Impairment of Endocrine and Exocrine Pancreatic Functions in Copper-Deficient Rats and the Amelioration Role of Aminoguanidine or/and N-Acetylcysteine

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Abstract: Copper (Cu) is an essential trace element that is found in a wide variety of tissues in the human and animal bodies. Copper is essential for several variety of biochemical processes in the body to operate normally, so it must be a part of the diet. Moreover, copper is involved in numerous enzyme systems that break down or build up body tissues. A deficiency of this element can cause a variety of disorders. This study was conducted on male albino rats to elucidate the harmful effects of copper deficiency on some biochemical variables of blood and liver tissues of rats which fed on a copper deficient diet. The same measurements were also measured in rats fed the same diet and supplemented with copper with or without AG or NAC separately or in combination for one month in order to assess their effectiveness for treatment the impacts of copper deficiency. The obtained results revealed that a significant decrease($p<0.05$) in the body weight, the pancreatic weight, DNA and RNA levels with a considerable decrease in the activities of pancreatic enzymes (amylase, lipase and trypsin) associated with a remarkable decrease in insulin level in copper-deficient rats group compared with those fed a copper sufficient diet. On the other hand, induced copper deficiency caused a significant ($p <0.05$) elevation in the protein content and glucose and HbA1C levels. When copper deficient rats group treated with copper alone, copper with aminoguanidine (AG), copper with N-acetyl cysteine (NAC) or copper with a mixture of AG and NAC led to a considerable amelioration in all previous studied parameters dependent on certain mechanisms which were discussed according to available references. The highest effectiveness in correcting the copper-deficiency induced perturbations was noticed when mixture was supplemented concomitantly with copper.

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1. Introduction:

Copper (Cu) is one of the relatively small groups of metallic elements which are essential for human and animal health**.** These elements along with amino and fatty acids as well as vitamins are required for normal metabolic processes ***(Aburto et al., 2001).*** This element (Cu) acts as a cofactor for several enzyme systems ***(Esipenko & Marsakova, 1995).*** The nature of the fundamental importance of copper is clearly revealed when the enzymes that require copper are considered. The presence of the same enzymes in man and in the animals used in studies on the function of copper enzymes ensures the relevance to man of information gained from such studies. Copper enzymes are widely distributed within the body; they perform several diverse functions including transport of oxygen and electrons, catalysis in oxidation - reduction reactions and the protection of the cell against damaging oxygen radicals ***(Heibashy & Amer, 2003*** and ***Heibashy et al., 2006)***. Since, copper is required for a number of enzyme systems and bodily processes, a deficiency in this element can cause a variety of disorders. Too little copper in the body can actually lead to disease.

A number of studies in experimental and domestic animals have demonstrated that copper (Cu) deficiency results in a selective and progressive atrophy of pancreatic acinar tissue with little or no effect on the pancreatic ductal system or islets ***(Smith et al., 1982; Fell et al., 1985 and Michael et al., 1989)***. This observation is best illustrated by the suggestion to employ copper deficiency as a research tool to improve the isolation of pancreatic interlobular ducts ***(Arkle et al., 1986)*** or islets ***(Weaver et al., 1986)***.

The human pancreas contains significant amounts of copper and the copper concentration declines with age (***Vuori et al., 1978).*** However, little is known about the physiological role(s) of copper in the pancreas. Moreover, ***Otsuki & Williams (1982)*** observed that copper *in vitro* stimulated the release of amylase from isolated rat pancreatic acini, in a manner independent of known secretagogue receptors or intracellular calcium. In addition, ***Smith et al. (1982)*** reported that severe copper depletion reduced pancreatic weight, markedly reduced pancreatic amylase concentration and reduced the secretory response to secretin and caerulein. Cytochrome oxidase activity was also found to be reduced in pancreas from cop-

per-deficient rats. Thus, it appears that copper metabolism may play an important role in exocrine pancreatic physiology ***(Smith et al., 1982).***

The pancreas is an organ containing two distinct populations of cells, the exocrine cells that secrete enzymes into the digestive tract and the endocrine cells that secrete hormones into the blood stream. The evidence is now overwhelming that both have a common origin in the early endodermal epithelium ***(Slack, 1995; Edlund, 1999; Percival & Slack, 1999 and Gu et al., 2002).*** The exocrine tissue is composed of the acinar cells which secrete the digestive enzymes (Amylase & lipase), together with the epithelial cells of the pancreatic ducts which transport the digestive enzymes from the pancreas to the small intestine. These ducts may be the site of residence of pancreatic stem cells that are responsible for renewing both tissues types ***(Bouwens, 1998)***. The endocrine cells are mostly organized into the islets of Langerhans and are subdivided into different cell types depending on the hormone that they secrete [α-cells, glucagon; ß-cells, insulin; δ-cells, somatostatin; PP (pancreatic polypeptide) cells, PP; γ-cells, ghrelin].

It has been shown that when rats are subjected to a copper-deficient diet the acini degenerate, whereas the ducts and the endocrine tissue persist ***(Rao et al., 1988 &1989).*** On refeeding with a normal diet the acini regenerate and this is accompanied by the appearance of foci of hepatocytes. These have been proposed to arise from pancreatic intralobular ducts, although experimental studies of the cell lineage have not been carried out. The development of the pancreatic hepatocytes in this model has been well documented, but the previous studies have not closely examined the effect of copper deprivation on the morphology of the endocrine pancreas. Although, ***Rao et al. (1988 &1989)*** suggested that islets and ducts are unaffected by copper deprivation, pancreatic tissue from copper-deprived rats does show an increase in the concentration of insulin ***(Fields & Lewis, 1997).*** Whether, the increase in insulin concentration within the pancreas is due to an increase in the synthesis of the hormone or to a change in the number of ß-cells has not been demonstrated.

Copper has been associated with pancreatic secretion although to varying degrees. ***Majumdar et al. (1989***) reported that marginal copper deficiency affects exocrine pancreatic structure and its responsiveness to physiological secretagogues. This is enhanced by a high fructose diet in copper deficient male rats ***(Lewis & Fields, 1989).*** Glandular atrophy can be produced by copper deficiency although this is more evident with severe rather than minor deficiency.

Aminoguanidine (AG) is one of the most extensively used inhibitors of AGEs accumulation. Besides its inhibitory action on AGE formation, AG acts as a competitive and selective inhibitor for inducible nitric oxide synthase (iNOS) ***(Ara et al., 2006).*** This action of AG has been known to be associated with reduction of peroxinitrite ($ONOO^-$), which has deleterious roles in inducing $NO^\bullet$ deficiency and cellular damages through degradation of eNOS cofactor, and inductions of inflammation, lipid peroxidation, protein nitrosylation and DNA fragmentation ***(Chowdhury et al., 2009 and Potenza et al., 2009).*** Previous investigations have also demonstrated that AG reduced hydrogen peroxide (H_2O_2) induced intracellular hydroxyl radical formation and apoptosis, further demonstrating a potential antioxidant activity ***(El-Khatibet al., 2001 and Thornalley, 2003).***

N-acetylcysteine (NAC) is derived from the sulfur-containing amino acid cysteine. It is found naturally in foods and serves as a powerful antioxidant. NAC is a precursor of intracellular glutathione which behaves as an antioxidant as well, functioning to remove toxic peroxides. NAC is readily absorbed, quickly converted to L-cysteine and then intracellular glutathione, thus, replenishing and maintaining healthy levels of glutathione ***(Lopez et al., 1991 and Heibashy, 2005).*** . In a study done by ***De Flora et al. (1986)*** noted that NAC induced an increase in oxidized glutathione reductase activity in rats. NAC and glutathione are very important antioxidant and detoxifying agents of the body.

The current investigation deals with recent achievements concerning the physiological role of copper and the problems of copper nutritional deficiency in the rats. The problem of copper deficiency and supplementation of aminoguanidine or N-acetylcysteine and their mixture to the animal diet is discussed and the possible amelioration effects of them on these alterations. Special attention has been paid to evaluate the impairment in the endocrine and exocrine pancreatic functions as a result of copper-deficient in rats.

2. Material and Methods

Sixty male albino rats *(Rattus rattus)* obtained from the animal House of Sera and Antigens Center , Inshas, were used in this study.

They were housed in the vivarum of Zoology Department, Women's Collage, Ain Shams University and kept under hygienic managerial and environmental conditions. They were fed to appetite on a standard laboratory animal diet according to ***NRC (1977).*** Fresh tap water was available at all times. Animals were then divided randomly into five groups, a control and four experimental groups, each of ten male albino rats on the base of the body weight, 140

g in average. The control group was fed on standard rodent diet [containing 10 mg copper sulfate ($CuSO_4$ $7H_2O$ / kg diet)].

Induction of copper deficiency:

Copper deficiency was induced in second generation rats by feeding the dams a low copper diet, one mg copper sulfate / kg diet (***Reinhold et al. ,1976)*** for two months. After two months, a comparison was occurred between five normal control diet rats (C) and five copper deficient (Cu-D) rats which considered as experimental one (Five rats in each one). This comparison was occurred to illustrate the impairment in the endocrine and exocrine pancreatic functions as a result of copper deficiency in rats.

In the second experiment, a comparison was occurred between normal control diet rats group (C) and four copper-deficient animals groups. However, the first one treated with a diet supplement 25mg copper sulfate/kg diet and expressed as Cu-D+Cu for one month, the second copper-deficient animals group received a diet supplemented 25mg copper sulfate/kg diet and a daily intraperitoneal injection of 50mg aminoguanidine (as hemisulfate)/kg diet ***(Saari et al.,1993)*** for one month and expressed as Cu-D+Cu+AG. The third copper-deficient animals group was given 25mg copper sulfate/kg diet with a daily intraperitoneal injection of N-acetylcysteine (25mg /100g b. wt.) according to ***Heibashy et al. (2005)*** for same previous period and expressed as Cu-D+Cu+NAC. The last deficient group (fourth) was fed on the diet supplemented with 25mg copper sulfate/kg diet with both aminoguanidine and N-acetylcysteine treatment for one month and expressed as Cu-D+Cu+M.

At the end of each experimental period, animals were sacrificed by head decapitation. Blood samples were collected in clean dry test tubes and serum was separated for the assessment of carbohydrate related parameters [glucose, and insulin] and pancreatic enzymes activities (amylase, lipase and trypsin). Also, fresh whole blood was obtained in heparinized tubes to determine the glycoslated haemoglobin ($HbA1_C$). The pancreases of all animals were frozen for DNA, RNA, total protein and lipid peroxidation (TBARS) estimations.

Processing of the pancreas and estimation of pancreatic parameters:

Pancreatic tissue, 0.1-0.2 g, was homogenized in 2 ml of 0.2 MTris-HCl (pH 8.0) containing 0.05 M $CaCl_2$ in a tissuemizer. DNA, RNA and proteins were extracted from $HC1O_4$ precipitates of an aliquot of the homogenate by the method of ***Wannemacher et al. (1965)***. DNA and RNA content were measured by the method of ***Burton (1956) and Ceriotti (1955)*** respectively. The protein level was determined by the method of ***Bradford (1976).*** The remaining pancreatic homogenate was centrifuged at 10,000 x g for 30 min at 2°C and the supernatant was assayed for the activities of amylase ***(Jung, 1985), trypsin (Walsh & Wilcox, 1970)*** and lipase ***(Lott et al., 1986).*** The activities of amylase, lipase and trypsin were estimated kinetically with the aid of commercial kits (Randox, LTD, UK).

Determination of serum copper level:

Serum copper level was determined with the aid of atomic absorption spectrophotometer (Perkin Elmer model No 3100, Norwalk CT , USA.) as described by ***Clegg et al. (1982)***

The assessment of carbohydrate related parameters:

Glucose was assayed enzymatically according to ***Trinder (1969)*** using commercial kits (Human Gesellschaft Für Biochemica and Diagnostica mbH, Germany). $HbA1_C$ was estimated according to ***Klenk (1991).*** The kit was purchased from Stanbio , USA . Insulin was estimated by radioimmunoassay (RIA) using solid phase component system according to ***Thorell & Lanner (1973)***. The kit was purchased from Millipore, St. Charles , Missouri , USA .

Estimation of lipid peroxidation:

Thiobarbituric acid reaction (TBARS) was assayed colorimetrically according to ***Ohkawa et al. (1979)*** using commercial ELISA kit (Cayman Chem. Co., USA).

Statistical analysis:

Statistical differences between the means were assessed by the student's t-test and analysis of variance (ANOVA) followed by Duncan's multiple range test according to ***Duncan (1955) and Snedecor & Cochran (1982)*** using a computer program (Costate). Values of $P < 0.05$ were considered statistically significant.

3. Results and Discussion

Copper has been found to behave as a molecular switch for activating growth rate, enzymes and cytokines. So, copper seems to act as an obligatory cofactor ***(Esipenko & Marsakova, 1995; Aburto et al., 2001 and Heibashy et al., 2006).*** Copper taken up by the cells (e.g. enterocytes, hepatocytes) is bound by intracellular peptides (cha-perones) and transported between intracellular Compartments. In the cells of the alimentary tract copper is bound by the HAH1 chaperone which enables its transport for binding with the ATP-7A transporter responsible for copper efflux into the circulation ***(Miyajiam, 2002).*** However, after entering the hepatocytes copper binds to different chaperones (CCS, COX17, HAH1) which provide copper for superoxide dismutase, cytochrome-c oxidase and ATP-7B transporter, respectively ***(Prohaska & Cybina, 2004).*** The authors also reported that adverse effects of copper de-

ficiency on genomic code (DNA or RNA) are not limited to the changes in DNA or/and RN sequences due to ROS production. These data are in harmony with those obtained in the current investigation (Table 1).

A high affinity copper transporter named Ctr1 is widely distributed in many tissues including the intestine, liver, and kidney and to lesser extent in the brain being responsible for dietary copper uptake by the tissues. The importance of Ctr1 in copper transport to the cells has been confirmed in mice completely deficient for Ctr1which exhibit profound growth and developmental defects and mostly die in utero ***(Lee et al., 2001)***

Cohen et al. (1982) have demonstrated that rats fed a copper-poor diet have a reduced insulin response to an oral glucose load in comparison with rats fed a copper supplemented diet. In addition, the increment in plasma glucose above the fasting level was significantly higher in rats maintained on a copper-poor diet suggesting impaired glucose tolerance in copper deficiency. The mechanism of the insulin-like effect of copper action in adipocytes has been studied by ***Cohen et al. (1985).*** The authors reported that copper stimulates glucose incorporation into lipids even after removal of the insulin receptor by enzymatic treatment of adipocytes. Thus, the authors have postulated that copper either affects cell membrane permeability or exerts oxidant action such as e.g. hydrogen peroxide formation which is also known to stimulate glucose uptake. Moreover, ***Vinci et al. (1995)*** and ***Field & Lewis (1997)*** noted that copper deficiency affects pancreas morphology with reducing the glandular mass and also causing islet hyperplasia associated with disorganized alpha and beta cell distribution with copper deficiency.

Several studies concerning molecular aspects of insulin-mimetic copper action have confirmed that copper ions, like insulin, strongly activate phosphoinositide-3-kinase/Akt pathway ***(Ostrakhovitch et al., 2002; Barther et al., 2007).*** Since, Akt is known to stimulate glycogen synthase, copper deficiency brings about a net depression of glycogen synthesis. However, Whole-body consequences of insulin-like copper effects on carbohydrate metabolism have been noted in rats fed a low-copper diet which reduce body weight due to greater fat but less carbohydrate utilization as energy source ***(Hoohveen et al., 1999)***.

From table (1), the obtained data showed that the copper deficiency in rats led to a marked depletion in the both of body and pancreatic weights associated with a significant decrease in the concentration of DNA & RNA and also a considerable depletion in the activities of pancreatic enzymes. These data are in harmony With the results obtained by ***Werman & Bhathena (1993); Vinci et al. (1995) Field & Lewis (1997) ; Barther et al. (2007) and Tosh et al. (2007).***

In all, the present data clearly establish that copper is an important element for normal exocrine pancreatic function. ***Vuori et al. (1978)*** found that the human pancreas contained a significant concentration of copper. Although, copper is known to act as a co-factor for a number of enzymes and to be an integral part of some proteins, the role of copper in exocrine pancreatic function is largely unknown. ***Otsuki & Williams (1982)*** reported that copper had multiple effects on inducing pancreatic amylase release. They concluded that changes in the oxidation state of protein sulfhydryl groups may be involved in pancreatic zymogene release.

Copper deficiency in the rats resulted in a profound reduced glandular mass of the pancreas, the pancreas continued low activities of lipase and amylase but excessive levels of insulin ***(Filed & Lewis, 1997).*** Reduced enzyme activity could be the consequence of less enzyme synthesis in the pancreas, less enzyme secretion by the pancreas. Many investigators studied the ability of the pancreas to adapt to changes in the level of dietary components ***(Poort & Poort, 1980).***

Moreover, copper is utilized by most cells as a component of enzymes involved in energy production (cytochrome oxidase) and in the protection of cells from free radical damage (superoxide dismutase). Copper is also involved with an enzyme that strengthens connective tissue (lysyl oxidase) and in brain neurotransmitters (dopamine hydroxylase and peptidyl alpha amidating monoxygenase) ***(Ostrakhovitch et al., 2002).***

Dietary copper deficiency in male rats led to a significant elevation in the concentration of glucose and $HbA1_C$ with a marked decrease n the level of insulin (Table 1). These data may be due to the destruction in the ß- cell of the pancreas as a result of elevation of free radical production, increment of lipid peroxidation levels represented by increasing in the glycation end products (GEPs), deficiency in the activities of enzymes antioxidants and decrease in the immune system. These results are parallel with those obtained by ***Dabeva et al. (1997); Devedjian et al. (1999); Barther et al. (2007)and Tosh et al. (2007).***

Chao & Allen (1992) recorded that the finding of reduced glucose tolerance in copper deficiency indicated that, because of relative elevation of blood glucose average blood glucose will be elevated by copper deficiency, this is supported by observation of elevated percentage of $HbA1_C$which is also regarded as index of chronic elevation of blood glucose. They found that in rats dietary copper deficiency had been shown to increase lipid peroxidation level.

Cohen et al. (1982) have demonstrated that rats fed a copper-poor diet have a reduced insulin response to an oral glucose load in comparison with rats fed a copper supplemented diet. In addition, the increment in plasma glucose above the fasting level was significantly higher in rats maintained on a copper-poor diet suggest-sting impaired glucose tolerance in copper deficiency. Impaired glucose tolerance in copper-deficient rats has been confirmed by ***Hassel et al. (1983).*** Moreover, *in vitro* glucose incorporation into diaphragm glycogen and into adipose tissue was stimulated by the addition of copper chloride to the incubation medium ***(Fields & Lewis, 1997).***

Dietary copper deficiency is due to elevate free radicals which are produced by normal metabolic activity. Free radicals such as superoxide radicals, hydroxyl radicals, peroxyl radicals and alkoxyl radicals when left unchecked wreak havoc on cells causing damage to membranes and DNA. Since, these free radicals have the potential for such devastating damage, they have been considered by scientists to be a major factor in the cancer and aging processes. On the other hand, it has been demonstrated that copper, despite its influence on glucose tolerance and disposal, affects islet function stimulating insulin release from perfused rat pancreas and preventing the inhibitory action of interleukin-1 on insulin secretion ***(Dabeva et al. 1997 and Devedjian et al.,1999)***. Additionally, it should be pointed out that copper deficiency affects pancreas morphology. ***Fields & Lewis (1997)*** have indicated reduced glandular mass, but ***Tosh et al. (2007)*** have shown islet hyperplasia and disorganized α and β-cell distribution with copper deficiency. .

Table (1): Biochemical alterations as a result of copper deficiency in male rats.

Parameters	Normal control (C) N=5 rats	Copper deficient (Cu-D) n=5 rats
Body weight (g)	253.572 ± 6.183	219.873 ± 7.418*
Pancreas weight (g)	0.894 ± 0.011	0.732 ± 0.007*
DNA (mg)	1.138 ± 0.017	1.019 ± 0.012*
RNA (mg)	5.352 ± 0.043	4.669 ± 0.051*
Protein (mg/g tissue)	55.719 ± 0.291	59.263 ± 0.327*
RNA/ DNA Ratio	4.703 ± 0.038	4.582± 0.034*
Protein/ DNA Ratio	48.962 ± 0.236	58.158 ± 0.278*
Amylase (IU/g)	22.836 ± 0.828	18.972 ± 0.714*
Lipase (IU/g)	16.394 ± 0.527	12.147 ± 0.395*
Trypsin (IU/g)	104.715 ± 2.761	82.937± 2.413*
Glucose (mg/dl)	116.587 ± 1.216	179.83 ± 1.738*
HbA_1C (%)	4.492 ± 0.019	5.312 ± 0.026*
Insulin (ng / ml)	1.138 ± 0.013	0.592 ± 0.009*
TBARS (nmol/g tissue)	139.625 ± 1.781	192.37± 2.803*

Values are expressed mean = SE. n=number of rats NC: normal control diet rats group.
Cu-D: copper deficient rats group. * Means a significant (P< 0.05).

It is well known that copper is an antioxidant nutrient because it is needed for an important enzyme that destroys superoxide, a harmful material produced by the body when it fights inflammation. Impaired defense against oxidation contributes to the increase in blood pressure, cholesterol and dotting found in copper deficiency ***(Arkle et al., 1986).***

A partial explanation for a general reduction for all of the pancreatic enzymes might be obtained from the histological observations of ***Fell et al. (1982) and Smith et al. (1982),*** which indicated that copper deficiency in rats caused selective atrophy of the exocrine pancreas with degeneration and necrosis of acinar cells. They also showed that cell size and cell number were reduced with most acini containing only a few cells. Zymogen granules were reduced, mitochondria were swollen and distorted and there was vesiculation of the endoplasmic reticulum. Conceivably, physiological consequence of reduced cell size and number in exocrine pancreas would be an overall reduction in hydrolase synthesis and secretion.

Antioxidants neutralize free radicals, which are produced by normal metabolic activity. Free radicals such as superoxide radicals, hydroxyl radicals, peroxyl radicals and alkoxyl radicals when left unchecked wreak havoc on cells causing damage to membranes and DNA. Since, these free radicals have the potential for such devastating damage. They have been considered by scientists to be a major factor in the diseases, cancer and aging processes ***(Thornalley, 2003; Ara et al., 2006; Heibashy et al., 2006 and Chowdhury et al., 2009).***

Aminoguanidine is capable of initiating lipid peroxidation by nucleophilic displacement of peroxy radicals, generated from fatty acid hydroperoxides, to yield a superoxide anion–like radical capable of promoting lipid peroxidation ***(Chao & Allen, 1992).*** So, aminoguanidine is able to join up with substances that cause links and to stop cross-links from developing. Therefore it may be able to help alleviate or prevent senile cataracts, thickening of the arteries, kidney failure, thinning bones, osteo-arthritis, skin wrinkles and many other signs of aging. Aminoguanidine's ability to stabilize the metabolism of glucose, it's role in reducing very low density lipoprotein cholesterol, and the evidence that it can improve blood flow, helping to reverse the conditions of arteriosclerosis and blood clots, indicates that aminoguanidine has a wide reaching ability to help prevent and treat a number of aging disorders ***(Ara et al., 2006).***

By reviewing tables (2 and 3), the supplementation of copper alone or with AG and NAC led to a considerable correction in all studied parameters dependent on the time of supplementation. These corrections may be contributed to the rebuilding and the improvement in the architect of pancreas cells due to decrease in the free radical production, amelioration in the antioxidant enzymes system, elevation in the auto-immune system. Also, these results may be due to antioxidant powerful of AG and NAC. The best correction in all studied parameters was recorded in the copper deficient animals group which received both antioxidants (AG and NAC) and this correction dependent on the time of supplementation. These data may be attributed to the Pharmacokinetics and pharmacodynamics properties of AG and NAC which act together to modulate the disturbance in the studied parameters as a result of dietary copper deficiency. These data are in parallel with those obtained by ***Smith et al. (1982); Saari et al. (1993); Heibashy et al. (2006) and Chowdhury et al. (2009).***

Table (2): Effects of supplementation of copper alone or with aminoguanidine or N-acetylcysteine and their mixture on some physiological parameters in copper deficient rats

Parameters \ Groups		C	Cu-D +Cu	Cu-D +Cu +AG	Cu-D +Cu +NAC	Cu-D +Cu +M
Body weight (g) dl)	15 days N = 5	286.138±7.387 $^{A}{}_{a}$	241.582±7.939 $^{B}{}_{a}$	257.193±8.639 $^{C}{}_{a}$	253.973±0.742 $^{C}{}_{a}$	275.326±0.625 $^{D}{}_{a}$
	30 days N = 5	317.437±8.541 $^{A}{}_{b}$	272.017±8.953 $^{B}{}_{b}$	298.884±0.607 $^{C}{}_{b}$	292.362±0.695 $^{C}{}_{b}$	310.142±0.563 $^{A}{}_{b}$
pancreatic weight (g)dl)	15 days N = 5	0.941 ± 0.011 $^{A}{}_{a}$	0.759±0.739 $^{B}{}_{a}$	0.792±0.704 $^{C}{}_{a}$	0.817±0.715 $^{D}{}_{a}$	0.861±0.691 $^{E}{}_{a}$
	30 days N = 5	1.215±0.296 $^{A}{}_{b}$	0.814±0.751 $^{B}{}_{b}$	0.854±0.665 $^{C}{}_{b}$	0.932±0.688 $^{D}{}_{b}$	1.071±0.608 $^{E}{}_{b}$
DNA (mg)	15 days N = 5	1.163±0.021 $^{A}{}_{a}$	1.047±0.019 $^{B}{}_{a}$	1.082±0.024 $^{C}{}_{a}$	1.097±0.026 $^{D}{}_{a}$	1.155±0.024 $^{E}{}_{a}$
	30 days N = 5	1.318±0.026 $^{A}{}_{b}$	1.088±0.0026 $^{B}{}_{b}$	1.117±0.026 $^{C}{}_{b}$	1.183±0.029 $^{D}{}_{b}$	1.276±0.022 $^{E}{}_{b}$
RNA (mg)	15 days N = 5	5.825±0.042 $^{A}{}_{a}$	4-982±0061 $^{B}{}_{a}$	5.104±0.074 $^{C}{}_{a}$	5.102±0.068 $^{C}{}_{a}$	5.348±0.082 $^{D}{}_{a}$
	30 days N = 5	6.176±0.048 $^{A}{}_{b}$	5.241±0.077 $^{B}{}_{b}$	5.753±0.068 $^{C}{}_{b}$	5.757±0.071 $^{C}{}_{b}$	6.023±0.652 $^{D}{}_{b}$
Protein (mg/g tissue)	15 days N = 5	64.361±0.049 $^{A}{}_{a}$	61.921±0.052 $^{B}{}_{a}$	63.917±0.066 $^{A}{}_{a}$	64.017±0.069 $^{A}{}_{a}$	68.813±0.074 $^{C}{}_{a}$
	30 days N = 5	85.027±0.061 $^{A}{}_{b}$	73.104±0.057 $^{B}{}_{b}$	79.638±0.068 $^{C}{}_{b}$	80.109±0.073 $^{D}{}_{b}$	84.616±0.082 $^{A}{}_{b}$
RNA/ DNA Ratio	15 days N = 5	5.009±0.042 $^{A}{}_{a}$	4.758±0.037 $^{B}{}_{a}$	4.717±0.038 $^{B}{}_{a}$	4.651±0.036 $^{C}{}_{a}$	4.630±0.045 $^{D}{}_{a}$
	30 days N = 5	4.686±0.037 $^{a}{}_{b}$	4.817±0.038 $^{b}{}_{b}$	5.150±0.034 $^{c}{}_{b}$	4.866±0.039 $^{d}{}_{b}$	4.720±0.044 $^{e}{}_{b}$
Protein / DNA Ratio	15 days N = 5	55.555±0.010 $^{A}{}_{a}$	59.141±0.017 $^{B}{}_{a}$	59.073±0.016 $^{B}{}_{a}$	58.356±0.016 $^{B}{}_{a}$	59.578±0.015 $^{B}{}_{a}$
	30 days N = 5	64.512±0.009 $^{A}{}_{b}$	67.191±0.018 $^{B}{}_{b}$	71.296±0.014 $^{C}{}_{b}$	67.717±0.017 $^{B}{}_{b}$	66.313±0.014 $^{B}{}_{b}$

- Values are expressed as mean ± SE. - n= number of rats. - NC: normal control diet rats group. - Cu-D: copper deficient rats group.
- Cu-D+Cu+AG: copper-deficient rats group received 25mg copper sulfate/kg diet and 50mg aminoguanidine /100g b.wt.
- Cu-D+Cu+NAC:.copper-deficient animals group was given 25mg copper sulfate/kg diet with 25mg /100g b. wt N-acetylcysteine.
Cu-D+Cu+M: :.copper-deficient animals group received 25mg copper sulfate/kg diet and 50mg aminoguanidine /100g b.wt. with 25mg /100g b. wt N-acetylcysteine as a mixture.
- $^{A, B, C, D, E}$ Means with a common superscript within a row are significantly different (P<0.05).
- $^{a, b}$ Means with a common subscript within a column are significantly different (P<0.05).

However, NAC affects on the immune system by increasing intracellular glutathione, which rapidly metabolized to intracellular glutathione. Glutathione acts as a powerful antioxidant in the body. Glutathione also detoxifies chemicals into less harmful compounds. NAC also protects the body from acetaminophen toxicity and is used in hospitals for patients with acetaminophen poisoning. It has also been shown to be effective at treating liver failure from other causes as well ***(Krakowski et al., 1999).*** Also, NAC has been used to regenerate oxidative phosphorylation complexes in mitochondria from age-related decline in function by sulfhydryl group action, rather than antioxidant effect and NAC protects against radiation damage by a direct radical scavenger action rather than by conversion to glutathione.

Table (3): Effects of supplementation of copper alone or with aminoguanidine or N-acetylcysteine and their mixture on some biochemical parameters in copper deficient rats.

Parameters \ Groups		C	Cu-D +Cu	Cu-D +Cu +AG	Cu-D +Cu +NAC	Cu-D +Cu +M
Amylase (IU/g)	15 days N = 5	$22.924\pm0.887^{A}{}_{a}$	$19.582\pm0.739^{B}{}_{a}$	$20.493\pm0.839^{C}{}_{a}$	$20.573\pm0.742^{C}{}_{a}$	$21.326\pm0.805^{D}{}_{a}$
	30 days N = 5	$24.173\pm0.890^{A}{}_{b}$	$21.017\pm0.853^{B}{}_{b}$	$22.284\pm0.797^{C}{}_{b}$	$22.392\pm0.695^{C}{}_{b}$	$23.142\pm0.863^{D}{}_{b}$
Lipase (IU/g)	15 days N = 5	$17.418\pm0.475^{A}{}_{a}$	$14.229\pm0.439^{B}{}_{a}$	$15.375\pm0.504^{C}{}_{a}$	$15.427\pm0.455^{C}{}_{a}$	$16.029\pm0.471^{D}{}_{a}$
	30 days N = 5	$17.735\pm0.496^{A}{}_{a}$	$15.414\pm0.451^{B}{}_{a}$	$16.698\pm0.565^{C}{}_{b}$	$16.712\pm0.528^{C}{}_{b}$	$17.538\pm0.498^{A}{}_{b}$
Trypsin (IU/g)	15 days N = 5	$107.163\pm0.147^{A}{}_{a}$	$94.447\pm0.391^{B}{}_{a}$	$98.824\pm0.327^{C}{}_{a}$	$98.597\pm0.367^{C}{}_{a}$	$103.105\pm0.296^{D}{}_{a}$
	30 days N = 5	$107.518\pm0.146^{A}{}_{a}$	$102.528\pm0.396^{B}{}_{a}$	$106.617\pm0.306^{A}{}_{a}$	$106.883\pm0.339^{A}{}_{b}$	$107.076\pm0.282^{A}{}_{b}$
Glucose (mg/dl)	15 days N = 5	$116.125\pm1.242^{A}{}_{a}$	$164.182\pm1.892^{B}{}_{a}$	$149.104\pm1.774^{C}{}_{a}$	$151.042\pm1.863^{C}{}_{a}$	$132.948\pm1.802^{D}{}_{a}$
	30 days N = 5	$113.876\pm1.248^{A}{}_{a}$	$145.331\pm1.911^{B}{}_{b}$	$130.453\pm1.689^{C}{}_{b}$	$131.244\pm1.788^{C}{}_{b}$	$110.463\pm1.652^{A}{}_{b}$
HbA1C (%)	15 days N = 5	$4.461\pm0.019^{A}{}_{a}$	$5.021\pm0.029^{B}{}_{a}$	$4.817\pm0.026^{C}{}_{a}$	$4.824\pm0.025^{C}{}_{a}$	$4.463\pm0.027^{A}{}_{a}$
	30 days N = 5	$4.459\pm0.021^{A}{}_{a}$	$4.804\pm0.026^{B}{}_{b}$	$4.698\pm0.038^{C}{}_{b}$	$4.702\pm0.027^{C}{}_{b}$	$4.488\pm0.031^{A}{}_{b}$
Insulin (ng / ml)	15 days N = 5	$1.138\pm0.011^{A}{}_{a}$	$0.676\pm0.009^{B}{}_{a}$	$0.865\pm0.016^{C}{}_{a}$	$0.872\pm0.016^{C}{}_{a}$	$1.003\pm0.015^{D}{}_{a}$
	30 days N = 5	$1.144\pm0.009^{A}{}_{a}$	$0.833\pm0.018^{B}{}_{b}$	$1.007\pm0.014^{C}{}_{b}$	$0.992\pm0.017^{C}{}_{b}$	$1.132\pm0.014^{A}{}_{b}$
TBARS (nmol/g tissue)	15 days N = 5	$141.734\pm1.721^{A}{}_{a}$	$180.526\pm2.617^{B}{}_{a}$	$169.325\pm2.016^{C}{}_{a}$	$169.522\pm2.007^{C}{}_{a}$	$154.493\pm1.715^{D}{}_{a}$
	30 days N = 5	$143.091\pm1.639^{A}{}_{a}$	$171.553\pm2.308^{B}{}_{b}$	$160.077\pm1.874^{C}{}_{b}$	$158.920\pm1.857^{C}{}_{b}$	$146.445\pm1.681^{A}{}_{b}$

- Values are expressed as mean ± SE. - n= number of rats. - NC: normal control diet rats group. - Cu-D: copper deficient rats group.
- Cu-D+Cu+AG: copper-deficient rats group received 25mg copper sulfate/kg diet and 50mg aminoguanidine /100g b.wt.
- Cu-D+Cu+NAC:.copper-deficient animals group was given 25mg copper sulfate/kg diet with 25mg /100g b. wt N-acetylcysteine.
Cu-D+Cu+M: :.copper-deficient animals group received 25mg copper sulfate/kg diet and 50mg aminoguanidine /100g b.wt. with 25mg /100g b. wt N-acetylcysteine as a mixture.
- $^{A, B, C, D, E}$ Means with a common superscript within a row are significantly different ($P<0.05$).
- $^{a, b}$ Means with a common subscript within a column are significantly different ($P<0.05$).

From the above cited data, it could be concluded that the copper deficiency diet could seriously alter the movement of nutrients through cell membranes. Also, copper is essential for life, which means that the human body must have copper to stay healthy. The supplementation of copper with aminoguanidine (AG) or N-acetylcystein (NAC) to copper deficiency rats group led to amelioration effects on the physiological and biochemical variables due to their antioxidants properties. The maximum amelioration effects was observed in dietary copper deficiency rats which received both antioxidants (AG and NAC) in the presence of copper due to their synergistic effects dependent on the time of supplementation (15 and 30 days).

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