RESEARCH ARTICLE

Effect of Zinc drug on amino acid concentration in liver of white mice, *Mus musculus*

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ABSTRACT

Zinc is one of the essential trace elements, it plays a key role in many biochemical and functional processes. It is less harmful than many other minerals, in the case of exposure to high doses of zinc, poisoning occurs, and this poisoning may mostly result from the accidental ingestion of household products containing zinc or nutritional supplements, this study was conducted to find out the effects of zinc on the concentration of amino acid.

A total of 30 adult white mouse males were taken and divided into three groups; the first group (control) of 10 mice taken with distilled water for 30 days, the second group includes 10 mice that were dose with Zn drug concentration of 50 mg/kg/day for 30 days, the third group includes 10 mice that were dose with Zn drug 100 mg/kg/day for 30 days. In the current study (18) amino acid was recorded in the liver of adult white mouse males as follow: asparagine (Asn), serine (Ser), glutamine (Glu), glycine (Gly), threonine (Thr), histidine (His), citrulline (Cit), alanine (Ala), proline (Pro), taurine (Tau), arginine (Arg), tyrosine (Tyr), valine (Val), methionine (Met), isoleucine (Ile), leucine (Leu), phenylalanine (Phe) and lysine (Lys). Statistical analysis showed high significant differences in the concentration of amino acids between the two groups of the zinc-treated experiment with a concentration (50 and 100) mg/kg/day and control group, as well as significant differences between the two groups of the zinc treatment experiment with a concentration of (50 and 100) mg/kg/day.

Keywords: Zinc, Amino acid, Liver, Mouse.

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INTRODUCTION

Zinc is a essential micronutrient found in almost all tissues of the body, less harmful than many other minerals, in the case of high doses of zinc poisoning, and this poisoning may mostly result from the accidental ingestion of zinc-containing household products or nutritional supplements.[1] Zinc plays a key role in many biochemical and functional processes. It is an essential component of more than 300 different enzymes. Its catalytic effect is due to its direct participation in substrate conversion and stabilization of its structure, in addition to zinc's extensive roles in immune response at multiple levels including gene expression as well as the differentiation and development of immune cells.[2] Zinc's human health implications are enormous, with nearly half of the world's population believed to be at risk of zinc deficiency.[3]

The liver is the main organ responsible for zinc metabolism, and various liver diseases may be affected by zinc deficiency, where zinc deficiency can lead to a range of clinical manifestations such as poor appearance, loss of body hair, changing taste and smell, delayed wound healing, immune weakness, and reduced ability to get rid of medications common in patients with chronic liver disease, especially cirrhosis.[4]

Amino acids form the cornerstone of the construction of proteins and peptides and play a key role as a means of food construction,[5] a group of organic compounds made up of the carboxyl group (COOH) with the amine group (NH_2), and the amino acid contains asymmetric carbon atom (except glycine) where they are D or L, and in all tissues are found in D form. There are more than 20 amino acids in nature.[6]

Amino acids are divided into two types.[7,8] Essential amino acids: the organism gets it through food and includes: tryptophan (Try), valine (Val), methionine (Met), leucine (Leu), threonine (Thr), isoleucine (Ile), histidine (His), arginine (Arg), phenylalanine (Phe) and lysine (Lys). Non-essential amino acids: made by living organisms through simple reaction pathways, derived either from pyruvate or oxaloacetate, glycerate 3-phosphate, ketoglutarate except tyrosine amino

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acid (Try) derived from amino acid phenylalanine (Phe) includes: proline (Pro), glutamine (Gln), glycine (Gly), aspartate, alanine (Ala), serine (Ser), tyrosine (Tyr), cysteine (Cys), glutamic acid and aspartic acid.

MATERIALS AND METHODS

Experimental Animals

The current study was conducted on 30 white male mice *Mus musculus* obtained from the Center for Drug Control and Research in Baghdad, at the age of 8 weeks.

Treatment

Zinc (Zn) was used in the current study and obtained from America Medic and Science (AMS).

Experimental Design

This study was conducted on 30 white male mice where they were divided into three groups, the first group (control) 10 mice were treated with distilled water for 30 days. The second group (experimental group) 10 mice were treated with Zn 50 mg/kg/day concentration for 30 days, group 3 (experimental group) 10 mice treated with Zn concentration 100 mg/kg/day for 30 days.

Analysis of Amino acid

The amino acid of the liver was separated and diagnosed in the white mouse based on its standard models, and using a high-performance liquid chromatography (HPLC) device, injecting the device with known concentrations of amount 20 µL per standard amino acid.The methods used according to as follows[9]

- Jones (1) g of mouse liver tissue with 20 mL of deionized water, then added 1.50 g of sulfosalicylic acid using manual homogeneous.
- Samples were centrifuged by centrifuge for 1 hour.
- HCL (pH 2.0) was added to the floating material.
- Samples were centrifuged by centrifuge at a speed of (3,000) cycles/minutes.
- Mix (10) microliters of the clear solution with 10 µL of the PITC Phenyl isothocyanate reagent, and after 1-minute added 50 µL of sodium acetate (0.1) (pH 7.0).
- Amino acid was measured using a HPLC.
- Depending on the concentration of amino acids using the following equation:

$$\text{Amino acid concentration} = \frac{\textit{Area of beam in sample}}{\textit{Area of standard beam}} \times \textit{Standard concentration} \times \textit{No. Dilution}$$

RESULTS

The current result of amino acid studied in the liver of the white mouse showed the presence of 18 amino acids, which are: asparagine (Asn), serine (Ser), glutamine (Glu), glycine (Gly), threonin (Thr), histidine (His), citrulline (Cit), alanine (Ala), proline (Pro), taurine (Tau), arginine (Arg), tyrosine (Tyr), valine (Val), methionine (Met), isoleucine (Ile), leucine (Leu), phenylalanine (Phe) and lysine (Lys).

The results observed high significant differences at the probability level ($p \leq 0.001$) between the two groups of experiments treated with Zn compared to the control group of all amino acid (Table 1 and Figure 1), the highest concentration of amino acid (Asn) was found to be in the control group it was (39.61 ± 3.80) and the lowest concentration found in the zinc treated group at a concentration of 100 mg/kg/day (28.28 ± 6.71). The highest concentration rate of (Ser) was found in the control group at (53.56 ± 5.88) and the lowest concentration was found in the zinc treated group at a concentration of 100 mg/kg/day at (36.72 ± 3.30), and in (Glu) the highest concentration in the zinc treated group appeared at 50 mg/kg/day at (100.06 ± 12.51) and the lowest concentration appeared in the zinc treated group at 100 mg/kg/day was (63.31 ± 10.50). In (Gly), the highest concentration was found to have appeared in the control group at (89.87 ± 6.28) and the lowest concentration in the zinc-treated group was 100 mg/kg/day at (59.81 ± 4.83). (Thr) had the highest concentration in the control group at (63.15 ± 2.54) and the lowest concentration in the zinc-treated group at 100 mg/kg/day (33.45 ± 4.46). (His) had the highest concentration rate in the control group at (59.22 ± 3.48) and the lowest concentration in the zinc-treated group at 100 mg/kg/day was (30.94 ± 3.02). In (Cit) amino acid, it found its highest concentration in the control group at (48.65 ± 4.46) and the lowest concentration found in the group with a concentration of 100 mg/kg/day (31.42 ± 4.28), while (Ala) found its highest concentration in the control group at (67.89 ± 3.27) and the lowest concentration found in the group with a concentration of 100 mg/kg/day (45.23 ± 5.76). In (Pro) amino acid, the highest concentration in the zinc-treated group was 50 mg/kg/day (39.19 ± 10.12) and the lowest concentration in the zinc-treated group was 100 mg/kg/day (30.46 ± 5.32). Tau amino acid had the highest concentration in the zinc-treated group at 100 mg/kg/day (1920.00 ± 250.18) and the lowest concentration in the control group at (928.48 ± 88.11). In Arg, the highest concentration in the zinc-treated group appeared at 100 mg/kg/day (246.04 ± 151.96) and the lowest concentration in the control group was 48.33 ± 4.94). In (Tyr) acid, the highest concentration in the zinc-treated group appeared at a concentration of 100 mg/kg/day (98.01 ± 44.75) and the lowest concentration in the control group was (33.98 ± 3.25). In Val amino acid, the highest concentration in the zinc-treated group appeared at 100 mg/kg/day (48.83 ± 15.88) and the lowest concentration appeared in the control group at 32.50 ± 4.95), In Met amino acid, the highest concentration in the zinc-treated group appeared at a concentration of 50 mg/kg/day (36.75 ± 7.70) and the lowest concentration in the control group was (28.59 ± 1.53). In Ile, the highest concentration in the zinc-treated group appeared at a concentration of 50 mg/kg/day (30.46 ± 1.63) and the lowest concentration in the control group was (26.96 ± 1.83). In Leu, the highest concentration in the zinc-treated group appeared at a concentration of 50 mg/kg/day (20.83 ± 0.78) and the lowest concentration in the control group was (19.14 ± 2.26), while in Phe amino acid the highest concentration in the zinc-treated group appeared at 50 mg/kg/day (20.40 ± 2.48) and the lowest concentration in the zinc treatment group was 100 mg/kg/day (15.89 ± 2.86). In Lys, the highest concentration was found in

Table 1: The concentration rate of amino acid in the adult white *Mus musculus* mouse liver of the control group and the two treatment groups treated with zinc Zn with a concentration (50 and 100) mg/kg/day

Amino acid	*Control group*	*Concentration 50 mg/kg/day*	*Concentration 100 mg/kg/day*
Asparagine (Asn)	39.61 ± 3.80	37.62 ± 1.23	28.28 ± 6.71
Serine (Ser)	53.56 ± 5.88	45.20 ± 2.82	36.72 ± 3.30
Glutamine (Gln)	87.11 ± 7.47	100.06 ± 12.51	63.31 ± 10.50
Glycine (Gly)	89.87 ± 6.28	73.61 ± 6.15	59.81 ± 4.83
Threonine (Thr)	63.15 ± 2.54	53.89 ± 7.11	33.45 ± 4.46
Histadine (His)	59.22 ± 3.48	47.45 ± 5.66	30.94 ± 3.02
Citrulline (Cit)	48.65 ± 4.46	42.08 ± 9.50	31.42 ± 4.28
Alanine (Ala)	67.89 ± 3.27	54.80 ± 9.16	45.23 ± 5.76
Proline (Pro)	36.86 ± 5.44	39.19 ± 10.12	30.46 ± 5.32
Taurine (Tau)	928.48 ± 88.11	977.84 ± 68.35	1920.00 ± 250.18
Arginine (Arg)	48.33 ± 4.94	105.98 ± 23.60	246.04 ± 151.96
Tyrosine (Tyr)	33.98 ± 3.25	69.48 ± 25.98	98.01 ± 44.75
Valine (Val)	32.50 ± 4.95	46.73 ± 16.93	48.83 ± 15.88
Methionine (Met)	28.59 ± 1.53	36.75 ± 7.70	35.51 ± 8.71
Isoleucine (Ile)	26.96 ± 1.83	30.46 ± 1.63	28.21 ± 4.01
Leucine (Leu)	19.14 ± 2.26	20.83 ± 0.78	19.97 ± 3.41
Phenylalanine (Phe)	18.92 ± 0.78	20.40 ± 2.48	15.89 ± 2.86
Lysine (Lys)	48.68 ± 2.87	32.76 ± 1.86	27.83 ± 3.88

the control group at (48.68 ± 2.87) and the lowest concentration found in the group was zinc treatment at a concentration of 100 mg/kg/day (27.83 ± 3.88).

The results also indicated highly significant difference at the probability level ($p \leq 0.001$) between the treatment group at a concentration of 50 mg/kg/day and the treatment group at a concentration of 100 mg/kg/day (1920.00 ± 250.18), of (Tua) amino acid, while in the zinc treated group with a concentration of 50 mg/kg/day with a concentration (977.84 ± 68.35). The rest of the amino acidity had no high significant differences between the two groups of experiments.

The results also showed that there were significant differences in the ($p \leq 0.05$) probability level of Arg between the two Zn-treated groups compared to the control group.

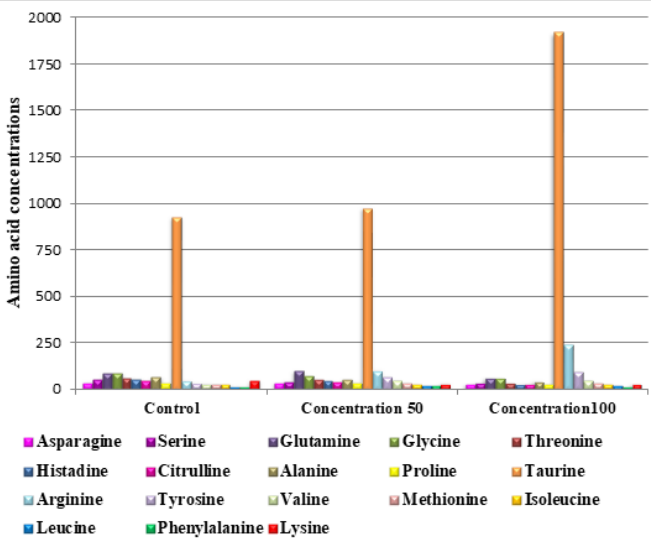

Figure 1: The relationship between the rate of amino acid concentrations in the adult white mouse *Mus musculus* liver of the control group and the two treatment groups treated with zinc Zn with a concentration (50 and 100) mg/kg/day.

The highest concentration of Arg amino acid in the treatment group was found at 100 mg/kg/day (246.04 ± 151.96) and the lowest concentration in the control group at (48.33 ± 4.94), while the rest of the amino acid had no significant differences between the two treatment groups. The results also indicated no significant differences in the amino acid in the liver at the probability level ($p > 0.05$) between the two groups of Zn-treated compared to the control group.

DISCUSSION

Amino acids are the essential structural component of nucleic acids, enzymes, tissue proteins and other nitrogen components that synthesize the cell. The supply of energy to construct and maintain these large and vital molecules is the primary purpose of the oxidation of carbohydrates and fats.[10]

The liver in vertebrates represents a site that creates many proteins of amino acids that play an important and effective role in the body, building muscles and cells, building enzymes and hormones, interfering with tissue repair, and forming collagen fibers of connective tissue.[11]

The amino acid was analyzed in the results of the current study, 18 amino acids found in the liver of adult white mouse males, but not within their study on a multi-change analysis of amino acids in plasma and mouse liver. During the infection of the malarial parasite Plasmodium yoelii, where they indicated that their liver number is (20) amino acid.[12] The same numbers were found in other vertebrates, for instance; in a study on the analysis of amino acids in the liver of a fetus and the adult chicken, where they found (18) amino acids.[13] In another study on the analysis of amino acids in the brain of a fetus and an adult amniotic bird quail found 16 amino acids in the bird's brain.[14]

The current result of amino acid also showed a highly significant increase ($p \leq 0.001$) between the two groups of experiments treated with zinc in concentration (50–100) mg/kg/day compared to the control group, as well as a significant increase ($p \leq 0.05$) between the two groups of the experiment treated with zinc in concentration (50–100) mg/kg. The current result found that the highest concentration of amino acid (Tau) in the zinc-treated group was 100 mg/kg/day at (1920.00 ± 250.18). Tau is found in all parts of the body, especially the heart, liver, retina, brain and platelets. It has a role in many functional and vital processes such as osmosis regulation, calcium stabilization, and the formation of gall bladder salt.[15] The amino acid Tau is a component of plasma membrane cells, which regulates the transport of nutrients through the cell membrane, protects cells from toxins, acts as a neurotransmitter, promotes muscle contraction, activates the heart muscle, and lowers blood pressure. The process of building Tau amino acid from cystine amino acid (Cys) is mediated by the cysteine-sulfinate decarboxylase (CSD) enzyme.[16] The increase in the current result may be related with the concentration of taurine acid when treated with zinc because of its role in protecting hepatic cells from the toxic effect of zinc.

The Arg is one of the primary amino acids that the body cannot synthesize and which plays a vital role in immune regulation and gene expression, as well as in hormone regulation.[17,18] Arginine acid is glycogenic because it can be converted into glucose.[19] The highest concentration in the zinc-treated group was 100 mg/kg/day at (246.04 ± 151.96). The increase in its concentration may be the result of a high concentration of glycogen due to treatment with the drug.

The Tyr acid plays an important role in regulating energy metabolism and motor activity. It also has an anti-inflammatory effect and reduces fatty degeneration in the liver when consumed.[20] The current result showed its highest concentration in the zinc-treated group at 100 mg/kg/day (98.01 ± 44.75) and may be due to the increase in its concentration to protect the liver from zinc-induced inflammation.

The Ile, Leu and Val are branched-chain amino acid (BCAA) and have a role in liver health and safety from diseases such as hepatic encephalopathy syndrome, a neuropsychiatric syndrome caused by liver failure such as cirrhosis.[21] The highest concentration of (Val) acid in the current result in the zinc-treated group was 100 mg/kg/day was (48.83 ± 15.88), while Ile and Leu showed their highest concentration. In the zinc treatment group at a concentration of 50 mg/kg/day, their concentration (30.46 ± 1.63, 20.83 ± 0.78), respectively, may be due to the increased concentration of these acids because of their importance in maintaining the integrity of liver tissue from the effects resulting of zinc toxicity.

The Gln amino acid has the highest concentration rate in the current result in the group treated with zinc at a concentration of 50 mg/kg/day was (100.06 ± 12.51), as it is considered an abundant amino acid in skeletal muscles, plasma and fetal fluids. It is the main pillar of immune system cells and helps multiply lymphocytes when responding to a stimulus through T-cells. It also prevents apoptosis and promotes cell growth and antibody production,[22] for which high concentration may have appeared in the current result as a consequence of an immune reaction to the harmful effects of zinc.

The Pro acid is a non-essential amino acid that the body can synthesize and has a role in the process of healing naturally after injury. It is responsible for skin safety and is involved in the synthesis of bones, cartilage, collagen and ligaments.[23] It has a role in regulating multiple biochemical processes in the cell,[24] with the highest concentration in the current result in the zinc treatment group at a concentration of 50 mg/kg/day (39.19 ± 10.12). The increase in its concentration in this result may be due to its role in regulating vital processes as well as healing liver tissue damaged by zinc effect.

The current result also indicated the highest concentration of (Phl) in the zinc-treated group at 50 mg/kg/day was (20.40 ± 2.48). In comparison, Met acid found its highest concentration in the current zinc treatment group at 50 mg/kg/day was (36.75 ± 7.70), that methionine acid is a essential amino acid obtained by the body through food, playing a key role in the biosynthesis of proteins, participating in cellular oxidation function.[25] Methionine-rich diets lead to homocysteine in the blood by increasing the level of circulation and causing vitamin deficiency B6 and B12 folic acid. It also stimulates hepatic cell injury by changing the metabolism of fat in the liver and stimulating oxidative stress.[26] The increase in its concentration in this result may be due to its role in stimulating hepatic cell infection towards zinc toxicity.

The Asn acid is a non-essential amino acid, acting as a neurotransmitter in the tissues of the neuroendocrine, and is important in protein synthesis and regulates the absorption of certain amino acids such as serine, arginine and histamine.[27] Asparagine plays an important role in immunity as it prevents programmed death and increases the growth of lymphocytes[22] and its highest concentration in the current score in the control group at (39.61 ± 3.80) and may have been the reason for high concentration.

The Ser has the highest concentration in the control group at (53.56 ± 5.88). (Ser) acid plays a role in the formation of (Gly) amino acid, as they pointed out,[28] and (Ser) have an important role in the development of hepatic diabetes.[29] Perhaps, the reason for his increased focus.

The Gly contributes to the synthesis of many functionally important molecules such as purines, nucleotides and glutathione.[30] It is also considered as an anti-inflammatory and immune-regulating substance that reduces inflammatory and pathogenic reactions in the body[22] with the highest concentration in this result is the control group at (89.87 ± 6.28). The high concentration of Glycine acid may have been found in the result because it is an antioxidant and protects the body from diseases.

The Thr amino acid is also one of the body's essential amino acids obtained through food.[31] It is an important acid in immune response for its active role in the central nervous

system, has a role in the production of collagen fiber and growth as well as in the muscles and heart with high concentrations, acts in the liver to reduce accumulated fat, and is included in the metabolism of the two amino acid Ser and Gly. Additionally, it enters in the structure of glycoproteins that are found in mucous substances in the digestive tract and therefore maintains intestinal health[32,33] and had its highest concentration in the control group at (63.15 ± 2.54). This increase in concentration may be due to reduced fat accumulation in the liver.

Histamine acid showed the highest concentration in the control group (59.22 ± 3.48), as it works in the process of regulating gene expression and bioactivity of proteins, regulates the function and structure of hemoglobin, also reduces high blood sugar, oxidation, inflammation and hyperlipidemia of blood for diabetes, and regulates the metabolism of glucose in the liver,[34] probably due to his high concentration because of its role in keeping the body from inflammation and oxidation.

The Cit is a non-essential amino acid as the liver and intestinal mucosa create it, and its concentration decreases in the case of acute inflammation, where it participates in energy regulation and metabolism, reduces fat deposition, is considered antioxidant,[35] and has found its highest concentration in the current result in the control group at (48.65 ± 4.46), and may be for its role as an antioxidant as well as reduces fat deposition.

The liver develops hepatic gluconeogenesis by forming glucose from the amino acid (Ala), so this acid is an important acid in the liver and also used (Ala) by exogenous hepatic cells such as immunocytes, which inhibit hepatic autophagy.[22,36] Ala also has an important role in protecting the liver from chronic diseases such as acute liver failure and in protecting hepatic cells from necrosis.[37] In the current study, the highest concentration of Ala in the control group was (67.89 ± 3.27) The increase in its concentration may be due to its important role in protecting hepatocytes and liver from diseases.

The Lys acid is an essential amino acid that the body gets through food. It has an immune efficacy against antniviral viruses and the treatment of herpes simplex virus infection, which supports fetal formation and healthy growth, and introduces the synthesis of collagen fiber and the synthesis of connective tissue of bones and protein,[36,38] and found its highest concentration in this result was in the control group at (48.68 ± 2.87) Perhaps he found a high concentration of it for its importance mentioned above.

In the current result, an increase in the concentration of a number of amino acids treated with zinc at a concentration of 100 mg/kg/day has been observed (Tau, Arg, Tyr and Val), and a concentration of 50mg/k G/day (Glu, Pro, Met, Ile, Leu, Phe), there are acids that have the highest concentration in the control group (Asn, Ser, Gly, Thr, His, Cit, Ala and Lys).

The increased concentration of amino acids in the treated groups of the drug and concentrations (50–100) mg/kg/day may have been due to its active role in regulating functional, vital and chemical processes, protecting liver cells from the toxic effect of the drug and maintaining the normal balance of cells, while amino acids that have seen an increase in their concentration in the control group may be due to metabolic and other functional aspects of mice.

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