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In vivo analysis of toxic effect of hydrose used in food preparations in Bangladesh

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PEER REVIEW

Peer reviewer

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Comments

The research work is exclusively important regarding public health and points out that using hydrose in food is very harmful for living being. The researchers examined the toxic effects of hydrose from food by observing biochemical parameters like serum urea, creatinine as well as histopathology. Results indicates that hydores in molasses could induce kidney failure.

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ABSTRACT

Objective: To evaluate the toxic effect of hydrose used in the molasses preparation in Bangladesh.

Methods: Molasses were collected from open markets in different parts of Bangladesh. The presence of hydrose in selected molasses was detected using commercial kit. To evaluate the toxic effect of hydrose, Swiss albino male mice were divided into four groups. Group I was used as control, while Groups II, III and IV received hydrose mixing food (5, 10 and 25 g/kg food), respectively, and these supplementations were continued to the end of the study (16 weeks). Blood was collected from thoracic arteries of the mice under ether anesthesia and then organs were taken. To determine the effect of hydrose on host, blood indices related to liver, heart and kidney dysfunctions were measured.

Results: Creatinine and urea levels were significantly ($P<0.05$) increased in a dose dependent manner in hydrose treated mice, whereas calcium level was significantly decreased in hydrose exposed mice compared to control mice. Histological study of kidney showed the glomeruler inflammation, increased diameter of renal glomeruli and enlargement of proximal tubular lumen of kidneys of mice exposed to hydrose compared to that of control animals.

Conclusions: The results of this study indicated that use of hydrose in molasses and other food preparations in Bangladesh may cause kidney impairment.

KEYWORDS
Hydrose, Molasses, Serum indices

1. Introduction

Adulteration of toxic chemicals in food harmful to health has reached an epidemic proportion in Bangladesh and these have dubbed it as the silent killer. Contamination of foods with toxic chemicals poses a serious threat to public health, especially in a country like Bangladesh as a result of poor health literacy and low level of awareness. Immediate effect of ingestion of such foods may cause life threatening diarrhoeal disease[1,2]. In the long run, these chemicals in food adversely affect vital organs such as liver and kidney resulting in organ failure and/or cancer. There is no database in the country about the above issue, but the recent surge in liver and kidney failure patients indicates the deteriorating situation. Food contamination and consumers exposure to food hazards have major implication on the food security and consumers heath in Bangladesh. Low level of awareness and weakness on existing food

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laws and regulation are also contributing to aggravate the country's food safety situation[3]. Harmful chemicals are allegedly being used indiscriminately in preparing molasses. It is alleged that the producers of molasses use hydrose ($Na_2S_2O_4$) in molasses to make it whiter and attractive so that they can get high price of the molasses. We visited several sugarcane producing areas of Bangladesh and made interviews with some of the molasses producers. According to them hydrose is used to make the molasses whiter.

Sodium hydrosulfite-sodium dithionite-hydrose ($Na_2S_2O_4$) is sulfur containing inorganic white crystalline powder with a weak sulfurous odor and also known as hydrose. Hydrose is used as reducing agent for the reduction of vat dyes and sulfur containing dyes[4]. Sodium hydrosulfite can also be used for water treatment, gas purification, cleaning and stripping. It can also be used in industrial processes as a sulfonating agent or a sodium ion source. In addition to the textile industry, this compound is also used in industries concerned with leather, food, polymers, photography, and many others.

Hydrose is a potent reducing agent has been used as an oxygen scavenger and also used to create hypoxia[5], in experiments studying the mechanism of hypoxic pulmonary vasoconstriction in cultured pulmonary artery myocytes[6,7], the sensing of oxygen in the carotid body[8,9], and the effects of anoxia on pH in cardiac cells[10]. It also deoxygenates hemoglobin and create hypoxia *in vitro*[11,12]. It is reported that the reduction of O_2 by hydrose not only lowers Po_2 but also yields hydrogen peroxide and a highly reactive SO_2^- radical[12]. Dithionite is a powerful nonspecific reducing agent that causes anoxia by reducing oxygen with the associated generation of large amounts of activated oxygen species (including superoxide anion)[5], and it can also reduce and thereby inactivate important enzymes, such as glutathione reductase[5].

Hydrose is indiscriminately used in Bangladesh for food preparations, especially in molasses. In country side as well as in city areas of Bangladesh, molasses is commonly used to make traditional cakes, payes *etc*. and these foods are being mixed with hydrose indirectly via molasses. In molasses preparation, hydrose is being used as a cleaning agent to brighten the color of molasses. There have two types of molasses available in Bangladesh. One type of molasses is made from sugarcane juice and another one from date tree's juice. Currently hydrose is massively used to manufacture both types of molasses. It is very difficult to find out either type of these sweeteners without such harmful chemical. Molasses manufacturers believe that this chemical is not harmful and no one ever hear that it is toxic and harmful. Unconsciously this toxic chemical is invaded in our food chain as well as in our body. Therefore, this study was undertaken to evaluate the effect of hydrose used in different foods preparation in Bangladesh through mice model.

2. Materials and methods

2.1. Detection of hydrose from molasses samples

Molasses were collected from different markets of Rajshahi, Sirajganj and Tangail districts. After collection, all the samples were kept separately in airtight zipper bags and stored at 4 °C.

Molasses solutions (20%) of different samples were prepared using distilled water. Hydrose present in foods was detected as described in the protocol provided by Global Complex Co., Ltd, Thailand. We slightly modified the protocol to detect the minor amount of hydrose present in molasses of Bangladesh. The presence of hydrose in the molasses was detected by addition of 3% cupric sulphate solution. The principle of the method is based on the formation of gray colored precipitation of CuO.

$$2CuSO_4 + Na_2S_2O_4 = 2CuO\downarrow + Na_2S_2O_6 + 2SO_2$$

Briefly, 1 mL of 3% cupric sulphate solution was added in 5 mL of 20% molasses solution and then stirred 2-3 min and kept it for 2-4 h. After that gray color ppt was appeared at the bottom of the test tube if the sample contained hydrose. For control 1 mL of 3% cupric sulphate solution was added in 5 mL of 0.25% hydrose solution and then stirred for 2-3 min and kept it for 2-4 h and clear ppt was observed.

2.2. Mice Experiment

Adult healthy (four weeks of age) Swiss albino male mice with average body weight of 20-22 g were purchased from International Centre for Diarrhoeal Disease Research, Bangladesh. Mice were housed in polycarbonate cages with steel wire tops and wood-cube bedding. After acclimatization to laboratory conditions for 7 d, the mice were randomly divided into four groups of six animals each. Group I consumed normal food while Group II, Group III and Group IV received normal food containing 5 g, 10 g, 25 g hydrose per kilogram food, respectively. Normal and hydrose containing food were provided to four different groups of mice for 16 weeks before sacrifice. Mice were maintained with 12 h: 12 h dark: light cycle with available supply of distilled water and food.

2.2.1. Ethical Permission

Ethical permission of this study was obtained from The Institute of Biological Sciences, University of Rajshahi, Bangladesh (42/320-IAMEBBC/IBSc).

2.3. Blood collection and preparation of serum from experimental mice

Blood specimens were collected from thoracic arteries of mice after anesthetization with diethyl ether. For coagulation,

blood was kept about 30 min at room temperature. After centrifugation at 4000 r/min for 15 min at 4 ℃, the serum was drawn off and stored at −80 ℃ until the experiments were performed.

2.4. Laboratory examination

Serum urea, creatinine and calcium were measured by commercially available kits from Human Diagnostic, Germany according to the manufacture's protocol with an analyzer (Humalyzer 3000, USA). All samples were analyzed in duplicate, and the mean values were taken.

2.5. Histological studies of kidney

Histopathology of kidney was performed to observe any changes in the cellular structures (degradation and regeneration) of the mice received hydrose at a dose of 5, 10 and 25 g/kg for 16 weeks with respect to control mice. After blood collection, kidneys from each mouse of four different groups were collected very carefully. Then the organs were stored in 10% formalin Buffer solution separately under room temperature for further study. For histological study kidney tissues were dehydrated through grades of alcohol. After that, it was cleared in xylene and finally embedded in paraffin wax. Using a rotary microtome, specimens were sectioned at 5 μm and sections were mounted on clean slides and stained with haematoxylin and eosin.

2.6. Statistical analysis

Statistical analyses were performed with SPSS for windows, version 15.0 (SPSS, Chicago, IL). Differences between the serum indices of different groups of mice were analyzed by using t-test. Data are expressed as mean±SD. A value of $P<0.05$ was considered statistically significant.

3. Results

3.1. Hydrose detection in molasses samples

A total of 170 molasses samples (113 from Rajshahi, 29 from Sirajganj and 28 from Tangail districts of Bangladesh) were analyzed for the qualitative detection of hydrose. Out of 113 samples, 91 samples from Rajshahi district were found to contain hydrose. From this qualitative detection it showed that 81% molasses of Rajshahi district contained this chemical and only 19% samples were free from hydrose contamination. From Sirajganj district 22 samples (76%) were found as contaminated with hydrose. Seventeen molasses samples (61%) from Tangail district contained hydrose. Results revealed that most of the producer mixed hydrose during the production of molasses and consumers obtained contaminated molasses with toxic chemicals from different markets.

3.2. Result of mice experiment

Creatinine is clinically important for renal function and measuring serum creatinine is the most common indicator of renal condition[13,14]. Therefore, we measured the creatinine level of experimental mice and evaluated the effect of hydrose on this parameter. The serum creatinine levels (mean±SD) of four groups of experimental mice were (0.27±0.08), (0.35±0.05), (0.47±0.10) and (0.57±0.14) mg/dL in Group I, Group II, Group III and Group IV, respectively (Table 1). A significant ($P<0.05$) elevation of serum creatinine levels was observed in Group III as well as in Group IV compared to the control group (Group I).

Table 1

Serum creatinine, urea and calcium levels of the groups of experimental mice. mg/dL.

Serum indices	Experimental groups			
	Group I	Group II	Group III	Group IV
Creatinine	0.27±0.08	0.35±0.05	0.47±0.10[a]	0.57±0.14[a]
Urea	17.67±3.08	21.00±2.61	24.67±4.92[a]	27.17±3.43[a]
Calcium	14.18±2.56	13.74±1.94	13.38±2.55	9.42±1.75[a]

Values are expressed as mean±SD, $n=6$ for each group of mice. [a]: Significantly difference compared with Group I at $P<0.05$.

Elevated levels of serum urea have been reported to be associated with renal dysfunction and excessive protein catabolism[15,16]. The blood urea levels in the four groups of experimental mice were (17.67±3.08), (21.00±2.61), (24.67±4.92) and (27.17±3.43) mg/dL in Group I, Group II, Group III and Group IV, respectively. This result suggested the dose-dependent action of hydrose on the elevation of serum urea levels in Group II, III and IV compared to the control group (Group I) and the elevation was statistically significant ($P<0.05$) in Group III and IV compared to the control group.

Calcium plays a key role in a wide range of biologic functions and hypocalcemia as well as hypercalcemia are deleterious to kidney function[17]. Therefore, we were very much enthusiastic to see whether hydrose could alter the serum Calcium levels. Intriguingly, we observed that serum Calcium level decreased in groups exposed to hydrose compared with the control group. Blood calcium levels in the four groups of experimental mice were (14.18±2.56), (13.74±1.94), (13.38±2.55) and (9.42±1.75) mg/dL in Group I, II, III and IV, respectively. A significant ($P<0.05$) reduction of serum calcium level was observed in higher concentration shown in Group IV compared with control mice.

3.3. Histopathological examinations of mice kidney

Normal structure of the cortex and medulla was observed

in the kidney of control mice (Figure 1A). An enlargement of proximal tubular lumen was observed in the hydrose treated mice. The diameter of renal glomeruli was increased and the glomeruler inflammation as well as leukocyte infiltration were observed in hydrose treated mice compared with control mice (Figure 1B, 1C, 1D). It has been shown that the histological changes in kidney cortex and medulla of Group III and IV are more serious than those observed in Group II. This indicated that hydrose has a toxic effect to impair kidney function.

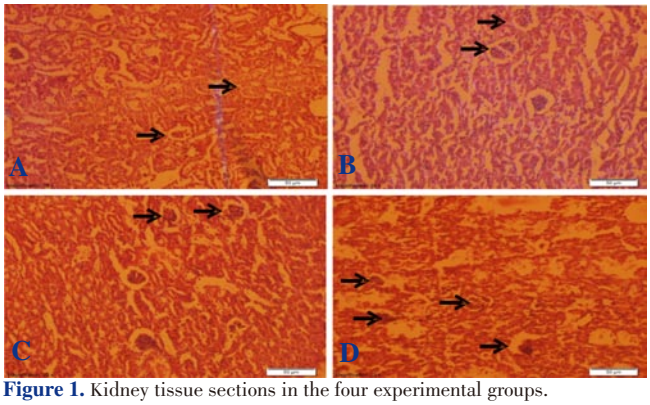

Figure 1. Kidney tissue sections in the four experimental groups.
A: Group I; B: Group II; C: Group III; D: Group IV. Arrow indicates glomerular inflammation. The section was stained with haematoxylin and eosin and examined by light microscopy.

4. Discussion

Food safety incidents are frequently reported and have a serious impact on public health in Bangladesh. General awareness about food safety and food hygiene in Bangladesh is very low and there is limited evidence or data on the dimensions of the food safety challenge faced by the country. Adulteration of food with toxic chemicals harmful to health has reached an epidemic proportion in Bangladesh. Carbide, formalin, textile colors, artificial sweeteners, dichlorodiphen yltrichloroethane, urea, hydrose and *etc*. are randomly used for this purpose[1,2].

In this study, we have focused on a chemical what is indiscriminately used in molasses preparation. Hydrose is a crystalline powder, usually used as a whitening agent in textile industries worldwide. We found that this compound was mixed with molasses to brighten the color of molasses in Bangladesh. So people obtained hydrose through molasses ingestion. In the first step of this study, the molasses samples collected from different markets of Rajshahi, Sirajganj and Tangail districts in Bangladesh have been analyzed and found that 81%, 76% and 61% molasses samples were mixed with hydrose of those districts. This result suggests that most of the producer mixed hydrose during the preparation of molasses and it is really difficult to get a chemical free from molasses in the markets of Bangladesh. There is no report about the toxicity and the presence of hydrose in food. Therefore this study tried to check its toxicity through mice model.

Several soluble enzymes, proteins or other metabolites of serum have been considered as indicators of cardiovascular diseases, hepatic and kidney dysfunctions. Pathogenic condition as well as organ dysfunction can be diagnosed by the alteration of serum indices. Measuring serum creatinine and urea are useful and inexpensive method of evaluating renal dysfunction and the increased level of blood urea nitrogen (BUN) is an integral indicator that reflects renal function[18]. Urea is a waste product formed from the breakdown of proteins and passed out in the urine. The blood urea becomes raised when the kidney tubules are prevented from removing urea and other waste products from blood. In this study, we found that the serum urea levels significantly ($P<0.05$) increased in mice treated with hydrose compared to control group that might be the indication of the adverse effects of hydrose on kidney. A high blood level of urea indicates that the kidneys may not be working properly[19]. Increased BUN is seen associated with kidney disease or failure, blockage of the urinary tract by a kidney stone, congestive heart failure, dehydration, fever, shock and bleeding in the digestive tract[20]. Creatinine is a breakdown product of creatine, usually excreted through urine and is clinically important for renal function test. Measuring serum creatinine levels is the most common indicator of renal function[13]. Mice treated with different concentration of hydrose caused the elevation of serum creatinine levels compared to control mice. The levels of creatinine in the mice of Groups III and IV were increased significantly ($P<0.05$) compared to the control mice. It has been reported that the increased level of serum creatinine might be due to reduce the ability of kidney to eliminate the toxic metabolic substances[21]. The diagnosis of renal failure is based typically on an elevation in the serum creatinine concentration which usually reflects a reduction in the glomerular filtration rate (GFR). However the elevation of the creatinine is not representative of a true reduction in GFR[22,23]. The GFR is considered as a best marker for renal function and renal function impairment are clinically silent and are diagnosed only by measuring GFR[24,25].

Chronic kidney disease is a common condition in which there is a loss of kidney function over time. It is associated with an increased risk of cardiovascular disease and chronic renal failure. Chronic hypercalcemia and acute hypocalcemia are both associated with increased mortality in male patients with moderate and advanced chronic kidney disease[26]. Reduction of calcium levels indicates a tendency to hypocalcemia in the mice exposed to hydrose. Serum calcium level is maintained within narrow range at least in part by actions of two calcium-regulating hormones, parathyroid hormone (PTH) and 1,25-dihydroxy vitamin D[27]. These two hormones increase serum calcium level through their actions on bone, kidney, intestine, and impaired

actions. Either of these is a major cause of hypocalcemia. Calcium and phosphate in the body react in opposite ways. As blood calcium levels rise, phosphate levels fall. A hormone called PTH regulates the levels of calcium and phosphorus in our blood[28]. The relation between calcium and phosphate may be disrupted by some diseases or infections. A high level of phosphate in the blood is usually caused by a kidney problem. The amount of phosphate in the blood affects the level of calcium[28]. In this study, we did not find any changes in serum phosphate levels of control and mice exposed to hydrose. Hypocalcemia can be divided into two categories with low serum phosphate level, and one changing from normal to elevated serum phosphate level[27]. A high serum creatinine level means kidney damage and high BUN usually means that kidney function is less than normal[29]. In this study, it has found that elevated serum creatinine and urea levels in the mice exposed to hydrose which indicated that kidneys may not work properly. The diameter of renal glomeruli was increased and the glomeruler inflammation as well as leucocyte infiltration were observed in mice exposed to hydrose compared with control mice. Histopathological study also reveals the abnormalities in kidney tissue of mice exposed to hydrose.

The present study reveals that the molasses samples available in the markets of Rajshahi, Sirajganj and Tangail districts are contaminated with hydrose. Through this mice model study, we have found that the blood indices related to kidney functions such as urea, creatinine levels are significantly ($P<0.05$) increased and calcium level significantly ($P<0.05$) decreased in hydrose treated mice. High levels of urea, creatinine and low level of serum calcium are associated with kidney dysfunction. Histological study of kidney also showed the increased diameter of renal glomeruli and the glomeruler inflammation as well as leucocyte infiltration in hydrose-treated mice. In this study, we have only detected hydrose in the molasses purchased from different markets of Rajshahi and its neighboring districts and studied the health effect of hydrose through mice model. Taking into account these results, it is found in this study that chronic hydrose administration through foods induces kidney dysfunction in mice.

Hydrose is available in the different markets of Bangladesh which is frequently mixed with molasses and subsequently molasses contained hydrose used to prepare some traditional foods in Bangladesh. In this study, we found the presence of high levels of hydrose in the molasses purchased from different parts of Bangladesh and evaluated their health effects through mice model approaches. Our present findings suggested that chronic hydrose administration through foods may cause kidney impairment. In addition, the abnormalities found in mice kidney tissues, further supported our notion. Moreover, decreased levels of serum calcium by hydrose may regulate PTH and vitamin D metabolism. In conclusion, further study should be taken to quantify the physiological toxic levels of hydrose in human.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

Hydrose, a chemical widely used in preparation of molasses in developing countries like Bangladesh. The principle underlying this research is to demonstrate the possible detrimental effects after using hydrose in food-stuffs.

Research frontiers

The present study demonstrated toxic effects of hydrose in experimental animal by studying biochemical parameters related to kidney and liver functions. They also examined the cellular architecture and tissue level damage induced by hydrose in mice.

Related reports

Toxicity of any chemical compounds or drugs was evaluated following the experimental design used in this study. Studying of liver and kidney functions is very important to judge the toxicity of xenobiotic in animal model because these substances are metabolized in liver and excreted by kidney.

Innovations and breakthroughs

Sodium hydrosulfite/sodium dithionite ($Na_2S_2O_4$) is also known as hydrose, is a toxic chemical and has harmful effects on living system especially in adulterated food. The authors showed the harmful effects on major organs of animal model.

Applications

The output from this research will make peoples more attentive about the usages of toxic substances in food items. The impact of this research may also contribute to improve public health and safety in general.

Peer review

The research work is exclusively important regarding public health and points out that using hydrose in food is very harmful for living being. The researchers examined the toxic effects of hydrose from food by observing biochemical parameters like serum urea, creatinine as well as histopathology. Results indicates that hydores in molasses could induce kidney failure.

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