

Original Research Article

Duodenal alterations due to administration of Odogwu Bitters in Wistar rats

Emeka C Okafor¹, Darlington N Onyejike^{1*}, Obioma C Ejimofor¹, Ifeoma M Onyejike¹, Oghenefego M Adheke², Goodness G Henry¹

¹Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, ²Department of Human Anatomy, Faculty of Basic Medical Sciences, Southern Delta University, Ozoro, Delta State, Nigeria

*For correspondence: **Email:** dn.onyejike@unizik.edu.ng; **Tel:** +234-8061131867

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Abstract

Purpose: To investigate the subacute effects of Odogwu Bitters on the duodenum of adult male Wistar rats, with a focus on histomorphological and morphometric alterations, as well as oxidative stress biomarkers.

Methods: Twenty adult male Wistar rats were randomly divided into four groups ($n = 5$ per group). The control group (A) was given standard feed and water, whereas the treatment groups (B, C, and D) received daily oral administrations of 0.2, 0.4 and 0.8 mL of Odogwu Bitters, respectively, for five weeks. Post-treatment, absolute and relative duodenal weights were recorded. The activities of superoxide dismutase (SOD) and glutathione peroxidase (GPX), as well as the concentration of malondialdehyde (MDA), were determined using colorimetric assay kits. Duodenal tissues were processed for histological evaluation using haematoxylin and eosin staining.

Results: A significant decrease in relative duodenal weight was observed in group D ($p < 0.05$). Superoxide dismutase (SOD) levels were elevated in the low-dose group (B) but showed a significant reduction in groups C and D ($p = 0.010$, $p = 0.020$). Malondialdehyde (MDA) levels followed a similar pattern. In contrast, glutathione peroxidase (GPx) activity was significantly elevated in groups C and D ($p = 0.034$, $p = 0.045$). Histologically, the treated groups exhibited mild-to-moderate intestinal metaplasia but showed no epithelial atrophy. A consistent immune cell infiltrate comprising lymphocytes, plasma cells, and occasional neutrophils was present.

Conclusion: A five-week exposure to Odogwu Bitters induce dose-dependent duodenal alterations, characterized by oxidative imbalance and metaplastic epithelial changes. While overt mucosal damage was absent, these findings highlight potential subclinical gastrointestinal effects associated with this specific herbal bitter, suggesting that even subacute exposure may not be without adverse consequences.

Keywords: Antioxidant enzymes, Epithelial remodelling, Gastrointestinal histology, Oxidative imbalance, Polyherbal formulation

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INTRODUCTION

According to the World Health Organization (WHO), herbal medicines are classified as

finalized, labelled products whose active constituents are derived from the aerial or subterranean parts of plants, or combinations thereof, whether in the crude state or as plant

preparations [1,2]. Herbal mixtures are widely used as treatment options by a significant portion of the population in Nigeria and other parts of Africa. This is often due to limited access to modern pharmaceutical medications or the heavy financial cost of such treatments, which disproportionately affects low-income households in these regions [3,4].

Herbal medicines are also commonly patronized in Nigeria due to their cultural beliefs and practices. In south-eastern parts of Nigeria, *Odogwu Bitters* is a common herbal medicine popularly used to treat a variety of gastrointestinal and other related diseases such as indigestion, peptic ulcers, and intestinal disorders [5,6]. *Odogwu Bitters* is a herbal preparation composed of ingredients such as ginger and honey [5]. The phytochemical constituents of *Odogwu Bitters* comprise saponins, alkaloids, terpenoids, flavonoids, carbohydrates, cardiac glycosides, and reducing sugar [5,7].

The duodenum performs critical functions, including receiving chyme from the stomach and continuing digestion via pancreatic enzymes and bile. It also regulates blood sugar and maintains fluid and electrolyte balance [8,9]. While *Odogwu Bitters* is often touted for its health benefits, there is limited research on its effects on the duodenum, which is a part of the gastrointestinal tract. Notwithstanding this lack of safety data, many people continue to use *Odogwu Bitters* and other herbal mixtures as part of their daily lives. Therefore, this study aimed at investigating the effects of *Odogwu Bitters* on the duodenum using adult male Wistar rats as experimental models.

EXPERIMENTAL

The ethics approval for this study was obtained from Nnamdi Azikiwe University animal research ethics committee with the approval number, NAU/AREC/2023/00125 dated 11th June 2023.

Study design

A total of 20 healthy adult male Wistar rats weighing between 131 and 189g, were utilized in this study. The animals were purchased and transferred to the animal house of the College of Health Sciences, Nnamdi Azikiwe University, Nnewi campus, Anambra State, Nigeria. A two-week acclimatization period with ad libitum access to feed and water was provided. After this period, the rats were weighed and allocated into four groups of five rats each, housed in large, meshed cages. Animal health was monitored

daily throughout the study by assessing physical activity, food/water intake, and fur condition. All the procedures were carried out in strict accordance with the institutional guidelines on the care and use of experimental animals.

Rats were randomly divided into four groups (A - D). The LD₅₀ for *Odogwu Bitters* was adapted from a published report [5]. Group A served as the control and received food and water only, while groups B, C, and D received daily oral administrations of *Odogwu Bitters* for five weeks at doses of 0.2, 0.4 and 0.8 mL, respectively. The doses represent low, medium, and high sub-toxic exposures for the assessment of subacute effects. All procedures were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals [10]. The experiment lasted for five weeks.

Assay of biochemical parameters

Glutathione Reductase (GPX) activity was determined using the Piggott & Karuso method [11]; superoxide dismutase (SOD) activity was assayed according to Misra and Fridovich [12], while the level of the lipid peroxidation marker malondialdehyde (MDA) was measured using technique of reactive constituents of malonylurea [13].

Histopathological analysis of the duodenum

The duodenal part of the intestines was removed from sacrificed rats to perform standard histopathological procedures and immediately preserved in 10 % formalin and dehydrated using ethanol grades (75, 90, 95 and 100 %). The samples were then cleared in two changes of xylene after dehydration. After that, samples were embedded and blackened after being impregnated with melted paraffin wax. Using a sled microtome, paraffin slices 5 µm thick were cut, mounted on glass slides, and stained using both haematoxylin and eosin in a stepwise manner. A NOVEX compound microscope with Hi-PLAN objectives was used for imaging, coupled with an Amscope 14MP digital camera to capture photomicrographs. Image labelling was performed with Photoscape v3.7. All histological procedures adhered to established protocols for rat experimental studies [14].

Statistical analysis

Analysis was carried out using the IBM SPSS Statistics software (version 25). Data were presented as mean ± standard deviation (SD). A *p*-value of less than 0.05 was considered

statistically significant. To assess the differences between the groups, a one-way analysis of variance (ANOVA) was performed, followed by a least significant difference (LSD) post hoc test.

RESULTS

Histological findings

Histological assessment of the duodenum revealed progressive epithelial remodelling across treatment groups. In the control group (Figure 1 A), the duodenal mucosa exhibited a well-differentiated, simple columnar epithelium with intact villi, an organized lamina propria, and well-preserved muscularis mucosae and submucosa. The submucosal layer contained prominent Brunner's glands and crypts of Lieberkühn. Immune cell infiltrates, composed predominantly of lymphocytes and plasma cells with scattered eosinophils and occasional neutrophils, were present without associated epithelial distortion. Mild intestinal metaplasia was observed in this group.

Group B (Figure 1 B) demonstrated largely preserved mucosal architecture with moderate intestinal metaplasia. The epithelial lining and submucosal structures remained histologically intact. Similarly, group C (Figure 1 C) displayed mild metaplastic changes within an otherwise structurally conserved villous framework. In group D (Figure 1 D), moderate intestinal metaplasia was again evident, yet the epithelial surface remained uninterrupted, and no features of atrophy or ulceration were detected.

Morphometric findings

Body weight, water intake, and feed intake did not differ significantly between Groups A–D, except for food intake (Table 1). Body weight and water intake remained statistically comparable across all groups. In contrast, feed intake was significantly higher in Groups C and D compared with Group A ($p < 0.05$), while no significant differences were observed in Group B ($p > 0.05$). These findings indicate that *Odogwu Bitters* had no measurable effect on body weight but was associated with reduced water consumption at high doses and increased feed intake at moderate and high doses.

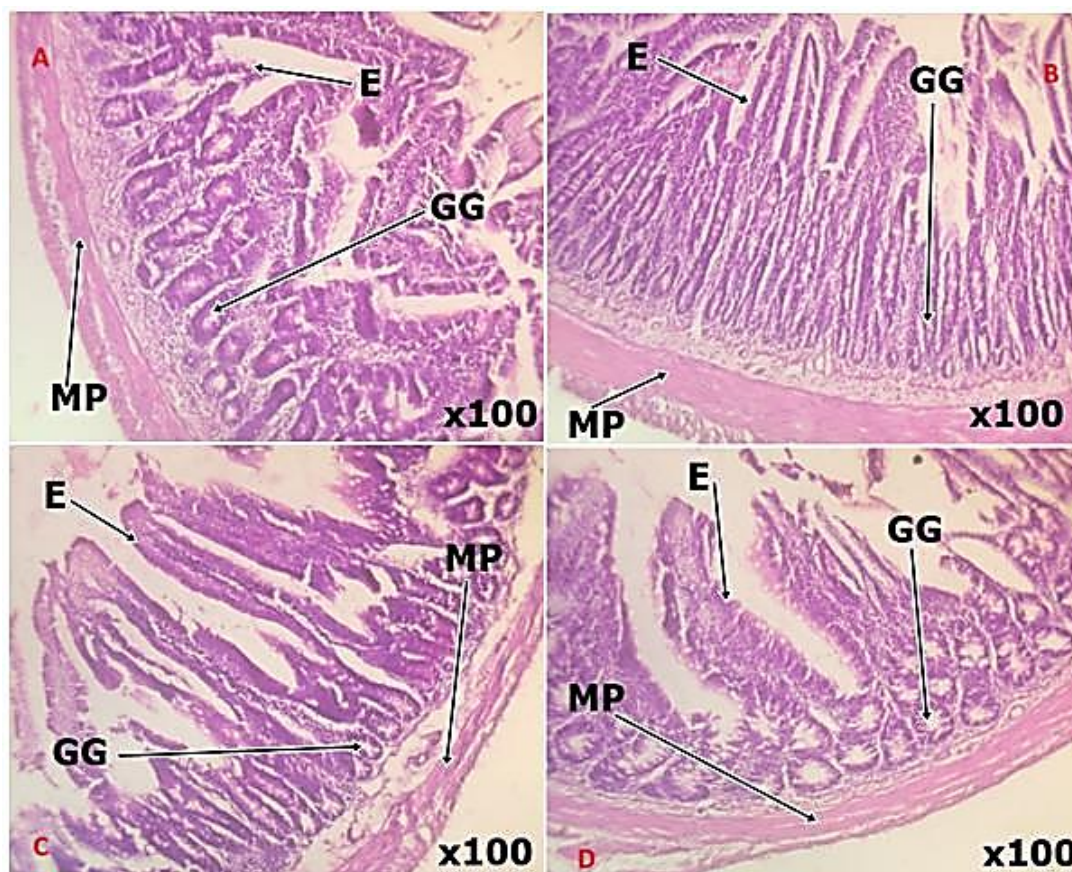


Figure 1: (a – d) Representative photomicrograph of the duodenum of rats in Groups A – D, respectively. E=Epithelium, GG= Submucosal gland, MP= Muscularis propria

Table 1: Effect of *Odogwu Bitters* on body weight, water intake, and feed intake

Group	Body weight (g)	Water intake (mL)	Feed intake (mg)
A	171.19±68.52	78.19±10.01	1101.03±63.12
B	177.35±11.28 [#]	85.45±9.63 [#]	1128.78±86.53 [#]
C	185.77±16.61 [#]	85.27±11.12 [#]	1202.53±115.71 [*]
D	159.36±12.73 [#]	81.22±6.25 [*]	1268.11±136.51 [*]

*: significant, #: not significant, values are mean ± standard deviation

The absolute duodenal weight showed no statistically significant changes across the treatment groups (Table 2). A non-significant decrease was observed in groups B ($p = 0.081$) and C ($p = 0.789$), while group D showed a non-significant increase ($p = 0.173$) compared to the control. In contrast, the relative duodenal weight was significantly decreased in group D. Although groups B and C also showed lower relative weights, these changes were not statistically significant.

Table 2: Effect of *Odogwu Bitters* on absolute and relative duodenal weight

Group	Absolute duodenal weight(g)	Relative duodenal weight (g)
A	0.41±0.08	0.0019±0.001
B	0.29±0.03 [#]	0.0016±0.001 [#]
C	0.39±0.10 [#]	0.0019±0.001 [#]
D	0.50±0.05 [#]	0.0030±0.001 ^{**}

** p = significant, # p = not significant, values are mean ± standard deviation

Table 3: Effect of *Odogwu Bitters* on duodenal oxidative stress markers and antioxidant status

Group	SOD (U/mL)	MDA (μmol/L)	GPx (U/mL)
Group A	11.53±2.11	44.10±5.77	0.68±0.09
Group B	12.50±0.46 [*]	45.70±1.01 [#]	0.59±0.08 [#]
Group C	8.22±0.35 [*]	36.98±1.58 [*]	0.84±0.01 [*]
Group D	8.69±0.95 [*]	35.96±0.66 [*]	0.82±0.07 [*]

*: significant, #: not significant, values are mean ± standard deviation, SOD = superoxide dismutase, MDA = malondialdehyde, GPx = Glutathione peroxidase

Biochemical findings

Analysis of oxidative stress markers revealed distinct patterns across the treatment groups. The mean SOD level was non-significantly elevated in group B ($p = 0.352$) but significantly reduced in groups C ($p = 0.010$) and D ($p = 0.020$) compared to the control. A similar pattern was observed for MDA, with a non-significant increase in group B ($p = 0.540$) and significant decreases in groups C and D. In contrast, GPx activity was non-significantly lower in group B ($p = 0.165$) but significantly increased in groups C ($p = 0.034$) and D ($p = 0.045$).

DISCUSSION

The histological findings from the histology analysis indicate that repeated oral exposure to *Odogwu Bitters* may elicit a low-grade, chronic inflammatory response that promotes epithelial remodelling in the form of metaplasia, independent of overt mucosal erosion or necrosis. The predominance of lymphoplasmacytic infiltration across treated groups further supports an adaptive mucosal response. Such histological alterations are consistent with findings from previous experimental models of chronic herbal ingestion, particularly those containing ethanol, where mucosal metaplasia developed in the absence of ulcerative pathology [15]. The emergence of intestinal metaplasia in this context reflects a compensatory or pre-neoplastic transformation often associated with sustained oxidative stress and chronic inflammation [16].

The alterations in superoxide dismutase (SOD) activity likely indicate an early compensatory activation of redox defence mechanisms, followed by enzyme inhibition or depletion under cumulative oxidative load. Similar SOD suppression has been documented in experimental models involving phytochemical-rich herbal preparations [17,18]. Oxidative stress is a principal mediator of gastrointestinal mucosal injury induced by xenobiotics, including polyherbal formulations [19,20]. Reactive oxygen species (ROS) generated during metabolism of bioactive herbal constituents are known to overwhelm cellular antioxidant systems, leading to oxidative imbalance, lipid peroxidation, and mucosal remodelling. The decline in SOD activity at higher exposures may therefore reflect compromised frontline antioxidant defence.

Interestingly, malondialdehyde (MDA) levels, an index of lipid peroxidation, showed an unexpected reduction despite SOD depletion. This paradox may be explained by enhanced glutathione peroxidase (GPx) activity which facilitates compensatory detoxification of lipid hydroperoxides. Indeed, GPx catalyses the reduction of hydrogen peroxide and lipid peroxides [21,22], and its upregulation likely represents a secondary antioxidant response

triggered by elevated peroxidative stress. While declining MDA concentrations in high-dose groups may be interpreted as a protective outcome, caution is warranted. Prior studies have shown that under severe oxidative burden, MDA levels may plateau or decline due to enzymatic saturation, thereby underestimating ongoing cellular damage [23]. The paradoxical antioxidant response observed in this study may be attributed to the dual pharmacological nature of polyherbal formulations. Phytochemicals such as flavonoids, alkaloids, tannins, and terpenoids present in *Odogwu Bitters* have been reported to exhibit both pro-oxidant and antioxidant properties, depending on concentration and exposure duration [24,25]. While some constituents may initially enhance antioxidant defences, chronic exposure or high doses may result in redox disequilibrium and cellular dysfunction. Furthermore, previously published findings have demonstrated that *Odogwu Bitters* significantly alters renal biochemical indices, as evidenced by increased levels of urea, creatinine, and uric acid, which points to systemic toxicity beyond the gastrointestinal tract [5,7]. This supports the view that sub-chronic intake of unregulated herbal mixtures poses potential multi-organ health risks, necessitating more rigorous safety evaluations and enhanced public health monitoring. The absence of significant changes in body weight across treatment groups suggests that *Odogwu Bitters* did not exert overt systemic toxicity severe enough to impair growth or cause wasting during the five-week exposure period. However, the significant reduction in water intake in the high-dose group indicates possible early effects on fluid balance or palatability, which could reflect gastrointestinal irritation or metabolic alterations. The dose-related increase in feed intake observed in Groups C and D may point toward an appetite-modulating effect of *Odogwu Bitters*, possibly mediated by its phytochemical particularly alkaloids, which are recognized for their ability to influence feeding behaviour and energy balance. For example, alkaloids such as berberine have been shown in animal and human studies to reduce food intake and body weight, likely through effects on gut hormones, glucose metabolism and central appetite pathways [26,27]. When considered alongside the duodenal histological changes and oxidative imbalance observed, these feeding and drinking behaviours suggest that *Odogwu Bitters* may subtly alter gastrointestinal physiology and energy regulation without immediately affecting gross body weight. In addition, the observed reduction in relative duodenal weight, in conjunction with histopathological evidence of epithelial metaplasia, may reflect subtle mucosal

atrophy or structural reorganization not appreciable at the gross anatomical level. These findings suggest that sub-chronic administration of high-dose *Odogwu Bitters* may induce latent morphological adaptation in the gastrointestinal tract. This highlights the potential for long-term metabolic and nutritional implications with chronic or high-dose use.

CONCLUSION

This study demonstrates that a five-week oral administration of *Odogwu Bitters* elicited dose-dependent alterations in the duodenum of adult male Wistar rats, characterized by mild-to-moderate intestinal metaplasia, significant dysregulation of oxidative stress biomarkers, and reduced relative duodenal weight in high-dose groups. Although overt mucosal erosion or necrosis was absent, the presence of metaplastic transformation, antioxidant enzyme dysregulation, and inflammatory infiltration suggest early-stage mucosal remodelling and oxidative imbalance. These findings underscore the potential for subclinical gastrointestinal effects associated with chronic or high-dose consumption of unregulated polyherbal formulations. Given these observations, caution is warranted in the prolonged and unsupervised use of *Odogwu Bitters* and similar herbal mixtures. Further long-term toxicity studies incorporating extended exposure durations are recommended to elucidate the potential for cumulative damage, including neoplastic progression and extra-gastrointestinal organ dysfunction. Furthermore, implementing stringent regulatory oversight and mandating accurate labelling for polyherbal products are critical public health measures to ensure consumer safety. Public health education campaigns may aid in mitigating misuse and informing evidence-based consumption practices across communities where such formulations are commonly used.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. All authors read and approved the final manuscript. ECO carried out the histology analysis, reviewed the final draft of the manuscript and assisted DNO in sourcing funding. DNO conceptualized the study, designed the study, wrote the experimental protocol, supervised the experiments, assisted OMA to write the first draft of the manuscript and sourced the funding of the project. OCE carried out the biochemical assay and data analysis. IMO curated the data and assisted GGH to carry out the experiment. OMA wrote the first draft of the manuscript and managed the literature searches. GGH carried out the experiment and managed the animals.

Availability of data and materials

The datasets generated during and/or analysed during the current study are available within the text.

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