal epithelial lining, gastric pit, lamina propria and muscularis mucosa with no sign of gastric injury

Alcohol-Only Group (Plate 2): Histological examination in Group B (alcohol only following sacrifice) showed a flask-shaped ulcer transecting the muscularis mucosa into the submucosa.

Alcohol + 0.5 ml AKP (Plate 3): Histological examination in Group C (alcohol plus 0.5ml AKP) showed a focal mucosal devitalization, uninterrupted mucosal lining, an intact muscularis mucosa and a well-defined muscularis propria.

Alcohol + 1 ml AKP (Plate 4): Histological examination in Group D (alcohol plus 1ml AKP) showed normal uninterrupted mucosal lining, intact muscularis mucosa, submucosa and muscularis propria as well as some pancreatic tissue.

Alcohol + 2 ml Omeprazole (Plate 5): Histological examination in Group E (alcohol plus Omeprazole) showed normal uninterrupted mucosal lining, intact muscularis mucosa, submucosa and muscularis propria.

Alcohol Recovery Group (Plate 6): Histological examination in Group F (alcohol and left to recover with no treatment) showed persistent irregular ulcers and transected muscularis mucosa and submucosa, indicating incomplete recovery without intervention irregularly shaped ulcer.

Table 1: Showing initial body weight in comparison to final body weight of experimental animals

GROUP	INITIAL BODY WEIGHT	FINAL BODY WEIGHT	P-VALUE
Control	187.404.98	191.608.89	0.423
Alcohol + Low Dose of <i>B. pinnatum</i>	193.404.85	180.809.40	0.299
Alcohol + High dose of <i>B. pinnatum</i>	187.406.06	189.006.16	0.656
Alcohol +_Omeprazole	192.404.87	185.806.38	0.113
Alcohol only (Natural Recovery)	193.405.98	183.2013.32	0.408
Alcohol only (Sacrificed next day)	190.758.20	191.255.15	0.890

*P<0.05 indicates significant difference in other groups compared with control.

3.4 Oxidative Stress

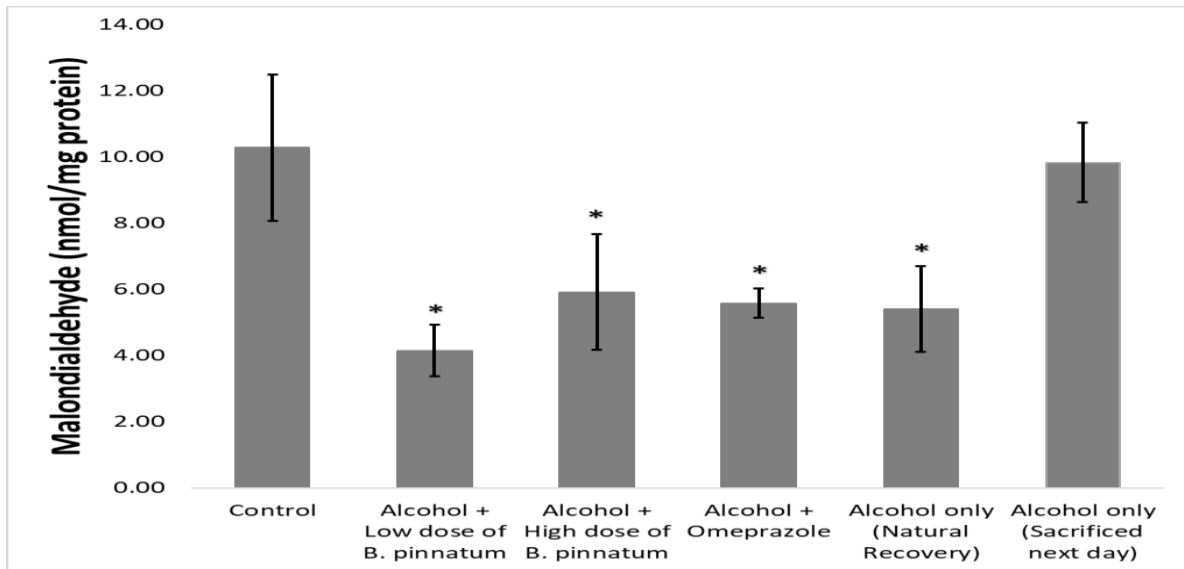


Figure 1: Chart showing the levels of malondialdehyde (MDA) across the groups.

*significantly different from the control group

There were significant decreases (P<0.05) of malondialdehyde in groups B, C, D and E, when compared to the control group.

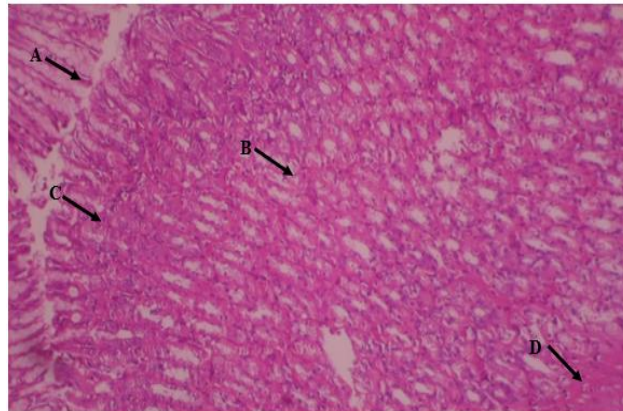


Plate 1: Rat stomach: Control group showing normal A, mucosal epithelial lining, B, gastric pit C, lamina propria, D, muscularis mucosa (HandE 100x).

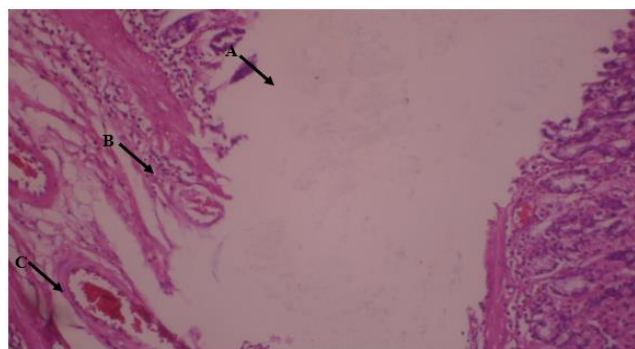


Plate 2: Rat given Alcohol only showing A, flask-shaped ulcer, transecting B, the muscularis mucosa into C, the submucosa (HandE 100x)

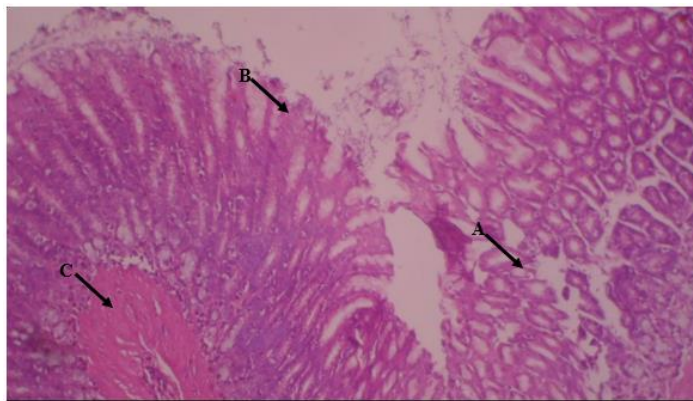


Plate 3: Rat given Alcohol plus 0.5ml AKA showing A, focal mucosal devitalization, B, uninterrupted mucosal lining and C, intact muscularis mucosa (H and E 100x)

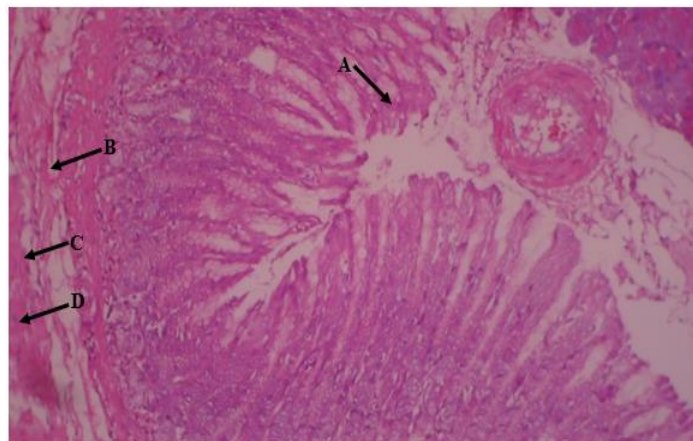


Plate 4: Rat given Alcohol plus 1ml AKA showing A, normal uninterrupted mucosal lining, B, intact muscularis mucosa, C, submucosa and D muscularis propria. (H and E 100x)

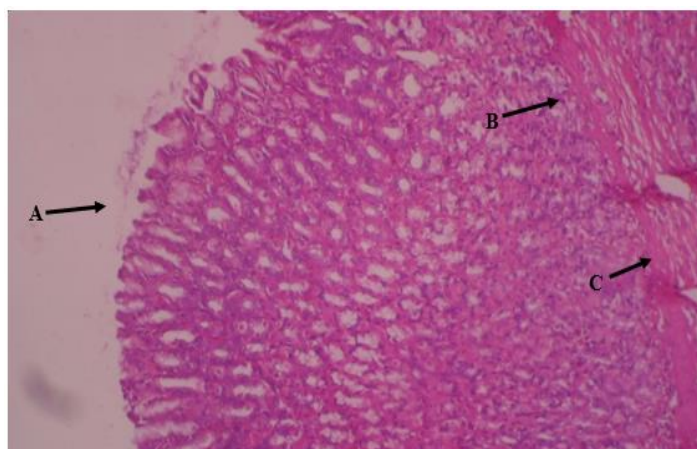


Plate 5: Rat given Alcohol plus 2ml Omeprazole showing A, normal uninterrupted mucosal lining, B, intact muscularis mucosa and C, submucosa (H and E 100x)

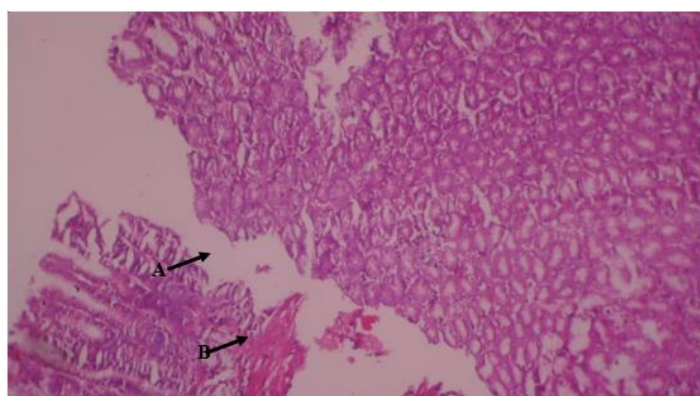


Plate 6: Rat given Alcohol and left to recover showing A, irregularly shaped ulcer, B, transected muscularis mucosa (H and E 100x)

4. DISCUSSION

Alcohol is one of the most widely used recreational drugs in the world, and about 33% of all humans currently drink alcohol (Griswold et al., 2018). Alcohol in copious quantities irritates the stomach lining, making it red, raw, and inflamed. Areas of bleeding may develop, this is caused by release of free radicals due to oxidative stress (Bishehsari et al., 2017). *Kalanchoe pinnata* leaves have several therapeutic characteristics, including antibacterial, antifungal, antihypertensive, antidiabetic, anti-inflammatory, analgesic, wound healing, anti-tumor, sedative, muscle relaxant, and possible treatment for leishmaniasis (Kendeson et al., 2021; Nagpal and Sharma, 2020). The results showed that there were no significant differences in the rats' body weights between the four treatment groups and the control. This shows that the administration of AKP and omeprazole had no deleterious effect on the animals' overall health and growth, indicating that observed differences in gastric health were related to the therapies themselves rather than general health degradation.

Malondialdehyde (MDA) is a key biomarker for lipid peroxidation, which occurs when free radicals attack polyunsaturated fatty acids in cell membranes. Elevated MDA levels are indicative of heightened oxidative stress, commonly observed in alcohol-induced tissue injuries. The study observed a significant reduction ($p < 0.05$) in MDA levels in groups treated with *Kalanchoe pinnata* (AKP) leaf extract (at doses of 0.5 ml and 1 ml) and Omeprazole (2 ml) compared to the control group. This is similar to a work according to where the level of malondialdehyde was decreased in the stomach of zingerone groups ($p < 0.05$) compared to the ethanol group (Karampour et al., 2019).

The reduction in MDA levels suggests that both AKP and Omeprazole possess antioxidant properties capable of neutralizing free radicals and inhibiting lipid peroxidation (Fatoki et al., 2022). This effect is critical in mitigating the damaging cascade triggered by oxidative stress, which contributes to tissue injury, inflammation, and delayed healing. The ability of *Kalanchoe pinnata* leaf extract to significantly lower MDA levels reinforces its antioxidant potential in mitigating alcohol-induced oxidative stress. These findings suggest that AKP could be a valuable natural alternative to conventional treatments like Omeprazole.

The histological findings revealed major disparities between the groups. The control group had normal histological architecture, including intact mucosal epithelial lining, gastric pits, lamina propria, and muscularis mucosa. In contrast, the ethanol-only group showed considerable gastric injury, as evidenced by a flask-shaped ulcer that crossed the muscularis mucosa into the submucosa, demonstrating the serious damage produced by ethanol. The group treated with ethanol and 0.5 ml AKP revealed some focal mucosal devitalization but mostly intact mucosal lining and muscularis mucosa, indicating that AKP had some protective benefits at this dose. The group treated with ethanol and 1 ml AKP showed normal uninterrupted mucosal lining, intact muscularis mucosa, and well-preserved submucosa and muscularis propria, showing a robust protective effect at this higher dose of AKP.

The study revealed that the gastroprotective effects of AKP, particularly at higher concentrations, are comparable to those of omeprazole, a well-established proton pump inhibitor. This aligns with studies on Wistar rats with gastric damage, where the plant's extract demonstrated ameliorative effects (Rodrigues et al., 2021; Sobreira et al., 2022). The untreated group, which was permitted to recuperate, had irregularly formed ulcers and transected muscularis mucosa, indicating chronic serious injury. The findings support the traditional usage of *Kalanchoe pinnata* in treating a variety of disorders, notably those linked to gastrointestinal health (Ehi-Omosun and Etunim, 2023; Fernández-Solà, 2020; Eteng et al., 2020; Sobreira et al., 2022; Rodrigues et al., 2021). The shown efficacy of AKP to considerably minimize ethanol-induced stomach ulcers validates its usage in traditional medicine and underlines its potential for incorporation into modern therapeutic methods.

5. CONCLUSION

The study confirms the gastroprotective potential of *Kalanchoe pinnata*, a natural remedy for gastric ulcers, highlighting the importance of integrating traditional knowledge with modern scientific methods as it demonstrated equal efficacy to omeprazole.

RECOMMENDATION

This study suggests future research on the gastroprotective effects of *Kalanchoe pinnata*, including clinical trials, comparative studies, and formulation development. These will help understand the biochemical mechanisms, evaluate the safety and efficacy of *Kalanchoe pinnata*

extracts, compare its effects with other anti-ulcer treatments, and ensure consistent therapeutic outcomes.

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