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Melamine Facilitates The Duodenal Contraction By Augmenting The Cholinergic Neuronal Pathway In Rat

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Abstract:

Now a days food adulterant is commonly used in food industry because of excessive production and preservation of food. Melamine is a food adulterant which has several industrial uses. There are various toxicological reports about the health effect of melamine on various organ of experimental and human system. There is a knowledge gap about the toxic effect of melamine on digestive system or duodenal contraction. The objective of our present study was to examine the probable toxic effect of melamine on contraction of duodenum. We used here male albino rat for our experiment for 5% and 10% of LD_{50} doses of melamine and 28 days treatment duration. We observed from our study that both frequency of contraction and force of contraction duodenum were increased in both the experimental doses compared to the duodenal contraction of control rat. We also observed significant degenerative changes in the cell layers of duodenum. From this results we may concluded that melamine facilitate the duodenal contraction by augmenting the cholinergic neuronal pathway.

Keywords: Melamine, duodenal contraction, cholinergic pathway, muscle layer, albino rat.

Introduction

Now a day, in the food industry, degrading the quality of food by adding various food adulterants components to the food for preservation and appearance for the extra demand and business profit. Since food adulteration is a huge concern in the worldwide. Approximately 57% of people suffered health problems due to ingestion of adulterated and contaminated food and annually 22% of foods are manually adulterated in world (1). Melamine, also called cyanuramide or triaminotriazine, a colorless crystalline substance belonging to the family of heterocyclic organic compounds. Because of its arrangement, it encloses 66% nitrogen in its atom. Melamine was first synthesized by the German chemist Justus von Liebig in 1834(2, 3). Melamine used as a binding agent in industrial purpose and it also used as a flame

retardant, and as a polymer in the construction of cooking utensils, dishware and plastics. It is a high nitrogenous compound so it mainly used in different nutritional substances for raises their protein levels. Melamine foam acts as a good sound insulator and also used in making plywood's. According to World Health Organization (4) and Food and Drug Administration (5), the standard acceptable label of melamine per day in body weight is 0.5mg/kgbw/day to 0.2mg/kgbw/day and from 0.63mg/kgbw/day to 0.063mg/kgbw/day (6). When the normal range exceeds, it shows the toxicological effects in the body. In China (2008), More than 290,000 people (most of them infant children) were poisoned, among 54000 were hospitalized and at least six babies are confirmed to have died from ingesting the melamine contaminated infant milk powder (7, 8). Consumption of melamine contaminated milk can causes various problems are observed in children, elderly, pregnant women and compromised persons. Chronic intake of melamine contaminated food; various clinical symptoms appeared in the body such as heart problem, cancer, kidney stones, and even death. There are several health problem of melamine exposure on excretory system (9), central nervous system (10, 11), reproductive system (12, 13), cardiovascular (14) and immune system (15, 16) are reported by various research works. But, there is knowledge gap of effect of melamine on the gastrointestinal system. So, we are reported here the adverse effect of melamine on duodenal contraction in rat model through experimental approach.

Materials and Methods

Reagents and Chemicals

All the reagents used were of analytical grade. Melamine (≤99%) was purchased from Sigma-Aldrich, USA. Sodium chloride (NaCl), Potassium chloride (KCl), Magnesium chloride ($MgCl_2$), Calcium chloride ($CaCl_2$), Sodium bicarbonate ($NaHCO_3$), Sodium dihydrogen phosphate (NaH_2PO_4), Dextrose, Eosin and Hematoxylin were procured from EMerck, India and SRL, India respectively.

Animal handling and care

Studies were performed on 3-4 months old albino rats of Charles Foster strain weighing about 90-100 gm. Animals were maintained in Departmental Animal House at Physiology Department as per national guidelines. The Animals were kept in equal light-dark cycle (12L: 12D) at a room temperature of 25ºC±2ºC and fed standard laboratory chow and water *ad libitum*. The animals were sacrificed by cervical dislocation on the 24th hour after the completion of last dosage.

Experimental set up

After acclimatization in the laboratory environment, the animals were randomly distributed into three groups (each group contains seven animals) for chronic melamine exposure. The different effective dosages of Melamine were selected in this study according to the graded percentage of LD_{50} value of Melamine in rat model (5).

Experimental grouping	Treatment doses	Treatment durations
Control	Received distilled water	28 days by oral gavage.
Treated I	Received 5% of LD_{50} of Melamine. LD_{50} of Melamine: 3850mg/kg/BW (rat, oral).	28 days by oral gavage.

Treated II	Received 10% of LD_{50} of Melamine LD_{50} of Melamine: 3850mg/kg/BW (rat, oral).	28 days by oral gavage.

Measurement of body weight and organ weight

The body weight of the rats was measured from the first day of the treatment up to the last day of the treatment period in every ten alternate days. The weight of rats taken on the day of the application of first dose was considered as the initial body weight, and the body weight taken on the day of sacrifice was considered as the final body weight (17). The body weight and organ weight of intestine of each rat was assessed using a sensitive balance (Wensar-Electronic balance, India).

Recording of duodenal contraction in single dose Ex Vivo Experiment

After overnight fasting, each rat was sacrificed by cervical dislocation. The abdomen of the sacrificed rat was then opened immediately and the duodenum was removed by transverse incision was placed longitudinally in 40 ml organ bath of Dale's apparatus (Recording has been taken through Kymograph instrument) containing Tyrode's solution consisting of 8.0 g/l NaCl, 0.2 g/l KCl, 0.2 g/l $CaCl_2$, 0.1 g/l $MgCl_2$, 1.0 g/l $NaHCO_3$, 0.05 g/l NaH_2PO_4 and 1.0 g/l glucose (pH-7.4). The temperature of the bath was maintained within a range of 37±0.5ºC and continuously bubbled with 95% O_2 and 0.5% CO_2 (18, 19).

Histological staining study

NBF fixed and paraffin impregnated uterine tissue sections were stained with hematoxylin-eosin stain according to the method of Bancroft et al, 2002 (20) with slight modifications. Images were observed under the microscope. Images were obtained by digital Camera fitted with light microscope.

Statistical Analysis

All the data obtained from this study were expressed as mean ± SEM. Statistical comparisons between the values obtained in control and in treated rats were evaluated by paired Student's t test or analysis of variance (ANOVA) whichever is applicable. $p \leq 0.05$ was considered as significant.

Results and Discussion

Effect of melamine on the mean body weight and organ weight of intestine

It has been observed from the Table 1, that the mean body weight of melamine treated groups of rats was increases significantly in all the treated groups in a dose dependent manner compared to control groups of rats (Table 1). It has also been seen that the absolute organ weights of intestine increases significantly in Melamine treated groups of rats in comparison with control group of rats (Figure 1).

Treatment grouping	Mean body weight (gm)	
	Initial body weight (gm)	Final body weight (gm)
Control	**90±1.58**	**100.4±2.18**
Treated I	**90±2.65**	**116±2.44 ****
Treated II	**90±1.33**	**129±4.13 ****

Table- 1: *Showing the body weight of Melamine treated and control rat groups. Values are represented as mean±SEM (n=7), *p<0.05 vs. control.*

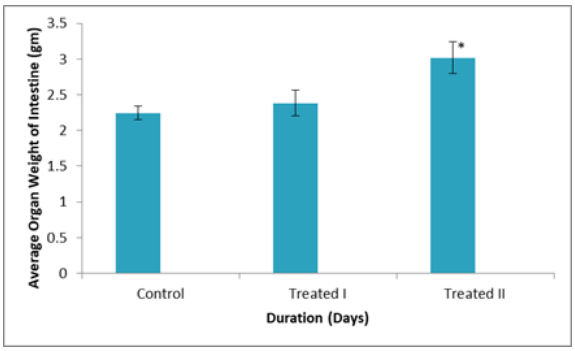

Figure 1: *Bar diagram represent the changes of organ weight of intestine of melamine treated groups of rat and control group of rats. Values are represented as mean±SEM (n=7), *p<0.05 vs. control.*

Effect of melamine on duodenal contraction in rat:

It is clearly seen that the height of contraction (amplitude) and force of contraction (frequency) has been increases significantly compared to height of contraction (amplitude) and force of contraction (frequency) of control duodenal movement (Figure 2, 3 and 4).

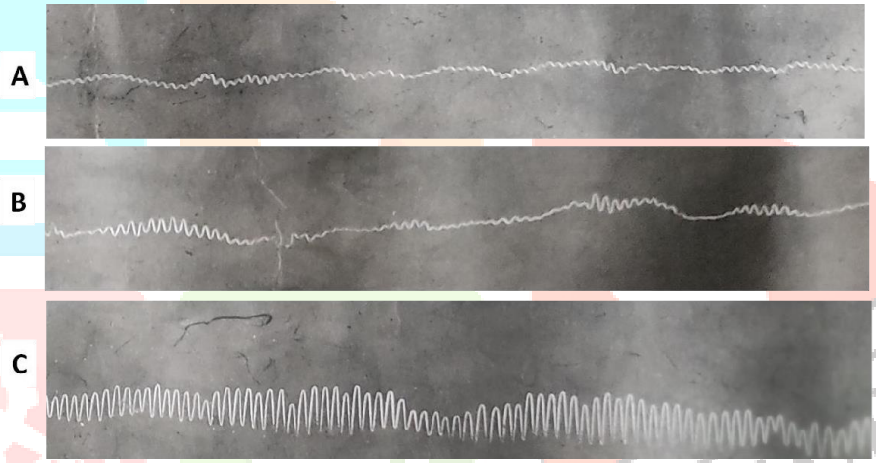

Figure 2: *Representative kymographic records of the isolated duodenal contraction of control (A) and melamine treated (B: Treated I and C: Treated II) groups of rats ex vivo for 28 days treatment durations.*

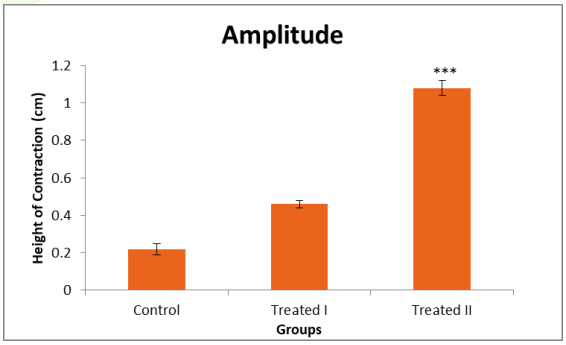

Figure 3: *Showing the changes in the amplitude of isolated duodenal contraction ex vivo of rats for 28 days treatment durations compared to amplitude of contraction of control rats. Values are as mean ±SEM (n=5),*p<0.05 Vs. Control.*

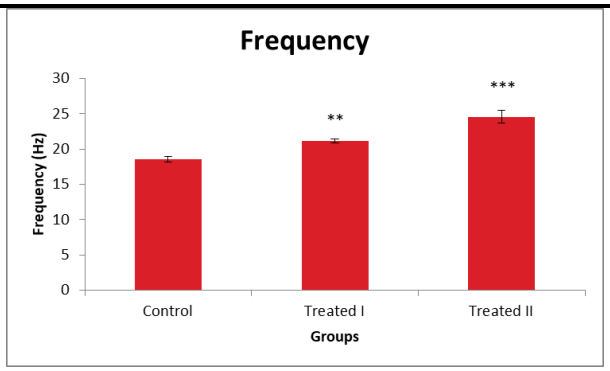

Figure 4: *Showing the changes in the frequency of isolated duodenal contraction ex vivo of rats for 28 days treatment durations compared to frequency of contraction of control rats. Values are as mean ±SEM (n=5),*p<0.05 Vs. Control.*

Effect of Melamine on histological change of duodenum:

Histologically there are four layers in the wall structure of duodenum (From innermost to the outermost layer, these are the mucosa, submucosa, muscularis, and serosa layers). We observed a significant degenerative change of the wall structure of duodenum in a dose dependent manner in melamine treated groups of rats compared to control rats (Figure 4).

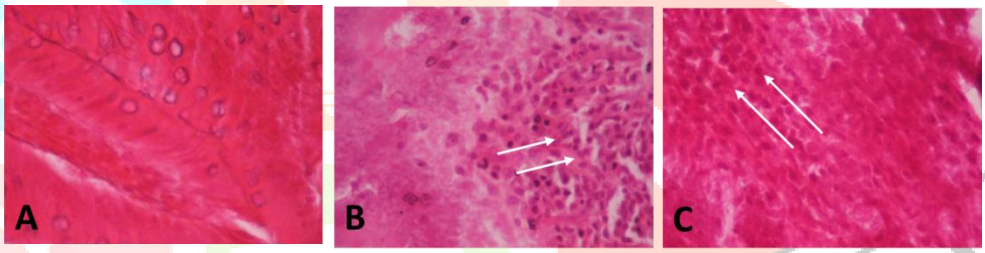

Figure 5: *Microphotographs of paraffin fixed transverse sections of intestine stained with hematoxyline and eosin showing the morphological alterations of intestine in melamine treated and control groups of rats of 28 days treatment durations. Arrow head indicates the degenerative alteration compared to control.*

Discussion

Adulteration in food is burning issue in different countries in India and Worldwide. We know that the melamine exposure in china by which infant, children and adults are affected with renal disorder, urinary stone (21). Even after this heath related problem arises the use of melamine has increased gradually in food industry (22-26) Although it was initially used in pet food in limited quantities, but now it is widely used to increase the protein level in milk as a result various harmful effects on our body. Melamine is a nitrogen rich, colorless, crystalline compound. Due to the high nitrogen contains compound, it mainly used in different nutritional substances for raises their protein levels.

The objective of the study was to examine the probable toxic effects of melamine on the duodenal contraction. In our study, the mean body weight of melamine exposed groups of rat increased significantly in a dose dependent manner compared to control groups of rats (Table 1). The absolute organ weight of intestine has been increased significantly in melamine exposed groups of rats compared to control groups of rats (Figure 1). This result suggests that melamine increases the body weight probably by impairing the set-point homeostatic mechanism for the body weight and organ weights of

intestine. We also observed from our study that melamine produced a significant potentiation of amplitude and frequency of the contraction of duodenum recorded ex vivo dose dependent manner in 28 days treatment durations (Figure 2, 3 and 4). This result suggests that melamine potentiate the duodenal smooth muscle contraction probably by augmenting the cholinergic neurotransmission from facilitatory cholinergic autonomic efferents to the smooth muscle. We also observed degenerative histological changes in the wall structure of the duodenum (Figure 5). Considering the entire results, we may conclude that melamine potentiates the amplitude and frequency of duodenal smooth muscles contraction probably by augmenting the cholinergic transmission in synapse en-passant junctions.

Conclusion

From our results it may be concluded that melamine facilitates the duodenal contraction by augmenting the cholinergic neuronal pathway in rat.

Conflict of Interest

There is no conflict of interest.

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