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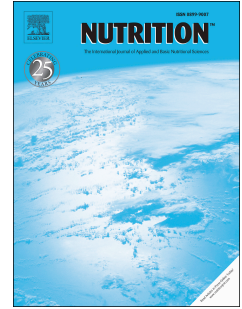
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Preparation of iron-enriched baker's yeast and its efficiency in recovery of rats from dietary iron deficiency

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Abstract

Background

Iron is an important metal, essential for the health and function of mammalian cells. Despite its key role, iron deficiency in humans is common worldwide, often leading to significant health issues within the population. In this study we aimed to evaluate the potential for using iron-enriched baker's yeast as a source of iron, especially for the protection and recovery from conditions related to anemia.

Methods

Iron-enriched yeast was prepared by cultivating cells on basal medium comprising different concentrations of iron. The effects of iron supplementation on animal health were assessed by feeding anemic rats with a variety of diets containing either inorganic iron, or iron-enriched yeast. Body weight, iron bioavailability, blood parameters, and the activity of iron containing enzymes (catalase) were studied.

Results

Iron accumulation in yeast cells increased with iron concentration, reaching a maximum of 15 mg/g when 32 mM Fe was applied. Rat groups fed on iron enriched yeast gave the highest feed efficiency, iron bioavailability and hemoglobin concentration. The source of iron supplementation influenced catalase activity in kidney tissues, increasing from 70 U/g tissue in anemic rats to 90 U/g tissue (inorganic iron salt), 110 U/g tissue (inorganic iron salt and non-enriched dry yeast), 145 U/g tissue (iron-enriched yeast 15 mg Fe/g) and 115 U/g tissue (iron-enriched yeast 30 mg Fe/g). The histological study of tissues from liver, kidney, heart, and spleen of rats from different groups showed that the damage observed in tissues of anemic rats, was not observed after feeding with iron-enriched yeasts.

Conclusion

The results demonstrate that ingestion of iron-enriched yeast is more efficient than inorganic treatment in recovery from iron deficiency, including tissue recovery in rats.

Key Words: *Saccharomyces cerevisiae*, Anemia, Fe, metal ions, malnutrition

Introduction

Although iron is an abundant metal, it has low bioavailability, and iron deficiency represents one of the most common nutritional deficiencies worldwide with significant implications to public health [1]. The average adult male (70 kg) has a total body iron content of approximately 4 grams, which remains relatively constant throughout adult life [2]. This is maintained by balancing loss of iron with assimilation via nutritional intake. Persistent deviation in iron intake can result in either iron deficiency, or iron overload (hemosiderosis and hemochromatosis) [2]. The most obvious manifestation of iron deficiency is anemia, but lack of iron can also have an adverse effect on the immune system [3, 4] and cognitive development [5, 6]. Iron is an essential nutrient since iron cofactors, including heme and iron-sulfur clusters, are required for the activity of a number of enzymes involved in a range of cellular processes. Furthermore, iron is also an important component of molecules that undergo redox reactions in cells [7, 8]. Anemia and iron deficiency may cause serious diseases, for example, anemia and iron deficiency are quite prevalent in patients with heart failure. In the past few years, there has been an enormous interest in the subject of iron deficiency and its management in patients with heart failure [9].

Since leavened bread, produced using *Saccharomyces cerevisiae* (baker's yeast), is a staple component of diets in many areas of the world, the potential for using yeast biomass enriched with iron as a feed supplement has the potential to deliver a safe and efficient mechanism for the prevention or recovery from anemia. Previous studies have shown that iron bound to organic carriers, such as cytochrome C, catalase and other proteins and enzymes in yeast cells, are more easily absorbed and have a reduced potential to be toxic [10]. In addition, yeast biomass is known to be a good source of protein, amino acids and vitamins, and has been shown to have a positive nutritional impact in animals and humans [10]. Despite the suitability of yeast for nutrient transfer, the uptake, storage and utilization of iron in *Saccharomyces cerevisiae* cells is tightly regulated. In particular, the metal uptake and assimilation processes are complex and dependent on the chemistry of metal ions, specific surface properties of the organism, cell physiology and the physico-chemical influence of the environment [10]. Consequently, in this study the capacity of a baker's yeast strain to assimilate iron was determined, and the resulting iron-enriched baker's yeast was evaluated for its potential in alleviating symptoms of iron deficiency in Wistar rats.

Materials and Methods

Preparation of iron-enriched yeast

A commercial baker's yeast strain was obtained and isolated from the local market (Aleppo, Syria). Yeast propagation was conducted in 250 ml Erlenmeyer flask containing 100 ml basal medium (20g/l glucose, 0.5g/l yeast extract, 5g/l ammonium sulphate, 1g/l potassium dihydrogen phosphate, 0.5g/l magnesium sulphate, pH adjusted to 5.2 with 5N NaOH). Yeast cultures were enriched with iron by supplementation with ammonium iron (III) citrate in concentrations of 0.5, 1, 2 and 4 % (W/V) corresponding to 16, 32, 64 and 128 mM Fe. The cultures were incubated at 30°C for 24 h, with shaking at 150 rpm. In each instance the entire cell culture was recovered by centrifugation, excess liquid was decanted and samples were desiccated using a drying oven (Memmert, Egypt). Dried yeast samples were collected and used for further analysis (iron content and as a nutritional supplement) as described below.

Evaluation of iron content in iron enriched yeast

After 24 h cultivation cultures were centrifuged (5 min, 4000 g) and the supernatant discarded. The pellet was washed three times with distilled water in order to remove loosely associated fraction of iron from yeast cell surface. Washed yeast biomass was dried at 105°C until a constant mass was achieved, and digested by adding 1 ml of 65 % (v/v) HNO₃ to 20 mg dry biomass, followed by heating for 30 min at 140°C. After cooling, samples were diluted with distilled water to a final volume of 5 ml and the content of iron was analyzed by Flame Atomic Absorption Spectrometry (FAAS) in the Agricultural Research Center, Giza, Egypt. The amount of total iron was calculated per gram of dry weight (mg Fe/g⁻¹ dry wt.) as described previously [10].

Animals and diets

Forty-eight newly weaned male and female Wistar rats (initial body weight, 75 - 85g) were obtained and housed individually in stainless steel, wire mesh bottom cages. A 12-h light: dark cycle and an environmental temperature of 25 ±2°C were maintained. Newly weaned rats were chosen since individuals of this age can be considered to be similar irrespective of gender; physiological, hormonal or metabolic changes are not observed between males and females at this early stage of life. This was verified in-house by analysis of data, which showed that there was no difference between young rats based on gender distinction (data not shown). Rats were randomly assigned to one of six dietary groups (eight rats per group) as illustrated in Fig.1. The control group was fed on basal diet containing 30mg of Fe per

kilogram of diet for 56 days (total experiment time). Precise composition of the basal diet used was based on Sun *et al* [11] and shown below.

Iron deficiency was induced as described by Hamdaoui *et al* [12] with minor modifications: a low iron basal diet (3mg/Kg) was provided in conjunction with bleeding of 1-2 ml of blood every week for four weeks until the Fe and Hemoglobin reached a designated low level (109-112 g/L Hemoglobin and 3 mg body iron). These anemic rats were assigned to five groups of 8 rats (four males and four females). Groups were examined by feeding on one of the following diets for an additional 28 days: Group 1 was fed on basal Fe-deficient diet (no iron added). Group 2 was fed on basal Fe-deficient diet plus FeSO₄.7.H₂O (30mg Fe/kg diet) Group 3 was fed on basal Fe-deficient diet plus FeSO₄.7.H₂O (30 mg Fe/kg diet) plus 1 g of non-enriched yeast per kg diet. Group 4 was fed on basal Fe-deficient diet plus iron-enriched yeast (containing 15 mg Fe/kg diet). Group 5 was fed on basal Fe-deficient diet plus iron-enriched yeast (containing 30 mg Fe/kg diet). Total food intake was determined at the end of the experiment; it should be noted that in each instance all animals were marginally under-fed in order to ensure that each individual ingested the total quantity of food provided.

The body weight of rats was measured every week throughout the 56 days of dietary treatment. Blood (0.5ml) was collected after 14, 21, 28, 42, and 56 days of the dietary program from their eyes into heparinized micro capillary tubes.

Basal Diet was prepared as follows: Skimmed milk(50% casein), 400 g/Kg diet; Corn starch, 603 g/Kg; Vegetable oil, 80 g/Kg; Sucrose, 50 g/Kg; Mineral mix, 40 g/Kg; Vitamin mix, 10 g/Kg.

The mixtures of minerals and vitamins were prepared according to [11] as follows:

Vitamin mix contained the following (g/kg mix): inositol, 25.0; ascorbic acid, 5.0; calcium pantothenate, 0.67; thiamine hydrochloride, 0.27; pyridoxine hydrochloride, 0.53; nicotinic acid, 1.0; menadione, 0.25; riboflavin, 0.27; *p*- amino benzoic acid, 0.50; folic acid, 0.067; biotin, 0.26; all-rac-a-tocopherol acetate, 3.60; retinyl palmitate, 0.034; cholecalciferol, 0.21; vitamin B-12 1 manitol, 3.33; choline chloride, (70% solution), 71.50; cerelose, 887.8.

Mineral mix contained the following (g/kg mix): CaCO₃, 139.7; CaHPO₄, 166.6; K₂HPO₄, 133.6; NaCl, 21.2, MgSO₄, 49.5; ZnCO₃, 0.8; MnSO₄.H₂O, 0.61; CuSO₄.5H₂O, 0.66; KI, 0.0033; CrK(SO₄).12H₂O, 0.048; Na₂SeO₃, 0.015; Na₂MoO₄.2H₂O, 0.0063, cerelose, 481.1

Blood analysis

Hematocrit was measured by centrifugation of blood and expressed as the volume percentage of red blood cells. Hemoglobin concentration was measured using a Hemoglobin determination kit (Biodiagnostic Inc., Egypt). Serum iron concentration and total iron binding capacity (TIBC) using iron and (TIBC) kit (Biodiagnostic Inc., Egypt). Transferrin saturation (TS) was calculated from the ratio of serum iron/TIBC.

Catalase assay

Catalase activity was determined in plasma and kidney tissue after homogenate preparation by tissue perfusion with a PBS (phosphate buffered saline) solution, pH 7.4 containing 0.16 mg / ml heparin to remove any red blood cells and clots prior to dissection. Tissue was homogenized in 5-10 ml cold buffer (50 mM potassium phosphate, pH 7.5. 1 mM EDTA) per gram of tissue; the homogenate was then centrifuged at 10,000 rpm for 15 minutes at 4 °C. The supernatant was removed and stored on ice. If the assay was not conducted on the same day, the sample was frozen at -80°C. Catalase activity was estimated using a catalase assay kit (Biodiagnostic Inc., Egypt).

Calculation of iron bioavailability and feed efficiency

The iron (Fe) bioavailability from test meals was calculated for each animal based on initial and final body weights, the initial and the final hemoglobin concentrations, and the amount of Fe consumed during the repletion period. The initial and final concentration of hemoglobin-Fe was computed according to equations 1, 2 respectively, assuming that 7.5% of the body weight is blood and that hemoglobin contains 3.35mg Fe per gram as defined previously [12].

The relative Fe bioavailability (equation 3) was calculated by dividing the % Fe bioavailability from the test meals by the % Fe bioavailability for ferrous sulfate [RBV = (Fe bioavailability from diets/Fe bioavailability from FeSO₄) × 100]. The relative liver weight was calculated by dividing the liver weight by the total body weight. The Fe status was evaluated by determining the Fe stored in the liver for each rat. Feed efficiency was also calculated according to equation 4.

Equation (1)

Initial Hb Fe [mg] = initial BW × (7.5 ml blood/100 g BW) × (initial Hb g/ 100ml) × (3.35mg Fe/1g Hb)

Equation (2)

Final Hb Fe [mg] = final BW × (7.5ml blood/100g BW) × (final Hb g/100ml) × (3.35mg Fe/1g Hb)

Where: BW = body weight, and Hb = hemoglobin.

Equation (3)

Fe bioavailability [%] = (final Hb Fe [mg] - initial Hb Fe [mg])/Fe consumed [mg]

Equation (4)

Feed efficiency % = BW gained/ total food intake

Where: BW gained = final BW – initial BW BW: body weight.

Tissue preparation for iron determination

After 56 days, animals were euthanized and their livers, hearts, kidneys, and spleens were removed. 1-2 g of tissue was transferred into borosilicate test tubes and concentrated nitric acid and perchloric acid were added to the contents. The contents were heated to 85°C for 24 h followed by 110°C for 5h. Digestion was determine to be complete by the appearance of a colorless solution [13]. The iron concentration of samples was measured by inductively coupled plasma emission spectrometry (ICPS-1000 II, Shimadzu, Faculty of Agriculture, Cairo University).

Histological examination of tissues

Tissues derived from liver, kidney, and heart were stained with Hematoxylin-Eosin (H&E) for histological examination, while the spleen was stained with Perls' Prussian blue to evaluate iron accumulated in different groups. Histological examinations of rat tissues were performed in Cairo University, Faculty of Veterinary medicine as follows: Tissue samples were fixed for 48 hours in neutral buffered formalin (NBF). Following the fixation procedure, tissues were dehydrated through increasing concentrations of ethanol and embedded in paraffin. Sections approximately 5-7 µm thick were cut from the paraffin blocks and stained with H&E for routine histological examination, and with Perls' Prussian

blue for iron pigment analysis.

Statistical analysis

All data obtained for yeast experiments reflects the mean of identical triplicate samples \pm the standard error. Where appropriate, statistical variation was determined using a two tailed Student T-Test. A significant difference was identified at the 5% confidence level ($P < 0.05$).

For rat analysis, in each instance sample groups were comprised of 8 newly weaned 'young' individuals, with males and females considered to be identical as described previously. Data was analyzed using a two tailed Student t-test to determine significant differences between trial and control samples. A significant difference was identified at the 5% confidence level ($P < 0.05$).

Results

Yeast growth and iron accumulation- Yeast cells were grown at different concentrations of iron in growth media in order to study the effect of iron concentration in growth medium on the yield of cells, iron accumulation in cells and one of main baking properties of the produced cells (leavening ability). Results clearly showed that the yield of *S.cerevisiae* cells increased as the concentration of iron increased until the iron concentration reached 32 mM Fe in medium (1% iron salt) but at higher concentrations of iron the yield of cells decreased as the iron concentration increased (**Fig.2A**). The amount of iron accumulated in yeast cells also increased and reached up to 15 mg/g at 32 mM Fe in medium (1% iron salt), while above that concentration (64 and 128 mM) it dropped again. Yeast cells accumulated about 11 mg Fe/g dry weight at 64 mM iron concentration, while the addition of 128 mM Fe inhibited iron uptake by yeast cells, which was only 7 mg Fe/g (**Fig.2B**).

Effect of iron concentration on leavening ability- Leavening ability was studied for yeast cells produced at different concentrations of iron. Data in **Fig.2C** revealed that all yeast cells grown on different iron concentrations gave leavening ability higher than the control (grown on basal medium). The leavening ability of yeast cells increased up to 32 mM Fe in medium, while above this concentration it dropped again but still higher than control and the concentration of 1 % (32 mM Fe) improved the leavening ability by 50 % than control.

Statistical evaluation of different feeding treatments- Results in **Table 1** indicate that the differences in final body weights of rats from different groups were non-significant at both 5 % and 1 %, so this suggests that there is no significant difference between all treatments. On the other hand, significant increases in body weights of rats were observed in all groups, although the increase in group 1 (fed on iron deficient diet) was the least significant increase. Moreover, **Table 2** shows that the increase of hemoglobin levels in all groups were significant except in group 3 (fed on diet containing iron salt plus yeast) which was non-significant at the level of 1 % and significant at 5 %. On the other hand, there was a significant increase in hemoglobin levels of rats from groups 4 and 5 when compared with other treatments groups 2 and 3; these results prove clearly that both groups 4 and 5 achieved better results than other treatments. Also when comparing hemoglobin results from groups 4 and 5 with those from control group, it was noticed that differences between hemoglobin levels of these groups and control group are

not significant but the result of group 4 was less significant (closer) to control group which give this group (treatment) the advantage on the other group (group 5).

Iron bioavailability and feed efficiency- Feed efficiency and iron bioavailability were studied and calculated during treatment period (4 weeks). Results indicate that animal groups fed on iron enriched yeast (4 and 5) gave the highest feed efficiency (10.9 %) when compared with other anemic groups (1, 2 and 3). These groups (4 and 5) gave 90.83 % of feed efficiency of control group (which was fed normally), also the feeding efficiency of groups fed on iron enriched yeast was increased 1.16 fold when compared with group 2 which was fed on basal medium plus inorganic iron salt(**Fig.3A** and **Table 3**).

Regarding iron bioavailability, results obtained from **Table 4** and **Fig.3B** show that the Fe in group 4 (fed on iron yeast 15 mg/g) was significantly more bioavailable compared to other groups ($p < 0.01$), also the iron bioavailability in group 5 was more than other treatments in groups 2 and 3. Results showed that Fe bioavailability increased to 22.46 % in group 5 when compared to that of control group where it was about 19.44 %, while Fe in groups 2 and 3 was less bioavailable than control group (16.11 % and 12.93 % respectively). The highest Fe bioavailability was obtained in group 4 reached to about 36.35 %. Relative iron bioavailability (RBV) gave the same trend of iron bioavailability and increased in groups 4 and 5 to give 186.98 and 115.53 % of control group, while (RBV) remained less than control in the other groups.

Blood parameters- Based on hemoglobin concentration and hematocrit value, iron in groups 4 and 5 (fed on iron enriched yeast) was more bioavailable in rats and cured iron deficiency when compared with other groups (1, 2 and 3), as shown in **Table 5**. In groups 2 and 3, hemoglobin concentration was less than that in the control group, whereas the hematocrit value was also slightly less than that in control group at the end of treatment period, indicating that the Fe bioavailability of the iron enriched yeast is better than other treatments for recovery from iron deficiency. The recovery of iron deficiency in groups 2 and 3 was poor when compared to that in groups 4 and 5, assuming that the iron sources of iron enriched yeast are more effective than iron salt such as ferric sulfate (group2). In the group supplemented with dry yeast besides iron salt (group 3) yeast cells might have functioned as absorption inhibitor decreasing the bioavailability of iron in rats When iron concentration in serum compared between different treatments (groups 2, 3, 4, and 5) results showed that group 4 was subjected to the best treatment (iron enriched yeast 15mg Fe/g dry weight) and the iron concentration in serum was similar to that of control group, whereas iron concentrations in serum of groups 2 and 5 were

nearly equal but less than control group. Treatment of group 3 was the poorest one among other treatments and only a slight increase of serum iron was observed when compared to negative control (group 1) fed on iron deficient diet. Similar results were obtained when comparing total iron binding capacity (TIBC) and transferrin saturation (TS) in different groups. Results obtained from hemoglobin analysis showed that groups 4 and 5 were subjected to the best treatments than other treatments, whereas group 4 also gave the best results in other blood parameters studied when compared to other groups.

Enzymatic activity of iron dependent enzyme (catalase activity)- Results shown in **Fig.3C** indicates that the iron restricted diet didn't affect the catalase activity in plasma during moderate iron deficiency anemia development (4 weeks) period in this experiment this may be because the period was not enough to affect the catalase activity in plasma. Catalase activity in kidney was also studied. **Fig.3D** shows the catalase activity in kidney tissues of rats from different groups. In contrast of plasma, results obtained from figure 3D showed that the catalase activity in kidney tissues of anemic rats decreased. Different treatments raised the catalase activity from 70 U/g tissue in anemic rats to 90 U/g tissue in group 2 (iron salt added), 110 U/g tissue in group 3 (iron salt and dry yeast added), 145 U/g tissue in group 4 (iron enriched yeast 15 mg Fe/g diet added) and 115 U/g tissue in group 5 (iron enriched yeast 30 mg Fe/g diet added). Although the improvement was less than aimed, the previous results suggest that the treatment applied in group 4 (iron enriched yeast 15 mg Fe/g diet) is the best treatment that can be used in the treatment of iron deficiency, but it may need more time than used in this experiment (4weeks) to achieve complete recovery of catalase activity in kidney tissues.

Iron accumulation in tissues- Data shown in **Fig.3E** shows that the reduction of iron concentration in liver tissues was about 34% (from 0.93 $\mu\text{mol/g}$ in control group to 0.61 $\mu\text{mol/g}$ in anemic rats group 1) but it was completely recovered in group 2 to reach 1.03 $\mu\text{mol/g}$, but the highest concentration of iron was accumulated in liver tissues of rats fed on iron enriched yeast in group 4 which was 1.29 $\mu\text{mol/g}$. On the other hand, iron concentration in spleen tissues shown in **Fig.3F** was gradually increased in different treatments from 1.93 $\mu\text{mol/g}$ in anemic rats (group 1) to 2.6 $\mu\text{mol/g}$ and 4.85 $\mu\text{mol/g}$ in groups 2 and 3 respectively, and reached its maximum of 5.9 and 6.1 $\mu\text{mol/g}$ in groups 4 and 5, respectively.

Histological examination of tissues- Analysis of rat liver sections (Figure 4A) indicated that livers from the control group showed no histological changes apart from hydropic degeneration of hepatocytes (Ctrl). However, liver of rat from group 1 (G1) revealed vacuolar degeneration of hepatocytes. Examined liver of rat from group 2 (G2) showed marked dilatation and

congestion of central vein as well as hydropic degeneration of hepatocytes. Meanwhile, liver of rat from group 3 (G3) revealed no histopathological changes. Liver of rat from group 4 (G4) showed vacuolar degeneration of some hepatocytes. Moreover, liver of rat from group 5 (G5) showed vacuolar degeneration of hepatocytes. Histological study of liver showed that a clear damage was observed in the livers of anemic rats (G2), but the damage was decreased by different treatments, and the best results were obtained in groups 4 and 5. Kidneys of rats from control group (Ctrl) revealed the normal histological structure of renal parenchyma. Meanwhile, kidneys of rats from group 1 showed peritubular leucocytic cells infiltration and perivascularitis (G1). However, kidneys from rats of group 2 revealed no histological changes (G2). Examined kidneys of rats from group 3 showed vacuolation of epithelial lining renal tubules and focal interstitial nephritis (G3). No histological changes were noticed in kidneys of rats from groups 4 and 5 (G4) and (G5). The previous results show that the kidneys of rats from groups 2, 4, and 5 were recovered from damage occurred due to anemia in group 2 (**Fig.4B**).

Examination of heart of rat from control group revealed no histopathological changes (Ctrl). On the other hand, heart of rat from group 1 showed myocarditis with mononuclear leucocytic cells infiltration (G1). However, heart of rat from group 2 showed no changes except slight intermuscular edema (G2). Vacuolations of some cardiac myocytes was noticed in heart of rats from group 3 (G3). Meanwhile, hearts of rats from groups 4 and 5 showed no histopathological changes (G4) and (G5). The results of microscopically studied heart sections showed that the severe damage occurred in hearts of anemic rats in group 1 (G1), was completely recovered in rats of groups 4 and 5 (**Fig.4C**).

Sections of spleens were stained with Perls' Prussian blue to evaluate iron accumulated in different groups. **Fig.4D** shows iron accumulated in spleens of rats from different groups. Examination of spleens of rats from control group showed marked deposition of greenish blue hemosiderin pigment, indicating iron accumulated in tissues (Ctrl). Examined spleens of rats from group 2 showed a very few deposits in spleen tissues (G1). Moreover, the iron deposition in groups 2 and 3 was increased but still less than control group, (G2) and (G3). However the iron deposition in spleen tissues from rats in groups 4 and 5 was clearly increased and recurred as control group, (G4) and (G5). These results support the results of iron accumulated in tissues of spleen of rats of different groups.

Discussion

Possible explanations for lower iron accumulation in yeast cells at higher concentrations in the medium could be found in some previous investigations. Lesuisse *et al.* [14] found out that higher concentration of Fe(III) in the medium caused decrease of negative potential at yeast cell surface due to saturation of negative groups near iron transporters, which otherwise participate in iron uptake. Consequently, the accumulation of iron would be inhibited, what is consistent with previous results, too. On the other hand, Tuttle *et al.* [15] discussed the possibility of iron efflux out of yeast cells at higher concentrations of iron in the cell interior. This could also be a reason for lower amounts of accumulated iron in *S.cerevisiae* at concentrations of ammonium Fe (III) citrate higher than 32 mM.

. In humans, the capacity of iron absorption from the diet depends on various factors including the amount of iron in the body, the rate of erythrocyte production, the amount and kind of iron in the diet, and the presence of absorption enhancers and inhibitors in the diet [16], [17].

The histological study of tissues from liver, kidney, heart, and spleen of rats from different groups showed that the severe damage occurred in tissues of anemic rats group 1, was completely recovered after treatment by iron enriched yeast in groups 4 and 5. However other treatments in groups 2 and 3 may require more time to achieve the same results obtained in treatment by iron enriched yeast, also only slight histological differences can be observed when comparing group 4 and group 5, leading that both treatments, 15 mg Fe/Kg diet (group 4) and 30 mg Fe/Kg diet (group 5), of iron enriched yeast have the same efficiency when used to cure moderate iron deficiency anemia.

In conclusion, this study demonstrates that the iron enriched baker's yeast prepared in presence of 32 mM Fe in growth medium has the best baking properties (leavening ability) of both parent and other Fe concentrations grown cells, with improvement of biomass produced. The used strain was also proved to accumulate the highest content of Fe (15 mg/g) at this concentration (32 mM) among other concentrations tested, and achieved the most bioavailability of iron when used in the diet of anemic rats ahead of other treatments by inorganic iron salts. Also the severe damage occurred in tissues of anemic rats was completely recovered after treatment by iron enriched yeast, However salt-based treatments may require more time to achieve the same results. It is suggested that the use of

iron-enriched yeast products may lead to the prevention of anemia in compromised individuals, or the ability to recover from moderate iron deficiency.

More advanced studies about the effects of iron enriched baker's yeast on the quality of bread loaf are undergoing for pave the way for the use of iron enriched yeast industrially for the production of bread.

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Highlights

- Iron enriched yeast was prepared by cultivation of yeast cells at different iron concentrations and best concentration was determined with no effect of yeast growth or baking properties.
- Anemic rats were fed on different diets containing either inorganic iron salt or iron enriched yeast as iron source.
- Feeding efficiency and different haematological incidences were followed up for all groups.
- Histological study was performed for liver, heart, kidney and spleen tissues in all groups.
- Feeding anemic rats with supplemented yeast leads to better results in recovery from iron deficiency when compared to other diets containing inorganic iron salt as iron source.

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Figure legends

Fig.1: Design of rat feeding experiments

Fig.2: Effect of growth media iron concentration on yield of yeast cells (A), iron accumulation in cells (B) and leavening ability (C). Data represents the average of triplicate samples and the standard error is indicated by the error bars.

Fig.3: Feed efficiency (A) , iron bioavailability (B), catalase activity in plasma (C) and kidney tissue (D), and iron concentrations in liver (E) and spleen (F) of each group of rats. Data represents the mean of 8 rats and the standard error is indicated by the error bars .

Fig.4

(A) Liver sections stained with H&E from each sample group. Red arrows indicate abnormalities in the tissue.

(B) Heart sections stained with H&E from each sample groups. Red arrows indicate abnormalities in the tissue.

(C) Kidney sections stained with H&E from each sample group. In each instance no major differences were observed.

(D) Spleen sections stained with Prussian blue from each sample group. Red arrows indicate abnormalities in the tissue.

Tables

Table.1: Initial and final mean values of rats' body weights (with *LSD values) from different groups during treatment period

Treatments	Time in days	
	21 (0 time)	56 (35)
Control	114.33±3.5	164.83±10.1
Group 1	97.80±8.1	126.80±11.1
Group 2	130.67±8.5	170.33±6.2
Group 3	120.33±8.2	161.50±10.8
Group 4	115.67±8.7	161.50±10.6
Group 5	115.50±7.8	161.40±11.2

Values are mean ± SE, n=8 all differences in body weights were significant at $p < 0.05$ for each group.

Table.2: Initial and final mean values of rats' hemoglobin (with LSD values) from different groups during treatment period

Treatments	Time in days	
	21(0 time)	56(35)
Control	143.50±6.4	162.00±8.2
Group 1	126.00±7.7	90.20±10.2
Group 2	109.60±10.6	130.30±12.5
Group 3	109.10±4.3	119.20±11.5
Group 4	112.60±10.5	160.00±9.8
Group 5	112.00±10.6	158.16±4.2

Values are mean ± SE, n=8 all differences in hemoglobin values were significant at p<0.05 for each group.

Table.3: Body weight, total food intake, and feed efficiency of rats. Rats were under-fed to make sure that all groups will consume the assigned amount of food. The food consumption of wistar rats investigated was 18g/day and 630 g over 35 days

Group	*Final B.W (g)	*Gained weight (g)	*Consumed food (g)	Feed efficiency %
Ctrl	164.8±13.2	50.5±9.8	420.00	12.0
Fe (-) Group (1)	126.8±14.3	29.0±8.6	420.00	6.9
Fe – S Group (2)	170.3±13.6	39.7±7.9	420.00	9.4
Fe – S Yeast Group (3)	161.5±28.3	41.1±9.1	420.00	9.7
Fe – Yeast 15 Group (4)	161.5±26.8	46.0±10.1	420.00	10.9
Fe – Yeast 30 Group (5)	161.4±15.7	45.8±9.7	420.00	10.9

*Values ± SE, p<0.05

Table.4: Indices of Fe bioavailability in different groups during treatment period

Group	Initial Hb Fe* mg	Final Hb Fe* mg	Fe Intake* mg	Fe bioavailabilit y* %	Relative Fe bioavailabilit y* (RBV) %
Ctrl	4.15± 0.3	6.60±0.4	12.60±0.5	19.44±3.1	100
Fe (-)	3.09±0.2	3.01±0.2	3.00±0.1	-2.60±1.1	-13.37
Fe – S	3.45±0.2	5.48±0.3	12.60±0.2	16.11±2.2	82.87
Fe – S Yeast	3.32±0.1	4.85±0.3	12.60±0.3	12.93±2.1	66.51
Fe – Yeast 15	3.28±0.3	5.57±0.2	6.30±0.4	36.35±4.2	186.98
Fe – Yeast 30	3.11±0.2	5.94±0.3	12.60±0.4	22.46±3.4	115.53

*Values ± SE, n=8, p<0.05

Table.5: Hemoglobin, iron concentration, TIBC, and TS in the serum of rats

Group	*Final Hemoglobin (g/l)	*Hematocrit (Volume fraction)	*Serum iron ($\mu\text{mol/l}$)	*Total iron binding capacity (TIBC) ($\mu\text{mol/l}$)	*Transferrin saturation TS (%)
Ctrl	162.0 \pm 13.5	0.44 \pm 0.06	26.7 \pm 2.8	60.5 \pm 3.5	44.1 \pm 2.1
Fe (-) Group (1)	90.2 \pm 10.3	0.35 \pm 0.02	11.6 \pm 2.3	30.7 \pm 2.1	37.7 \pm 1.8
Fe – S Group (2)	130.3 \pm 12.6	0.39 \pm 0.07	22.9 \pm 1.5	43.5 \pm 3.8	52.5 \pm 3.1
Fe – S Yeast Group (3)	119.2 \pm 11.5	0.33 \pm 0.09	14.5 \pm 2.1	35.3 \pm 2.4	41.5 \pm 2.9
Fe – Yeast15 Group (4)	158.2 \pm 15.4	0.42 \pm 0.06	26.2 \pm 3.8	48.9 \pm 3.6	53.0 \pm 3.6
Fe – Yeast30 Group (5)	160.0 \pm 10.3	0.46 \pm 0.08	21.9 \pm 3.6	40.9 \pm 3.3	56.5 \pm 3.4

*Values \pm SE, n=8, p<0.05

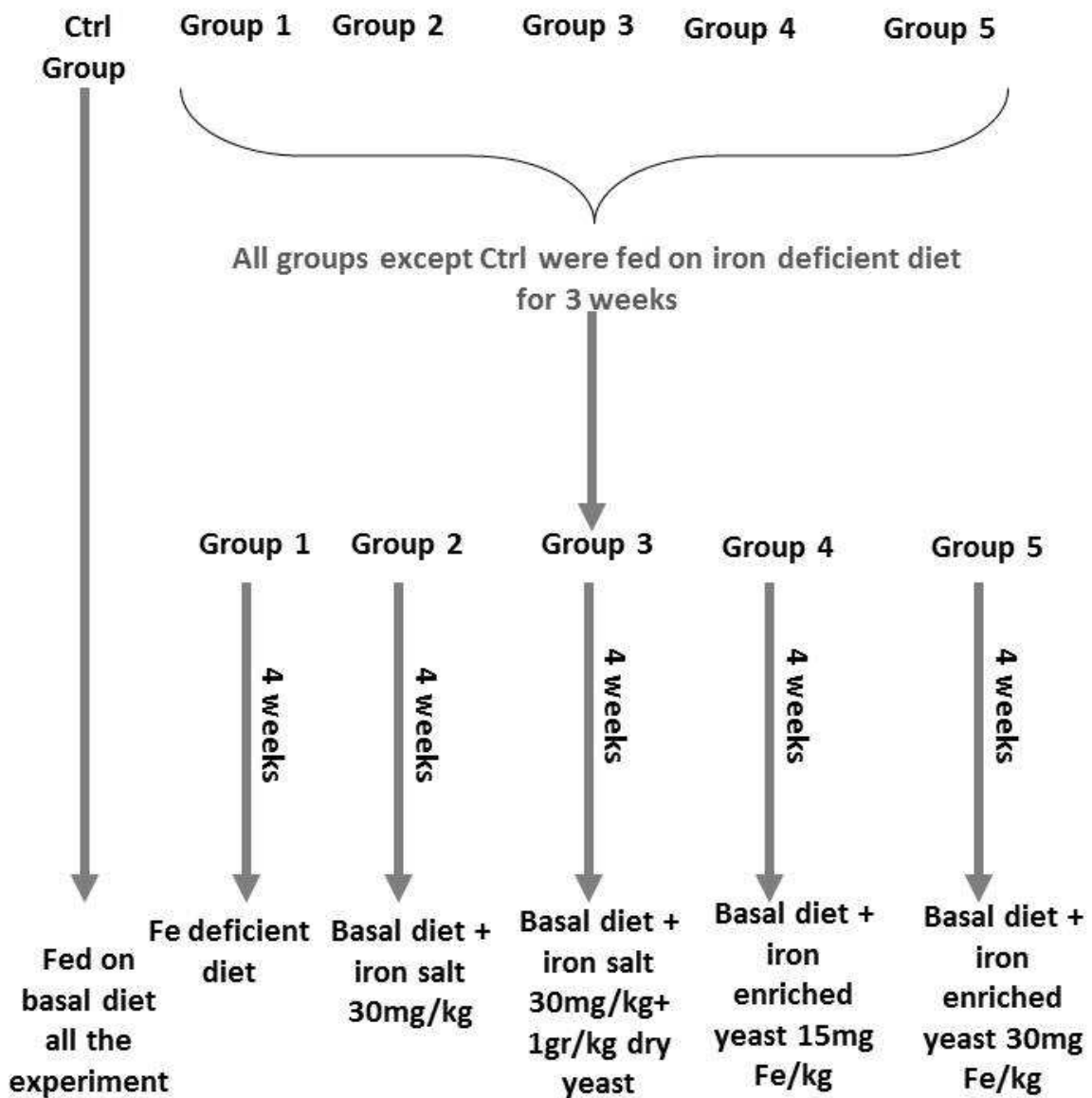


Fig.1: Design of rat feeding experiments

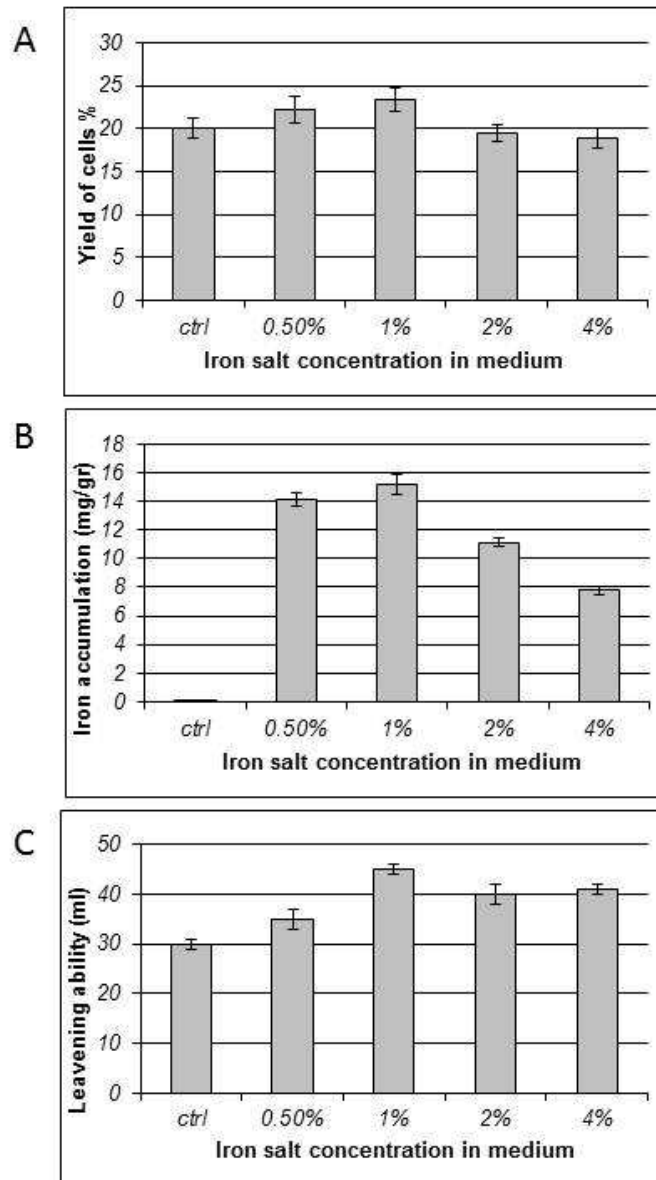


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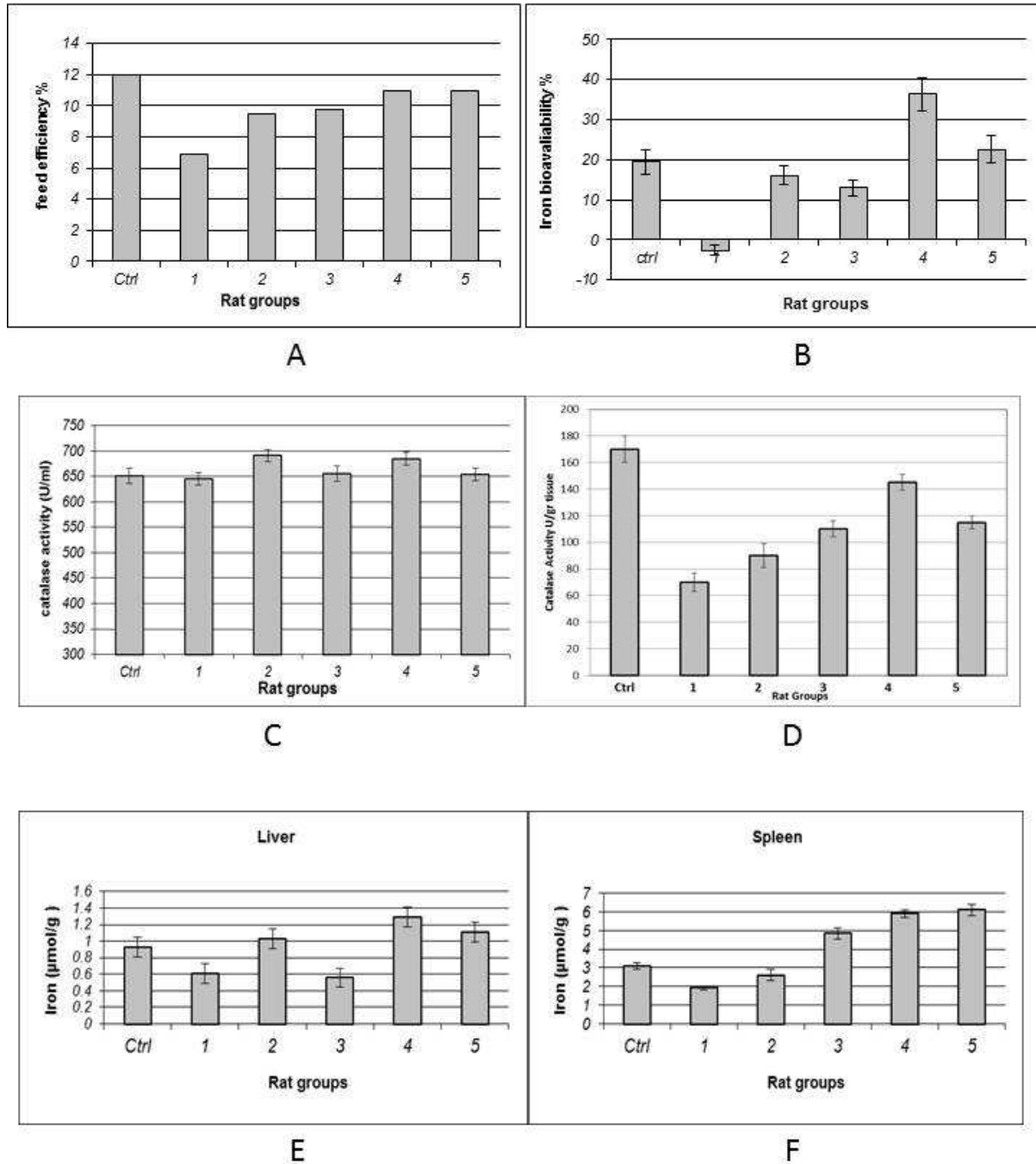


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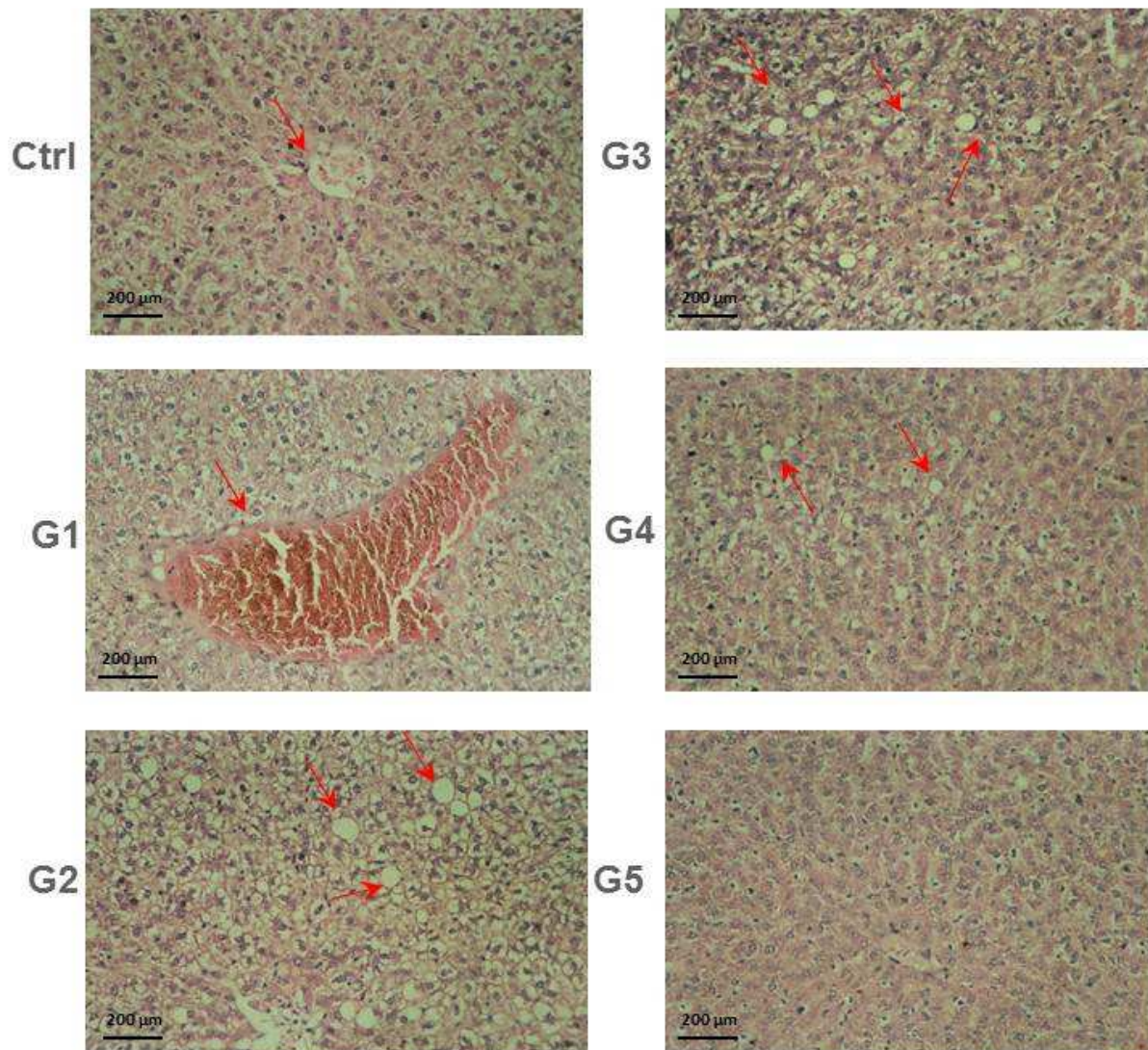


Fig.4 (A) Liver sections stained with H&E from each sample group. Red arrows indicate abnormalities in the tissue.

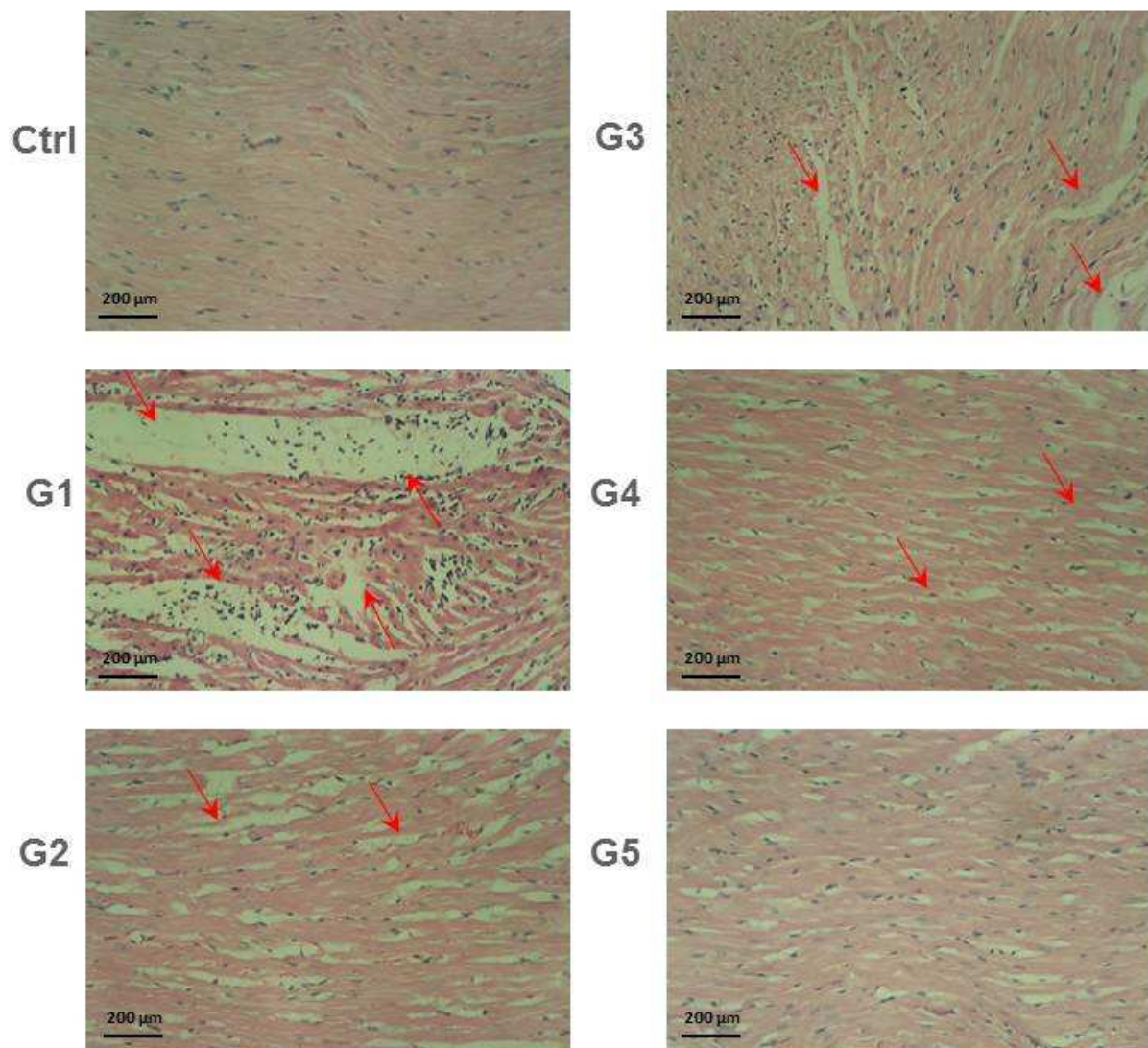


Fig.4 (B) Heart sections stained with H&E from each sample groups. Red arrows indicate abnormalities in the tissue.

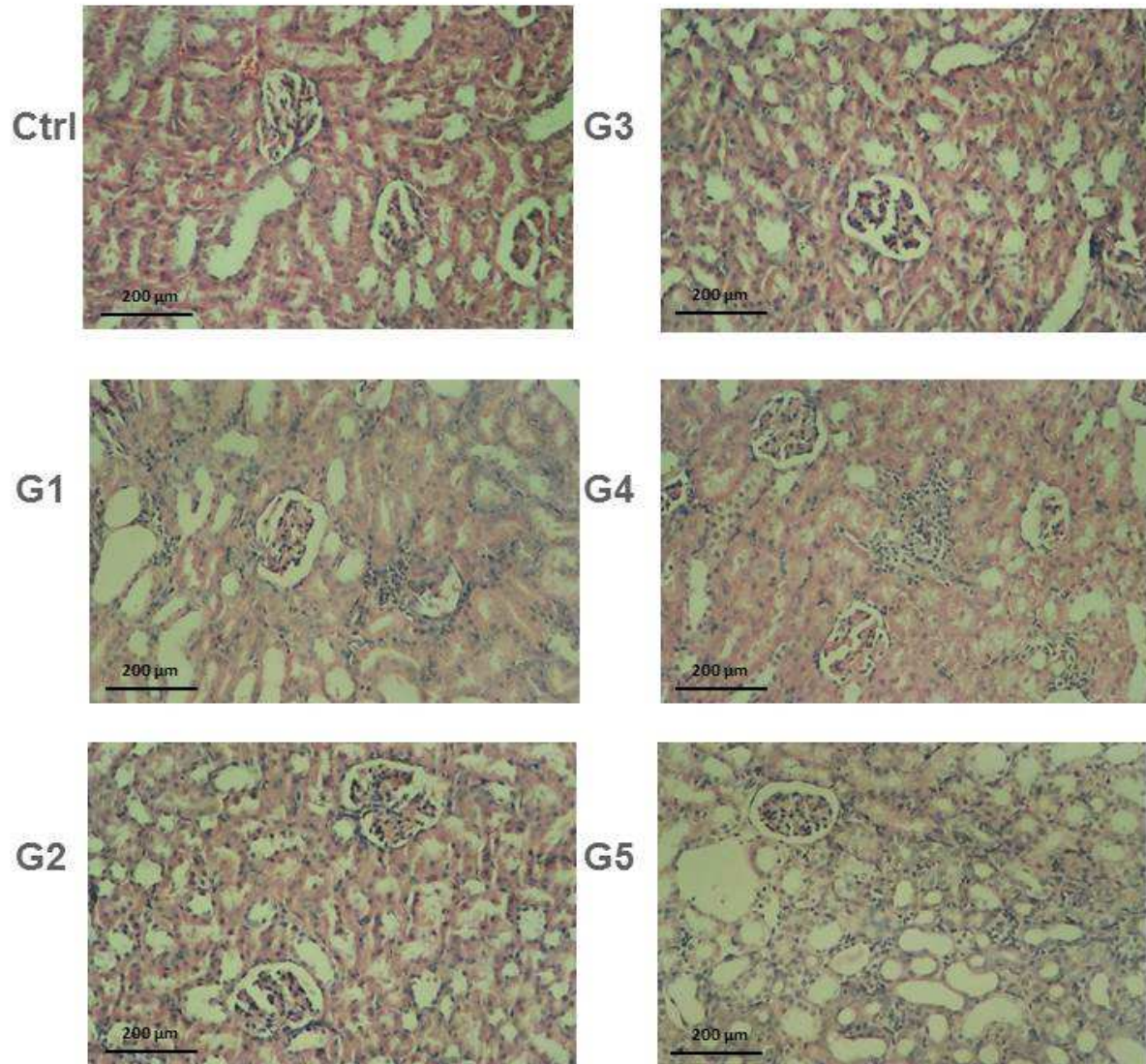


Fig.4 (C) Kidney sections stained with H&E from each sample group. In each instance no major differences were observed.

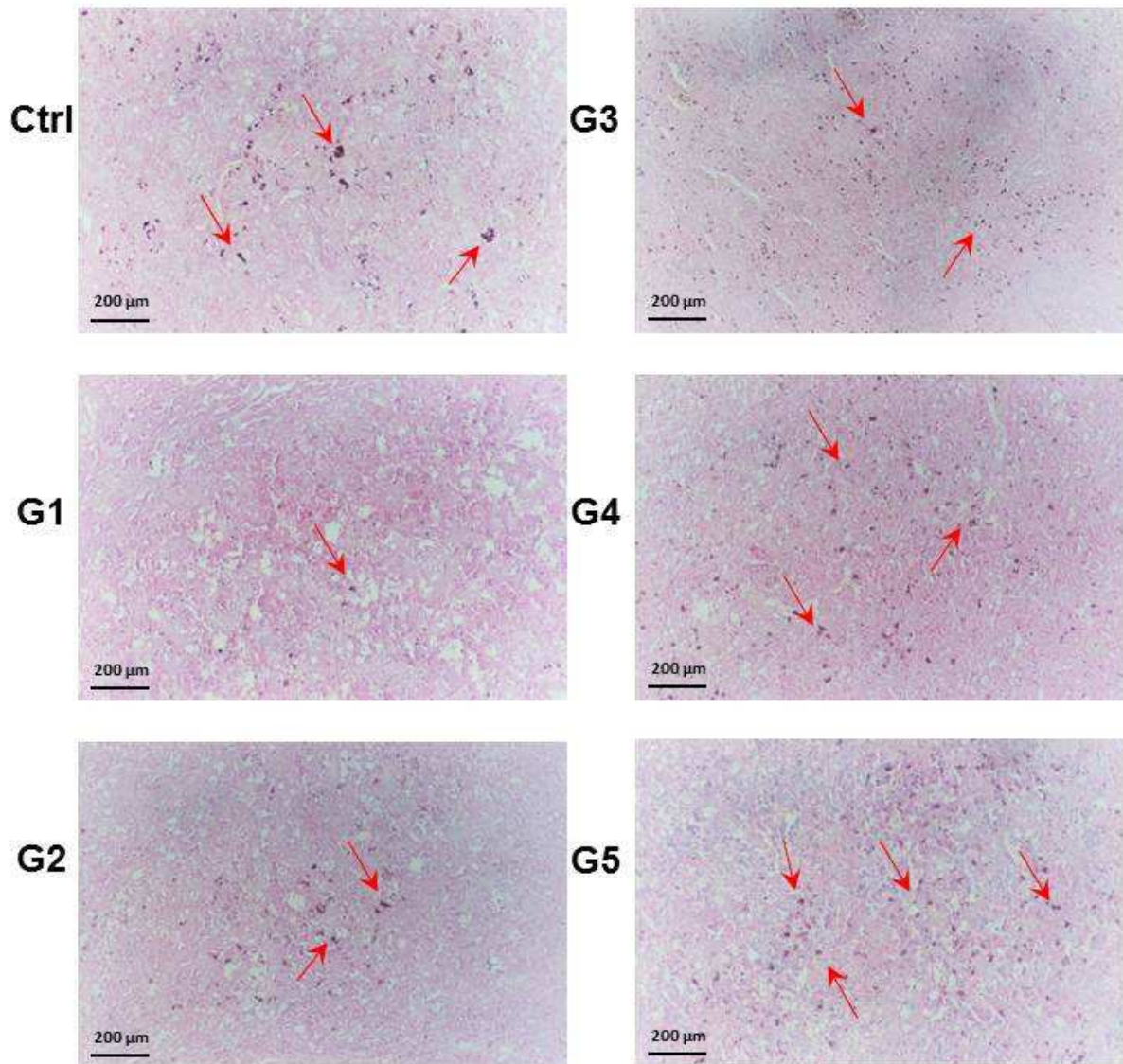


Fig.4 (D) Spleen sections stained with Prussian blue from each sample group. Red arrows indicate abnormalities in the tissue.