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College of Pharmacy

COMPARATIVE STUDY OF HISTOPATHOLOGICAL EFFECTS OF ANTI FREEZING AGENT IN TOOTHPASTE ON PIGEON

Graduation Project

Submitted to the Council of College of Pharmacy in the University of Basra in Partial Fulfillment of the Requirement for the Degree of BSC in Pharmacy

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Abstract:

This study was carried out in the college of pharmacy /Basrah university to compare the histopathological effects of an anti-freezing agent polyethylene glycol (PEG) found in toothpaste on the kidney of pigeons, which were obtained (20) from Al Zubair, weights were (240-250) g.

The experiment was divided in to administering orally a specific amount of toothpaste containing the anti-freezing agent to a group of pigeons and observing the changes in the kidneys of the birds. Another group of pigeons were the control group with distal water.

Histopathological changes were observed in the kidneys section for pigeons that administered with toothpaste included glomeruli and tubular necrosis, swelling in endothelial layer of tubles, absent of glomeruli in some section accompanied by infiltration of inflammatory cells, congestion in the glomeruli, decrease in diameter lumen of tubules, absent of space of Bowman`s capsule, enlargement of glomeruli, absent of the histological structure characters of the glomeruli and tubules, enlargement of some tubules nuclei were detected in comparison with the control groups.

In conclusion, the results of this study suggest that the use of anti-freezing agent in toothpaste can have significant negative effects on the kidneys of animals if ingested. Therefore, it is important to limit exposure to such agents to decrease potential harm.

Introduction:

A toothpaste is a semi solid material for removing naturally occurring deposit from teeth, it's have several ingredients act as oxidation agents (1)

Toothpastes generally contain water (20–40%), Abrasives (50%), Fluoride (usually 1450ppm), Detergents and Humectants including glycerol, xylitol, sorbitol, polyethylene glycol, and propylene glycol. (2).

Polyrthylene glycol (PEG) have expanding range of consumer products that may contain 1,4-dioxane as a contaminant, no data describe the effects of exposure to 1,4-dioxane on children or immature animals. It is likely that children would show the same health effects as adults. (3).

Isolating and concentrating 1,4-dioxane from the commercial samples is critical to achieve resolution at the sub-ppm level and to minimize interference from contaminants present in the sample matrix. (4)

The FDA permits vacuum stripping as a method for the reduction of 1,4-dioxane from ethoxylated compounds, but this does not fully remove dioxane from the final product. (5) While, WHO clarified the 1,4-dioxane lethality due to an occupational exposure that lead to haemorrhagic nephritis, centrilobular liver necrosis, severe epigastric pain, convulsion and coma were found as the major effects depend on levels or length of exposure.

1,4-Dioxane has been considered as a carcinogen based on long-term animal experiments, in rats chronically exposed to 1,4-dioxane, damages were observed in the central nervous system, liver, and kidney. However, the in vivo molecular target of 1,4-dioxane and the action mechanism remain to be understood. (6 ,7)

The developed method was applied to several types of toothpaste, making identification and quantification of DEG and other polyethylene glycols (PEG) feasible with very little sample manipulation, as only extraction with water is required. (8)

REVIS et al, gives answer for why birds will be good choice for nephrotoxicity who strongly suggesting that the pigeon may be a useful animal model to study the yet unanswered question due to relationship of exposure via the drinking water to

tissue deposition and the relationship of kidney lead to histopathological changes in the pigeon kidney are comparable to those observed in human. (9).

However, several recent studies have raised concerns about safety issues related to renal impairment following PEG bowel preparation. A case reported port has raised the possible association of the use of PEG and acute renal failure. (10)

Materials and methods:

Pigeons (*Columba livia*) were buy from local market (18 pigeons) and separated for six group with three birds for each group.

In a 30-day study, pigeons received toothpaste solution in drinking water for five brands: Colgate, Dr. dabur kids, Dentakleen kid, Kodomo kid and Amber at concentrations up to 800 ppm as shown in below equilibrium as shown in Fig. (1) (11):

$$\text{PPM} = \frac{\text{mass of solute (g)}}{\text{mass of solvent (g)}} \text{ x } 10^6$$

Document the body weights of all pigeons to make sure they are similar Initiate an experimental period of 30 days in which the pigeons are exposed to the respective toothpaste formulations orally once a day.

Histopathological equipment (microtome, staining equipment, etc.) Laboratory animals' care and use guidelines were focused on after fixated with formalin and dehydration to make block and by use microtome for sectioning (3 micron) and stained with eosin/hematoxylin for full body and examine the heart cells and tissue after perfusion, the extracted organs (like liver, lungs, kidneys, and heart) .

Figure (1): Show the dose preparation and rout of administration of toothpaste solution.

Results and discussions:

Drying test: Place 2g of toothpaste sample accurately weighed in a plastic dish and exposed to air for about 2 hours at 37°C and calculate the drying time for each brand to that give indicate and predict the ant freezing material that effect on drying time according standard specifications (12). as shown in table (1).

Table (1): Show the drying time for each toothpaste brand.

Toothpaste brand	**Drying time/h**
Colgate	Not Dry
Dr. dabur kids	24
Dentakleen kid	30
Kodomo kid	20
Amber	48

Clinical signs: The body weight of pigeons in the control group did not show any significant signs at the end of the study period while, the body weight of the pigeons in the experimental groups showed a gradual decline in body weight that was more pronounced in the groups that received Colgate toothpaste compared with other brands.

Histopathological finding:

Dentakleen slide show blocked of collecting tubules by dense hyaline cast of precipitated light chain that give a sign of atrophy and loss of the epithelial cells in many places with disruption of the tubules wall as shown in Image (1- A). While, in image (1-B) the clear invasion of infiltrated inflammatory cell between tubules were observe that lead to myelomatosis as a main cause of renal failure.(13)

This neoplastic over growth of plasma cells usually confined to the hematopoietic bone marrow that give a rise of tumor. **(14)**

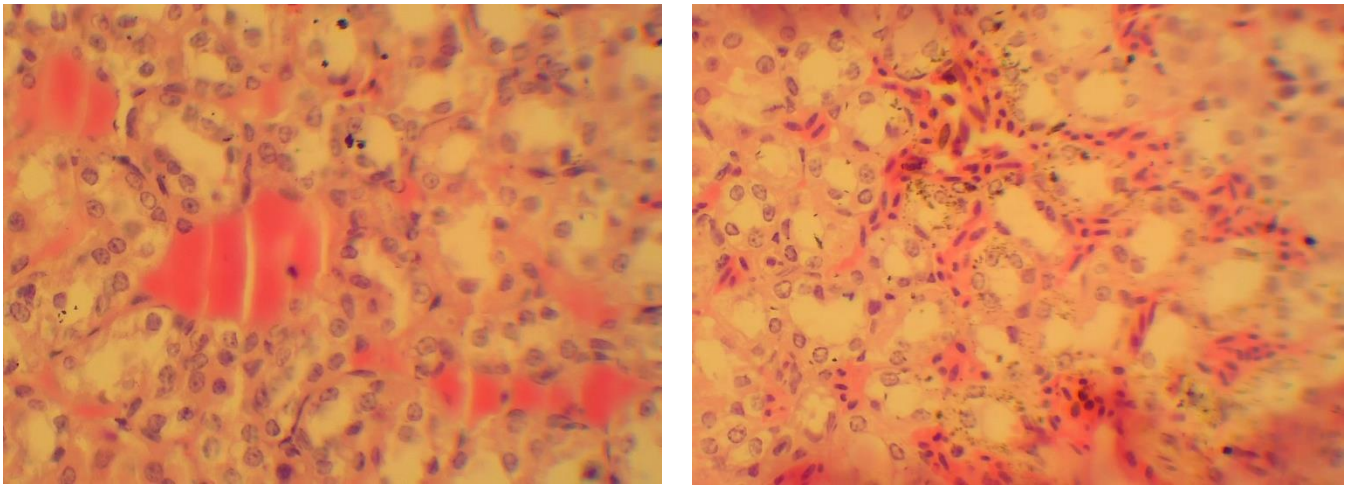


Image (1)for Dentakleen toothpaste sample: A, on the left side and B on the right side (EH 40x).

On the other hand, the Colgate sample was obviously clarified sever lesion related with its contain of PEG as antifreeze with cloudy swelling of tubules characterized by enlargement of tubules cells wall that lead to narrowing of renal tubule lumen (Image 2-A). However, the clear diabetic glomerulosclerosis with Kimmelstiel-Wilson lesion with hyaline material that deposit practically acellular, nodule in the glomerular tuft (Image 2-B). **(15)**

Other lesion in Colgate sample shown in image 2-C with malignant hypertension in the afferent glomerular arterioles consisting of a fibroid necrosis of arterioles wall that commonly extend to the glomeruli as a result of arterioles sclerosis due to diffuse change which affect all coat of the artery in the kidney by beginning of essential hypertension as a result of hypertrophy of muscular media as shown in image 2-D. **(16)**

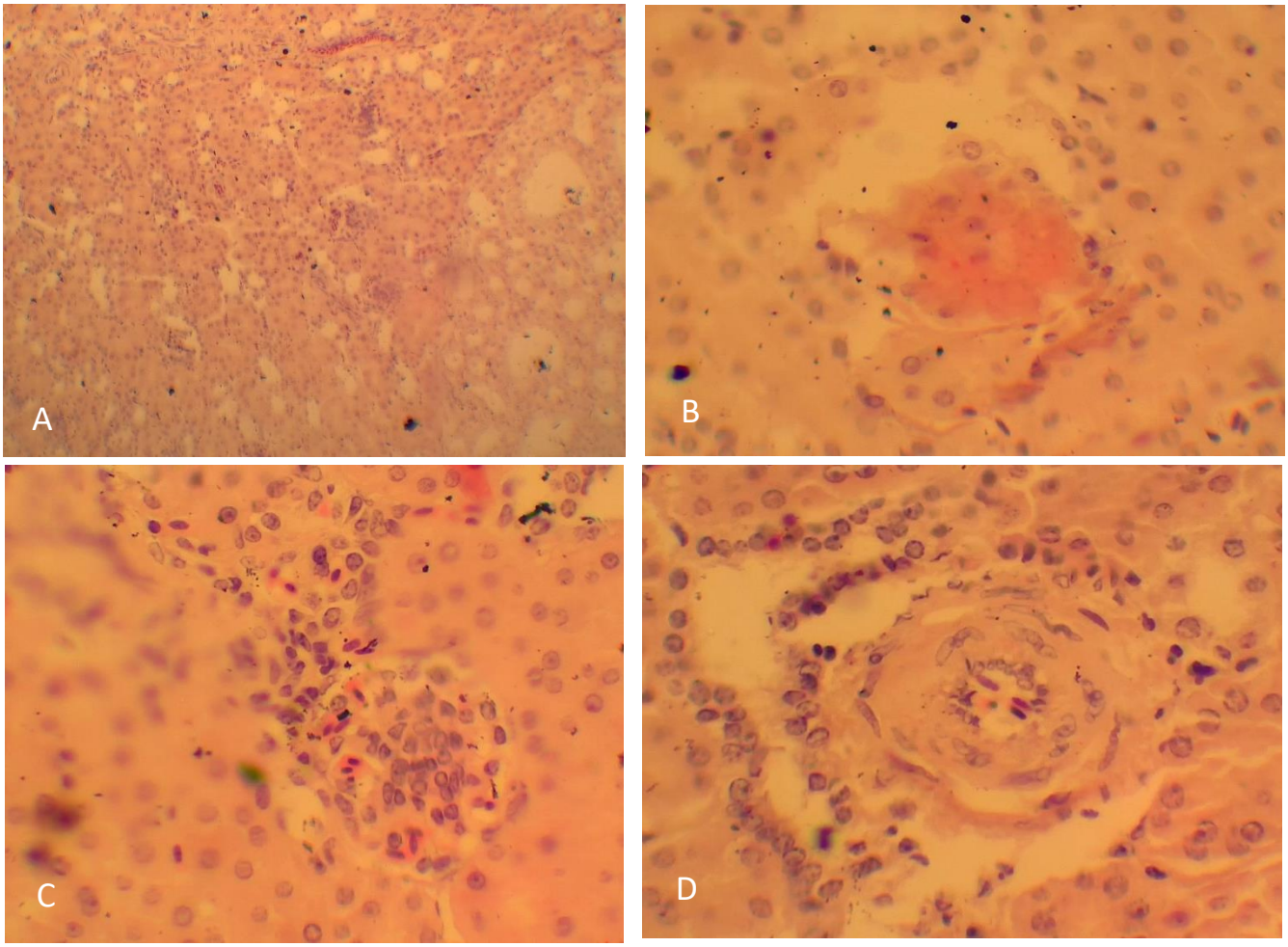


Image (2) for Colgate toothpaste sample (EH 40x).

Kodomo toothpaste lesion affected pigeon kidney with clearly segmented and membrane proliferative glomerulonephritis with lobular parts and necrotic and containing many nuclear fragments as shown in image 3-A. Its adhere to the small epithelial crescent of Bowman capsule with increase of mesangium cell, while marked increase of inflammatory cells as a result of above as shown in image 3-B. **(17)**

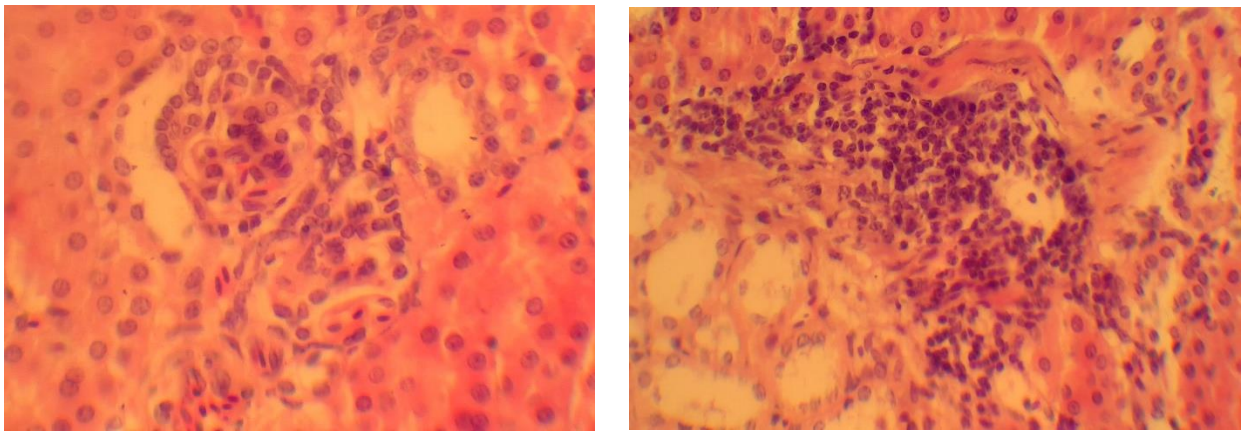
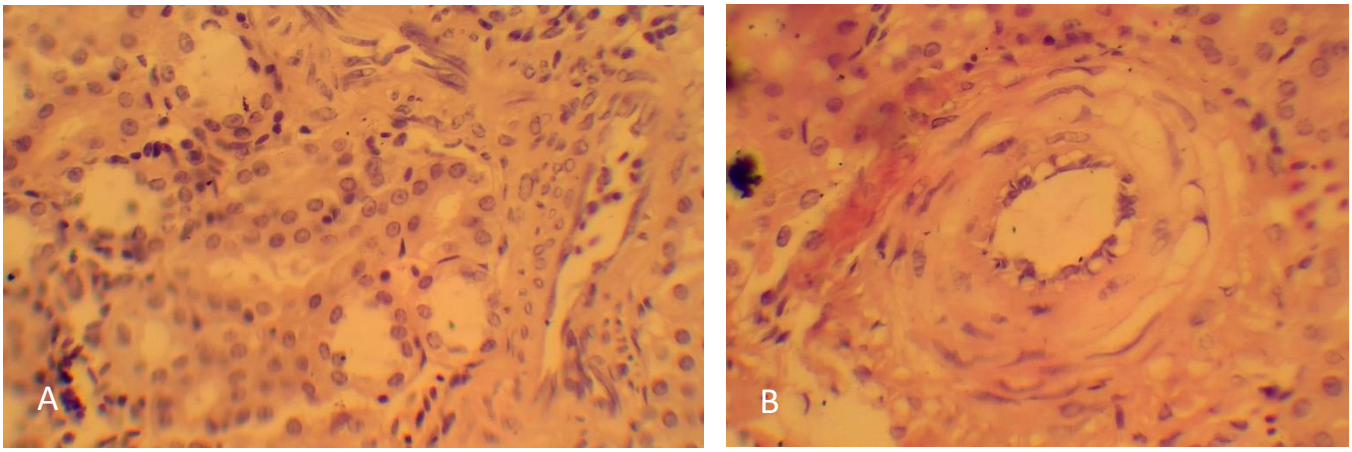


Image (3) for kodomo toothpaste sample (EH 40x).

Image 4-A related with Dr. Daber toothpaste that show cloudy swelling of tubules that lead to narrowing of tubule. On the other hand, image 2-B clarify arterioles sclerosis **(18).**



Amber toothpaste have close lesions like in Kodmo and Dr. Daber with both acute tubular necrosis and segmented glomerularnephritis consequently as shown in Image 5 A and B. (19)

Conclusion:

In this study, we evaluate the toxic effects of dioxane that released from toothpaste antifreezing materials on pigeon kidney. Exposure to daily dioxane from antifreezing materials such as PEG, DEG did induce a pathological change with significantly lesions. These findings suggest that although with clinical changes were observed, a high level of dioxane might also induce imperceptible injury to kidney, with significantly decreased the body weight gain, increased the relative kidney weight, that disrupted various metabolic pathways in kidney and simultaneously altered the metabolic profiles of urine.

The best toothpaste that recommended which have glycerin as a antifreezing material that can be used for both adult and child to minimize the injury of kidney due to combine with oxidizing materials like fluoride and bromide.

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