

Effect of subacute administration of orange juice (*Citrus sinensis*) on duodenal structure in paediatric Sprague-Dawley rats (*Rattus norvegicus*)

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ABSTRACT: Acidic foods often pose concerns regarding their effects on gastrointestinal health, particularly in developing organisms. This study explored the effects of orange juice consumption on the histological and anatomical structures of the duodenum in post-weaning rats, a critical site for nutrient absorption and digestion. Utilising orange juice at pH 3, we administered varying dosages ranging from 0 g/kg to 20 g/kg body weight to Sprague-Dawley rats aged 4–6 weeks over two weeks. Our detailed histopathological evaluation focused on critical parameters, such as the density of crypts of Lieberkühn and the dimensions (length, height, width, and depth) of the villi, measured in micrometers. Anatomical assessments also included measurements of duodenal length, width, and mean colour optical density. This investigation revealed no significant alterations in the histopathological or anatomical integrity of the duodenum after orange juice administration. This absence of a significant change suggests that young rats possess an inherent ability to adapt to the acidic properties of orange juice without detrimental effects on their gastrointestinal health. These findings provide valuable reassurance that short-term intake of acidic substances, such as orange juice with a pH of 3, does not pose substantial risks to intestinal integrity during critical periods of growth and development.

Keywords:

acidic changes, duodenum, histopathological changes, oranges, paediatric

■ INTRODUCTION

The gastrointestinal tract is integral to digestion, nutrient absorption, and waste elimination, and the duodenum plays a pivotal role in the digestion and absorption of nutrients (Celi *et al.* 2017). In paediatric research, understanding how the duodenum develops and responds to various stimuli is essential, particularly when evaluating the effects of oral drugs in young animals.

Sweet oranges (*Citrus sinensis*), known for their nutrient and phytochemical richness, offer notable health benefits (Momoh & Loyibo 2021). However, their acidic nature may cause dental enamel erosion and exacerbate symptoms such as ulcers or gastroesophageal reflux disease (Gebishu *et al.* 2022). This study examined the subacute effects of orange juice, as a model for acidic oral drugs, on duodenal maturation in post-weaning rats. Over two weeks, we assessed the histopathological and anatomical changes in duodenal tissue from repeated exposure to orange juice,

shedding light on its influence on the development of the gastrointestinal system.

■ MATERIALS AND METHODS

This study, conducted at SVMBS IPB under approval number 159/KEH/SKE/I/2024, involved 25 male Sprague-Dawley rats aged 4–6 weeks divided into five groups receiving varying doses of freshly squeezed orange juice (0, 5, 10, 15, and 20 g/kg body weight) daily for 14 days, following BPOM 2022 guidelines. Post-euthanasia, duodenal sections were stained with Hematoxylin and Eosin for microscopic examination of anatomical and histopathological parameters, including color, size, microvilli, and crypts of Lieberkühn dimensions. Data were

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analyzed using ANOVA and ImageJ software to ensure precise measurement conversions, providing insights into the duodenal response to acidic exposure.

■ RESULTS AND DISCUSSION

In this study, we explored the impact of administering orange juice with a pH of 3 to paediatric rats via feeding tubes and assessed the histological changes in the duodenum over two weeks. The experiment involved varying dosages, with results indicating increased villus surface area and height in most groups, except for the 15 g/kg BW group, where no significant changes in crypt depth or villus width were observed.

Histopathological analysis revealed preservation of duodenal integrity with no tissue degeneration and maintained crypt-villus ratios across various dosages of orange juice. Sweet oranges (*Citrus sinensis*), rich in bioactive substances such as flavonoids, carotenoids, and vitamin C, influence systemic and gastrointestinal metabolism, underscoring their potential health benefits (Azzini *et al.* 2017). Flavonoids, in particular, have been associated with beneficial changes in gastrointestinal histopathology, such as modifications in the crypts of Lieberkühn, which are crucial sites for stem cell activity and nutrient absorption (Kim *et al.* 2018, Shaker & Rubin 2010, Porter *et al.* 2002). These structures are essential for growth and gastrointestinal health, with villus deterioration potentially leading to severe nutrient deficiency or intestinal failure (Farrell & Kelly 2002, Simon *et al.* 2019).

Despite the acidic challenge, our findings revealed that young rats possess an inherent resilience to the acidic nature of orange juice, suggesting that such short-term exposure does not compromise gastrointestinal integrity or health. This resilience provides reassurance regarding the safety of acidic substances during critical developmental stages, underscoring the robust adaptive mechanisms of the young gastrointestinal tract (Beasley *et al.* 2015, Gelberg 2018).

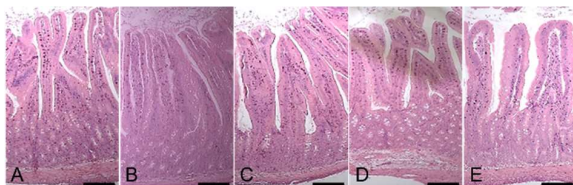


Figure 1. Histopathological duodenum of rats given various doses of orange juice: (A) 0 g/kg BW, (B) 5 g/kg BW, (C) 10 g/kg BW, (D) 15 g/kg BW, (E) 20g/kg BW. Stained with Hematoxylin and eosin (HE) and bar = 50 μ m.

Table 1. Histopathological analysis of the duodenum in relation to different doses of orange juice

Dosage (g/kg BW)	Number of Lieberkuhn crypts	Length of surface area (μ m)	Depth of crypts (μ m)	Height of villi (μ m)	Width of villi (μ m)
0	159 \pm 96	180 \pm 10	292 \pm 75	654 \pm 56	136 \pm 69
5	225 \pm 120	233 \pm 44	359 \pm 93	869 \pm 258	119 \pm 27
10	160 \pm 18	250 \pm 74	251 \pm 52	531 \pm 67	166 \pm 46
15	321 \pm 89	115 \pm 66	518 \pm 88	776 \pm 89	114 \pm 11
20	167 \pm 31	211 \pm 21	343 \pm 190	805 \pm 484	124 \pm 32

Note: No significant differences were found between the treatment groups in the observed parameters across the columns

Table 2. Anatomical pathology analysis of duodenal histopathology in response to varying orange juice dosage

Dosage (g/kg BW)	Length of duodenum (cm)	Width of duodenum (cm)	Colour mean (OD)
0	4.82 \pm 1.03	0.54 \pm 0.05	0.63 \pm 0.04
5	5.60 \pm 1.12	0.59 \pm 0.08	0.47 \pm 0.33
10	4.20 \pm 1.04	0.51 \pm 0.10	0.77 \pm 0.12
15	3.89 \pm 0.95	0.44 \pm 0.05	0.76 \pm 0.06
20	5.23 \pm 0.68	0.54 \pm 0.07	0.58 \pm 0.08

■ CONCLUSION

The absence of significant histopathological changes in the duodenal tissues of young rats exposed to acidic orange juice suggests their robust adaptation, indicating that short-term acidic challenges do not compromise gastrointestinal health.

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