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Biochemical, Histopathological and Cytogenetic Evaluation of Fortified Milk and Yoghurt with Zinc and Iron Salts in Male Albino Rats

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Abstract: Milk and its products are among the most important sources of nutrients for humans diets along their life, but they are poor in some other elements particularly, Iron and Zinc. Iron or Zinc fortification of dairy products may cause problems in many products and disposers to the consumers. The work herein aimed to study the effect of zinc and iron salts fortification of the feed on biochemical, Histopathological and cytogenetic parameters in rats. Results individually or a in combination at concentrations of 20 and 40 mg/kg/b.w. ferrous chloride and Zinc acetate at a daily dose for 8 weeks, caused remarkable increase in the activity of liver enzymes (AST and ALT). The microscopical examination of liver tissues revealed that moderate to marked changes in hepatocytes, congestion in portal vein, fibrous tissue and proliferated bile ducts. Cytogenetic results indicated that ferric chloride and Zinc acetate salts exhibited significant increase in the frequencies of micronucleated polychromatic erythrocytes (MNPCEs) than control. The degree of micronucleated polychromatic erythrocytes is directly proportional to the doses used for ferric chloride and zinc acetate. It was concluded that based on, cytogenetic studies that ferrous chloride and Zinc acetate salts may have a mutagenic activity in bone marrow cells of rats.

Key words: Biochemical · Histopathological · Cytogenetic studies · Fortified Milk · Zinc and Iron Salts · Yoghurt · Male Albino Rats

INTRODUCTION

Milk or other dairy products are close to ideal food that contains all nutrients required for newborn adults and olders. Milk is considered as a good source of proteins, fat, carbohydrates as well as vitamins, calcium and phosphorus, however, it is generally poor source for trace elements [1].

Traces metals like zinc, manganese, copper and iron have special importance among milk constituents. Variations in milk composition occur due to various factors such as maternal trace elements, intake and status, maternal age, parity, residing area. This variability, in essentials elements could result in inadequate elemental nutrition of infants feeding on human milk. The use of fortified human milk produces adequate growth in premature infants and satisfies the specific nutritional requirements of these babies [2].

The popularity of dairy products make them as natural sources to iron and zinc fortification considering their ability to deliver rare combination of iron, zinc and calcium. Despite its importance, iron and zinc fortification of dairy products may affect the quality and storage stability fortified products. Iron (Fe) deficiency in the diet leads to anemia which is still the most prevalent nutritional problems in the world including Egypt, among preschool children, whose rapid growth increase their need for Iron. [3].

The recommended dietary allowances (RDA) of iron, 10 mg/day for olderly women and men [4]. Zinc deficiency in human includes loss of appetite, growth retardation, skin changes and immunological abnormalities [5].

To meet the needs of practically all healthy persons, including those who habitually consume diets with ion-zinc bioavailability, RDA for adult women 12 mg/day and for men is set at 15 mg zinc/day.

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Iron (Fe) is a common chemical element that is essential for organisms as a co-factor in oxygen transport, but high Fe amounts presents a significant risk of neurodegenerative disorders. Ferrous chloride causes alteration and inhibition of DNA synthesis only in proliferative cells, which explain the concomitant occurrence of mutagenicity and cytotoxicity [6].

It was demonstrated that the degree of chromosomal damage induced by three compounds of zinc (zinc chloride, zinc sulfate and zinc acetate) was directly proportional to the concentrations used for zinc sulfate and zinc acetate but not for zinc chloride [7].

In view of the a forementioned data the objectives of this study were to evaluate the effect of fortification of milk with iron and zinc depending upon the changes in some biochemical, histophathological and cytogenic parameters in experimental animals.

MATERIALS AND METHODS

Materials

Source of Milk: Fresh whole buffalo's milk was obtained from the herd of Faculty of Agriculture, Al-Azhar University, Mostorod, Cairo, Egypt.

Yoghurt Culture: Mixed starter culture, consisted of *Lactobacillus delbrueckii subsp*. *Bulgaricus* and *Lactococcus salivarius subsp, Thermophiles* were purchased by CHR-Hansen's lab A/S Copenhagen, Denmark.

Iron and Zinc Salts: Food grade salts were used. Ferrous chloride and ferrous sulphate (Merck Chemicals Company, Germany).

Zinc sulphate and zinc acetate (El-Nasr Pharmaceutical Chemicals Company, Egypt).

Experimental Animals: A total of 42 adult male albino rats weighing about 100-120 g were used in experiment. Animal were kept under normal laboratory conditions in animal house of Natinal Organization of Drug Control and Research (NODCAR) for 2 weeks before the initiation of the experiments. Rats were allowed free access of water and fed on uniformly diet.

Methods

Iron and Zinc Salts Preparations: In order to achieve the accuracy and the complete distribution of fortified salts, preparation of 10000 mg/kg. of iron and zinc salts were prepared by dissolving theses salts in distilled water. Then the real concentration of iron ad zinc salts was determined using atomizer these preparations were kept in refrigerator and monthly renewed.

Pilot Experiment

Solubility of Iron and Zinc Salts Against Heating: The solubility of any fortified element is considered as a limiting factor for its absorption in body. Heating at 85°C for 5 min is essential treatment for dairy industry. Therefore, this experiment was devoted to investigate the effect of heating on solubility of iron and zinc salts either in separate addition or in combinations. At the rate of 40mg /kg

The following results were obtained

Type of salt 40 mg/kg permeate	Observation
Ferrous chloride	Clear
Ferrous sulphate	Clear
Zinc sulphate	Clear
Zinc acetate	Clear
Ferrous chloride + Zinc sulphate	Turbid
Ferrous chloride + Zinc acetate	Clear
Ferrous sulphate + Zinc sulphate	Turbid
Ferrous sulphate + Zinc acetate	Turbid

From the above table, it could be say that used iron and zinc salts could added to milk and dairy products as separate salt, however only ferrous chloride and zinc acetate could be added together without expecting their precipitation.

Organoleptic Assessment

Yoghurt: Yoghurt was organoleptically evaluated according to the score suggested by Nelson. and. Trout [8] with total score of 100 points as follows:

- Falvor (50 points)
- Body and texture (40 points)
- Appearance (10 points)

Yoghurt Manufacture: The full fat fresh buffalo's milk of (5.5-6%) fat was heated to 85°C for 20 min. then iron and zinc salt concentrations were added to milk while heating. Milk was cooled to 42°C inoculated with 3% yoghurt starter and incubated for about three hours. Yoghurt samples were chemically, microbiologically and organoleptically examined when fresh and after 3,6,10 ad 15 days of refrigerating at 5°C.

Experimental Design: Six rats served as a normal control (without treatment with minerals) and other 42 rats were divided randomly into equal groups (6 rats each) and subjected to the fortification with minerals treatments as follows:

- Group1: normal control, (basal diet)
- Group2: 20 mg/kg. b.w. ferrous Chloride in milk.
- Group3: 40 mg / kg. b.w ferrous chloride in milk
- Group 4: 20 mg/ kg. b.w. zinc acetate in milk
- Group5: 40 mg / kg. b.w. zinc acetate in milk
- Group6: 40 mg / kg / b.w zinc acetate + 40 mg / kg/b.wt ferrous chloride in milk
- Group 7: 40 mg / kg/b.w ferrous chloride + 40 mg / kg zinc acetate / kg/ b.w in yoghurt.

The previous dose were daily ingested to adult male albino rats for 8 weeks

Biochemical Analysis: Venous blood samples (2 ml from each rats) were collected from retro-orbital plexus veins before treatment (0 time) and then after 4 and 8 weeks. Serum samples were separated and the subsequent biochemical parameters were performed as follows:

Transaminases Alanine aminotransferase ALT (GPT) and Aspartate Aminotransferase AST (GOT) [9].

Histopathological Examination: At the end of experiments (8^{th} weeks), the animals were scarified and the post mortem examinations were carried out. Samples of liver and kidney were taken for microscopically [10,11].

Cytogenetic Techniques

Micronucleus Analysis: A micronucleus (MN) formed during the metaphase/anaphase transition of mitosis (cell division). It may arise from a whole lagging chromosome (aneugenic event leading to chromosome loss) or an acentric chromosome fragment detaching from a chromosome after breakage (clastogenic event) which do not integrate in the daughter nuclei. The bone marrow of five animals of control and all the treated groups were extracted, smear preparations made by using fetal calf serum according to the method of Salamone *et al*., [12] and stained in 10% phosphate buffered Giemsa (pH 6.8) for 5min. Polychromatic erythrocytes scored for micronuclei under the microscope. All slides should be independently coded before microscopic analysis. At least 2,000 immature erythrocytes per animal scored for the incidence of micronucleated immature erythrocytes.

Chromosomal Aberrations: Animal were injected IP with colchicines solution two hour later, animals were sacrificed by cervical dislocation and chromosomes of bone marrow cells were prepared and examined according to Yosida and Amano [13].

Statistical Analysis: The obtained data were subjected to analysis of variance (ANOVA) according to Snedecor and Cochran [14]. Least significant differences (LSD) were used to compare between means of treatments according to Walter and Duncan [15] (at probability 5%).

RESULTS AND DISCUSSIONS

Table (1) shows that the results of the effect of fortification with iron and zinc salts on the organoleptics properties of yoghourt. The obtained data revealed that Yoghurt could be acceptable when fortified with ferrous chloride at the rate of 20 and 40 mg/kg and also when fortified with ferrous sulphate, zinc acetate and zinc sulphate at the rate of 20 mg/kg only. A close result was previouslyobtained [8].

The Effect of the Short Term Feeding of a Milk Iron-zinc Diet to Rats: Alanine aminotransferase ALT (GPT) and Aspartate Aminotransferase AST (GOT) are considered to be a good markers for the state of the liver function. Any increase in the activity of these enzymes is considered to be a good sign for liver abnormalities and extensively used in the diagnosis of liver diseases. Thus detection of these enzymes is a valuable indication of the clinical activity of hepatic disease [16].

Essential trace metals like zinc, manganese, copper and iron have special importance among milk constituents. Variations in milk composition occur due to various factors such as maternal trace elements, intake and status, maternal age, parity, residing area. This variability, in essentials elements could result in inadequate elemental nutrition of infants feeding on human milk [17].

In this study, short term oral ingestion of buffalo's milk fortified with two essential trace elements (iron and zinc) was carried out. These rates of fortification were chosen according to results of organoleptic assessment of yoghurt samples, in the meantime the results of pilot experiments. (as described in materials and methods)

The objective of this experiment was to evaluate the safety utilization of fortification in milk and milk products with the minerals understudy to extenuate the disorders in health which may be occur.

Short term orally administration of ferrous chloride in doses of 20 and 40 mg/kg body weight for 8 weeks was carried out by ingestion on daily dose incorporated with milk.

Table 1: Organoleptic assessment of yoghurt fortified with iron and zinc salts.

Storage period (days)	Organoleptic properties	control	Treatments mg/kg: Ferrous chloride 20 mg	Ferrous chloride 40mg	Ferrous chloride 60mg	Ferrous sulphate 20 mg	Ferrous sulphate 40mg	Ferrous sulphate 60mg	Zinc sulphate 20 mg	Zinc sulphate 40mg	Zinc sulphate 60mg	Zinc acetate 20 mg	Zinc acetate 40mg	Zinc acetate 60mg
Fresh	Flavor (50)	48	48	40	37	44	36	31	38	31	R	38	25	R
	Body and texture (40)	34	32	34	33	35	34	27	33	30	R	34	27	R
	Appearance(10)	9	9	7	7	9	8	7	9	9	R	9	8	R
	Total (100)	91	89	81	77	88	78	65	80	70	R	81	60	R
3	Flavor (50)	48	47	38	36	40	33	25	35	29	R	38	25	R
	Body and texture (40)	32	31	32	34	34	31	27	32	30	R	31	27	R
	Appearance(10)	9	8	7	6	9	8	7	8	9	R	9	8	R
	Total (100)	89	86	77	67	83	72	59	75	68	R	78	60	R
6	Flavor (50)	42	46	34	33	35	28	25	32	29	R	34	23	R
	Body and texture (40)	32	30	27	27	30	22	20	27	27	R	27	27	R
	Appearance(10)	9	8	6	6	9	7	8	7	7	R	7	5	R
	Total (100)	83	84	67	66	74	57	53	66	63	R	68	55	R
10	Flavor (50)	40	44	34	30	33	26	22	30	26	R	32	20	R
	Body and texture (40)	31	31	24	27	30	20	21	25	25	R	26	25	R
	Appearance(10)	8	7	6	6	7	7	7	7	6	R	7	5	R
	Total (100)	79	82	64	63	70	53	50	62	57	R	65	50	R
15	Flavor (50)	38	42	34	29	31	22	23	28	21	R	30	20	R
	Body and texture (40)	30	29	23	25	28	21	20	25	23	R	24	25	R
	Appearance(10)	8	7	6	5	6	7	7	6	6	R	6	5	R
	Total (100)	76	78	63	59	65	50	50	59	50	R	60	50	R

Table 2: Effect of fortified milk and yoghurt with iron and zinc on liver functions in male albino rats

	parameters: ALT (IU / L) ± S.E			AST (IU/L) ± S.E		
	Time intervals / weeks					
Groups	0	4weeks	8week	0	4weeks	8weeks
Group (1) (control)	30.00± 2.99	34.00±0.57	37.00± 0.57	117.0±4.83	123.66±6.03	131.16±6.0±*
Group (2)	31.41±1.45	63.00±0.73**	78.00±11.29**	108.33±6.70	148.83±15.01**	161.83±11.52**
Group (3)	34.17±3.44	47.33±3.57*	47.00±1.06*	110.33±10.25	132.0±12.40*	177.83±28.83**
Group (4)	31.83±2.45	35.83±2.71*	57.66±3.17**	124.33±165.25*	165.83±6.25*	172.33±9.67**
Group (5)	37.83±1.11	67±4.43**	83.83±9.96**	128.66±17.20	153.5±17.58*	187.5±15.53**
Group (6)	26.66±1.85	31.66±0.76*	38.66±1.22**	96.83±8.50	136.16±6.24**	155.5±10.84**
Group (7)	27.21±2.22	46±2.12*	57±4.54**	100.5±9.45	139.83±13.22**	165.83±15.0**

*Stistically Significant form corresponding control group (0 Time) at $p < 0.01$

** Stistically very Significant form corresponding control group (0 Time) at $p < 0.001$

Group(1): normal control, (basal diet).

Group (2): 20 mg/kg. b.w. ferrous Chloride in milk.

Group (3): 40 mg / kg . b.w ferrous chloride in milk.

Group (4): 20 mg/ kg . b.w. zinc acetate in milk.

Group (5): 40 mg / kg . b.w . zinc acetate in milk.

Group (6): 40 mg / kg / b.w zinc acetate + 40 mg / kg/b.wt ferrous chloride in milk.

Group (7): 40 mg / kg/b.w ferrous chloride + 40 mg / kg zinc acetate / kg/ b.w in yoghurt.

Table (2) shows the effect of ferrous chloride on the activity of serum ALT and AST, a daily dose of 20 mg of ferrous chloride/kg body weight of tested animals caused a remarkable significant ($p<0.05$) increased in the activity of ALT and AST after 8 weeks of treatments (78.00± 11.29 and 161.83± 11.52 IU/L compared with normal group 37.0±.057 and 131.16±6.0 IU/L respectively).

Ferrous chloride in higher dose (40 mg /kg) after 8 weeks of treatments caused the same increase in the two enzymes namely ALT and AST. The increase was 47.0 ± 1.06 and 177.83±28.83 IU/L compared with normal group 37.0±.057 and 131.16±6.0 IU/L, respectively.

Regarding to, the effect of zinc acetate after 0, 4, 8 weeks of treatment on the activity of serum (ALT) and (AST) as shown in table (2). Zinc acetate in low dose (20 mg /kg) after 8 weeks of treatment caused an increase in serum ALT and AST being 57.66± 3.1 and 172±9.6 IU/L respectively. Moreover, the higher dose of zinc acetate (40 mg /kg) increased the activity of ALT and AST to 83.83±996 and 187.5±15.53 IU/L.

Mixture of the two minerals dissolved in milk in equal concentrations 40 mg /kg for each, showed a remarkable increase in ALT and AST whereas activities were 38.66± 1.22 and 155.5±10.84 IU/L, respectively.

The same trend of results as shown in fortified milk with salts understudy, was obtained in the case of feeding on daily diet yoghurt incorporated with on equal concentrations of the two salts, 40 mg /kg for each. An increase in the two enzymes namely ALT and AST was found to be 57.0±4.54 and 165.83±15.0, respectively.

According to the proceeding view, it was evident that the ingestion of fortified milk with trace minerals caused remarkable increase of ALT enzyme in case of low and high dose of ferrous chloride. In the meantime the same trend was noticed in the manipulated animals with zinc acetate separately and incorporated with ferrous chloride comparing with the second one in two prementioned concentrations.

The disorders upon manipulation of groups with ferrous chloride were more obvious than iron salts after 8 weeks. The obtained results concerning with the side effect of fortification with minerals are in agreement with that found by [18] in respect with the supplementation of iron which caused a deleterious effect on tissues the isolated organs from experimental animals. The same toxic findings were observed by [19] in the case of utilization of higher dose of zinc in feeding *in vivo* experiment. On the other hand, the obtained data revealed that, the fortification of yoghurt with ferrous chloride and zinc acetate caused a remarkable increase in ALT and AST enzymes than in milk; these disorders may be due to the effect of acidity in availability of salts understudy. The obtained finding may be due to that found by [20], who showed that milk fermentation or acidification, caused an increase in the bioavailability of some minerals such as ferrous chloride.

The disorders upon manipulation of groups with ferrous chloride were more obvious than iron salts after 8 weeks. The obtained results concerning with the side effect of fortification with mineral salts understudy are in agreement with that found by [21] in respect with the supplementation of iron which caused a deleterious effect on tissues the isolated organs from experimental animals. The same toxic findings was observed by [22] in case of utilization of higher dose of zinc in feeding *in vivo* experiment. On the other hand, the obtained data revealed that, the fortification of yoghurt with ferrous chloride and zinc acetate caused a remarkable increase in ALT and AST enzymes than in milk, these disorders may be due to the effect of acidity in availability of salts understudy. The obtained finding may be due to that found by [23] who showed that milk fermentation or acidification caused an increase in the bioavailability of some minerals such as ferrous (Fe).

Effect of Fortified Milk and Yoghurt with Iron and Zinc Salts on Liver and Kidney as Detected by Histopathological Examination

Histopathological Examination of the Liver: A cross section in normal liver of rat is shown in fig (1). The group of animals treated for (8 weeks) with low dose (20 mg/kg) of ferrous chloride revealed moderate histopathological changes in the blood vessels in the form of congestion in central and portal veins. Moreover, dilatation in sinusoids were also seen (Fig 2). The group of animals treated with high dose (40 mg /kg) of ferrous chloride showed marked changes in hepatocytes with homogenous cytoplasm and pyknotic nuclei. Marked hyperemia in sinusoids and congested thickened wall of central vein were also observed (Fig 3).

The treatment of rats with low dose (20 mg /kg) of zinc acetate showed dilatation and thickened wall of portal vein. Also, proliferation in bile ductless and lymphocytic infiltration were seen around portal vein (Fig. 4) Samples of liver treated with high dose (40 mg /kg) of zinc acetate revealed marked degenerative changes in hepatocytes with marked, thickened hylinized wall of portal vein. Fibrous tissue and lymphocytic infiltration were also observed (Fig.5).

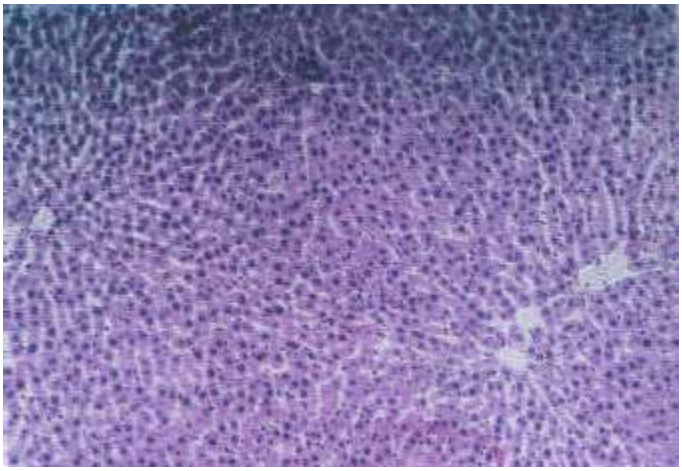

Fig. 1: Cross section in liver of normal control rat showing normal hepatocytes in cords from the central vein. (H and E, x 150)

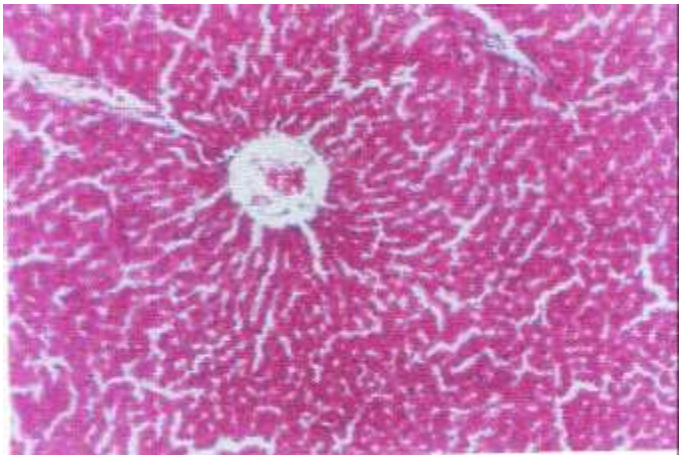

Fig. 2: Cross section in liver treated rats with ferrous chloride low dose (20mg/kg) showing mild dilatation in sinusoids and congestion in vascular channels.(H and E, x 150)

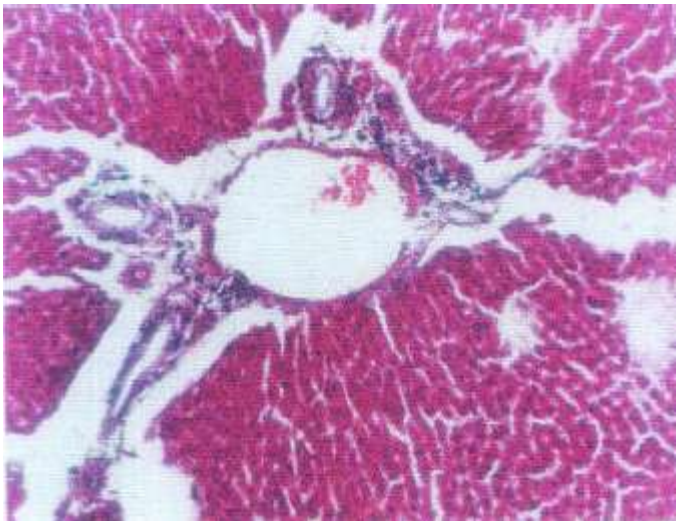

Fig. 3: Cross section in liver treated rats with zinc acetate at low dose (20mg/kg) showing dilated and proliferated bile ductless with lymphocytic infiltration around portal vein,. (H and E, x 150)

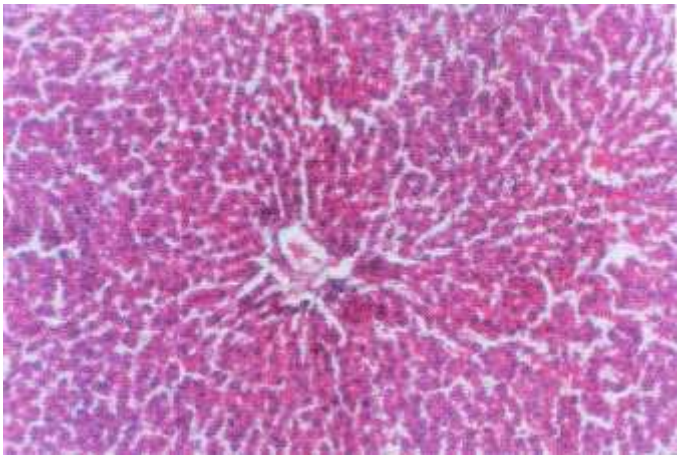

Fig. 4: Cross section in liver treated rats with ferrous chloride at high dose (40mg/kg) showing marked hyperemia in sinusoids and thickened wall of central vein,. (H and E, x 150)

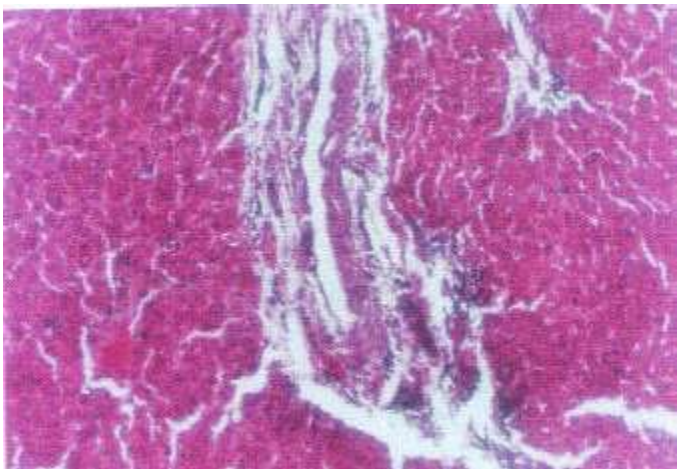

Fig. 5: Cross section in liver treated rats with zinc acetate at high dose (40 mg/kg) showing fibrous tissue around portal vein with pyknotic nuclei hepatocytes, (H and E, x 150)

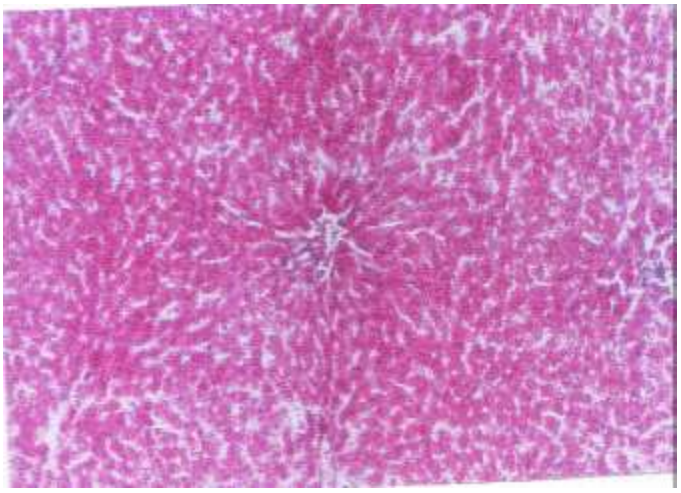

Fig. 6: Cross section in liver treated rats with mixed ferrous chloride and zinc acetate at doses (40 mg/kg) for each mineral showing lymphocytes in focal area and pyknotic nuclei with degenerative hepatocytes,. (H and E, x 150)

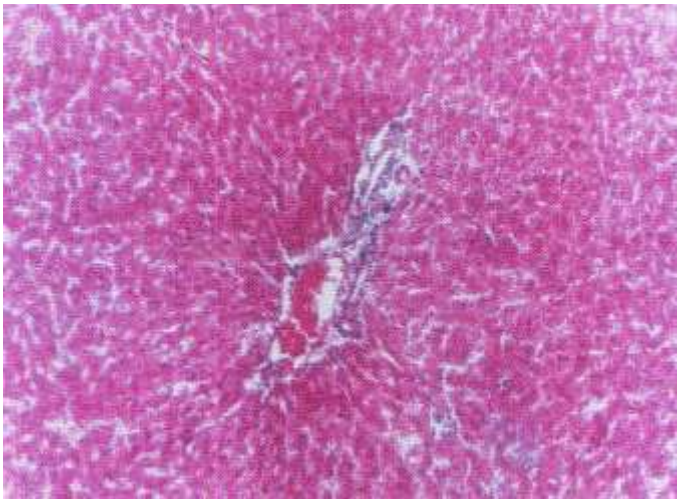

Fig. 7: Cross section in liver treated rats with mixed ferrous chloride and zinc acetate at doses (40 mg/kg) for each revealing pyknotic, karryolysis nuclei without nucleus in individual numbers of hepatocytes with congestion in portal vein,. (H and E, x 150).

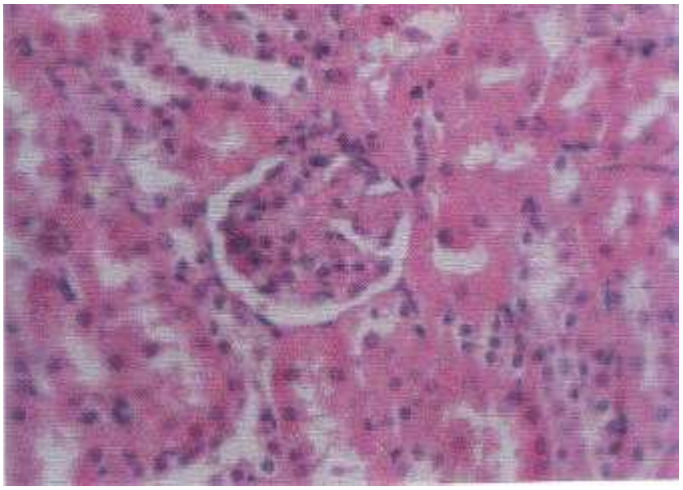

Fig. 8: A photomicrograph of a kidney of normal control animal showing a glomerulus with its capillary tufts surrounded by capsular space and Bowman's capsule. The proximal and distal convoluted tubules were also observed,. (H and E, x 600).

The treatment of rats with a mixture of ferrous chloride and zinc acetate in concentrations 40 mg for each in milk showed a leukocyte infiltration in focal area and degenerative changes in hepatocytes (Fig. 6)

In the group of animals which were treated with a mixture of Ferrous chloride and Zinc acetate 40 mg for each incorporated with yoghurt showed marked degenerative changes of hepatocytes, congestion in portal vein and proliferated bile ducts in perivascular channels. Moreover, pyknotic nuclei and homogenous cytoplasm were appeared (Fig.7)

Histopathological Examination of the Kidney: Control section of the normal is shown in fig. (8).

In group of animals treated with ferrous chloride at low dose (20 mg /kg), kidney revealed congested, hypocellular glomerular tuft and wide capsular space in Bowman's capsule. In some areas, there were atrophy in renal tubules (Fig 9): After administration of ferrous chloride at high dose (40 mg /kg), homogenous cytoplasm of tubular cells and tubulated mesangial cells of glomerular tuft and cytoplasmic vacillation in renal tubules were observed in some regions (Fig. 10)

The administration of zinc acetate at low dose (20 mg/kg) showed degenerative changes in renal tubules, atrophy in glomerular tuft and hypercellular in the others. Also, fibrous tissues were seen around thickened blood vessel (Fig 11). On the other hand, the treated rats with

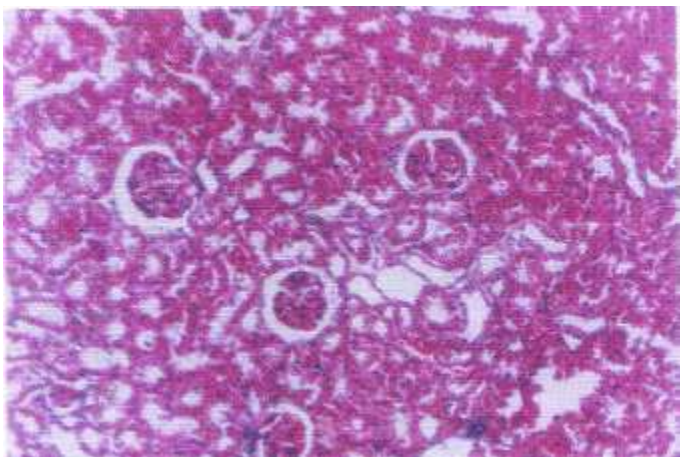

Fig. 9: A photomicrograph of a kidney of treated rats with ferrous chloride at low dose (20 mg/kg) showing hypocellular and congested glomerular tuft and degenerative changes in renal tubules in some area,. (H and E, x 150)

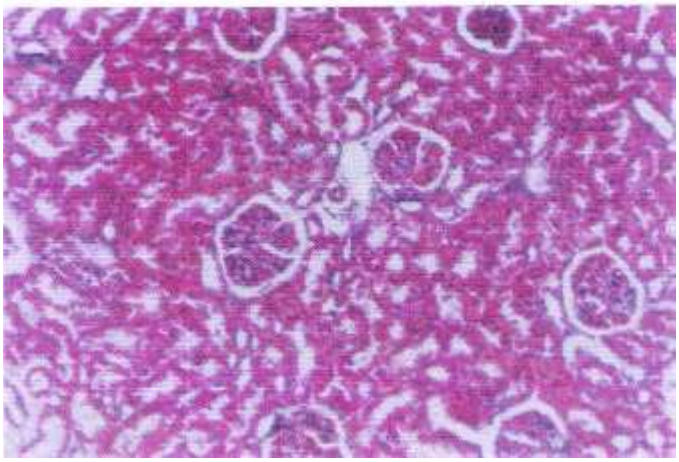

Fig. 10: A photomicrograph of a kidney of treated rat with zinc acetate at high dose (20 mg/kg) showing lobulated mesangial cells of glomerular tuft and homogenous cytoplasm of renal tubules cells,. (H and E, x 150)

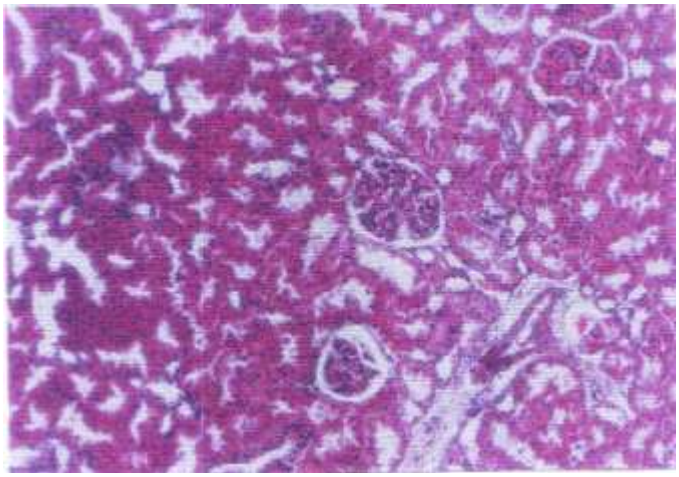

Fig. 11: A photomicrograph of a kidney of treated rat with ferrous chloride at low dose (40 mg/kg) showing congested and atrophy of glomerular tuft and fibrous tissue around thickened blood vessel,. (H and E, x 150)

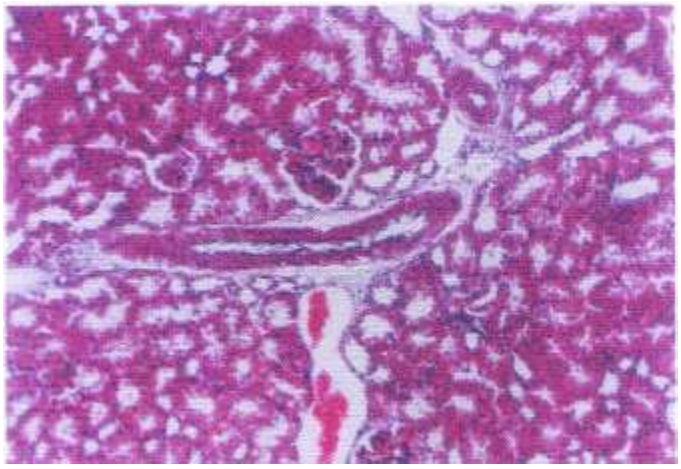

Fig. 12: A photomicrograph of a kidney of treated rat with zinc acetate at high dose (40 mg/kg) showing thickened wall and congestion of blood vessels, atrophy in glomerular tuft and intracellular vacuoles involving many of the tubular cells,. (H and E, x 150)

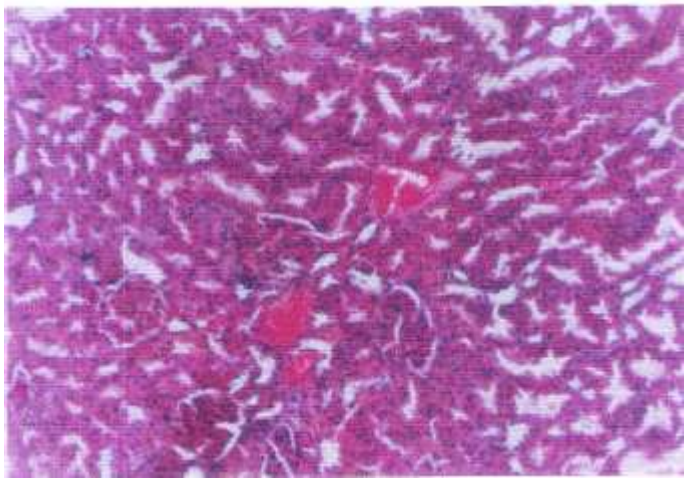

Fig. 13: A photomicrograph of a kidney of treated rat with mixed ferrous chloride and zinc acetate (40 mg/kg) for each showing congestion of blood vessels and homogenous cytoplasm of epithelial of renal tubules, (H and E, x 150)

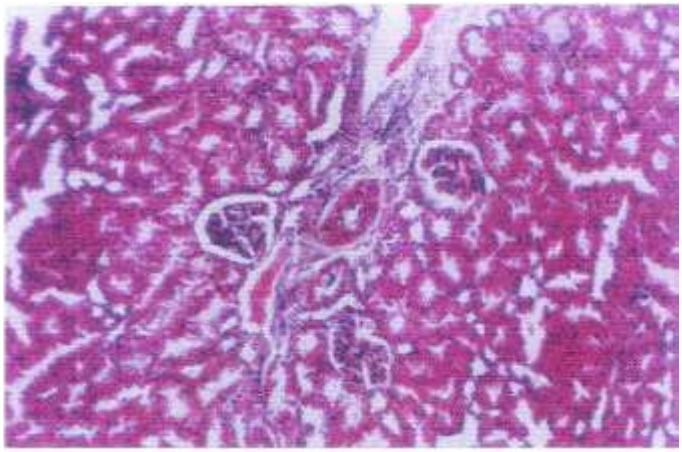

Fig. 14: A photomicrograph of a kidney of treated rat with mixed ferrous chloride and zinc acetate for each showing numerous intracellular vacuoles involving many of the tubular cells and fibrosis (fibrous tissue) around thickened blood vessel and hypocellular in glomerular tuft, (H and E, x 150)

zinc acetate at high dose (40 mg /kg), marked atrophy of glomerular tuft, congested and thickened wall of blood vessels and fibrous tissue around it were observed (Fig 12).

After administration of mixture of ferrous chloride and zinc acetate at doses (40 mg /kg) for each in milk revealed congestion in blood vessels and cloudy swelling of renal tubules with atrophy in glomerular tuft (Fig 13).

In the meantime a fibrous tissue was congested and thickened blood vessels, atrophy in glomerular tufts and were noticed in fortified yoghurt with the mixture minerals. Also, cytoplasmic vacillations in renal tubules were observed in some tubular cells (Fig. 14). The obtained results concerning the influence of fortified dairy products with minerals on the isolated organs are in agreement with that previously obtained [18] who found a deleterious effect of iron supplementation on liver tissues. Also, the same results are in agreement with those obtained by ot6hger aut6hors [19] who suggested that the higher dose of zinc may produce toxic effect on various tissues and organs.

Effect of Fortified Milk and Yoghurt with Zinc and Iron Salts on Cytogenetic Examination

Micro Nucleated Polychromatic Erythrocytes: Table (3) represents the means percentages of total count of micronucleated polychromatic erythrocytes of control and treated groups. The result of the present study showed that animals of the group (2) treated with 20 mg ferrous chloride administrated with milk showed non significant increase in the mean value of MNPCEs (0.24± 0.029) in bone marrow cells when compared with control (0.18±0.026). However, there was a significant increase ($p<0.05$) in the mean values of micronucleated polychromatic erythrocytes (MNPCEs) of animals treated with 40 mg ferrous chloride administrated with milk (group 3) and 20 mg Zinc acetate administrated with milk (group 4) (0.36 ±0.029 and 0.35 ± 0.035, respectively) when compared with control group (0.18 ± 0.026). Also, there is a significant increase ($p< 0.05$) in the mean value of MNPCES of animals treated with 40 mg Zinc acetate administrated with milk (group 5) (0.52 ± 0.046) than the control (0.18 ± 0.026). In addition, results revealed that the animal administration combination of 40mg Ferrous chloride + 40mg Zinc acetate (groups 6 and 7) in milk and yoghurt, had significant increase ($p<0.05$) in the mean value of MNPCES (0.85 ± 0.043 and 0.85 ± 0.047, respectively) than the control group (1) (0.18 ± 0.026). However, there was no significant difference between the mean value or MNPCEs in group 7 combined group 40 mg ferrous chloride and 40 mg Zinc acetate in yoghurt (0.85 ± 0.047) and that of 40mg Zinc acetate only (0.84 ± 0.043) and group (6) combind group in milk (0.84 ± 0.043). Meanwhile, results showed that the high dose of zinc acetate caused significant increase in the mean value of MNPCEs ($p<0.05$) than that of ferrous chloride.

Finally, cytogenetic results, in the present study, indicated that ferrous chloride and zinc acetate salts exhibited significant increase in the frequencies of micronucleated polychromatic erythrocytes (MNPCEs) than control. The degree of micronucleated polychromatic erythrocytes is directly proportional to the doses used for ferric chloride and zinc acetate. This means that ferric chloride and zinc acetate salts may have a mutagenic activity in bone marrow cells of rats.

This result was supported with [20]. They indicate that the frequencies of basophilic stippled erythrocyte (BSE) and MPCEs in the Zn high group were significantly higher than those in the control group ($P<0.05$). The levels of serum Glutamic Oxalacetic Transaminase (GOT) and serum Triiodothyronine (T3) in the Zn high groups decreased significantly, compared with the control group ($P<0.01$ or 0.05). Moreover, he also observed that the level of serum cortisol, another adrenal corticoid hormone in rats, was increased by zinc acetate in a dose-dependent manner that exposure to zinc, especially at higher doses may produce toxic effects on various tissues and organs including the hematopoietic system, cytogenetics, biochemistry and endocrine system function.

Table 3: Frequencies of micro-nucleated polychromatic erythrocytes (MNPCEs) in all rat bone marrow cells in ail experimental groups

Animal groups	Total Counted PC Es / animal	MNPCEs %
controlG 1	10000	0.18d±0.026
G2	10000	0.24 d± 0.029
G3	10000	0.36 c ±0.029
G4	10000	0.35 c± 0.035
G5	10000	0.52b ±0.046
G 6	10000	0.84 a ± 0.043
G 7	10000	0.85 a ± 0.047
LSD at a 0.05		0.108

Table 4: Mean values of different chromosomal aberrations induced in bone marrow of all experimental groups

	Structural chromosomal aberrations %				Numerical chromosomal aberration %				
	Chromatid Gaps	Chromatid breaks	Deletions	Centromerie attenuations	Hypoplodis	Hyperplodis	Polyploldy	Total aberrations with gaps %	Total aberrations excluding gaps %
G1 Control	1.50 a±0.07	0.50d±0.10	2.00 c±0.17	4.00 c±0.25	0.50 c±0.06	1.50a±0.06	0.50c±0.06	10.50 c±0.96	9.00 c±0.91
G2	1.00b±0.09	1.00 c±0.09	2.50 be±0.07	4.50 be±0.58	1.00 b±0.07	0.50 c±0.06	1.00 b±0.09	11.50 bc±0.53	10.50 bc±1.10
G3	1.50 a±0.08	0.50 d±0.06	2.00 c±0.24	4.50 bc±0.24	1.00 b±0.05	1.00 b±0.07	0.50 c±0.06	11.00 c±0.84	9.50 c±0.65
G4	1.50 a±0.06	1.50 b±0.12	2.00 c±0.18	4.50 bc±0.25	1.00 b±0.06	1.00 b±0.09	0.54 c±0.06	12.00 bc±0.77	10.50 bc±1.10
G5	1.00 b±0.05	1.50 b±0.18	3.00 b±0.20	5.50 b±0.32	1.50 a±0.07	0.50 c±0.06	1.00 b±0.09	14.00 b±0.86	13.00 b±1.02
G6	1.50 a±0.06	1.50 b±0.12	2.50 bc±0.45	5.00 be±0.38	1.50 b±0.08	0.50 c±0.06	1.50 a±0.06	14.00 b±0.75	12.50 b±0.88
G7	1.50 a±0.06	3.00 a±0.20	5.50 a±0.58	8.00 a±0.94	0.50 c±0.06	0.50 c±0.06	1.00 b±0.09	20.00 a±1.15	18.50 a±1.07
LSD at a 0.05	0.207	0.403	0.820	1.469	0.192	0.200	0.215	2.671	2.951

The means followed by the same alphabetical letters were not significantly different at the probability level of 0.05.

The results are presented as mean value ± SE.

Group (1): normal control, (basal diet).

Group (2): 20 mg/kg. b.w. ferrous Chloride in milk.

Group (3): 40 mg / kg. b.w ferrous chloride in milk.

Group (4): 20 mg/ kg. b.w. zinc acetate in milk.

Group (5): 40 mg / kg. b.w. zinc acetate in milk.

Group (6): 40 mg / kg / b.w zinc acetate + 40 mg / kg/b.wt ferrous chloride in milk.

Group (7): 40 mg / kg/b.w ferrous chloride + 40 mg / kg zinc acetate / kg/ b.w in yoghurt

Therefore, it is suggested that zinc should be used carefully, especially by high risk groups such as children and pregnant women despite its use as a food additive or in self-medication. At the same time, it is necessary to investigate and research further these toxicities of zinc with long-term administration of low dosage.

The means flowed by the same alphabetical letters were not significantly different at the probability level of 0.05. The results are presented as mean value ± SE

Chromosomal Aberrations: Table (4) represents the frequencies of different chromosomal aberrations observed in bone marrow cells of control and all treated groups. Structural chromosomal aberrations types were chromatid gap, chromatid break, deletion and centromeric attenuation. Numerical chromosomal aberrations were hypoploidy, hyperploids and polyploidy. Data of the study showed that there is no significant difference between the groups (2) and (3) compared with control (Table 4).

In the case of groups (5) and (6) there was increase in the mean percentage of the total aberrations (14.0 ± 0.86 and 14.0 ± 0.75, respectively) than the control (10.5± 0.96) at ($p< 0.05$). In case of group (7) there was a high significant increase in the mean percentage of the total aberrations (20.0 ± 1.15) than the control (10.50 ± 0.96). In addition, the high dose of zinc acetate (40 mg/ kg) group (5) showed a significant increase in the frequencies of chromatid break, centromeric attenuations, deletion, polyploidy, hypoploidy and hyperploidy than the control.

Also, the frequencies of chromatid break, deletion, centromeric attenuations, hypoploidy and polyploidy of ferrous chloride (40mg/kg) group (3) showed a significant increase ($p < 0.05$) than control.

The results obtained in this study suggested that the high dose of ferrous chloride and zinc acetate separately and in combined induced mutagenic effect in bone marrow cells of male rats.

It was noted that, Iron is a potent oxidant that can lead to the formation of genotoxic lipid peroxides. Ascorbic acid, which enhances dietary iron absorption, has been suggested to enhance the oxidant effects of iron and to directly lead to the formation of lipid peroxides [21]. The results of the bone marrow micronucleus test revealed that the high iron diet resulted in an increased frequency of micronucleated polychromatic erythrocytes (MNPCEs) as compared to low iron.

It was demonstrated that the degree of chromosome damage induced by three compounds of zinc (zinc chloride, zinc sulfate and zinc acetate) was compared in human leucocytes *in vitro*. Three concentrations of each salt was 3.0 x 10(-5) M, 3.0 x l0 (-4) M 1.5 x 10/ (-3) M, were added to leukocyte cultures. The cells were harvested after 48 and 72 h and chromosome spreads were prepared following a colchicine-hypotonic-fixation-air drying-Giemsa staining schedule. The end point screened was chromosome aberrations [7]. All three salts were lethal at the highest concentration. The degree of chromosome damage was directly proportional to the concentrations used for zinc sulfate and zinc acetate but not for zinc chloride.

Also, there was a significant increase in the frequencies of chromosomal aberrations between high doses of Fe and Zn combined group and the control one.

This result was supported with [6], they indicated that Iron (Fe) is a common chemical element that is essential for organisms as a co-factor in oxygen transport, but that in height amounts presents a significant risk of

neurodegenerative disorders. Fe causes alteration and inhibition of DNA synthesis only in proliferative cells, which explain the concomitant occurrence of mutagenicity and cytotoxicity, respectively.

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