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Assessment of the Effect of Melamine on Some Biochemical Parameters in Adult Albino Rat

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Abstract: ***Melamine is an organic compound with the formula is $C_3H_6N_6$. Melamine is sometimes legally added to food product in order to increase the apparent protein content. The adverse effect of melamine on some animal model has been reported but the effects of melamine on biochemical parameters have not been reported. So, in this study we report here the adverse effect of melamine on some biochemical parameters in rat model. The dosages of melamine selected according to the LD_{50}. There are two experimental groups for 28 days exposure duration. We observed from our experimental study that melamine increases the level of total protein, SGOT and SGPT and no significant alteration of serum ALP level were observed compared to control. Histologically, melamine causes significant degenerative alteration in the wall structure of heart and liver. From this study, it may be concluded that melamine produces hazardous adverse effect in different blood parameters.***

Keywords: Melamine, Serum glutamic oxaloacetic transaminase, Serum glutamate pyruvate transaminase, Alkaline phosphatase, Rat

1. Introduction

Melamine is a nitrogen - rich heterocyclic triazine with the chemical formula $C_3H_6N_6$ and chemical structure 1, 3, 5 - triazine - 2, 4, 6 - triamine. Melamine has many uses in industrial applications, such as in fire retardant products, adhesives for woods, molding compounds and fertilizer urea mixtures [(1).] Melamine resin or melamine formaldehyde is widely used in the manufacturing of plastic - ware because it is heat - resistant and durable [(2).] However, melamine - ware, though non - flammable can decompose under extreme heat; thus, at temperature above 300C, it is not suitable for use in the conventional ovens [(3).] Over the years, many issues have been raised on the migration of chemical substances into foods during packaging, and chemical migration from food contact materials and utensil [(4).] Currently, there is an increasing public awareness of melamine contamination in foods since the 2007 incident where melamine - tainted pet food caused renal failure and death in cats and dogs in the United States and Europe [(5).] Another major incident in China in 2008, causing death and inflicting serious kidney damage in infants and children who consumed melamine - tainted milk formulae, has increased fear and anxiety among the public [(6).] Melamine toxicity has been reported in animal studies and chronic exposure may cause reproductive damage, excretory problem, cardiovascular and gastro intestinal damage [(7 - 11).] So, we are reported here the adverse effect of melamine on some biochemical parameters in rat model.

2. Materials and Methods

2.1 Reagents and Chemicals

All common chemicals used for the study were of analytical grade. Melamine (≤99%) was purchased from Sigma - Aldrich Co (USA). Eosin and Hematoxylin, Paraffin wax (58˚ - 60˚C) and Xylene etc. were procured from E. Merck, India.

2.2 Animal handling and care

Studies were performed on 3 - 4 months old female virgin albino rats of Charles Foster strain weighing about 90 - 100 gm. Animals were maintained in Animal House at Physiology Department as per national guidelines. The Animals were kept in equal light - dark cycle (12L: 12D) at a room temperature and fed standard laboratory chow and water ad libitum.

2.3 Animal grouping and treatment schedule

After one week of acclimatization to 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 [(12 - 15).] Control: received distilled water, Treated I: received 5% of LD_{50} of melamine (3850mg/kg/BW, rat, oral), Treated II: received 10% of LD_{50} of melamine (3850mg/kg/BW, rat, oral), for 28 days exposure durations by oral gavage route.

2.4 Sample collection and preparation

After the completion of the treatment duration, the animals were sacrificed by cervical dislocation on the 24th hour after the application of last dose. For the biochemical study serum was prepared following the centrifugation of whole blood at 4000 rpm for 10 minutes and kept in - 20˚C.

2.5 Biochemical study

Serum protein contents were estimated by the method of Lowery et. al, 1951 [(16).] Serum ALP (Alkaline phosphatase), SGOT (Serum glutamic oxaloacetic transaminase) and SGPT (Serum glutamate pyruvate transaminase) levels were estimated by using commercial kits (ALP by Kind & king's Assay; SGOT and SGPT by 2, 4 DNPH Assay respectively) according to the kit instructions.

2.6 Histological study

Neutral buffered formalin (NBF) fixed and paraffin impregnated uterine tissue sections were stained with hematoxylin - eosin stain according to the method of Bancroft et al., 2002 with slight modifications [17]. Briefly, 5µm paraffin section of uterine tissue was kept sequentially in xylene and graded ethanol and stained with hematoxylin for two minutes. After removing the excess color the slide was counterstained with eosin for 30 seconds and then the stained slides were dehydrated with graded ethanol, cleared with xylene and mounted with DPX and were observed under the microscope (100X magnification). Images were obtained by digital Camera fitted with light microscope.

2.7 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.

3. Results

3.1 Effect of Melamine on Total Protein Level

We found significant increase in the concentration of serum total protein in a dose dependent manner in treated rats compared to control groups of rats (Figure 2).

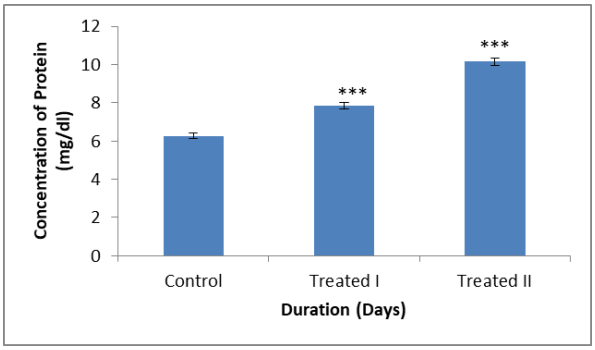

Figure 2: Effect of Melamine on Total protein level of rat. The values represented as mean ± SEM (n=7) for statistical analysis***p<0.001 Vs Control

3.2 Effect of Melamine on SGOT Level

We found significant increase in the level of SGOT in a dose dependent manner in melamine treated groups of rats compared to control groups of rats (Figure 3).

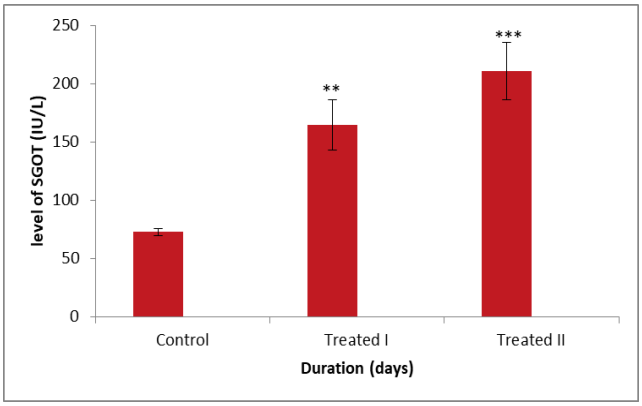

Figure 3: Effect of Melamine on SGOT level of rat. The values represented as mean ±SEM (n=7) for statistical analysis *p<0.05 Vs Control

3.3 Effect of Melamine on SGPT Level

We found significant increase in the level of SGPT in a dose dependent manner in melamine treated groups of rats compared to control groups of rats (Figure 4).

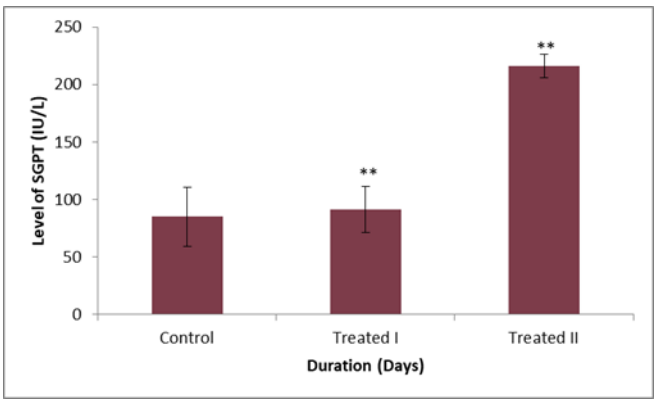

Figure 4: Effect of Melamine on SGPT level of rat. The values represented as mean ±SEM (n=7) for statistical analysis *p<0.05Vs Control.

3.4 Effect of Melamine on ALP Level

No significant alteration was observed in the level of ALP in melamine exposed groups of rats compared to control (Figure 5).

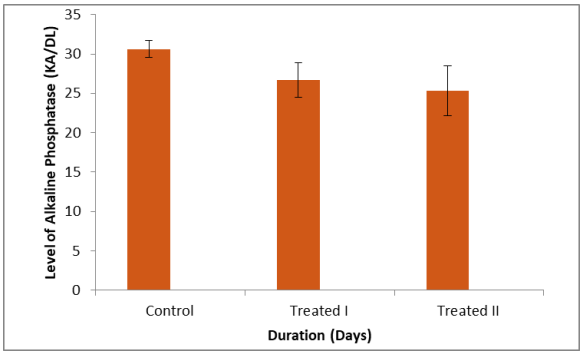

Figure 5: Effect of Melamine on ALP level of rat. The values represented as mean ±SEM (n=7) for statistical analysis.

3.5 Effect of Melamine on histological change in Heart and Liver

To study the melamine induced impairment, we observed the histological characteristics of heart and liver of melamine exposed and control groups of rats. We observed significant degenerative alterations in the wall structure of heart of melamine exposed group of rats (B and C) compared to control (A) group of rat. We also observed from our result that significant degenerative alterations in the wall structure of liver of melamine exposed group of rats (E and F) compared to control (D) group of rat (Figure 6).

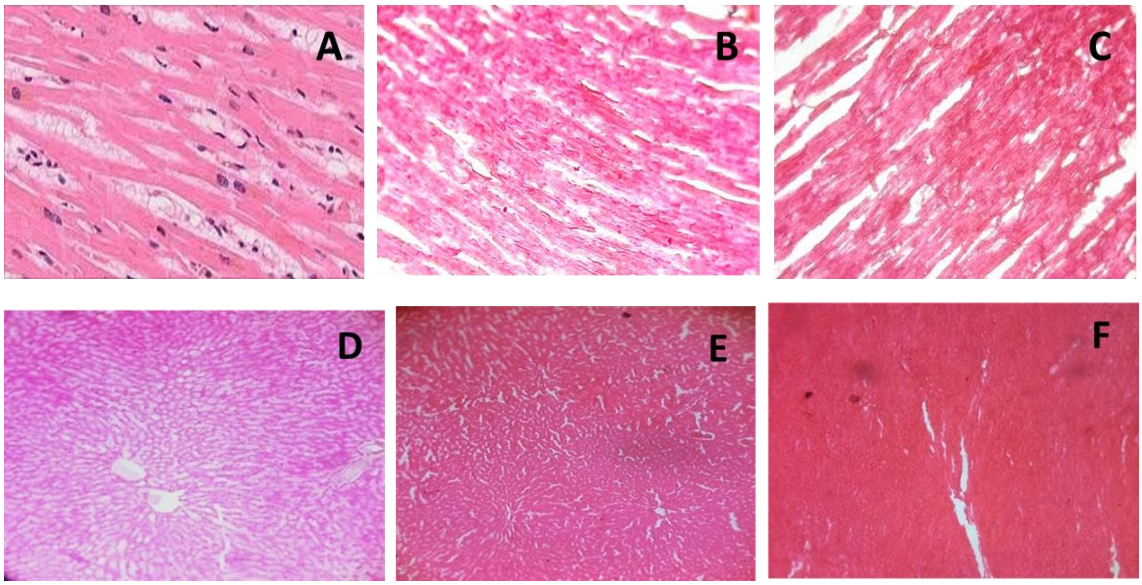

Figure 6: Microphotographs of paraffin fixed transverse sections of the heart and liver stained with hematoxyline and eosin showing the morphological alterations in melamine exposed and control groups of rats of 28 days exposure durations (10X magnification). A: Control (heart); B: T1 (heart); C: T2 (heart); D: Control (liver); E: T1 (liver); F: T2 (liver).

4. Discussion

Melamine is added into milk to increase the protein content at less cost for extra profit at business. It is a nitrogen - rich compound which has been used for producing plastics, adhesives, laminates, paints, permanent - press fabrics, flame retardants, textile finishes, tarnish inhibitors, paper coatings and fertilizer mixtures ([17 - 20]). Our experimental study we observed that melamine significantly increases the level of serum total protein, SGOT and SGPT when compared to control. The liver is a vital organ in the human body, and is responsible for various metabolic functions in the body. One of the most important roles of the liver is to detoxify the blood by processing different waste products in the hemoglobin. To conduct all of these functions properly, the liver produces and secretes several types of enzymes for optimal regulation. SGOT and SGPT are two of the most common enzymes produced by the liver which are acts as biomarker for liver. In our study the level of SGOT and SGPT were elevated in the melamine treated groups of rats which may cause severe liver damage in the experimental groups. In the histological study we observed significant degenerative changes in the wall structure of lever and heart compared to control groups of rats. In over - exciting the brain cells with melamine on a regular basis has a devastating effect on the nervous system which directly alters the level of above discussed biochemical parameters.

5. Conclusion

In conclusion, it may be suggested that melamine impairs the functions of liver and heart in rat probably by altering the biochemical parameters.

Conflict of Interest

There is no conflict of interest.

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