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ROLE OF DIETARY COPPER SUPPLEMENTATION AS AN ANTIOXIDANT IN THE INDUCED HYPER URICEMIC RAT MODEL

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Abstract

Free radicals are capable of triggering chain reactions which can damage the different cell constituents. Oxygen, most vital for the human survival has on the same time deleterious potential .This is attributed to the formation, in vivo, of free radicals. Urate radicals do not react with oxygen to form another peroxy radical, thus increasing the efficacy of various metal like zinc and cupper as an antioxidant. Therefore, this study is designed to measure the level of coper and find out the association of coper in induced hyperuricemic rat model.

Methodology:

Study subject are forty male albino rats with an average weight of 180 ± 2 g were selected. The rats were fed on standard diet and given tap water ad libitum until treatment. They were divided into four groups. Group A were control, group B rat fed on 60% fructose with standard diet, group C were fed on fructose, standard diet and intraperitonially oxonic acid 250 mg/kg and group D only on injection intraperotonially oxonic acid 250 mg/kg. From rat heart 10 mL of blood was drawn and coper level were measured

Results:

Mean plasma level of copper of Control was found to be 19.59 mg/dl[±1.74] .Group "F"[fructose] showed mean plasma copper of 12.38 mg/dl[±1.39] .This reflects that copper fell down to 58% in rats which were exposed to diet comprising 60% Fructose than control. On comparing both groups i.e Control with Group "F" significant statistical correlation [P <0.01] was observed

Conclusion:

Fructose diet produce hyperuricemia in rat. Copper level were statistically significant and correlated between control and other group.

Keywords: Uric Acid; Copper; Albino Rats; Fructose Induced Hyperuricemia

Introduction

The Free radicals formation takes place during several important redox reactions therefore there are several studies which have pointed out their biological significance .Examples are of nitric oxide and Superoxide &related ROS.[1]. Nitric oxide is involved in smooth muscle relaxation [control of vascular tone] platelet inhibitorand various other cGMP-dependent functions[2]. While superoxide [O_2] and related ROS are involved in erythropoietin production, smooth muscle relaxation, signal transduction from various membrane receptors/enhancement of immunological functions &the maintenance of redox homeostasis. Superoxide through its derivative hydroxyl radical and hydrogen peroxide can induce second messenger cGMP, T-cell growth factor interleukin 2, heme oxygenase [3,4]. Thus the delicate balance between the advantageous and detrimental effects of free radicals is clearly an important aspect of life. They are part of normal cellular metabolism so exist in certain measurable concentrations in cells[5,6]. Their concentrations are determined by the balance between their rates of production and clearance by various antioxidant compounds and enzymes.[7] They can be classify into three main groups: antioxidant enzymes, chain breaking antioxidants, and transition metal binding proteins.

Antioxidant Enzymes:

Catalase, Glutathione peroxidase& Glutathione reductase, Superoxide dismutase

Chain breaking Antioxidant:

They are further divided into lipid phase chain breaking and Aqaous chain breaking antioxidants.

Lipid phase chain breaking antioxidants include Vitamin E, Carotenoids, Flavonoids, Ubiquinol. Aqaous phase chain breaking antioxidants comprises of Vitamin C,*Uric acid* ,Albumin bound bilirubin, Thiol group etc.

Transition metal binding proteins:

Ferritin, transferring, lactoferrin etc.

Uric acid now is not considered as merely a metabolic waste. It has been proposed that increase in life span observed in human evolution to some extent might be due to protective action of uric acid[8].Uric acid along with albumin and ascorbic acid account for more than 85% of total antioxidant activity[9,10,11]. Increase uric acid levels have been found in

oxidative stress and ischemia which might be compensatory mechanism of protection against free radicals [12,13].Uric acid cause inactivation of Nitric oxide and peroxynitrite radicals [14,15].Along with dopamine ,uric acid also help in repair of oxidative free radical induced damage of DNA in certain brain cells[16,17].

In present study we have tried to elaborate whether hyperuricemia is the precursor or resultant feature of metabolic syndrome. We have also seen uric acid relationship with other antioxidants so that uric acid role as antioxidant may be established.

Copper is an essential trace element in all living organisms and serves as a cofactor of key metabolic enzymes that regulate physiological processes, including cellular respiration, antioxidant defense, and iron metabolism in eukaryocytes [18,19].Copper plays numerous important roles in body including wound healing, angiogenesis, etc. Copper chelation prevents tumour growth and non-intemal thickening after vascular injury [20,21,22].

MATERIALS and Methods

Collection and storage of ANIMALS:

Locally bred forty[40] male Albino rats as shown in figure 22-23 with an average weight of 180±20g were purchased. The rats were grouped and housed in environmentally controlled room[ambient temperature 24±2°C and relative humidity of 55±5%] in the animal house and acclimatized for 07 days. The animals were fed standard diet and given tap water ad libitum until treatment. The protocols for experimentation was approved and performed in strict accordance with the Guide for the care and use of laboratory animals [Institute of Laboratory Animal Resources on Life Sciences, US National Research Council,1996] and the Institutional Animal Ethical Committee [IAEC] of Baqai Medical University, Karachi. Pakistan All animals housed in standard conditions were initially fed standard diet and allowed adaptation of one [01] week. Albino rats were divided in four[04] groups; A,B,C & D.

Group A:

Ten [10] male albino rats as Control were kept as control and were fed standard diet and water ad libitum for 10 weeks.

Group B:

Ten [10] male albino rats [F]were fed 60% fructose mixed in standard diet and water ad libitum for 10 weeks.

Group C:

Ten [10] male albino rats [FO]were fed 60% fructose mixed in standard diet and water ad libitum for 10 weeks. They were also injected intraperitonealy oxonic acid 250mg/kg every third day for 10 weeks.

Group D:

Ten [10] male albino rats [O]were injected intraperitonealy oxonic acid 250mg/kg every third day for 10 weeks. They were fed standard diet and water ad libitum for 10 weeks.

The amount of diet was measured before giving and then subtracted from the amount of food left over daily.

Collection of Blood: Approximately 10 mls of blood was drawn from heart using disposable syringe. This solution was diluted with deionized water, filtered and the volume was made up to 10mls with deionized water. Estimation Of Serum Uric Acid was done by Pap method *[23]*.

MEASUREMENT OF TRACE ELEMENTS:

Trace element analysis was carried out on a Hitaci Z-8000 atomic absorption spectrometer equipped with Zeeman background correction and a data processor.flame atomization was used for Copper, Zinc and Magnesium estimation.

Statistical analysis:

Using SPSS 17 WAS carried out.

Results:

Figure 1.1 shows the comparison of mean plasma Uric acid levels of all groups engaged in this study. When plasma uric acid levels of these groups were checked and compared with each other following results were obtained;

1.1.1. COMPARISON OF PLASMA URIC ACID LEVELS OF CONTROL WITH OTHER GROUPS:

Table 1.1.1 shows the comparison of mean plasma uric acid levels of Control with rest of the groups. Mean plasma level of uric acid of Control is found to be 1.97 mg/dl[±0.09] .Group "F"[fructose] showed mean plasma uric acid of 3.15 mg/dl[±0.17] .This reflects that uric acid was raised to 37% in rats which were exposed to diet comprising 60% Fructose than control. On comparing both groups i.e Control with Group "F"[highly significant statistical correlation[P <0.001] was observed.

The mean plasma uric acid levels of Group "O" [oxonic acid] was 3.63 mg/dl[±0.22]which is 45% higher than Control. The probability calculated was highly significant [P<0.001] when both groups were evaluated.

While comparing Group "F+O"[Fructose +Oxonic acid] with Control, highly significant correlation was observed[P<0.001].It was due to high mean plasma serum uric acid level of Group "F+O" which was 4.41mg/dl[±0.14] .The combination of fructose with uricase inhibitor, Oxonic acid raises uric acid to 55% from control and this level is highest of all these groups.

COMPARISON OF PLASMA URIC ACID LEVELS OF GROUP "F" WITH OTHER GROUPS:

Table 1.1.2 exhibits the correlation between mean plasma uric acid of Group "F"[with rest of the groups. The Group "F"[shows highly significant association [P<0.001] with Control as described in section 1.1.1. The mean plasma uric acid level in Group "O" 3.63mg/dl[0.22] which is only 13% more than Group "F"[, therefore the P value calculated were non- significant [P>0.01].

Group "F+O" mean plasma uric acid i.e 4.43 mg/dl were comparatively higher than Group"F", therefore the highly significant correlation was observed [P<0.001] between these two groups. This revealed that Fructose induced hyperuricemia augmented by uricase inhibitore raised urate level to 28% more than Group "F".

1.1.3. COMPARISON OF PLASMA URIC ACID LEVELS OF GROUP O WITH OTHER GROUPS:

Table 1.1.3 shows the statistic connection of Group "O" [Oxonic Acid] with the rest of the groups. The comparison of Group "O" [with control was highly significant [P<0.001] and with Group F was non-significant [P>0.01] as describe before.

The Group "O"showed highly significant correlation [P<0.001] with Group F+O as the mean serum uric acid levels of Group "O"are 18% lower than Group "F+O" reflecting that uric acid levels raised significantly in rats which were treated with oxonic acid along with dietary fructose in comparison to rats which were injected with Oxonic acid alone.

1.1.4. COMPARISON OF PLASMA URIC ACID LEVELS OF GROUP F+O WITH OTHER GROUPS: Table 1.1.4 reflects the statistical comparison of Group F+O with the rest

of the groups. Mean plasma uric acid of this group[F+O] were found to be highest of all groups. The statistic correlation were found to be highly significant [P<0.001] when Group F+O was evaluated both with Control and Group F while significant [P<0.01] when compared to Group O.

Table 1.1.2: COMPARISON OF URIC ACID LEVELS [MG%] OF GROUP "F" WITH OTHER GROUPS

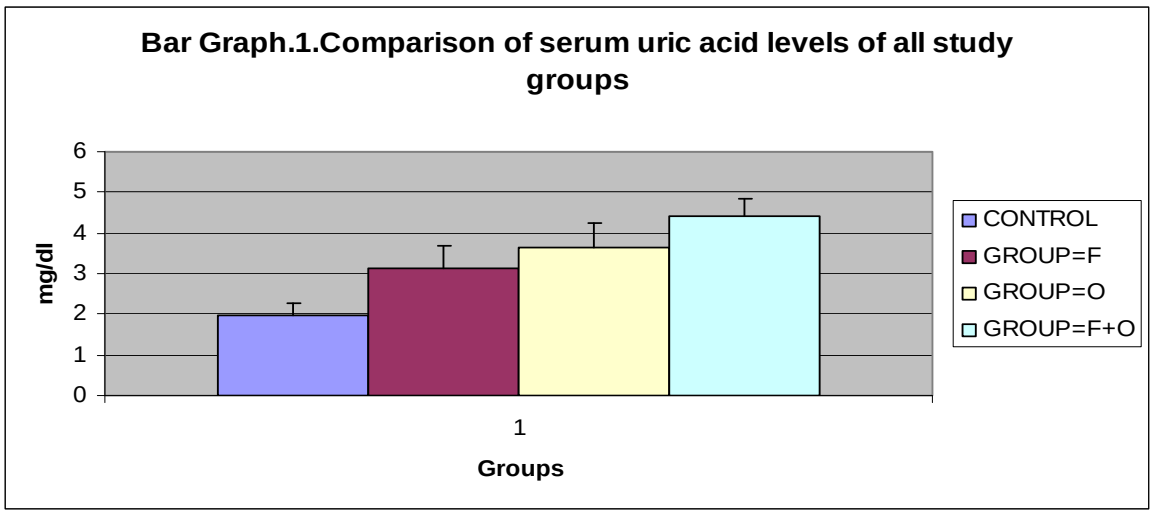
GROUPS	***MEAN VALUES & SEM[Standard Error of Mean]***	***P-Values***
GROUP FRUCTOSE[F]	3.15 SEM±0.17	
GROUP CONTROL[C]	1.97 SEM±0.09	Gr "F" Vs Gr "C" P<0.001
GROUP OXONIC ACID[O]	3.63 SEM±0.22	Gr "F" Vs Gr "O" P>0.01
GROUP FRUCTOSE+OXONIC ACID[F+O]	4.41 SEM±0.14	Gr "F" Vs Gr "F+O" P<0.001

TABLE 3.1.3: COMPARISON OF URIC ACID LEVELS OF GROUP "O" WITH OTHER GROUPS

GROUPS	***MEAN VALUES & SEM[Standard Error of Mean]***	***P-Values***
GROUP OXONIC ACID[O]	3.63 SEM±0.22	
GROUP CONTROL[C]	1.97 SEM±0.09	Gr "O" Vs Gr "C" P<0.001
GROUP FRUCTOSE[F]	3.15 SEM±0.17	Gr "O" Vs Gr "F" P>0.01
GROUP FRUCTOSE+OXONIC ACID[F+O]	4.41 SEM±0.14	Gr "O" Vs Gr "F+O" P<0.01

TABLE 3.1.4: COMPARISON OF URIC ACID LEVELS OF GROUP "F+O" WITH OTHER GROUPS

GROUPS	***MEAN VALUES & SEM[Standard Error of Mean]***	***P-Values***
GROUP FRUCTOSE+OXONIC ACID[F+O]	4.41 SEM±0.14	
GROUP CONTROL[C]	1.97 SEM±0.09	Gr "F+0" Vs Gr "C" P<0.001
GROUP FRUCTOSE[F]	3.15 SEM±0.17	Gr "F+O" Vs Gr "F" P<0.001
GROUP OXONIC ACID[O]	3.63 SEM±0.22	Gr "F+O" Vs Gr "O" P<0.01



SERUM URIC ACID

GROUPS	CONTROL	GROUP=F	GROUP=O	GROUP=F+O
M.V	1.97	3.15	3.63	4.43
S.D	0.3	**0.55**	**0.63**	**0.43**

3.7.COMPARISON OF PLASMA COPPER LEVELS BETWEEN CONTROL AND STUDY GROUPS :

Figure 3.7 shows the comparison of mean plasma Copper levels of all groups engaged in this study. When plasma copper levels of these groups were checked and compared with each other following results were obtained;

3.7.1. COMPARISON OF PLASMA COPPER LEVELS OF CONTROL WITH OTHER GROUPS:

Table 3.7.1 shows the comparison of mean plasma copper levels of Control with rest of the groups. Mean plasma level of copper of Control was found to be 19.59 mg/dl[±1.74] .Group "F"[fructose] showed mean plasma copper of 12.38 mg/dl[±1.39] .This reflects that copper fell down to 58% in rats which were exposed to diet comprising 60% Fructose than control. On comparing both groups i.e Control with Group "F" significant statistical correlation[P <0.01] was observed. The mean plasma copper levels of Group "O" [oxonic acid] was 20.6 mg/dl[±1.40]which is only 5% higher than Control. The probability calculated was non-significant [P>0.01] when both groups were evaluated.

While comparing Group "F+O"[Fructose +Oxonic acid] with Control, significant correlation was observed[P<0.01].It was due to 63% decrease in plasma serum copper levels bringing levels to 11.95mg/dl[±1.45] in Group F+O.

3.7.2. COMPARISON OF PLASMA COPPER LEVELS OF GROUP F WITH OTHER GROUPS:

Table 3.7.2 exhibits the correlation between mean plasma copper of Group "F"[with rest of the groups. The Group "F"shows significant association [P<0.01] with Control as described in section 3.7.1.

The mean plasma copper levels in Group O were 20.6mg/dl[±1.40] which is 40% more than Group "F", therefore the P value calculated were highly significant [P<0.001].

Group F+O mean plasma copper was found to be 11.95mg/dl which is only 03%more than Group"F", therefore the non-significant correlation was observed [P>0.01]between these two groups.

TABLE 3.7.1:COMPARISON OF COPPER LEVELS [mg%] OF GROUP "C" WITH OTHER GROUPS

GROUPS	***MEAN VALUES & SEM[Standard Error of Mean]***	***P-Values***
GROUP CONTROL[C]	19.59 SEM±1.74	
GROUP FRUCTOSE[F]	12.38 SEM±1.39	Gr "C" Vs Gr "F" P<0.01
GROUP OXONIC ACID[O]	20.6 SEM±1.40	Gr "C" Vs Gr "O" P>0.01
GROUP FRUCTOSE+OXONIC ACID[F+O]	11.95 SEM±1.45	Gr "F" Vs Gr "F+O" P<0.01

TABLE 3.7.2:COMPARISON OF COPPER LEVELS [mg%] OF GROUP "F" WITH OTHER GROUPS

GROUPS	***MEAN VALUES & SEM[Standard Error of Mean]***	***P-Values***
GROUP FRUCTOSE[F]	12.38 SEM±1.39	
GROUP CONTROL[C]	19.59 SEM±1.74	Gr "F" Vs Gr "C" P<0.01
GROUP OXONIC ACID[O]	20.6 SEM±1.40	Gr "F" Vs Gr "O" P<0.001
GROUP FRUCTOSE+OXONIC ACID[F+O]	11.95 SEM±1.45	Gr "F" Vs Gr "F+O" P>0.01

3.7.3. COMPARISON OF PLASMA COPPER LEVELS OF GROUP "O" WITH OTHER GROUPS:

Table 3.7.3 shows the statistic connection of Group "O"[Oxonic Acid] with the rest of the groups. The comparison of Group "O" with control was non- significant [P>0.01] and with Group F was highly significant[P<0.001] as describe before.

The Group "O"showed highly significant correlation [P<0.001] with Group F+O as the mean serum copper levels of Group "F+O"are 72% lower than Group "O" reflecting that copper levels decreased significantly in rats which were treated with oxonic acid along with dietary fructose in comparison to rats which were injected with Oxonic acid alone.

3.7.4. COMPARISON OF PLASMA COPPER LEVELS OF GROUP F+O WITH OTHER GROUPS:

Table 3.7.4 reflects the statistical comparison of Group F+O with the rest of the groups. Mean plasma copper of this group [F+O] were found to be highest of all groups. The statistic correlation were found to be significant [P<0.01] when Group F+O was evaluated with Control, nonsignificant [P>0.01] with Group F while highly-significant when compared to Group O. [P<0.001]

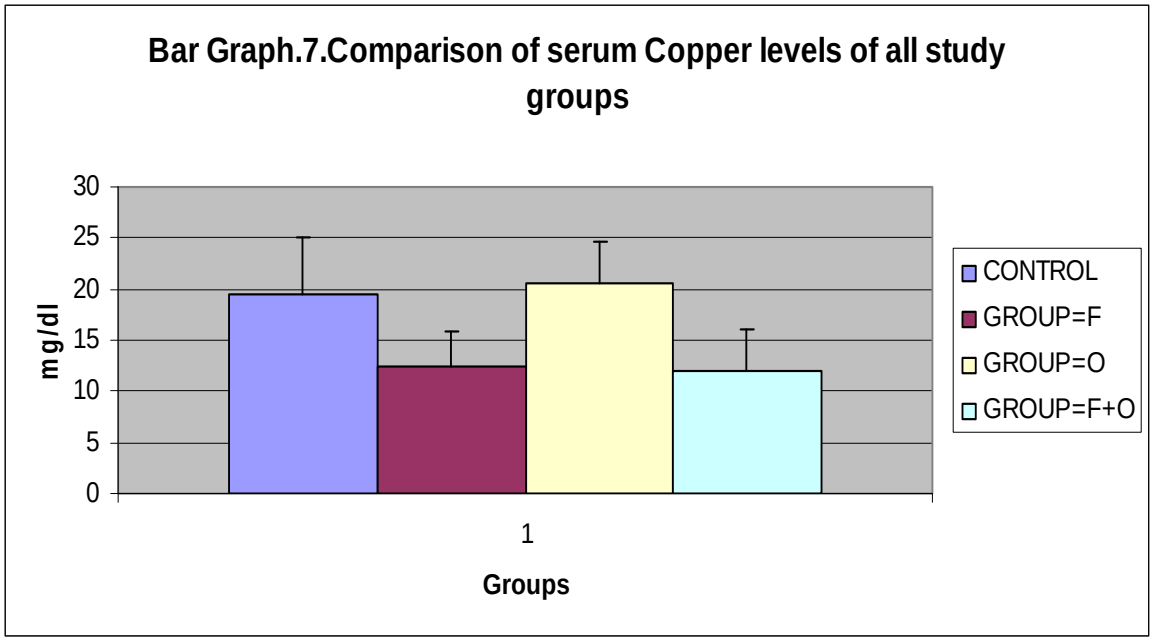
TABLE 3.7.3:COMPARISON OF COPPER LEVELS OF GROUP "O" WITH OTHER GROUPS

GROUPS	***MEAN VALUES & SEM[Standard Error of Mean]***	***P-Values***
GROUP OXONIC ACID[O]	20.6 SEM±1.40	
GROUP CONTROL[C]	19.59 SEM±1.74	Gr "O" Vs Gr "C" P>0.01
GROUP FRUCTOSE[F]	12.38 SEM±1.39	Gr "O" Vs Gr "F" P<0.001
GROUP FRUCTOSE+OXONIC ACID[F+O]	11.95 SEM±1.45	Gr "O" Vs Gr "F+O" P<0.001

TABLE 3.7.4:COMPARISON OF COPPER LEVELS OF GROUP "F+O" WITH OTHER GROUPS

GROUPS	***MEAN VALUES & SEM[Standard Error of Mean]***	***P-Values***
GROUP FRUCTOSE+OXONIC ACID[F+O]	11.95 SEM±1.45	
GROUP CONTROL[C]	19.59 SEM±1.74	Gr "F+0" Vs Gr "C" P<0.01

GROUP FRUCTOSE[F]	12.38 SEM±1.39	Gr "F+O" Vs Gr "F" P>0.01
GROUP OXONIC ACID[O]	20.6 SEM±1.40	Gr "F+O" Vs Gr "O" P<0.001



COPPER GROUPS	CONTROL	GROUP=F	GROUP=O	GROUP=F+O
M.V	19.59	12.38	20.6	11.95
S.D	5.5	**4.38**	**3.97**	**4.35**

Discussion:

One of the important features of this study is the method by which hyperuricemia have been induced in animal model. First group [G=Fructose] was given fructose , second group[G=Oxonic acid] was treated with "oxonic acid" and third group was offered both fructose and oxonic acid[G=Fructose+Oxonic acid]. The principle hyperuricemic factor in this study is fructose as it is extensively used in beverages and food .Its a rather controversial factor as number of studies both animals and human, are in the favour that fructose can induce hyperuricemia [24,25]but many studies have opposed this hypothesis[26,27] and even mixed response has been shown[28] .Present investigation has tried to verify this theory. Very few studies have used this combined model of fructose plus oxonic acid.In order to make conditions similar to human, uricase inhibitor oxonic acid was

incorporated to abolish the effect of this enzyme in rats . Also these different regimens were used to establish the extent of hyperuricemia caused by fructose.

In present study uric acid was found to be increased in all three groups,G=Fructose, G=Oxonic acid and G=Fructose+Oxonic acid when the levels were compared with the Control[C],but considerable variations in levels were observed in these groups as shown in Tab3.1.1 and Fig 3.1 in the section of results.The mean serum level of uric acid in G=Fructose+Oxonic was highest of all three study groups being 55% more than control,28% more than Fructose treated only and 18% higher than Oxonic acid treated hyperuricemic rats . These findings are in consistent with several other studies which have shown that fructose can increase uric acid levels [29,30,31] . The proposed mechanism by which fructose might have increased uric acid production is that fructose is rapidly phosphorylated by fructokinase to fructose-1-phosphate on entering the hepatocytes by passing the regulatory step of glycolysis. [32].ADP is generate due to donation of Phosphate by ATP during this reaction. This ADP is then furthur metabolized to uric acid[33].Fructose may also increase latcate production which is a competitive inhibitor for urate excretion [34,35]. In addition to this Fructose might also have role in hyperinsulinemia which may also have contributed to impairment in urate excretion by promoting renal reabsorption [36]. Finaly resultant hyperuricemia due to fructose itself impaired its own excretion as demonstrated in several studies by causing endothelial dysfunction and renal vasoconstriction[37]. The different magnitude of hyperuricemia observed might be due to the reason that in rats hepatic enzyme uricase [urate oxidase] is present which is responsible for converting uric acid to allontoin.Due to this reason the normal levels are kept in range of 0.5 to1.5mg/dl[38]. This was well demonstrated in present study in the G= Oxonic acid and G= Fructose+ Oxonic acid in which uric acid considerably increased when uricase inhibitor “Oxonic acid” was added.

During minerals estimation in the present study, the levels of Copper were found to be decreased in all groups when compared with the control and lowest levels of 11 mg/dl observed in the G=F+O followed by G=Fructose which 12 mg/dl as shown in Taband Graph 3.7.It is interested to be noted here in both groups the common factor is fructose. These findings are in agreement with Boyd L O’Dell in 1993[39] who pointed out that fructose decreases copper bioavailability when it forms 60% of the diet.This is augmented by Koh et al[40] who demonstrated in their experimental study that in copper level was found to be higher in those group of rats in which copper was injected in comparison to rats in which copper was given orally .These reflects that fructose might be interfering with the copper

absorption at intestinal level as both groups were supplemented with dietary fructose. The possible explaination as proposed by M.Fields et al[41] research in which it was proposed that high concentration of fructose in diet might reduce copper resulting its precipitation as cuprous oxide. This form is highly insoluble so its transport across the intestinal cells can be hampered.

Conclusion

Copper level between the groups are significantly correlated

Recommendation

Antioxidants such as copper and zinc and some vitamins like C ,E and A are important to include in diet. It is recommended to include copper containing vegetables and fruit daily to reduce their deficiency.

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