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Effects of Butylated Hydroxy Toluene on the Kidney of Swiss Albino Mice

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Abstract

The present studied aimed to investigate the possible effects of a common food antioxidant butylated hydroxy toluene (BHT) toxicity on histological parameters in swiss albino mice. Mice were orally administered with the BHT at the rate of 225mg/kg body weight daily in single dose for 30 and 60 days. Histopathological examination showed dramatic dose related increase in the incidence and severity of toxic nephrosis as indicated by several tubular lesions (distal and proximal tubular dilatation and cysts). On the basis of these findings the study suggests that butylated hydroxy toluene has direct toxic effects on the kidney.

Keywords: Butylated hydroxy toluene, Swiss albino mice, Kidney, Degeneration

INTRODUCTION

Butylated hydroxy toluene (BHT) is commonly used as food antioxidant. It stops the chemical breakdown of food that happens in the presence of oxygen. BHT is extensively used in oils, chewing gum, dried potato flakes and nuts. Unsaturated fatty acids in oils and lipids are particularly susceptible to autooxidation. This antioxidant functions by interrupting the free radical chain mechanism involved the lipid oxidation. They are effective even in small concentration (Ito.et.al 1986).

BHT can also improve the stability of pharmaceutical fats, soluble, vitamins and cosmetics (FDA 1986). In1978 Mayer et. al., reported adverse effects on the kidney of female rats given 25,100and 500mg/kg body weight of BHT. 1.6% BHT in the diet of pregnant rats caused drastic loss of weight and fetal death as well (Ames et al. 1956).

Inaiet al. (1988) reported in $B_6C_3F_1$ mice receiving butylated hydroxy toluene in diet for 10 weeks revealed marked starvation atrophy of the spleen, heart and kidneys

Takahashi (1992) reported that in male ddy mice receiving 0.135%, 1.75%, 2.28%, 2.96%, 3.85%or5.00% BHT in purified diet for 30 days 7/10 of the high dose animals showed misshapen kidney compared to other treated or control groups.

MATERIALS AND METHODS

Male Swiss albino mice with average body weight of 25-30 grams were selected for the experimental study. The animals were housed at controlled environmental condition 22 ± 2C, relatively humidity

50±10% and 12-hour dark light cycle. Animals were housed in polypropylene cages and allowed free access of food and water. The mice were divided into two groups. There was the control group and a treated group. The control group were not treated with any chemicals and therefore supposed to have normal basic structure of kidney. The treated group were fed with 225mg/kg bw of BHT for 30 days and 60 days. After the experimental period was over. The animals were sacrificed from each group for histological study. The selected organ was dissected and fixed in10% neutral formalin fixative and tissue were processed and stained with hematoxylin and eosin (H&E) examined under microscope. The procedures followed are those of Drury and Wallington (1967).

All the experimental data were expressed as mean ±SD difference was considered significant, if P<0.05. The changes in the diameter of the different parts of the kidney in the treated group were compared to the control and significance analyzed statistically by T-test.

RESULTS

Table -1 Diameter of glomerulus (in mm) of the kidneys of mice after oral administration of BHT.

Duration → Dose↓	30 days	60days
Control	0.052187 ± 0.0045825	0.053879 ± 0.0043931
225mg/kg bw /day	0.049466 ± 0.004734	0.0047433* ± 0.0038987

*P<0.05

Table-2 Diameter of Bowman's capsule (in mm) of the kidneys of mice after oral administration of BHT.

Duration→ **Dose↓**	30 days	60 days
Control	0.079962 ± 0.00478539	0.07955 ± 0.00304302
225mg/kg bw /day	0.075786* ± 0.00325576	0.072355* ± 0.0415932

*P<0.05

Table-3Diameter of proximal convoluted tubules (in mm) of the kidneys of mice after oral administration of BHT.

Duration → Dose ↓	30 days	60 days
Control	0.032786 ± 0.00012409	0.033653 ± 0.0016643
225mg/kg bw /day	0.034545* ± 0.0017088	0.034997 ± 0.00219087

*P<0.05

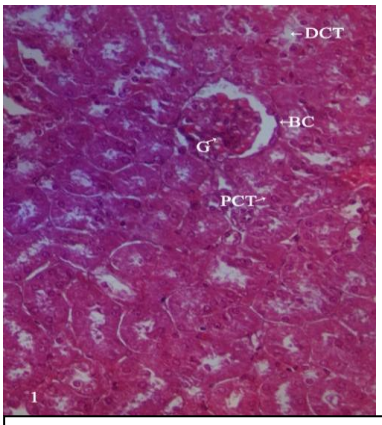
Table-4Diameter of distal convoluted tubules (in mm) of the kidneys of mice after oral administration of BHT

Duration → Dose ↓	30 days	60 days
Control	0.029672 ± 0.002426	0.029788 ± 0.0018275
225mg/kg bw /day	0.031375 ± 0.0013453	0.032907* ± 0.00186815

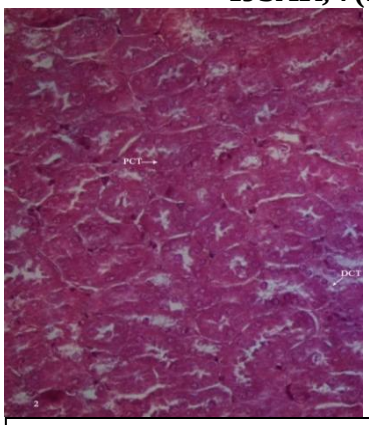
*P<0.05

Table-5 Diameter of collecting ducts (in mm) of the kidneys of mice after oral administration of BHT.

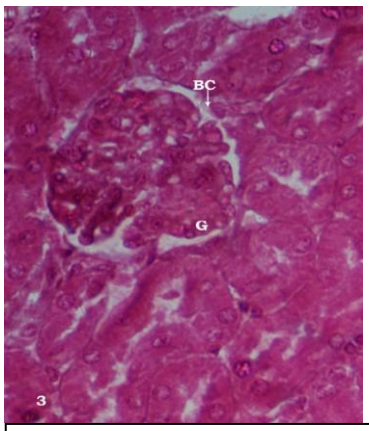
Duration → Dose ↓	30 days	60 days
Control	0.023434 ± 0.001378	0.025139 ± 0.002677
225mg/kg bw /day	0.026457 ± 0.0020469	0.02857 ± 0.0023108



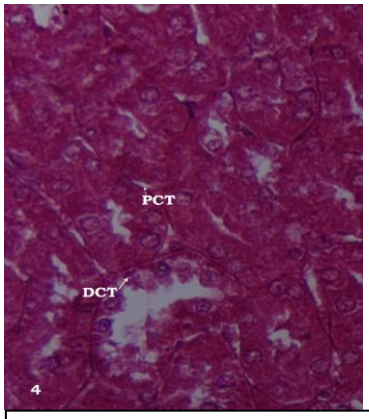
1. Untreated control mice. It shows normal glomerulus(G), Bowman's capsule (BC), Proximal convoluted tubules (PCT)and distal convoluted tubules (DCT) X 400.



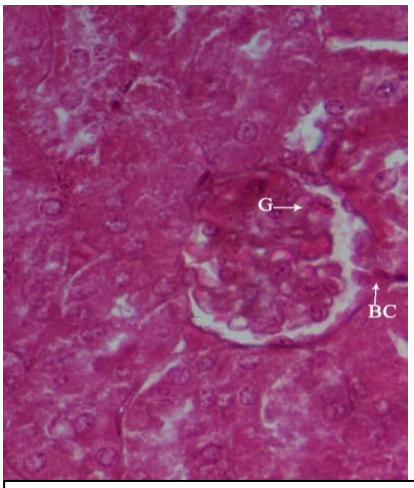
2. Untreated control mice. The proximal convoluted tubule (PCT)appeared normal. The star shaped lumen of it can be easily observed. The distal convoluted tubules (DCT) are visibly distinctly. The nuclei of renal tubules seen normal in number and shape X 400.



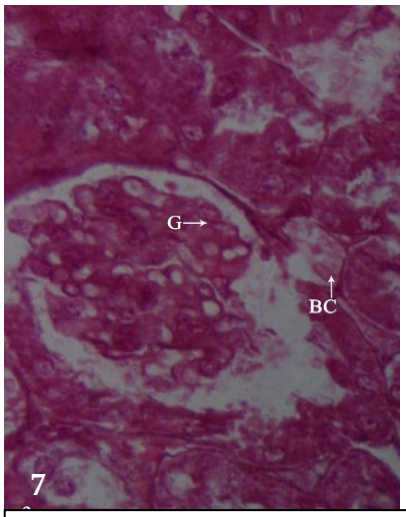
3. Fig 1 in higher magnification X1000.



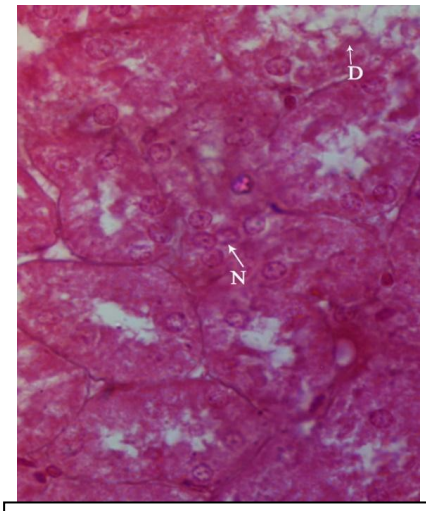
4. Fig 2 in higher magnification X1000.



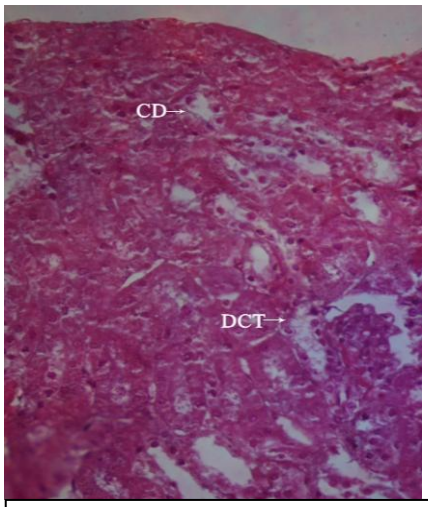
5. Treated with butylated hydroxy toluene 225mg/kg bw orally for 60 days. It shows clumped glomerulus and necrosis of epithelium of bowman's capsule. Necrosis of individual tubular epithelial cells and irregular spacing seen X 1000.



7. Treated with butylated hydroxy toluene 225mg/kg bw orally for 30 days. It shows necrosis of glomerulus(G) and degeneration of epithelium of bowman's capsule. lumen of proximal convoluted tubule appeared irregular n shape due to degeneration of brush border X1000.



6. Treated with butylated hydroxy toluene 225mg/kg bw orally for 60 days. It shows degeneration (D)of tubular epithelial cells. Agglutination of the epithelial cell nucleus (N) of renal tubules observed X1000.



8. Treated with butylated hydroxy toluene 225mg/kg bw orally for 30 days. It shows dilated distal convoluted tubules (DCT) and collecting ducts (CD) as well as degeneration of renal tubules also appeared X 1000.

Discussion

In the treated group changes in the different parameters were noted. The deviation from the normal basic structure was observed in different parameters of the kidney. The glomerulus is target of toxic effects of a number of drugs and chemical agents that may cause changes in glomerular morphology. The epithelium of glomerulus undergoes irreversible damage. The capacity of these cell types to repair damaged tissue is restricted as compared to the other cell types such as proximal convoluted tubular cell. Although glomerular and Bowman's capsule are together referred to as renal corpuscles but they differ greatly in extent of damage as well as repair. In the present study there was a significant change observed in the glomerulus at the 225 mg/kg bw oral dose of BHT per day after 60 days treatment at $P<0.05$. But there was no significant change observed in the diameter of glomerulus after 30 days treatment at $P<0.05$. In histopathological study clumped glomerulus were found.

The kidneys of aluminum chloride treated mice showed shrunken glomeruli, intraglomerular congestion, mesangial hyperplasia and obliteration of the filtration slits (Kahtani ,2010).

Imane et al. (2011) in the study of tartrazine at the dose of 1,2,3% for three weeks reported intercapillary sclerosis and atrophy of glomerulus in guinea pigs.

On the treatment with 225mg/kg bw butylated hydroxy toluene orally per day there was a statistically changes observed in the diameter of bowman's capsule after 30 days and 60 days duration. In histopathological study necrosis of Bowman's capsule epithelium was evident at 60 days treatment.

A single dose of BHT (1000mg/kg) in male Fisher 344 rats produced renal damage, reduced accumulation of p aminohippuric acid, proteinuria and enzymuria in addition to hepatic damage (Nakagawa and Tayama,1988)

Took (1996) reported that permanent vasodilation and congestion causes cellular hypoxia and cell death.

Because of the large volumes of fluid entering and being reabsorbed by proximal convoluted tubules, its many and varied transport mechanisms and its mitochondrial and cytoplasmic enzyme activities and associated high metabolic rate, the proximal convoluted tubules is a target for a wide variety of toxicants. In the present study 225 mg/kg bw butylated hydroxy toluene per day given orally showed significant change in diameter of proximal convoluted tubule after treatment of 30 days at $p<0.05$. In histological study necrosis of epithelial cells of proximal convoluted tubule, tubular dilatation and agglutination of epithelial cell nuclei of renal tubules was also seen.

The increase in diameter of proximal convoluted tubules, may be attributed to tubular dilatation with flattening of cells. Dilatation of tubular lumina can be explained as resulting from increased intracellular pressure according to Olsen and Solez (1982).

Takahashi (1992) reported that in male ddy mice receiving 0.135%, 1.75%, 2.28% 2.96%, 3.85%or5.00% BHT in purified diet for 30 days 7/10 of the high dose animals showed misshapen kidney compared to other treated or control groups. Histopathology of kidney revealed a dramatic dose related increase in the incidence and severity of toxic nephrosis as indicated by a number of tubular lesions,distal and proximal tubular degeneration, distal tubular necrosis, distal tubular dilatation and cysts.

There was a significant increase in the diameter of distal convoluted tubule after 60 days treatment but there was no significant change found at 30 days of treatment at $p<0.05$. In histopathological study degeneration of individual tubular epithelial cell was clearly seen. The distal convoluted tubules were greatly affected by the treatment of butylated hydroxy toluene. As it is known intraluminal concentration of

toxicants are likely to be higher in the distal regions of the renal tubules providing a greater concentration gradient to drive passive diffusion from lumen to the cell. The distal tubule is characterized by relatively high electric resistance, a feature that impede diffusion of solute.

In Wistar rats 1% butylated hydroxy toluene for 13-48 days pronounced nephrocalcinosis was found (Mayer et al.,1989).

Bulger et al. (1983) reported that administration of mercuric chloride to rats results in few changes in the distal convoluted tubules which may however show some degree of degeneration.

There was no marked change observed in collecting duct of untreated mice during the course of experiment. On treatment with 225mg/kg bw butylated hydroxy toluene per day orally showed significant increase in the diameter of collecting duct after 30 days of treatment at $p<0.05$. In histological studies the collecting ducts were dilated and necrosis of cells lining of the duct were observed. Damage due to tetracyclines may occur in these segments (Hook &Hewitt 1986).

Faine et al. (2006) showed that long term administration of butylated hydroxytoluene is capable of inducing oxidative and metabolic alterations in Wistar rats after intraperitoneal injection of 150 mg/kg bw.

After the present study it was concluded that continuous use of butylated hydroxy toluene and also its use in excess quantity is harmful to the kidneys.

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