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Research Article

TO STUDY THE EFFECT OF MONOSODIUM GLUTAMATE ON THE URINARY BLADDER

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ABSTRACT

Objective: This study evaluates the appropriateness of statistical tests (Z-test, t-test, and Mann–Whitney U test) for small-sample analyses, addressing common misconceptions and providing evidence-based guidelines for test selection in low-data scenarios.

Methods: A comparative framework was developed to assess the performance of parametric and non-parametric tests under varying sample sizes (n<10 to n=30) and distributional assumptions (normal vs. skewed). Normality was tested through Shapiro–Wilk tests, and simulated datasets with controlled variance and outliers were analyzed.

Results: Z-tests produced inflated type I errors for n<30 due to reliance on known population variance, a rarely feasible assumption in small samples. T-tests maintained robustness for n≥15 with normal distributions, whereas Mann–Whitney U tests outperformed parametric alternatives for n<15 or non-normal data (skewness >2).

Conclusion: Researchers should default to t-tests for small samples with approximate normality and use Mann–Whitney U tests for highly skewed data or n<15. Transparent reporting of sample sizes, normality checks, and test rationale is critical to ensure methodological validity.

Keywords: Aspartame, Swiss Albino mice, Urinary bladder, Histology.

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INTRODUCTION

Consumers in both developed and emerging countries, such as India, are increasingly concerned about the quality and safety of various dietary products, including artificial sweeteners, flavorings, colorings, preservatives, taste enhancers, and dietary supplements. Today, aspartame has become an integral part of the modern diet and is used as a food additive in more than 5000 food articles, such as tabletop sweeteners, bakery food, and carbonated beverages [1]. Aspartame is an attractive sweetener because it is 180 times sweeter than sugar. Mere one pellet of sugar-free gives the sweetness of one teaspoon full of cane sugar and only 0.2% calories. Chemically, aspartame is N-Aspartyl-L-phenylalanine, 1-methyl ester. Commercial aspartame is a mixture of three chemicals, namely aspartic acid, phenylalanine, and methanol, in proportions of 50%, 40%, and 10%, respectively [2]. In dry formulations, it persists long, but in circumstances such as solution form, prolonged storage, high temperature, and high pH, it breaks down into its constituents. Dilution and increased temperature lead to the formation of Aspartylphenylalanine diketopiperazine and free phenylalanine (an amino acid). This makes the use of aspartame highly unsuitable in soft drinks, fluid beverages, and cooked products. However, in the absence of statutory warnings in this regard, the use continues in households and commercial organizations. It is well known that reactive oxygen species are by-products of methanol metabolism. Imbalanced anti-oxidant systems may lead to oxidative stress in the body, which might lead to catalytic damage to cellular membranes [3]. Excessive amounts of phenylalanine, an excitatory neurotransmitter in the brain, can make individuals more susceptible to seizures and decrease their appetite. It can also decrease levels of serotonin – the mood regulator, leading to depression [4]. In view of the alleged widespread toxic role but sporadic reports of effects of aspartame on the histological structure of mammalian tissues, we chose the urinary bladder in the present study as a target organ. The principal aim of the study was to observe the histological changes in the urinary bladder of Swiss albino mice as an effect of prolonged oral intake of aspartame on mice.

METHODS

The present study was carried out in the research laboratory of our department. For this, a case–control-based animal experimental model was designed. Statistical methods were used for critical analysis of the result of Morphometric parameters. Prior approval of "Institutional Ethics Committee" and "Animal Ethics Committee" was duly obtained before starting the work.

Animals dosing

Adult Swiss albino mice above the age of 25 days of either sex were obtained from registered animal breeders. Commercial preparation of aspartame was in the form of pellets (sugar-free gold' – each pack has 100 pellets of approximately 18 mg each). Commercial rat food pellets were fed to the animals and libitum, along with hygienic drinking water. Sexes were kept separate to prevent mating. Distilled water was used for the reconstitution of the aspartame solution. The weight of animals of both groups was recorded before dosing and before sacrifice.

Animals were sacrificed by euthanasia with a single dose of injection thiopentone sodium intraperitoneally. After setting saline perfusion, the urinary bladder was dissected out and subjected to histological processing. 7-micron thick histological sections were stained with Hematoxylin and Eosin and with Masson's trichrome stain for qualitative study and photomicrography. Pre-calibrated linear micrometer scale used for histomorphometry. As per the methods adopted, we paid

attention to the mucosal layer of the urinary bladder only. The histomorphometric parameters studied were the height of epithelium, the number of nuclear layers of transitional epithelium, and the thickness of the glycocalyx coat over the epithelium. Statistical analysis was performed using the "Z" test.

RESULTS

The weight record of the mice of two groups is shown in Table 1.

The qualitative study was based on apparent changes seen in the epithelium and underlying lamina propria of the urinary bladder of the experimental group as compared to the control group (Figs. 1 and 2). There was an apparent increase in the thickness of epithelium as well as glycocalyx coat in the experimental group (Figs. 3 and 4). There was no evidence of epithelial damage, ulceration, degeneration, metaplasia, dysplasia, or altered nucleo-cytoplasmic ratios. The number of epithelial folds and thickness of connective tissue of lamina propria were also found to be increased (Fig. 5); however, there was no evidence of cellular infiltration, vascular congestion, or hemorrhages. For quantification of these features, the height of epithelium was measured in two sets of bladders, and values are recorded in Table 2 (Fig. 5**)**.

The mean value of epithelial height was statistically compared, and we found a statistically significant difference in the epithelial thickness, as shown in Table 2. We further counted the number of cell layers in the transitional epithelium of the bladder of two groups and the mean value for each animal, and then each group is entered in Table 3.

DISCUSSION

As far as human consumption of aspartame is concerned, we primarily ingest it orally in daily life. To correlate potential adverse effects of aspartame across mammalian species, we administered it orally to adult Swiss albino mice. While some studies have used varying doses (e.g., 5–20 mg/kg in mice or 3.5–350 mg/kg for cytogenetic effects), we selected 100 µg/g (100 mg/kg body weight [BW]), which is well below the established toxic dose of 4 g/kg for rodents per WHO guidelines [7]. However, to contextualize this dose for human relevance, a human equivalent dose conversion – using body surface area normalization (mouse-to-human Km factor ratio: 3/37) – translates this to ~8.1 mg/kg/day. This remains far below the FDA/WHO safety limits of 50 mg/kg/day and 40–60 mg/kg/day, respectively, for humans. Thus, while our dose was subtoxic for rodents, it also aligns with human safety thresholds, reinforcing its relevance for risk assessment.

We focused on structural rather than biochemical changes, as seen in prior work [5,6,8,11-13]. Weight loss in treated mice (Table 1) may align with phenylalanine-induced appetite suppression or hyperglycemia-linked glycogen depletion [13,14]. Urinary bladder changes were limited to urothelial hyperplasia (increased layers, glycocalyx, and stroma), consistent with Kitahori *et al*. [5], but showed no pre-neoplastic features (dysplasia, polyps). This hyperplasia may reflect urinary epidermal growth factor (EGF) stimulation from aspartame metabolites, though pH/sodium interactions warrant further study.

The dose of aspartame given to small laboratory mammals in different studies markedly differed, such as 5–20 mg/kg in Swiss albino mice for the demonstration of structural changes in the kidney of mice [6]; 2.5% and 5% solutions of aspartame in drinking water in rats; 3.5, 35 and 350 mg/kg BW aspartame orally for demonstration of cytogenetic effects in mice [8]. Aspartame preparation to be administered for animal experimentation was either a saline solution [9] or an aqueous solution in distilled water [10]. We have used an aqueous solution of aspartame as a vehicle for oral administration. In the present study, we

Table 1: Difference in body weight of two groups of animals

Time of weight recorded	Mean body weight (control)	Mean body weight (experimental)	"z" value
At the beginning of the study	25.66 g	25.58 g	0.1867 (insignificant)
At sacrifice	26.28 g	24.03 g	
Difference	(+) 0.66	(−) 1.55	

*(z-value<1.96 makes "p" value insignificant)

Table 2: Comparison of the mean height of epithelium (in microns) of urinary bladder

Control	Experimental	Difference	"z" value	Remarks
49.11	64.11	15.08	13.1834	Significant*

* "z" value>1.96, so the "p" value is significant (p<0.05)

Table 3: Comparison of the mean number of cell layers of epithelium of the urinary bladder

Control	Experimental	"z" value	Remarks
6.23±0.61	7.1±1.18	3.57	"p" value, highly significant (p<0.01)

*("z" value>1.96), so the "p" value is highly significant (p<0.01)

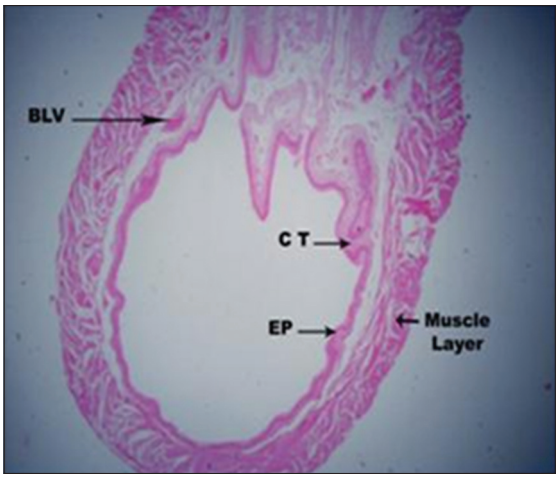

Fig. 1: Photomicrograph shows a panoramic view of the cut section of the urinary bladder. CT: Connective tissue of lamina propria, EP: Transitional epithelium, BLV: Blood vessel (H&E ×40, Control group)

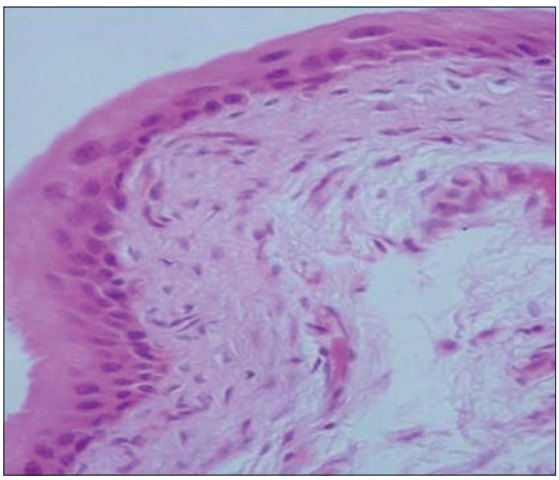

Fig. 2: Urinary bladder shows urothelium with its wall (Control, H&E ×400)

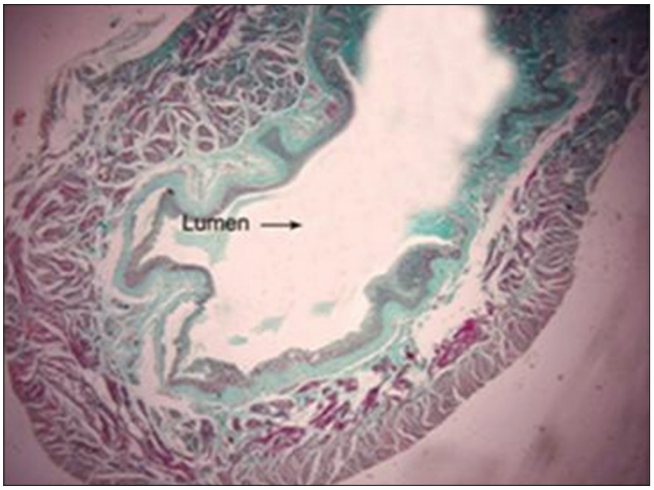

Fig. 3: Photomicrograph shows a panoramic view of the cut section of the urinary bladder (Masson's trichrome ×40, Experimental group)

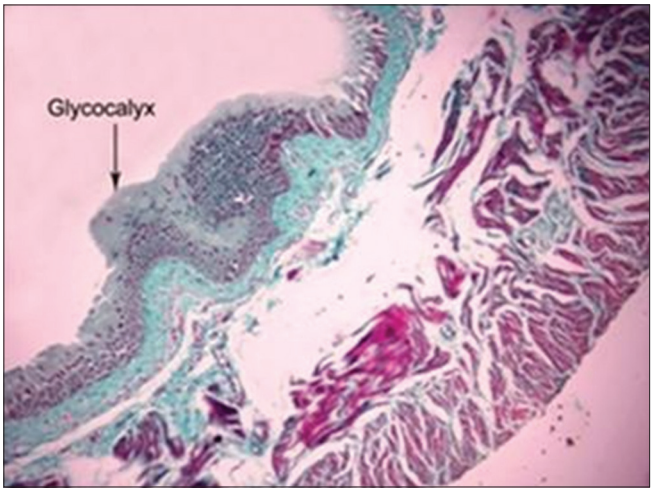

Fig. 4: Photomicrograph shows thickened epithelium (cell layers as well as glycocalyx) of the bladder (Masson's trichrome ×400, Experimental group)

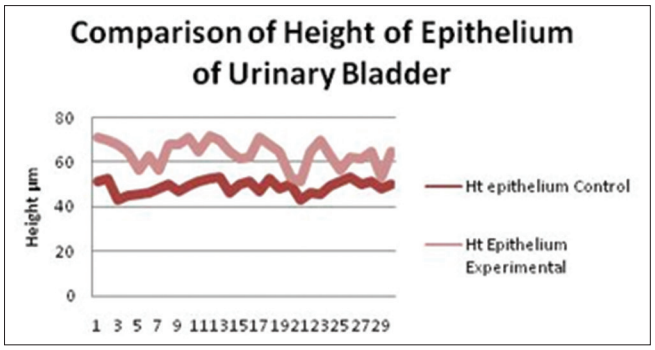

Fig. 5: Diagram shows the difference in heights of the transitional epithelium of the bladder in two groups of mice

have emphasized more on gross and microstructural changes rather than biochemical changes which were beyond the scope of the present study. Iman [11-13] has also demonstrated structural changes only. Kitahori *et al*. demonstrated structural changes in the urinary system of rats along with urinalysis and blood biochemistry, which are shown [5]. The weight of mice has declined in the experimental group of animals, as shown in Table 1. Martins *et al*. (2007) suggested that the reduction in weight is due to loss of appetite as a central nervous system (CNS) toxicity of phenylalanine, an ingredient of aspartame [13,14], and further commented that aspartame administration in rats led to hyperglycemia by depleting the hepatic stores of glycogen and prolonged hyperglycemia suppressed the appetite [13]. The changes seen in the wall of the urinary bladder in the present study were pertaining to the urothelium only. We found several-fold increases in the thickness of the urothelium of the bladder which was attributable to an increase in the number of cell layers as well as an increase in glycocalyx and connective tissue stroma of lamina propria. Our results match favorably with (Kitahori *et al*.) who found transitional cell hyperplasia in 12% of male rats and 8% of female rats following aspartame treatment with 5% solutions of monosodium aspartame [5,15,16]. They also found this feature in 24% of male and 25% of female rats in the renal pelvis. They stated that epithelial proliferation was an effect of stimulation of the EGF, which was further dependent on the pH of the urine and the presence of sodium salts. Since aspartame is principally eliminated by the urinary route, it probably affects urothelium. In the present study, there was no alteration in the nucleo-cytoplasmic ratio of the epithelial cells and no incoherence was there among the epithelial cells so that the epithelial architecture was not disturbed. Thus, the hyperplasia was well within normal limits, and we did not find any trend toward pre-neoplastic change in the form of polyps, dysplasia, or metaplasia.

CONCLUSION

The use of aspartame should be judged with potential benefits. Although it is a low-caloric sweetening agent and highly popular among calorie-conscious individuals, its use should be specifically restricted in children, pregnant ladies, CNS disorders, people on anticoagulants, and renal compromised individuals. No carcinogenicity or lethal effects have been documented so far, but this potential of the drug should be kept in mind before its prolonged use is advocated.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest, financial or otherwise.

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