Evaluation Of The Toxic Effect Of Butylated Hydroxy Toluene (BHT) After Long Term Exposure With Special Reference To Its Effect On Male Fertility In Albino Rats

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ABSTRACT

Because of the wide spread use of BHT in food products and as a consequence of long term exposure of animals and humans, it is important to investigate the potential health risks associated with its dietary intake. Thus the purpose of this study is to test the toxic effects resulted from BHT feeding in ration of male albino rats for 65 days. Twenty four mature male albino rats weighing about (180- 200 g) were distributed randomly into two equal groups. The first group was used as control group and fed on feed additives free ration C (control group). The second fed on ration containing the antioxidant butylated hydroxytoluene (BHT) 0.5% and 1% for 65 days (sub groups B1 and B2). animals from each group were scarified, after 65 days. Blood was collected and the sera were separated for biochemical changes. The semen was collected for studying the sperm motility, number, viability and abnormalities. Hepatic and testicular samples were used for estimation of DNA and RNA contents. Lung, liver and testes were preserved in formalin for histopathological changes. It was revealed that BHT resulted in a significant decrease in body weight gain and a significant increase in liver weight at 65 days. There was a significant decrease in the motility percentage of BHT 0.5% and 1% treated male rats, non significant decrease in sperm cell concentration, high increase in percentage of sperm abnormalities, significant decrease in live % in 0.5% and 1% BHT fed male rats compared with the control group, and a significant decrease in testosterone level in BHT 0.5% and 1% treated male rats compared with the control group. There was a significant increase in the S.O.D and G.R.D and non significant increase in MDA levels, a significant increase in hepatic and testicular DNA and RNA contents .

INTRODUCTION

Food preservatives are utilized to decrease the rate of degradation of food during processing and storage, which include antioxidants. They occur either in natural compounds such as vitamin A, E, and C or synthetic antioxidants which are mostly phenolic and include butylated hydroxyanisole (BHA); butylated hydroxyltoluene (BHT) and tertiary butyl hydroquinone (TBHQ) or 200-500 ppm of gallates for stabilization of fats and oils *(1)*. BHT used as an antioxidant in food stuff with ADI of 0-0.3 mg/kg body weight /day has been established *(2)*. In veterinary field BHT is a common food preservative, and also used against Newcastle disease in chickens. Quantity of added BHT (100–200 ppm of total diet), as a feed additive, is equal to its amount necessary for inhibition of Newcastle disease virus in chicken *(3)*. Moreover, BHT has been used to counteract many of the deleterious effects caused by aflatoxin B1 in turkeys *(4)*. Recently, some synthetic antioxidants such as BHT and BHA have been suspected to be dangerous to human health, they are not believed to be a health hazard, but they have been found to trigger behavior *(5)*, they weaken the immune system *(6)*, and are tumors promoters *(7)*. There have been several reports indicating that BHT and BHA might have both beneficial and deleterious effects *(8)*. A number of food additives can cause adverse reactions such as BHT and BHA are antioxidants substances that prevent food becoming rancid, they aggravate urticaria in occasional cases *(9)*.

There is an urgent need to search and evaluate synthetic food additives either after short or long periods. Therefore, much attention should be directed to study the undesirable effects of ingested synthetic food additives to select the most suitable and safe ones for their use in both animal and human food, pharmaceutical, and cosmetic industries.

MATERIALS AND METHODS

Animals:- Twenty four male albino rats weighing about (180- 200 g) were used; the animals were obtained from faculty of veterinary medicine, Zagazig University (laboratory animal's housing unit). The animals were clinically healthy, kept under hygienic condition, housed in metal cages with hard wood shavings as bedding. They were maintained on basal ration and given water ad-libitum for two weeks of acclimatization before use.

Chemicals:-Butylated hydroxytoluene (BHT) was obtained from El-Nasr Pharmaceutical Chemicals Company (Egypt).

Dose of BHT:- BHT was used in the dose of 0.5% and 1% according to *(10)*.

Methods:- Bleeding time was determined according to *(11)*. Clotting time in rats was determined according to method of *(12)*.PT was determined according to *(13)*. Semen picture The epididymal contents was obtained in a suspension that was handled as the semen according to the method of *(14)*, and used for determination of Sperm motility assay .It was described by *(15)*.Sperm cell concentration per ml of semen according to *(16)*. Sperm abnormalities and live % of spermatozoa determined by the method of *(17)*. Biochemical studies of serum samples were used for biochemical analysis to determine testosterone hormone. The liver homogenates used for estimation of superoxide dismutase (S.O.D), glutathione reductase (G.R.D) activities and malondialdehyde MDA concentration. Testosterone was determined according to *(18)*. S.O.D activity was assayed by *(19)*, G.R.D activity was determined by *(20)*. MDA concentration was determined according to *(21)*. Hepatic and testicular DNA contents were determined by *(22)*. Hepatic and testicular RNA contents were measured by *(23)*. Routine histopathological examination according to *(24)*.The data were analyzed using one-way **ANOVA** to determine the statistical significance of differences among groups.

RESULTS

- Effect of BHT on clotting factors assay: -
From table (1), the present study revealed that BHT feeding in diet at 0.5% and 1% of male rats for 65 days resulted in a non significant change in bleeding time (112± 0.02and 136±0.23sec.) respectively if compared with the control group (100±0.005sec.). The results revealed that BHT 0.5% and 1% caused a significant increase in clotting time (79±0.02 and 130±10.5sec.) respectively if compared with clotting time in control group (28±2.00 sec.). BHT feeding in diet at 0.5% and 1% of male rats at 65 days resulted in non significant change in prothrombin time PT (23.83 ±0.30 and 25.66±1.62sec.) respectively if compared with the control group (21.54±0.58 sec.)

- Effect of BHT on body and liver weight: -
From table (2), rats of BHT fed male albino rats at 0.5% and 1% in ration for 65 days showed anorexia and decreased mean value of body weights of treated groups than the control. The present study showed that after there was a significant decrease in mean body weights values of BHT 0.5% and 1% fed male rats after 65 days (254.83±2.79 and 242.76±3.41gm) respectively if compared with control (275.16±2.6gm) respectively. Regarding to the effect of BHT on liver weights, BHT 0.5% and 1% caused a significant increase in liver weight (10.9±0.48 and 12.4±0.34gm) if compared with that of the control group (6.5±0.05 gm).

Post mortem changes:- There were congestion in lung of male albino rats fed on

BHT at 0.5% and 1% and degree of severity was dose dependent fig.(3). Also, liver showed marked enlargement and congestion in all treated BHT rats if compared with that of the control fig. (4).

Effects of BHT on male fertility in rats:- From table (3), regarding to the motility percentage there was a non significant decrease in BHT 0.5% fed male rats (80.00±2.58%) and a significant decrease in motility % in BHT 1% fed male rats (77.50±1.11%) if compared with the control group (86.66±1.66%). Concerning the mean values of sperm cell concentration, there was a non significant decrease in sperm cell concentration of BHT 0.5 % and 1 % fed male rats (23.5±2.43 and 23.0±2.62 Sp.C.C/ml x 125 $x10^5$) respectively if compared with the control group (26.33±0.88 Sp.C.C/mlx 125 $x10^5$). The obtained results also, revealed that there was high increase in percentage of sperm abnormalities in BHT 0.5 % and 1% fed male rats were (16.26±3.40 and 16.53±3.40%) if compared with control groups (6.4±0.61%). Concerning to live % of male rats fed on 0.5% and 1% BHT for 65 days, there was a significant decrease in live % (84.21±5.54 and 81.58±4.02%) respectively if compared with the control group (93.22±0.94%).Concerning with testosterone level, the results of this study revealed that BHT 0.5% and 1% fed male rats showed a significant decrease in testosterone level at 65 days in BHT 0.5% and 1% fed male rats (1.09±0.05 and 0.456±0.006 ng/ml) if compared with the control group (1.92±0.71ng/ml).

- Effects of BHT on some biochemical parameters:- From table (4), the present results revealed that BHT 0.5 % and 1% fed male rats showed altered S.O.D activity, there was a significant increase in the enzyme activity (72.16±4.04 and 92.00±16.7 U/mg) respectively if compared with that of the control (30.53±2.67 U/mg). BHT 0.5% and 1% fed male rats showed altered G.R.D enzyme activity, there was a significant increase in the enzyme activity (0.615±0.06 and 0.645 ±0.03 U/mg) respectively if compared with that of the control (0.208±0.009 U/mg) . BHT 0.5 % and 1% fed male rats showed non significant increase in the MDA concentration(1.48±0.02 and 1.63±0.07 µ mol/L) if compared with that of the control group (1.46±0.09 µ mol/L).

- Effects of BHT on nucleic acid contents:- Regarding to the obtained results in table (5), there was a significant increase in hepatic DNA content in BHT 0.5% and 1% fed male rats (3.39±0.05 and 3.66±0.6 mg/g) respectively if compared with control (3.05±0.07 mg/g) , there was a significant increase in hepatic RNA content in BHT 0.5% and 1% (12.24±0.29 and 12.84±0.39 mg/g) respectively if compared with control (10.23±0.04 mg/g). There was a significant increase in testicular DNA content in BHT 0.5% and 1% fed male rats for 65 days (3.9±0.3 and 4.26±0.02 mg/g) respectively if compared with the control group (3.20±0.03 mg/g), there was an non significant change in testicular RNA content in BHT 0.5% fed male rats (19.36±0.16 mg/g) if compared with control group (19.84±0.16). BHT 1% caused a significant increase in testicular RNA content (20.27±0.35 mg/g) if compared with control group (19.45±0.15 mg/g).

***-Histopathological findings*:-** The hepatocytes of rats fed on BHT 0.5% revealed necrosis of some hepatocytes with hemorrhages . Also, the hepatic cells revealed swelling with partial or complete cytoplasmolysis (hydropic degeneration) or apoptosis with hyperplastic kuffer's cells and congested hepatic sinusoids. Portal fibrosis, proliferation of bile ductules and hyalinized wall of hepatic arterioles beside mild portal lymphocytic infiltration could be seen in rats fed on 1% BHT, the surrounding hepatic cells suffered from degeneration or pressure atrophy. The hepatic capsule was thickened by fibrous tissue with sub capsular fibrosis. The pulmonary tissue revealed thickened interlobular septa, by leukocytes and

perivascular edema, Moreover; mild hyperplastic bronchial epithelium could be seen. Somniferous tubules are apparently normal, spermatogonial cells and mild spermatogenesis in rats fed on 0.5% BHT. Mild spermatogenesis with mild edema and congestion in the interstitium were seen in rats fed on BHT 1%. Mild degenerated or dissociated spermatogonial cell layer could be seen in some somniferous tubules. Moreover, few blood vessels revealed endotheliosis. The pulmonary tissue revealed thickened interlobular septa, by leukocytes and perivascular edema, Moreover, mild hyperplastic bronchial epithelium could be seen.

Table 1. Changes in bleeding, clotting and prothrombin time (seconds) of male albino rats fed on 0.5% and 1% BHT containing ration for 65 days. (Mean± S.E.).

Duration	Treatment	Group	Bleeding time (sec)	Clotting time (sec) S.O.D	PT time (sec)
	Free ration	C	100±0.005 [a]	28±2.00 [c]	21.54±0.58 [a]
65 days	BHT 0.5%	B 1	112±0.02 [a]	79±0.02 [b]	23.83 ±0.30 [a]
	BHT 1%	B 2	136±0.23 [a]	130±10.5 [a]	25.66±1.62 [a]

Means in the same row having the same superscript were not significantly different (P< 0.05)

Table 2. Changes in mean body and liver weight (g) of male albino rats fed on ration containing 0.5% and 1% BHT after 65, days. (Mean± S.E.)

duration	Groups	Treatment	Liver weight	Body weight	Mean body weight at the begining
65 days	C	Free diet	6.5 ±0.05 [c]	275.16±2.6 [a]	182± 3.5 [a]
65 days	B1	BHT 0.5%	10.9 ±0.48 [b]	254.83±2.79 [b]	185 ±6.08 [a]
65 days	B2	BHT 1%	12.4 ±0.34 [a]	242.76±3.41 [b]	184 ±4.04 [a]

Means in the same row having the same superscript were not significantly different (P< 0.05).

Table 3. Changes in epididymal sperm characters of male albino rats fed on 0.5% and 1% BHT containing ration for 65 days and their sera testosterone level after 65 days (Mean± S.E.).

Group	Motility %	Live %	Abnormalities %	Sp.C.C/ml x 125 x10^5	Testosterone level (ng/ml). 65 days
C	86.66±1.66 ^{a}	93.22±0.94 a	6.4±0.61 ^{b}	26.33±0.88 ^{a}	1.92±0.71 ^{a}
B 1	80.00±2.58 ^{ab}	84.21±5.54 ^{b}	16.26±3.40 ^{a}	23±2.62^{a}	1.09±0.05 ^{b}
B 2	77.50±1.11 ^{b}	81.58±4.02^{b}	16.53±3.40 ^{a}	23.5±2.43 ^{a}	0.456±0.006 ^{b}

Means in the same row having the same superscript were not significantly different (P< 0.05).

Table 4. Changes in antioxidant enzymes activity in liver homogenate of male albino rats fed on 0.5% and 1% BHT containing ration for 65 days (Mean± S.E.).

Group	duration	S.O.D (U/mg protein)	GRD (U/gm tissue)	MDA (µmol/L homogenate)
C	65 days	30.53±2.76 $^{b.}$	0.208±0.009 $^{b.}$	1.46±0.09 ^{a}
B 1	65 days	72.16 ±4.04 ^{a}	0.615±0.06 ^{a}	1.48±0.02 ^{a}
B 2	65 days	92.00 ±16.7 ^{a}	0.645 ±0.03 ^{a}	1.63±0.07 ^{a}

Means in the same row having the same superscript were not significantly different (P< 0.05)

Table 5. Hepatic and testicular DNA and RNA contents (mg/g wet. Tissue) in male albino rats fed on 0.5% and 1% BHT containing ration for 65 days (Mean± S.E.).

Group	duration	Hepatic (contents mg/g)		Testicular (contents mg/g)	
		DNA	RNA	DNA	RNA
C	65 days	3.05±0.07 ^{c}	10.23±0.04 ^{b}	3.20±0.03 ^{c}	19.45±0.15 ^{b}
B 1	65 days	3.39±0.05 ^{b}	12.24±0.29 ^{a}	3.9±0.3 ^{b}	19.36±0.16 ^{ab}
B 2	65 days	3.66±0.6 ^{a}	12.84±0.39 ^{a}	4.26±0.02 ^{a}	20.27±0.35 ^{a}

Means in the same row having the same superscript were not significantly different (P< 0.05).

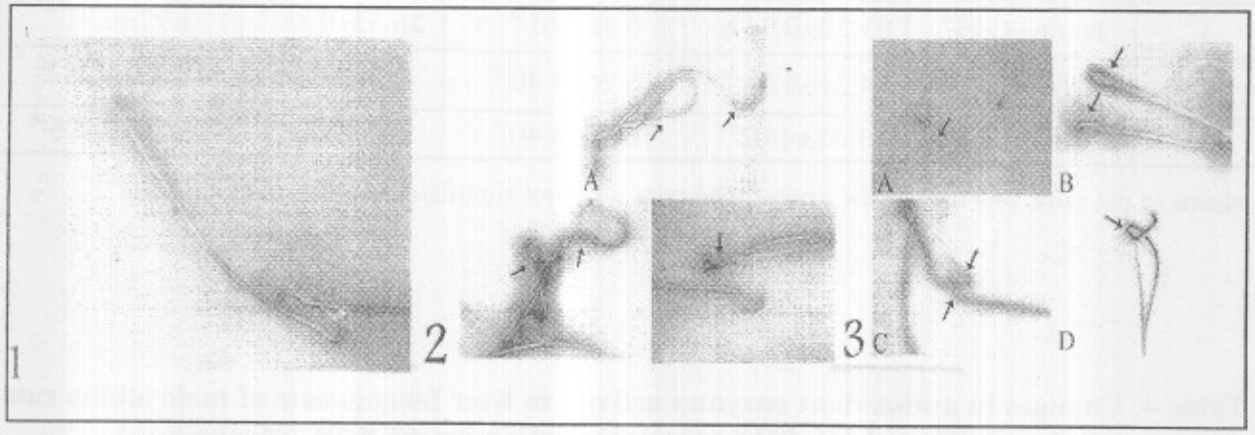

Fig. 1. Sperm of control show normal hock shape spermatozoa 2)spermatozoa of male albino rats fed on 0.5% BHT containing ration for 65 days showing abnormalities in the form of A) looped sperm and bent tail B)coiled tail C)abnormal hock shape. 3) Spermatozoa of male albino rats fed on 1% BHT containing ration for 65 days A) detached head and mid piece B) abnormal hock shape C) detached bent tail, bent mid piece D) coiled mid piece and bent tail.

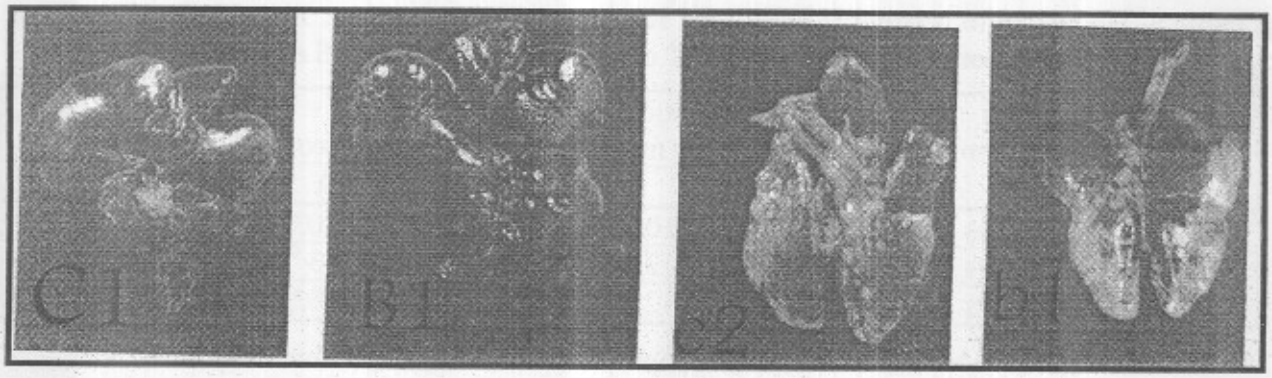

Fig. 2. Gross picture of Liver and lung of male rats fed on BHT 0.5% and 1% containing ration showing congestion in lungs, congestion and enlargement of liver . C1) liver of control B1) liver of BHT treated male rat for 65 days showing moderate congestion c2) lung of control b1) lung of BHT treated male rat for 65 days showing moderate congestion

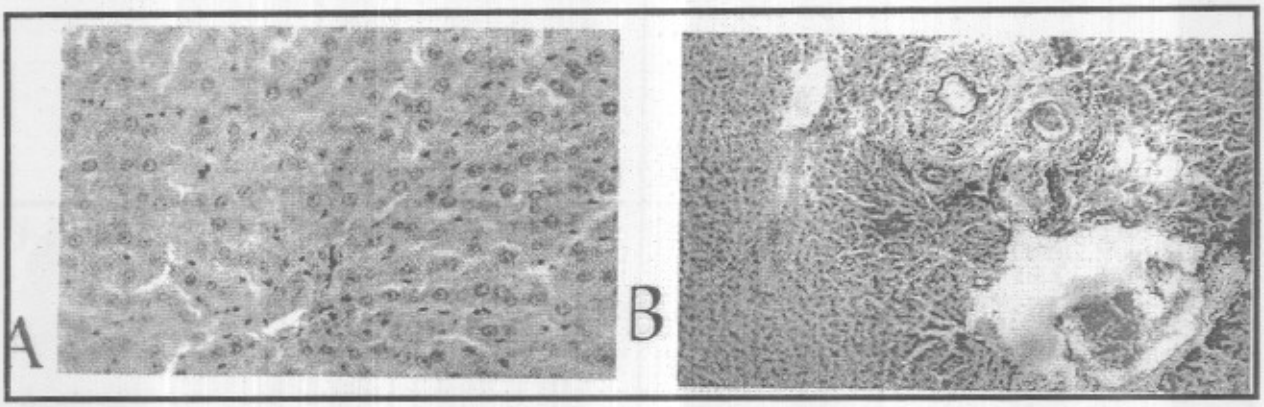

Fig. 3. Photomicrograph of section of rat liver A) rat liver fed on BHT 0.5 % containing ration for 65 days showing hydropic degeneration or apoptosis of the hepatic cells. (H&E x1200). B) rat liver fed on BHT 1% containing ration for 65 days showing portal fibrosis and proliferation of bile ductules. (H&E x 300).

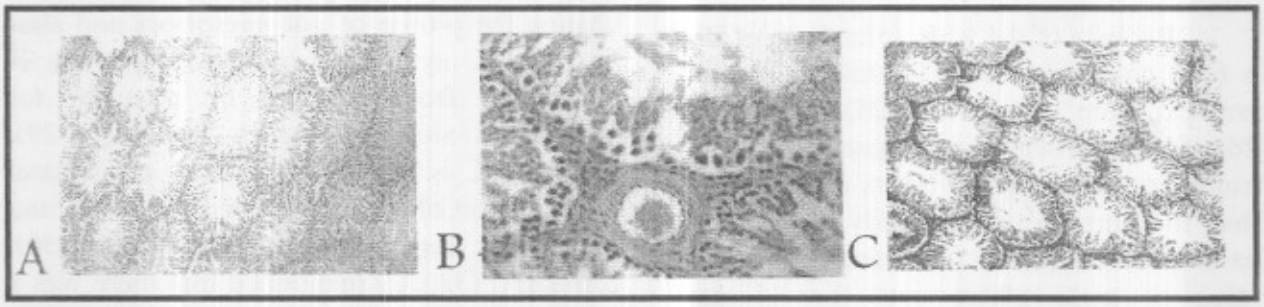

Fig. 4. Photomicrograph of section of rat testes fed on BHT 0,5 % and BHT 1% containing ration for 65 days showing A) testes of control B) testes of BHT 0.5% showing mild spermatogenesis with congested interstitial blood vessels (endotheliosis). (H&E x 300). C)testes of BHT 1% showing mild spermatogenesis with mild edema and congestion in the interstitium(H&E x1200).

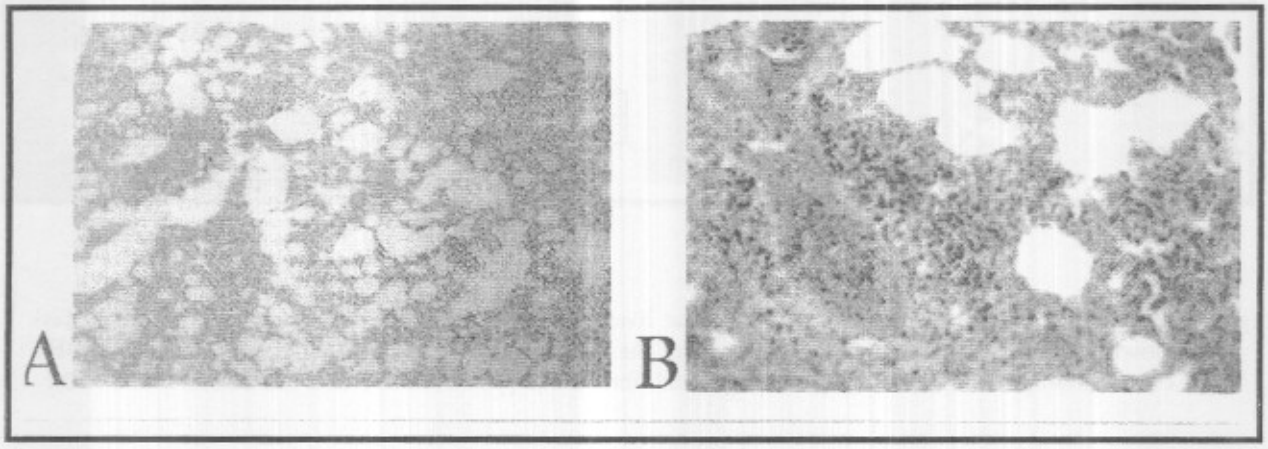

Fig. 5. Photomicrograph of section of rat lung showing A) lung of control B) lung of rat fed on BHT 1 % containing ration for 65 days thickened inter lobular septa, perivascular edema and hyperplastic bronchial epithelium. (H&E x 300).

DISCUSSION

Because of the wide spread use of BHT in food products and as a consequence long term exposure of animals *(25)* and humans *(26)*, it is important to investigate the potential health risks associated with its dietary intake. Thus the purpose of this study is to test the toxic effects resulted from feeding BHT in ration of male albino rats for 65 days. Concerning the effect of BHT on haemostatic mechanism, there were a significant increase in PT time and clotting time but there is a non significant increase in bleeding time. These results agreed with that obtained by *(27-30).* This may be attributed to BHT caused vitamin K deficiency by blocking of redox cycling of vitamin K or by inhibiting vitamin K absorption from the intestine or its uptake by the liver as stated by *(1).* Also BHT could change the profile of gut microflora and alter vitamin K synthesis, influence vitamin K absorption from the gut or compete for vitamin K storage sites in the liver *(29).* Vitamin K is required for post transitional modification of clotting factors II, VII, IX and X in their conversion to active forms *(30).* Similarly, *(31, 32)* mentioned that there was a dose dependent increase in PT time and decrease in vitamin K concentration in the liver of male rats after receiving dietary BHT for 2 weeks and increase in fecal excretion of vitamin K. In addition, BHT may affect haemostasis by disruption of hepatic functions itself which indicated by elevated AST and ALT enzymes activity. Decreased plasma concentration of clotting factors is manifested

by prolonged PT and clotting time ***(33)***. Concerning to the effect of BHT on the body weights of rats, the results of present study showed that BHT feeding in rations at 0.5% and 1% to male albino rats for 65 days resulted in a significant decrease in body weights if compared with control group. These results are in agreement with ***(27, 34-36)***. This may be attributed to hyperactivity of thyroid gland following BHT feeding which consequently increased the basal metabolism and reduced body weight as mentioned by ***(2, 37, 38)***.Regarding to liver weight, the male albino rats fed on 0.5% and 1% BHT, there were a significant increase in liver weight if compared with the control group at 65 days. These results are in agreement with that given by ***(1, 5, 27, 35, 39, 40)***. This may be due to proliferation of smooth endoplasmic reticulum, increased lipid droplets within the hepatic cells and activity of many microsomal enzymes including glutathione reductase, epoxide hydrolase, glutathione -S- transferase. Similarly, an increase in hepatic lipid contents followed BHT feeding as reported by ***(41, 42)***. Regarding to the effect of BHT on spermatogenesis, the present study showed that BHT caused a significant decrease in sperm cell concentration, motility % and significant increase in sperm abnormality % of male rats fed on BHT for 65 days. These results could be supported by the findings of ***(43)*** who mentioned that BHT act as a respiratory inhibitor and uncoupler of oxidative phosphorylation in rat liver mitochondria and might be the cause of testicular damage and decrement of spermatogenesis. Moreover, chronic administration of BHT causes human prostate tumor as mentioned by ***(44)***. This is supported by our histopathological findings in testes and somniferous tubules. Concerning the effect of BHT on serum testosterone, the obtained results showed that BHT caused a significant decrease in serum testosterone level of male rats fed on 0.5% and 1% BHT for 65 days. This may be attributed to decreased vitamin K whereas; BHT inhibits its intestinal absorption, its uptake by liver or increased fecal excretion in rats ***(45)***. This is confirmed by ***(46)*** who mentioned that the testosterone concentration is significantly decreased in Vitamin K deficient rats whereas, testicular vitamin K plays an important role in the gene expressions which involved in the biosynthesis of steroid hormones. Regarding to the effect of BHT on antioxidant enzyme activities, the present study showed that BHT caused a significant increase in enzyme activities in liver homogenate of male rats fed on 0.5% and 1% BHT for 65 days. BHT increased S.O.D enzyme activity and this in agreement with ***(47)*** who mentioned that BHT resulted in increased SOD and other antioxidants enzymes activities. Similarly, ***(34)*** mentioned that BHT can induce oxidative stress in rats. Regarding to the effect of BHT on G.R.D enzyme activity, there were a significant elevation in this enzyme activity and these results are in agreement with ***(48)*** who mentioned that BHT increased level of G.R.D in liver and lungs. This may be attributed to the oxidant effect of BHT as mentioned by ***(49)*** who mentioned that several known antioxidant might have both antioxidant and oxidant action dependant on its concentration. On the same side BHT is known for its antioxidant activity whereas, higher doses may exert a prooxidant effect on the organism ***(50)*** Regarding to the effects on the nucleic acid content, the present study revealed that BHT revealed an increase in both hepatic and testicular nucleic acids and these results are in agreement with ***(51, 52)*** who mentioned that BHT resulted in a significant increase in DNA content and its enlargement. This may be due to the increased serum total protein level. Concerning the histopathological finding detected in present study caused by BHT. The results showed hepatic centrilobular necrosis was observed after BHT. Similar types of lesions were observed by ***(53)***. This is

confirmed by *(5, 54)* who reported that BHT is likely to be hepatotoxic in rodents. Histopathological findings in lungs of BHT treated male rats are similar to that described by *(55, 39)*. This may be attributed to an overproduction of BHT-QM metabolite which can covalently bind to protein and nucleic acid, which lead to acute hepatotoxicity, pneumotoxicity and enhance oxidative stress in other organs *(56)*. Moreover, oxidative stress is considered to play an important role in the cause of many diseases, including inflammation, aging and cancer *(57)*.

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الملخص العربي

تقييم التأثير السمي بعد التعرض للبيتيوليتيد هيدروكسي تولين بالاشارة الي تاثيره علي الخصوبة في الفئران البيضاء

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قديما استخدم الإنسان العديد من إضافات الأغذية الطبيعية كمواد حافظة ومكسبات طعم و رائحة في كثير من المنتجات الغذائية و غيرها ، حديثاً تزايد استخدام بعض الإضافات الغذائية المخلقة صناعياً سواء في أطعمة الإنسان وعلائق الحيوان لذلك فمن الأهمية الحيوية دراسة التأثير السام لهذه الإضافات الصناعية و تستهدف هذه الدراسة تقييم تأثير الجرعات المختلفة لأحد من المواد الحافظة وهو مضاد الأكسدة البيتيوليتيد هيدروكسي تولين بعد استخدامه لفترات مختلفة علي الفئران البيضاء. أجريت هذه الدراسة علي عدد ٢٤ من ذكور بالفئران البيضاء و كانت الأوزان تتراوح بين (١٨٠- ٢٠٠جم) قسمت إلي مجموعتين الأولي كمجموعة ضابطة (١٢ فأر) والمجموعة الثانية . تم تقسيمها إلي مجموعتين فرعيتن كلا منهما (٦ فئران) و تم تغذيتها علي عليقة تحتوي علي ٠,٥%و ١% من مضاد الأكسدة البيتيوليتيد هيدروكسي تولين لمدة ٦٥ يوما و قد اشتملت الدراسة علي دراسة التغيرات المحتملة في وزن الجسم والكبد و تأثيرها علي تكوين الجلطة الدموية و دراسة تأثيرها علي الخصوبة في ذكور الفئران و دراسة بعض التغيرات البيوكيميائية في الدم و أنسجة الكبد و تأثيرها علي المعدل الكمي للحامض النووي الديؤكسي ريبوسي والريبوسي في كلا من أنسجة الكبد و الخصية و بعض الدراسات الهستوباثولوجية لأنسجة الجسم المختلفة .

أظهرت النتائج إن البيتيوليتيد هيدروكسي تولين ٠,٥ و١ % احدث زيادة معنوية في زمن التجلط عند ٦٥ يوما و احدث زيادة غير معنوية في زمن النزف ٦٥ يوم وكذلك زيادة غير معنوية في زمن البروثرومبين عند ٦٥ يوما و احدث نقص معنوي في حركة الحيوانات المنوية و نقص غير معنوي في عدد الحيوانات المنوية وزيادة معنوية في نسبة التشوهات بها وكذلك احدث نقص معنوي في عدد الحيوانات المنوية الحية و نقصا معنويا في مستوي هرمون الذكورة (التستوستيرون) أوضحت النتائج أن البيتيوليتيد هيدروكسي تولين أحدث زيادة معنوية في نشاط إنزيم السوبر اوكسيد ديسميوتاز و الجلوتاثيون ريدكتاز في أنسجة الكبد بينما سجل زيادة غير معنوية في تركيز المالونداي الديهايد

أظهرت النتائج ان البيتيوليتيد هيدروكسي تولين ٠,٥ %و ١% احدث زيادة معنوية في معدل كلا من الحمض النووي الديؤكسي ريبوسي و الحمض النووي الريبوسي في أنسجة الكبد و الخصية .

اظهر الفحص الميكروسكوبي أن هناك تغيرات في الكبد و الرئة و الخصية و الكلي ممثلة في مناطق متعددة من النخر التخثري و مناطق متعددة من النزف و تنكسات مختلفة لخلايا الكبد مع زيادة في عدد الخلايا المبطنة للقنوات المرارية. أما في الرئة وجد أن هناك زيادة في سمك الحواجز بين الحويصلات الهوائية. و في الخصية وجد أن هناك تنكس في الخلايا المبطنة للأنابيب المنوية وهناك تنخر خثري في الأنابيب الكلوية و وجود نزف بينها .