CARCINOGENICITY, CYTOGENICITY AND HISTOPATHOLOGICAL ASPECTS FOLLOWING LONG TERM ADMINISTRATION OF HIGH DOSES OF TARTRAZINE AND BUTYLATED HYDROXANISOL TO ALBINO RATS

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ABSTRACT

Food additives are non- nutritive substances which are added to food to preserve freshness, enhance flavor or appearance and aid in preparation of foods. Coloring agents are added to enhance the product image. Antioxidants are materials, which prevent oxidation of food, as well as oxidative reaction in edible fat processed foods. Long-term consumption of some food additives was accused in initiating different toxic reactions. Food additives used in this study were tartrazine and butylated hydroxy anisole. Fifty-six Albino rats of both sexes were divided into four groups each of fourteen rats. First group was given oral dose of 40 mg/kg.b.w. tartrazine; second group was given dist .water twice a week for 20 consecutive weeks. Third group was given oral dose of 50 mg./kg.b.w. butylated hydroxy anisol; fourth group was given corn oil twice a week for 20 consecutive weeks. Half the number of rats was slaughtered after 10 weeks and the other was slaughtered at end of 18 weeks. Both tartrazine and BHA increased aspartate amino transferase and Alanine amino transferase levels in rats treated for10 or 20 weeks. Globulin level was increased in-group administered tartrazine for 20 weeks. Total protein was decreased in rats administered butylated hydroxy anisol for 20 weeks . Deoxy ribonucleic acid level in wet liver tissue was decreased in group given butylated hydroxy anisol for 20 weeks. Alpha- fetoprotein level was increased in-group given butylated hydroxy anisol for 20 weeks. Tartrazine administration showed mutagenic effect after 10 or 20 weeks of administration. Such effects were in the form of deletion, break, rang, end to end and sticky chromosomes. Histopathological examination of the liver showed hydropic degeneration, fatty changes, some cells were enlarged with vaculated cytoplasm in rats treated with tartrazine for 20 weeks. Liver of rats treated with butylated hydroxy anisol for 20 weeks showed fibroblastic proliferation with increase number of bile ductules in portal area .

INTRODUCTION

A number of food additives, especially preservatives, dyes and flavoring agents, can induce a wide range of adverse reactions in sensitive individuals by a variety of mechanisms (Moneret and Andre 1983). Tartrazine is added to drugs in both solid and liquid preparations and to foods for identification purposes and to produce an attractive appearance (Lockey,1977). (F.D.A, 1979) estimated that the number of sensitive persons ranged from 50.000 to 100.000 in the United State. Juhlin (1981) studied 330

cases of recurrent urticaria and attributed 33% to food additive induced reactions; 18% reacted to azo dyes, 15%P to the antioxidant food preservatives (butylated hydroxyanisol and butylated hydroxy toluene) and 10% to annato .Tse (1982) and Rohl, et al (1987) and Josephr, (1990) mentioned that many manufactured food products contain tartrazine. The list includes beverages (particularly orange and lime drinks) ice cream and sherbets, gelatins, salad dressings, cheese, cake mixes seasoned salt and confections (butter, flavor, banana and pine apple extract) and many miscellaneous items such as candies and fruit chews and 300 drug items.Juhlin, (1984) reported that average daily consumption of dyes is 15 mg 85% of which is tartrazine. Henschler and Wild (1985) reported that tartrazine given to rats was transformed into urinary metabolites which exert dose-dependent mutagenic activities. Murdoch, et al (1987) reported that tartrazine caused increase in urinary prostaglandin E2; thromboxan b2 and prostaglandin 6 –keto f1.

Munzner and Wever, (1987) and Maekawi, et al., (1987) stated that tartrazine is not carcinogenic in rats when administrated continuously at doses of up to 2% in the drinking water for up to two years. Amount of tartrazine required to produce sensitivity in susceptible persons varies from 0.85ug to 25mg (Weiner and Bernstein, 1989). The same authors stated that tartrazine sensitivity reaction might be due to an action on a metabolic system or to other mechanisms, such as the release of local control substances or hormones. Giri,et al (1990) reported that significant increase in sister chromatid exchange and chromosomal aberrations were observed in bone marrow cells of mice and rats taking tartrazine in diet in high doses .Josephr, (1990)stated that tartrazine contains a sulfanil group; which may spilt in the body to yield sulfanilic acid . A case of leukocytoclastic vasculitis was recorded due to food additives (Niels,et al 1991). Tartrazine inhibited dopamine sulphotransferase activity of human liver (Bamforth, et al 1993) . Aromatic amine benzidine was detected in "tartrazine "; introduced during manufacture of color additive (Davis and Bailey, 1993 Prival, et al 1993). Inflammatory cellular infiltrate within hepatic lobules ,vacuolated nuclei of hepatic cells and hyperplasia of bile duct were detected in liver of rats administered tartrazine(Madiha, 1995)Tartrazine inhibited mitochondria respiration in mitochondria isolated from rat liver and kidney (Reyes, et al ,1996) .

Butylated hydroxy anisole is a phenolic compound (Denz and Llaurado, 1957). Butylated hydroxy anisole was found in oil, margarine and butter (Yamada, 1990). Unsaturated fatty acids in liver and heart used for prevention of lipid peroxidation were increased after administration of butylated hydroxy anisol " hence aging of the organism".None of the antioxidants was previously considered to possess carcinogenic activity (Hirose, et al., 1980 and Shirai, et al. 1982). BHA caused papilloma and carcinoma in rodents (Ito et al., 1983).BHA caused proliferation of tritiated thymidine (a specific DNA precursor) (Neura, et al 1984).BHAshown to induce a range of proliferative lesions (Altmann, et al.1985)there is increased incidence of liver tumors in rat fed BHT for 33 months (Olsen, et al., 1986) .Lindenschmidt et al. (1986) reported that male (not female) animals had an increased incidence of hepatic tumors compared to animals kept on BHT-free diet. Chronic administration of the antioxidant: (BHT) was described to enhance the development of neoplasm (Williams, 1979). Butylated hydroxy anisol anti-oxidant stimulate synthesis of DNA resulting in increase in mitotic

index (Walker and Quattrucci, 1988)Thompson et al (1989) reported that butylated hydroxy toluen (antioxidant) can enhance the formation of carcinogen induced lung tumors in mice. Phenolic anti-oxidant caused subcellular liver damage (Clapp et al 1973), hepatocellular vacuolation, degeneration and necrosis in the liver enlargement (Takahashi and Hiraga, 1981 and Gaunt et al 1985 and Takahashi, 1987). Walker and Quattrucci, (1988) stated that toxic effect of BHA are mediated by the parent or metabolite compound and tissue specific.

This work aims to study and evaluate either tartrazine or butylated hydroxy anisol (common food additives) considered to posses carcinogenic and mutagenic activities in experimental Albino rats.

MATERIAL AND METHODS

Test material

A) Tartrazine: Tartrazine (C.I.19140) orange yellow fine powder; was obtained from Aldrich Chemical Co. Ltd.

B) Butylated hydroxy anisol: White crystals of Butylated hydroxy anisol (B.H.A.) was obtained from Kamena Products Corporation Cairo, Egypt.

Animals:

Fifty-six Albino rats with weight from 146-170 g of both sexes obtained from the laboratory animal house, Faculty of Veterinary Medicine, Moshtohor. Rats were kept under hygienic conditions, fed on a balanced ration and water ad-libtum. Rats were divided into four groups each of fourteen rats.

Experimental design

First group: Tartrazine was dissolved in distilled water and administered orally by a stomach tube at 40 mg/kg.b.w.,every 48h. for 20 consecutive weeks according toGrzelewaske,et al.,(1986).

Second group: This group was given oral administration of an equivalent volume of distilled water that was given to the first group every 48 h for 20 consecutive weeks (control for first group).

Third group: Butylated hydroxy anisol was dissolved in corn oil and administered orally by stomach tube to rats at 50 mg/kg.b.w.according toWartzen, (1993) every 48h. for 20 consecutive weeks.

Fourth group: This group was given an equivalent volume of corn oil that was given to the third group by the same route and in the same time (control for third group).

Half the number of rats from each group was slaughtered after 10 weeks of experiment. Other rats were slaughtered after 20 weeks of experiment.

Samples

Blood was obtained and allowed to clot then serum was derived . Serum value of aspartate amino transferase, alanine amino transferases were determined according to methods described by Reitman and Frankel (1957) and Bessey et al., (1946) respectively. Serum levels of total protein, albumin were measured colorimetrically by using kits according to Doumas, et al.(1981) and Pinell and Northam (1978) respectively; while serum globulin was detected according to Coles, (1974) .Liver

tumor markers as Alphaphetoprotein by Electro-chemolumensis technique on ELECSYS.1010 apparatus according to Ramsy and Wug,(1995) Deoxy ribonucleic acid in liver tissue was detected according to Melmed et al .(1975)and Abdel salam, (1983). Bone marrow was collected from femur for detection of chromosomal apparition according to (Macgregor and Varley, 1983). Specimens from liver was collected and fixed in 10% formalin solution. Paraffin sections of 5- micron thick were prepared and stained by H&E (Brancroft and Stevens,1990).

Statistical analysis: The data obtained in this study were calculated as mean ± standard error and were statistically analyzed by the student's (t) test. All statistical analysis were carried out according to Johnston, (1972).

RESULTS

Table (1) illustrate the serum levels of alanine amino transferase; and aspartate amino transferase . First group (administered 40 mg/kg.b.w. tartrazine) showed significant increase in the serum levels of ALT and AST in comparison to second group (control). ALT serum levels for first group were 65.7±2.8 u/ml and 74.57± 1.7 u/ml after 10 and 20 consecutive weeks respectively compared to 53.42± 3.01 and 60.86± 1.55 u/ml for second group (control). Serum aspartate amino transferase levels in first group were 89.14± 2.97 u/ml and 111.85± 2.19 u/ml after 10 and 20 weeks. An increase in serum levels of alanine amino transferase and aspartate amino transferase were detected in group administered oral dose of butylated hydroxy anisol for 10 or 20 weeks in comparison with forth group (administered corn oil).

Table (2) shows the effect of oral administration of tartrazine and butylated hydroxy anisol on total protein, albumin and globulin. The results indicat that tartrazine and BHA. caused non-significant effect on total protein, albumin and globulin after 10 weeks. Oral administration of 40 mg/ kg.b.w. for 20 consecutive weeks caused increase in globulin levels. Globulin levels were 3.74± 0.126gm/dl in first group compared with 2.4 ± 0.39 gm/dl of control group. Oral administration of butylated hydroxy anisol for 20 weeks showed high significant decrease in total protein compared with control group. Total protein in third group (administered 50 mg/ kg.b.w) was 5.57 ± 0.209 gm/dl compared with 7.515± 0.472 gm/dl of control group.

Deoxy ribonucleic acid content in liver tissue of Albino rats administered tartrazine or butylated hydroxy anisol for 10 or 20 consecutive weeks were showed in table (3). The results indicate decrease in liver DNA content in group taking BHA for 20 consecutive weeks. DNA liver content in third group after 20 weeks was 2.37±0.156 mg/g in comparison with 1.61± 0.09 mg/g tissue of control group (administered corn oil).

Table (4) reveales that serum Alpha-fetoprotein levels were not affected by oral administration of 40 mg/kg.b.w. tartrazine for 10 or 20 consecutive weeks. Similar pattern occurred with oral administration of butylated hydroxy anisol for 10 consecutive weeks. High significant increase was recorded in Alpha-fetoprotein level in-group administered 50 mg /kg.b.w.butylated hydroxy anisol for 20 consecutive weeks. Alpha-

fetoprotein levels was 16.620± 3.56 iu/ml for third group in comparison with 5.729± 1.915 iu/ml for control group.

Table (5) showed the structural and numerical chromosomal aberrations of bone marrow cells of rat administered tartrazine or butylated hydroxy anisol for 10 consecutive weeks. First group (taking 40 mg/ kg.b.w. tartrazine) is the only group showed deletion, ring chromosomal aberration as clear in fig.(1)and polyploidy as compared to second group (control group). Structural and numerical chromosomal aberration of bone marrow cells after 20 consecutive weeks were clear in table (6) First group (administered 40 mg/kg.b.w. tartrazine) showed an increase in abnormal cells, fragment, deletion, break, ring, end to end and sticky chromosome in addition to polyploidy in comparison with control group are fig.(2,3,4,5,6). Oral administration of butylated hydroxy anisol for 20 consecutive weeks-showed non-significant effects on structural and numerical chromosomal aberration.
Histopathological findings of liver in rats given tartrazine for 9 or 18 weeks revealed leukocytic infiltration in portal area, kupffer cells, bile duct proliferation ,hydropic degeneration at peripheral, fatty change, focal areasof coagulative necrosis, some cells were enlarged and vesiculated with vaculated cytoplasm(Fig.7 and 8) .Liver in rats given butylated hydroxy anisol for 9 or 18 weeks showed hydropic degeneration ,few fibroblastic proliferation in portal areas and in between hepatic cells in addition to thickness in portal area ,increase number of bile ductules and disassociation of hepatic cells with necrosis (Fig.9 and 10).

DISCUSSIONS

It is well recognized that a number of food and drug additives can induce a wide range of adverse reactions elicited by a variety of mechanisms. Serum levels of alanine transferase and aspartate amino acid transferase showed increased levels in group administered tartrazine. These results could be related to deterioration of hepatic functions as a resulted from toxic effect of tartrazine on the liver that induce inhibition of dopamine sulphotransferase activity (Bamforth, et al.1993 and Reyes, et al, 1996).The increased ALT and AST could be due to the inflammatory cellular infltrate within hepatic lobules vaculated nucelei in hepatic cells. (Madiha,1995). This interpretation was supported by the histopathological changes of the liver. the increased serum levels of alanine tranferase and aspartate amino acid transferase in group administered BHA for 20 weeks could be due to hepatic damage as a result of chronic toxic effect of phenolic antioxidant BHA (Clapp et al 1973, National institute of health, 1979, Takahashi and Hiraga, 1981, Gaunt, et al, 1985 and Takahashi, 1987). The increased serum ALT and AST values might discussed due to the BHA shown to induce a range of proliferative lesions in liver (Altmann ,et al.1985).This effect are mediated by the parent or metabolite compound of BHA antioxidant (Walker and Quattrucci,1988). The interpretation was supported by histopathological finding.

Serum levels of total protein, albumin, and globuline showed no change in-groups administered tartrazine of butylated hydroxy anisol for 10 weeks. Administration of tartrazine for 20 weeks caused increased levels of globulin .These results may be due to the sensitivity reactions of tartrazine as mentioned by Juhlin, (1981). Sensitivity

reactions of tartrazine are due to tartrazine metabolites as sulfanilic acid food additives or their metabolites act as hapten (Johnson, et al, 1975) or due to pharmacological mechanism that lead to mediator release (Murdoch, et al, 1987). Tartrazine caused histamine release (Timbrell, 1989).. Butylated hydroxy anisol caused a decrease in total protein ;which might be possible due to liver damage from chronic BHA administration as mentioned by Clapp et al (1973), Takahashi and Hiraga (1981), Gaunt, et al (1985) and Takahashi (1987).

Deoxy ribonucleic acid and alpha-phetoprotein levels showed non-significant effects in-group administered tartrazine either for 10 or 20 consecutive weeks. highly significant increase in DNA content was detected in group administered butylated hydroxy anisol for 20 weeks . Alpha-fetoprotein was increased in group administered BHA for 20 weeks. These results do not agree with Hirose, et al (1980) and Shira, et al (1982). Our results were in agreement with those of Ito ; et al (1983) and Lindenschmidt, et al (1986),Altmann et al (1985),Ito, et al (1986), Olsen, et al (1986); Williams, (1979) and Thompson, et al (1989). These results might be related to proliferation of tritiated thymidine (a specific DNA precursor) as reported by Neura, et al (1984) and stimulation of DNA synthesis resulting in an increase in mitotic index (Walker and Quattrucci, 1988). This effect is mediated by the parent or metabolite compound and tissues specific that led to tumor promoting effects (Walker and Quattrucci, 1988 and Thmpson, et al 1989).

Effect of tartrazine on structural and numerical chromosomal aberrations showed a presence of deletion, ring form and polyploidy after 10 weeks of treatment ;the effect was more evident after 20 weeks of administration. These results agree with Henschler and Wild (1985) and Giri et al (1990) These results are attributed to tartrazine metabolized by gut flora giving rise to several metabolites, which exert dose-dependent mutagenic activity (Henscher and wild, 1985) or due to other mechanisms such as the release of local control substances or hormone (Weiner and Bernstein, 1989) Butylated hydroxy anisol showed non-mutagenic effect on rat bone marrow.

As regard to histopathological findings, the liver of rats given tartrazine, in the present work, showed leukocyte infiltration in portal area, bile duct proliferation, degeneration, fatty change, vacuolated cytoplasm similar results obtained by (Madiha, 1995). Inflammatory cellular infiltrate within hepatic lobules, vacuolated nuclei of hepatic cells and hyperplasia of bile duct were detected in liver of rats given tartrazine. Liver of rats treated with BHA showed hydropic degeneration, fibroblastic proliferation in portal area, increase number of bile ductules and dissociation of hepatic cells with necrosis. These findings were in agreement with (Ford et al, 1979 and Powell et al 1986) . Degenerative changes and necrosis in liver may be attributed to toxic effects of tartrazine and BHA.

Conclusion: *According to the above-mentioned findings we can conclude that both tartrazine and BHA have a remarkable toxic effects on hepatic tissue. So we recommended that food additives must be used only when it is vitally essential and should not exceed the acceptable daily intake, permitted by the joint FAO/WHO. Name and types of additives must put on the container of food. The Egyptian nutritional habits as their dependence on the carbohydrates and vegetables oils there should be limit to use of antioxidants. Food and sweet manufacture must be put under control especially those made without permission.*

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Table (1): Effect of oral administration of tartrazine and butylated hydroxy anisol (40 and 50 mg/kg.b.w. respectively) on some biochemical parameters after administration to Albino rat three time /week for 10 or 20 consecutive weeks (mean ± SE).

Groups	Doses	ALT(u/ml) at 10 weeks	AST (u/ml) at 10 weeks	ALT(u/ml) at 20 weeks	AST (u/ml) at 20 weeks
Group 1	40 mg/kg.b.w.	65.7*±2.8	89.14*±2.97	74.57**±1.7	111.85**±2.19
Group 2	1ml ist.H2O (control for group)	53.42±3.01	74.85±2.95	60.86±1.55	83.14±1.62
Group 3	50mg/kg.b.w. butylated hydroxy anisol.	67.43*±3.59	90.14*±2.77	76.29**±2.02	106.429**±1.94
Group 4	1ml corn oil	56±3.17	81.14±1.83	65.4±1.645	88.14±2.25

*Significant P≤ 0.05 ** Significant at p≤ 0.01

Table (2): Effect of oral administration of tartrazine and butylated hydroxy anisol (40 and 50 mg/kg.b.w.respectively) on total protein, albumin and globulin after administration to Albino rat three time /week for 10 or 20 consecutive weeks (mean ± SE).

Groups	Doses	Total protein gm/dl at 10 w.	Albumin gm/dl at 10 w.	Globulin gm/dl at 10 w.	Total protein gm/dl at 20 w.	Albumin gm/dl at 20 w.	Globulin gm/dl at 20 w.
Group 1	40 mg/kg.b.w.	5.97±0.43	3.4±0.195	2.51±0.35	7.19±0.39	3.4±0.44	3.74**±0.126
Group 2	1ml dist.H2O (control for group)	6.14±0.31	3.74±0.18	2.4±0.39	6.37±0.34	3.96+0.09	2.4±0.392
Group 3	50mg/kg.b.w. butylated hydroxy anisol.	6.93±0.42	3.6±0.16	3.34±0.24	5.57**±0.21	2.94±0.09	3.65±0.19
Group 4	1ml corn oil	6.77±0.32	3.91±0.14	2.8±0.23	7.52±0.47	3.37±0.16	2.214±0.14

*Significant P≤ 0.05 ** Significant at p≤ 0.01

Table (3): Effect of oral administration of tartrazine and butylated hydroxyanisol (40 and 50 mg/kg.b.w.respectively) on Deoxyribonucleic acid (mg/g) content after administration to Albino rat three time /week for 10 or 20 consecutive weeks(mean ± S.E.).

Groups	Doses	Deoxyribonucleic acid (DNA) mg/g wet tissue	Deoxyribonucleic acid(DNA)mg/g. wet tissue
Group 1	40 mg/kg.b.w.	1.62±0.18	3.64±0.29
Group 2	1ml dist.H2O(control for group)	2.08±0.12	3.47±0.25
Group 3	50mg/kg.b.w.butylated hydroxy anisol.	1.93±0.124	1.16**±0.09
Group 4	1ml corn oil	1.84±0.13	2.37±0.16

*Significant P≤ 0.05 ** Significant at p≤ 0.01

Table (4): Alpha-fetoprotein serum levels of Albino rats following oral administration of 40mg/kg.b.w. tartrazine or 50 mg/kg.b.w.butylated hydroxyanisol for 10 or 20 consecutive weeks compared to control groups. (Mean ± S.E.)

Groups	Doses	AFP at 10 weeks	AFP at 20 weeks
Group 1	40 mg/kg.b.w.	4.029±0.464	7.329±0.453
Group 2	1ml dist.H2O(control for group)	4.714±0.53	6.292±0.658
Group 3	50mg/kg.b.w. butylated hydroxy anisol.	6.36±0.621	16.62**±3.56
Group 4	1ml corn oil	4.27±0.74	5.729±1.92

*Significant P≤ 0.05 ** Significant at p≤ 0.01

Table (5): Structural and numerical chromosomal aberrations of bone marrow cells of rat following oral adminestration of 40 mg/kg.b.w. tartrazine and 50 mg/kg.b.w.butylated hydroxyanisol for 10 consecutive weeks compared to control groups .(Mean± S.E.)

Groups	Doses	Examined cell No.	Abnormal cells No.	Structural chromosomal aberrations							Mitotic index	Numerical aberation		
												Aneuploidy		Poly-ploidy
				Fragment No.	Deletion No.	Break No.	Ring No.	End to End No.	Centromeric attenuation No.	Sticky No.		Hypo-diploide	Hyper-diploidy	Poly-pioidy
Group 1	40 mg/kg. b.w. tart-razine	250	4.28 ± 0.96	3.286 ± 0.606	4.57* ± 0.84	5.14 ± 0.74	10.14** ± 0.74	4.43 ± 0.65	4.86 ± 0.91	3.86 ± 0.95	1.91 ± 0.24	6.429 ± 0.75	5 ± 1.1	7.57** ± 1.21
Group 2 control for 1st. group	1ml dist. water	250	3.14 ± 0.86	3.571 ± 0.61	2 ± 0.9	3.57 ± 0.57	2 ± 0.44	2.57 ± 0.65	4 ± 0.69	3.86 ± 0.98	1.83 ± 0.26	4.29 ± 0.89	3.1 ± 0.40	2.86 ± 0.67
Group 3	50 mg/kg. b.w BHA	250	4.57 ± 0.65	3.43 ± 0.65	3.857 ± 0.59	3.71 ± 0.52	3.43 ± 0.37	3.29 ± 2.65	3.29 ± 0.86	4.86 ± 1.01	1.93 ± 0.13	7.71 ± 3.35	4.57 ± 0.84	3 ± 0.93
Group 4 (control for 3rd. group)	1ml of corn oil	250	4.14 ± 0.79	3.29 ± 0.71	3.71 ± 0.87	3.86 ± 0.51	2.57 ± 0.84	3.57 ± 0.84	2.29 ± 0.42	3.43 ± 1.07	1.8 ± 0.12	5.57 ± 2.07	3.86 ± 1.01	1.71 ± 0.57

*Significant at p≤ 0.05 ** Significant at p≤ 0.0.01

Table (6): Structural and numerical chromosomal aberrations of bone marrow cells of rat following oral administration of 40 mg/kg.b.w. tartrazine and 50 mg/kg.b.w.butylated hydroxyanisol. For 20 consecutive weeks compared to control groups .(Mean± S.E.)

Groups	Doses	Examined cell No.	Abnormal cells No.	Structural chromosomal aberrations							Mitotic index	Numerical aberration		
				Fragment No.	Deletion No.	Break No.	Ring No.	End to End No.	Centromeric attenuation No.	Sticky No.		Aneuploidy: Hypo-diploide	Aneuploidy: Hyper-diploidy	Polyploidy
Group 1	40 mg/kg b.w tartrazine	250	15.8** ± 0.66	7.71** ± 0.89	1.4** ± 0.98	7** ± 0.98	14.71** ± 1.209	18.57** ± 01.29	13.14 ± 0.86	19.57** ± 01.77	1.98 ± 0.22	2.7 ± 0.29	2.14 ± 0.59	18.28** ± 2.45
Group 2 (control for 1st group)	1ml dist water	250	4.29 ± 0.92	2.571 ± 0.53	2 ± 0.9	2.71 ± 0.75	4.57 ± 0.840	5.28 ± 01.016	4 ± 0.76	4.29 ± 0.68	1.93 ± 0.25	1.43 ± 0.83	1.57 ± 1.06	4 ± 0.75
Group 3	50 mg/kg b.w BHA	250	5.43 ± 0.78	6.14 ± 0.828	4.857 ± 0.88	3.43 ± 0.895	4 ± 0.786	5 ± 0.76	5.86 ± 01.06	4.71 ± .87	2.44 ± 0.21	2.286 ± .42	2.14 ± 0.81	4.57 ± 1.04
Group 4 (control for 3rd group)	1ml of corn oil	250	4.57 ± 0.84	5.29 ± 01.06	3.29 ± 0.421	4.29 ± 0.87	4.71 ± 0.87	4.14 ± 0.595	4.143 ± 0.962	2.29 ± .52	2.08 6± 0.21	2 ± .31	1.85 ± .34	2.14 ± 0.51

*Significant at p≤ 0.05 ** Significant at p≤ 0.0.01

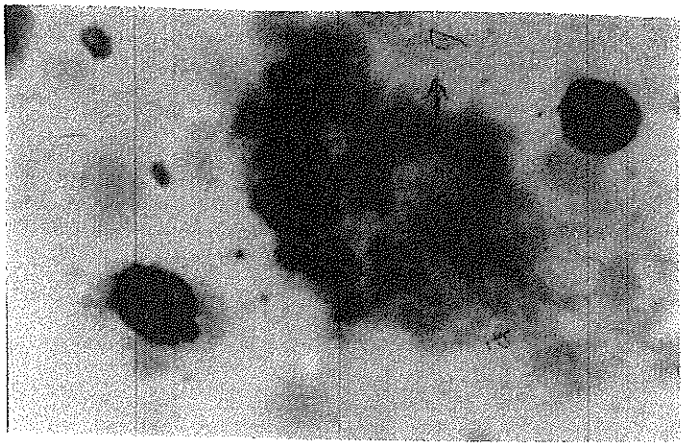

Fig.(1): Metaphase chromosomes of bone marrow cells of rats treated with tartrazine after 10 weeks showing. a –Ring chromosome. b –Chromatid deletion.

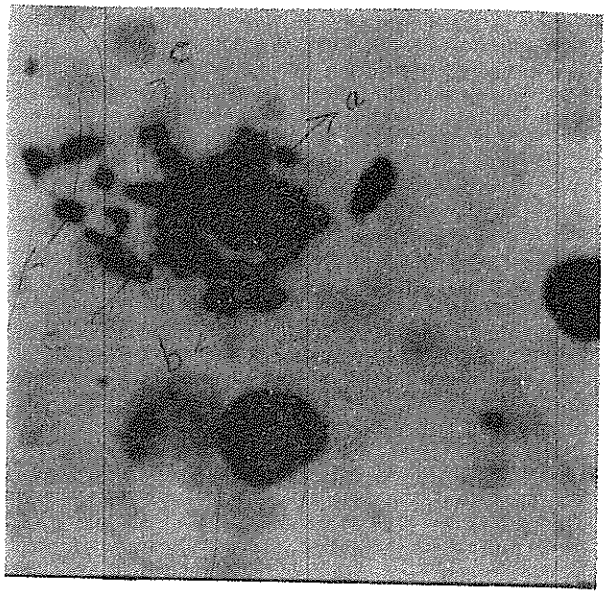

Fig.(2): Metaphase chromosomes of bone marrow cells of rats treated with tartrazine after 20 weeks showing. a-Chromosomal fragment. b-Ring chromosome. c-Deletion.

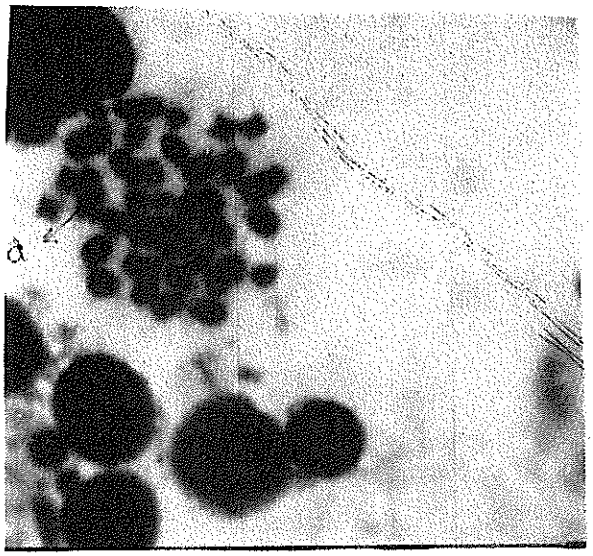

Fig.(3): Metaphase chromosomes of bone marrow cells of rats treated with tartrazine after 20 weeks showing. a-End to end chromosome

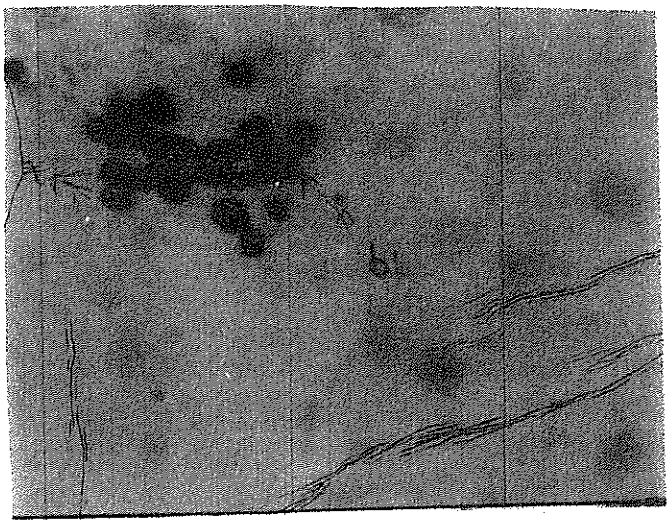

Fig.(4): Metaphase chromosomes of bone marrow cells of rats treated with tartrazine after 20 weeks showing. a-Ring chromosomes. b-Deletion chromosome

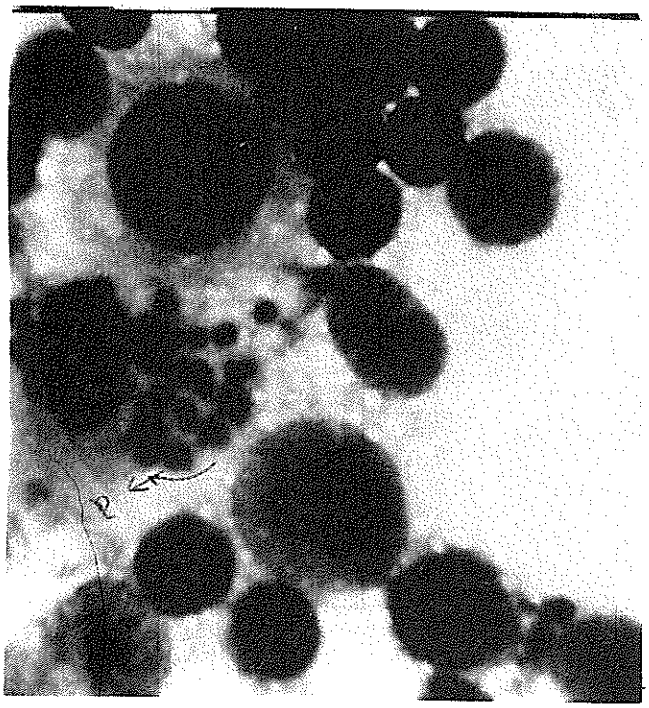

Fig.(5): Metaphase chromosomes of bone marrow cells of rats treated with tartrazine after 20 weeks showing. a-Centromeric attenuation chromosomes.

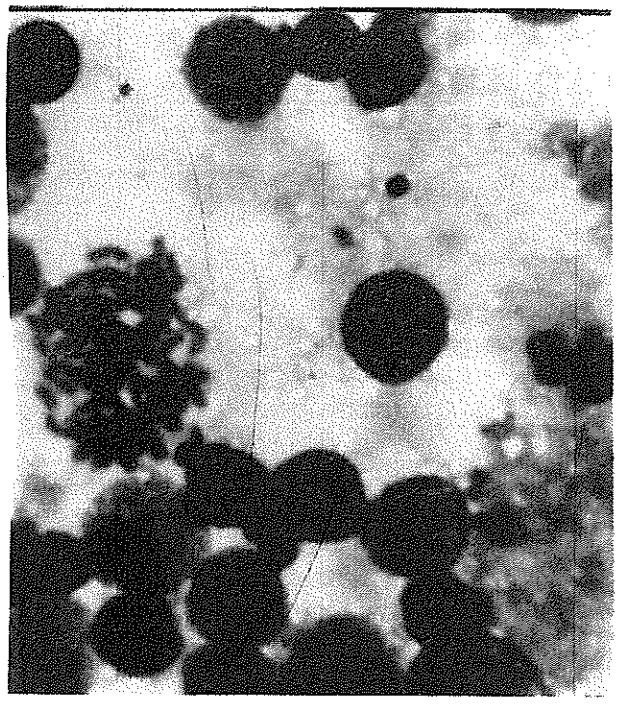

Fig.(6): Metaphase chromosomes of bone marrow cells of rats treated with tartrazine after 20 weeks showing sticky chromosomes.

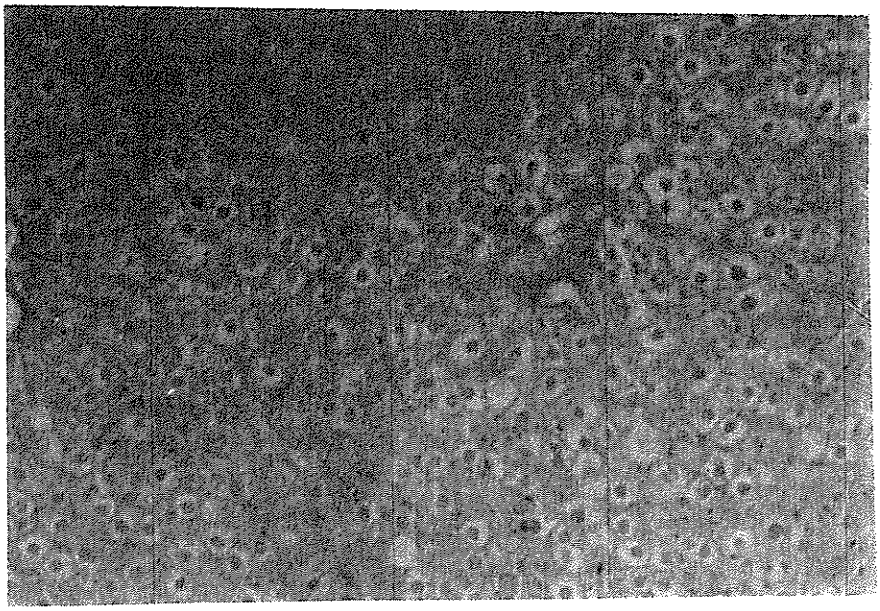

Fig.(7): hydropic degenration together with congestion of the central vein. (H&E stain X1200).

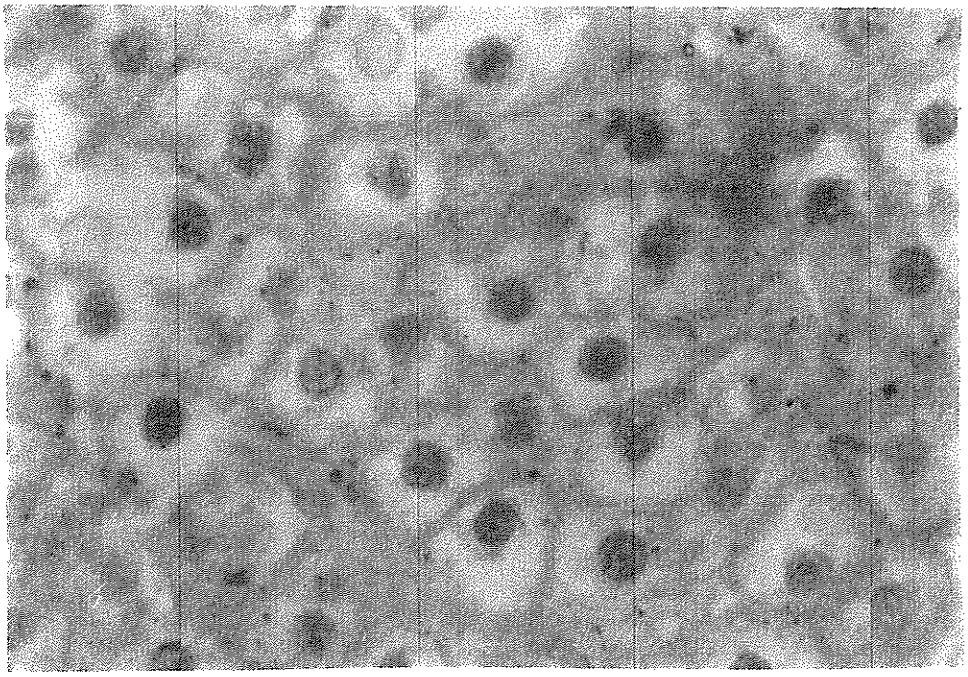

Fig.(8) : Liver in rats given tartrazine for 20 weeks showing fatty change , some cells were enlarged and vesiculated with vaculated cytoplasm..(H&E stain X 300)

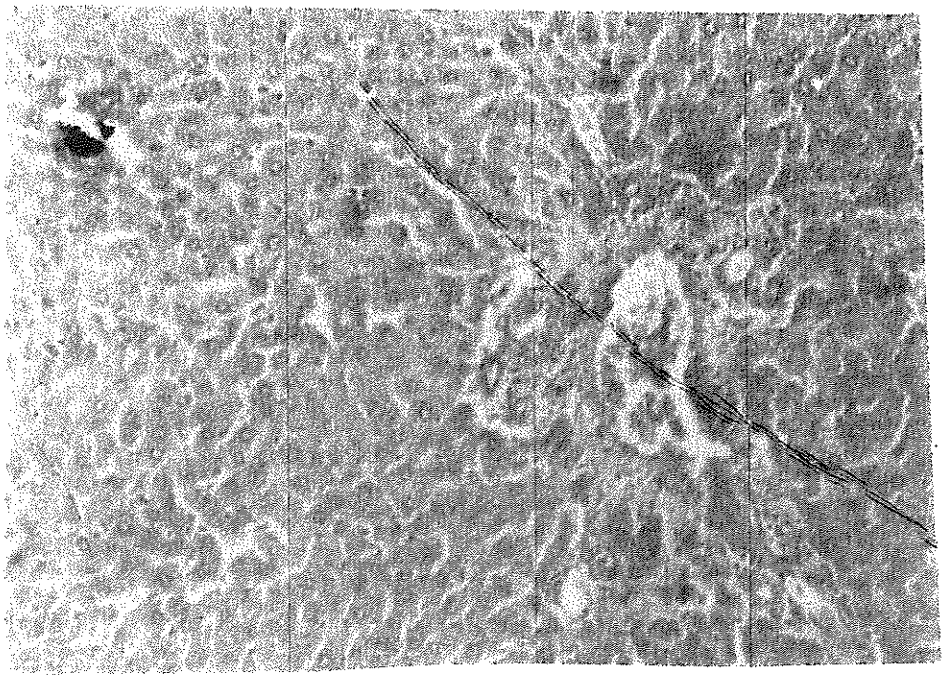

Fig.(9): Liver of rats given Butylated Hydroxy anisol showing fibroblastic proliferation with increase number of bile ductules in portal area .(H &E stain X 300).

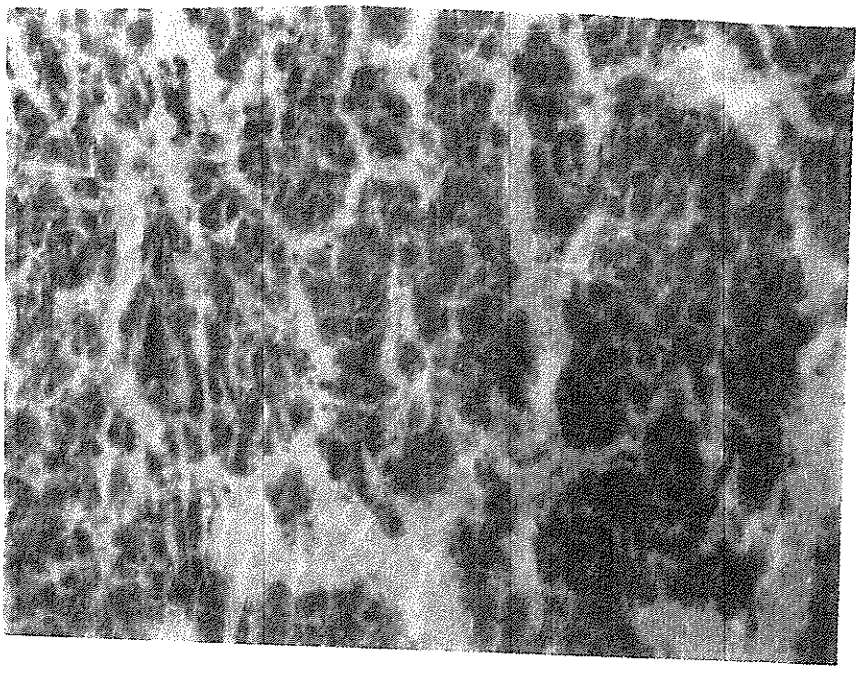

Fig.(10) : High power for the previous figure.

الملخص العربى

التأثير السام والمسرطن والهستوباثولوجى لجرعات عالية من الترترازين والبيتيوليتيد هيدروكسى أنيزول على الفئران البيضاء

نبيلة محمود عبد العليم و ممدوح محمد توفيق**

جامعة الزقازيق- فرع بنها.-قسم الطب الشرعى والسموم- كلية الطب البيطرى مشتهر

**قسم الباثولوجى-معهد بحوث صحة الحيوان.فرع الزقازيق.

ازداد فى الفترة الأخيرة استخدام اضافات الأغذية بدون رقابة محكمة سواء فى المصانع المصرح لها او تلك البعيدة عن الرقابة الصحية.بعض المواد يضاف الى الأطعمة كمكسبات لون (الترترازين) أو كمضادات أكسدة مثل(البيتيوليتيد هيدروكسى أنيزول).لذلك هدفت الدراسة الى تقييم التأثيرات السامة لجرعات عالية من تلك المواد.استخدم عدد ٥٦فأر أبيض قسمت الى أربعة مجموعات كل منها ١٤ فأر.جرعت المجموعة الأولى ٤٠مجم لكل كيلو جرام من وزن الجسم ترترازين.جرعت المجموعة الثانية ماء مقطر (استخدمت مجموعة ضابطةللمجموعة الأولى) . جرعت المجموعة الثالثة بمادة البيتيولتيد هيدروكسى أنيزول المذابة فى زيت الذرة بجرعة ٥٠ مجم لكل كيلو جرام من وزن الجسم عن طريق الفم جرعت المجموعة الرابعة بزيت الذرة (استخدمت مجموعة ضابطةللمجموعة الثالثة) .تم التجريع كل ٤٨ ساعة لمدة ٢٠ أسبوع متتالية.تم ذبح نصف الفئران عند الأسبوع العاشر من التجربة .بينما تم ذبح النصف الأخر عند الأسبوع العشرين .أخذت عينات مصل وعينات من نخاع عظام الفخذ وكذلك أنسجة من الكبد لأجراء بعض التحليلات.أظهرت النتائج أن كل من مادتىالترترازين والبيتيولتيد هيدروكسى أنيزول تحدث زيادة فى نسبة انزيمى أسبرتيت ترانسفيريز و الألانين أمينوترانسفيريز بعد ١٠ أو ٢٠ عشرون أسبوع. كما أحدث الترترازين زيادة فى نسبة الجلوبين بعد ٢٠ أسبوع بينما أظهرت المجموعة الثالثة انخفاض فى نسبة البروتين الكلى بعد ٢٠ أسبوع بالمقارنة بالمجموعة الضابطة. كما أظهر حقن البيتيولتيد هيدروكسى أنيزول الى ارتفاع نسبة الفافيتوبروتين فى السيرم .بالأضافة الى زيادة فى كمية الحمض النووى الديزوكسى ريبوزى بعد عشرين أسبوع بالمقارنة بالمجموعة الضابطة.أظهرت المجموعة الأولى (جرعت بمادة الترترازين) الى ظهور تغييرات فى كروموسومات نخاع العظام والتى كانت أوضح ما يمكن بعد مرور ٢٠ أسبوع. كما أظهر الفحص الباثولوجى لنسيج الكبد وجود حرض شحمى و تضخم فى الخلايا وكان أكثر وضوحا فى المجموعة التى جرعت بمادة الترترازين لمدة ٢٠ أسبوع.وجد زيادة فى القنوات المرارية وكذلك خلايا الفيبروبلاست فى المجموعة التى جرعت بالبيتيوليتيد هيدروكسى أنيزول لمدة ٢٠ أسبوع.