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Original Research Article

EFFECTS OF ASPARTAME ON URINARY BLADDER OF ADULT SWISS ALBINO MICE

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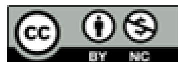

Abstract

Background: Aspartame is one of the most widely used artificial sweeteners in world. It is a high intensity but low caloric sweetener making popular amongst diabetics and calorie conscious people. The studies on effect of aspartame on mammalian tissues are scarcely reported. **Materials and Methods:** The study was conducted on 60 adult Swiss Albino mice, 30 as control and 30 as experimental. Aspartame, reconstituted as aqueous solution, was given to experimental set of animals at a dose of 100µg/g of body weight by intragastric route, daily, for a period of 8 weeks. Control group of animals received equivalent quantity of distilled water (as vehicle base) by same route. Mice of both sets were sacrificed at the end of 8 weeks and urinary bladder was dissected out for histological processing. Qualitative and quantitative histological parameters were recorded in two groups and statistically assessed for significance. **Result:** Height of transitional epithelium of urinary bladder, number of cell layers in transitional epithelium and thickness of glycocalyx coat was increased in experimental group of mice. **Conclusion:** The findings are suggestive of untoward and potential toxic role of aspartame on bladder wall of mice.

INTRODUCTION

Consumers of developed as well as on the rise countries like India in particular, are more and more concerned about the quality and safety of many dietary products such as artificial sweeteners, flavorings, colorings, preservatives, taste enhancers and dietary supplements. Today aspartame has become an integral part of modern diet and used as a food additive in more than 5000 food articles like table top sweetener, bakery food, carbonated beverages etc.[1]
Aspartame is an attractive sweetener because it is 180 times sweeter than sugar. Mere one pellet of Sugarfree gives 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 3 chemicals namely aspartic acid, phenylalanine and methanol in proportion of 50%, 40% and 10% respectively.[2] In dry formulations it persists long but in circumstances such as in solution form, prolonged storage, high temperature and high pH, it breaks down into its constituents. Dilution and increased temperature lead to formation of Aspartyl phenylalanine diketopiperazine (DKP) and free phenyl alanine (an amino acid). This makes the use of Aspartame highly unsuitable in soft drinks, fluid beverages and cooked products. However in absence of statutory warnings in this regard, the use continues in households and commercial organizations.
It is well known that reactive oxygen species (ROS) are by-products of methanol metabolism. Imbalanced anti-oxidant system may lead to oxidative stress in the body which might lead to catalytic damage of cellular membranes.[3]
Excessive amount of Phenylalanine, an excitatory neurotransmitter in brain, can make individuals more susceptible to seizures and decrease the appetite. It can also decrease levels of serotonin- the mood regulator, leading to depression.[4]

Aim of Study

In view of the alleged widespread toxic role but sporadic reports of effects of aspartame on histological structure of mammalian tissues, we chose urinary bladder in the present study, as a target organ. The principal aim of study was to observe the histological changes in urinary bladder of Swiss

albino mice as an effect of prolonged oral intake of aspartame on mice.

MATERIALS AND METHODS

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

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 ad libitum along with hygienic drinking water. Sexes were kept separate to prevent mating. Distilled water was used for reconstitution of Aspartame solution. The weight of animals of both groups was recorded before dosing and before sacrifice.

Animals were sacrificed by euthanasia with single dose of injection Thiopentone sodium intraperitoneally. After setting a saline perfusion, urinary bladder was dissected out and subjected to histological processing. 7 micron thick histological sections were stained with Haematoxylin and Eosin (H & E) 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 urinary bladder only. The histo-morphometric parameters studied were height of epithelium, number of nuclear layer of transitional epithelium and thickness of glycocalyx coat over the epithelium. Statistical analysis was performed using 'Z' test.

RESULTS

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

The qualitative study was based on apparent changes seen in epithelium and underlying lamina propria of urinary bladder of experimental group as compared to control group [Figure 1,2].

There was an apparent increase in thickness of epithelium as well as glycocalyx coat in experimental group [Figure 3,4]. 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 [Figure 5] however there was no evidence of cellular infiltration, vascular congestion or haemorrhages.

For quantification of these features the height of epithelium was measured in two sets of bladder and values were recorded in [Table 2], [Figure 5]. The mean value of epithelial height was statistically compared and we found a statistically significant difference of the epithelial thickness as shown in [Table 2].

We further counted the number of cell layers in transitional epithelium of the bladder of two groups and the mean value for each animal and then each group was entered in [Table 3].

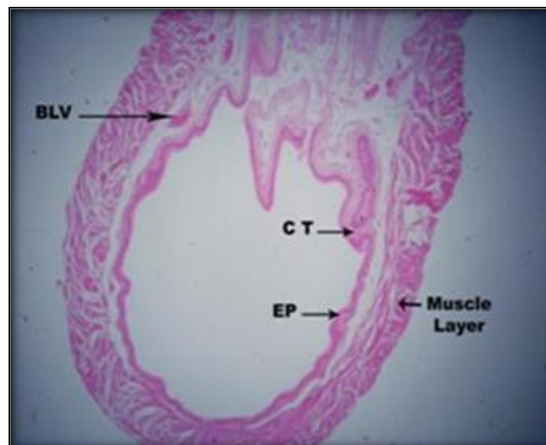

Figure 1: Photomicrograph showing a panoramic view of cut section of urinary bladder. CT= connective tissue of lamina propria; EP= transitional epithelium; BLV= blood vessel [H & E x 40, Control group]

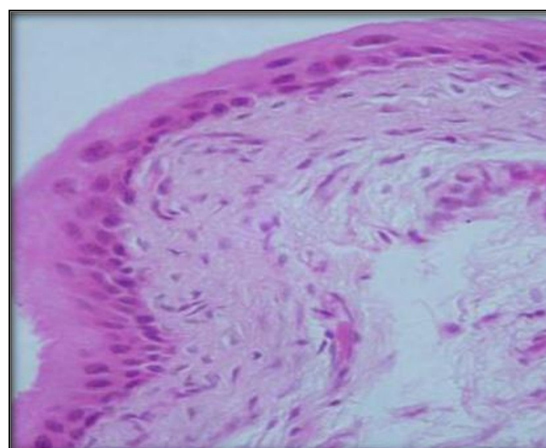

Figure 2: Urinary Bladder showing urothelium with its wall (Control, H&E X400)

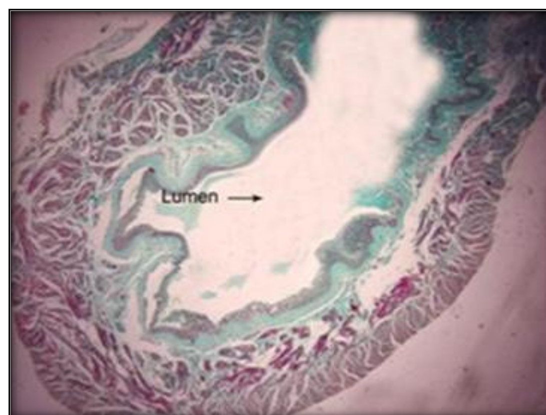

Figure 3: Photomicrograph showing a panoramic view of cut section of urinary bladder [Masson's trichrome x 40, Experimental group]

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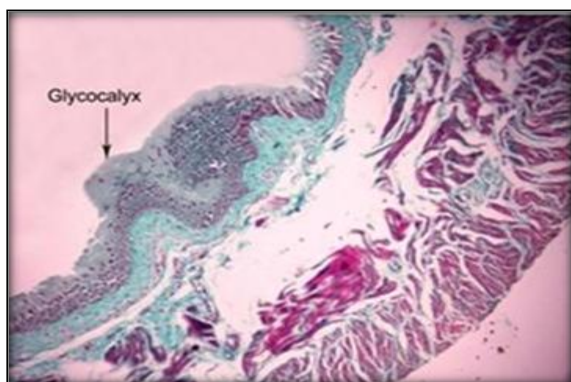

Figure 4: Photomicrograph showing thickened epithelium (cell layers as well as glycocalyx) of bladder [Masson's trichrome x 400, Experimental group]

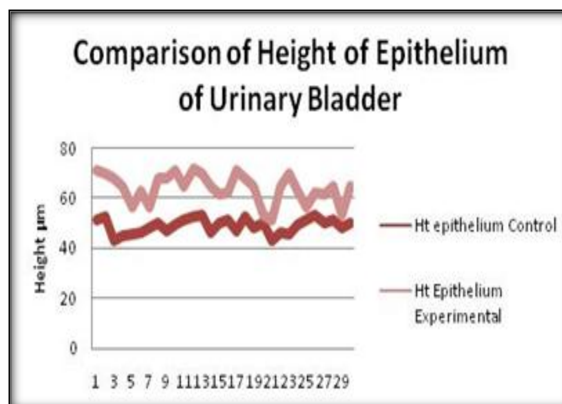

Figure 5: Diagram showing difference in heights of transitional epithelium of bladder in two group of mice

Table 1: Difference in body weight (b.w.) of two groups of animals.

Time of Weight recorded	Mean B.W. (control)	Mean B.W. (experimental)	'z' value
At beginning of 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 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 'P' value significant(P<0.05)

Table 3: Comparison of Mean number of cell layers of epithelium of 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 'p' value highly significant(p<0.01)

DISCUSSION

As far as human consumption of Aspartame is concerned, we mostly consume it orally in day to day life. To have a correlation of the possible ill effects of Aspartame in other mammalian species, we preferred study in adult Swiss albino mice by administering Aspartame orally. In some other studies researchers administered aspartame orally for demonstration of structural changes in urinary system of rats and mice respectively[5,6] According to WHO guidelines the safe limit for daily intake of aspartame for human, is below 60 mg per kilogram body weight per day while, dose free of toxicity in rat, as per WHO food additive series- 16 has been worked out to be 4g/kg body weight (i.e. 4mg/g b.w.).[7] We administered 100µg/g (equivalent to 100mg/kg) aspartame orally, much below toxic dose for small laboratory mammals. The dose of Aspartame given to small laboratory mammals in different studies markedly differed such as 5 to 20 mg/kg in Swiss albino mice for the demonstration of structural changes in kidney of mice;[6] 2.5% and 5% solutions of aspartame in drinking water in rats; 3.5, 35 and 350 mg/kg b.w. aspartame orally for demonstration of cytogenetic effects in mice.[8]

Aspartame preparation to be administered for animal experimentation was either saline solution,[12] or aqueous solution in distilled water.[13] We have used aqueous solution of aspartame as a vehicle for oral administration. In the present study we have emphasized more on gross and micro structural changes rather than biochemical changes which were beyond the scope of present study. Iman et al and others.[8-10] have also demonstrated structural changes only. Kithiri et al demonstrated structural changes in urinary system of rats along with urinalysis and blood biochemistry are shown.[5] Weight of mice has declined in experimental group of animals as shown in [Table 1]. Abdallah et al (1989) suggested that the reduction in weight is due to loss of appetite as a 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 urinary bladder in present study were pertaining to the urothelium only. We found several fold increase in the thickness of urothelium of 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 that transitional cell hyperplasia in 12% male rats and 8% female rats following aspartame treatment with 5% solutions of monosodium aspartame (MSA).[5] They also found this feature in 24% male and 25% female rats in renal

pelvis. They stated the epithelial proliferation was an effect of stimulation of the epidermal growth factor (EGF) which was further depended on the pH of the urine and presence of sodium salts. Since aspartame is principally eliminated by urinary route it probably affected urothelium. In the present study there was no alteration in 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 towards pre-neoplastic change in the form of polyps, dysplasia or metaplasia.

CONCLUSION

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

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