HEPATOTOXIC EFFECT OF COPPER SULPHATE AND COBALT CHLORIDE AS FEED ADDITIVES IN ALBINO RATS.

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A B S T R A C T

This study is designed to investigate the hepatotoxic effect of copper sulphate and cobalt chloride, which are used as feed additives. One hundred rats classified to five groups. First group used as control; second and third groups were given 1/10 and 1/5 LD_{50} of $CuSo_4$ respectively, fourth and fifth groups were given 1/10 and 1/5 LD_{50} of $CoCl_2$ respectively (orally via stomach tube). Body weight was measured weekly. Serum samples used for detection of liver functions. Liver tissue was homogenized for detection of oxidative stress (Reduced glutathione (GSH), Glutathione-s-transferase (GST) and maloneldehyde MDA)). Histopathological examination was performed in liver tissue. Results showed increase level of serum Alanine aminotransferase (ALT), Aspartat aminotransferase (AST), alkaline phosphatase (AP), gamma glutamyl transferase (GGT) and MDA. Decrease level of GSH and GST was detected. It can be concluded that ingestion of copper and cobalt in high doses has hepatotoxic effect on albino rats.

Key words: cobalt hepatotoxicity- copper hepatotoxicity- liver enzymes- oxidative stress- heavy metals.

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1. INTRODUCTION

Copper and cobalt are essential elements in humans and animals and have both beneficial and harmful effect on human health. They are necessary for life at very low concentrations, but when their concentrations increase, they become toxic and interfere with cell metabolism (Kjoss et al., 2005). Copper is beneficial for human as a trace dietary mineral because it is a key constituent of the respiratory enzyme complex cytochrome c oxidase (Johnson and Larry 2008). It is incorporated into a number of metalloenzymes involved in hemoglobin formation, carbohydrate metabolism, catecholamine biosynthesis, the cross- linking of collagen, elastin, and hair keratin, and the antioxidant defense mechanism. (Linder, 2002). It helps in the production of blood hemoglobin (Heath, 1995). Copper has hepatic effects ranging from increases in alanine aminotransferase activity to hepatocellular necrosis and renal effects (protein droplets in proximal tubules) was detected in rats exposed to high doses of copper sulfate in the diet. Simillary increase in activity of plasma ALT and AST in copper loaded mice was recorded by (Galhardi et al., 2004). Cobalt is beneficial because it is part of vitamine B12 (cobalamine) which is essential to maintain health (Institute of Medicine. 2000). Cobalt is widely distributed naturally, always in association with nickel; moreover, cobalt has been used for the coloring of pottery and glass and for manufacture of alloys (Kiec-Swierczynska, 1990). Occupational exposure to cobalt occur in dental technicians (Sherson et al., 1990). Exposure of rats and guinea pigs to cobalt results in liver lipid peroxidation and reduced levels of glutathione, superoxide dismutase, catalase, heme oxygenase, and glutathione peroxidase (Christova et al.,

2002). In light of these facts, the main goal of the present study was to evaluate the effect of copper sulphate and cobalt chloride toxicity on body weight, liver function and oxidative stress on white albino rats, after chronic exposure for six month.

2. MATERIAL AND METHODS

2.1. Experimental animals:

One hundred apparently healthy male albino rats (western strain) weighted 175-182 g were obtained from Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt. The animals housed in stainless steal wire bottom cages and kept under constant environmental conditions and fed on fresh standard pellet and given tap water through out the study. All animals were acclimatized for 1 week before the beginning of the experiment.

2.2.Tested substance:

Copper in the form of copper sulphate pentahydrate ($Cu\ SO_4.5H_2O$) crystals and Cobalt in the form of cobalt chloride hexahydrate ($CoCl_2 \cdot 6H_2O$) crystals are the tested substances.

2.3. Experimental design:

In this study 100 male albino rats (western strain) were divided in to five groups each one contain 20 rats. First group was kept as control; while second and third groups were given 1/10 and 1/50 LD_{50} of $CuSo_4$ (Abu- Zinadah and Hussein 2010) respectively. Fourth and fifth groups were given 1/10 and 1/50 LD_{50} of $CoCl_2$ (National Institute For Occupational Safety and Health (NIOSH) 2003).
respectively (orally by stomach tube) 3 days per week for 6 months.

2.4.Body weight:

Mean body weight was calculated according to Broody (1945) by weighting of animals every weeks and given mean body weight every month.
Weight gain (g) = Final rats weight (g)- Initial rats weight (g)

2.5. Biochemical analysis:

At the end of 2^{nd}, 4^{th} and 6^{th} month , five rats were sacrificed from each group and samples were collected. Serum samples collected for biochemical analysis. Alkaline phosphates (AP) was performed according to (Belfield and Goldberg 1971), while ALT and AST according to (Schumann and Klauke 2003) and serum gamma glutamyl transferase (GGT) level according to (Teitz 1987).

2.6. Detection of liver oxidative cascade:

After slaughter, the liver was dissected and washed by normal physiological saline. One gram of each liver was homogenized in 5 ml of cold solution of Potassium dihydrogen phosphate (KH_2PO_4) using sonicator homogenizer. A liquots of liver homogenates centrifuged by cool centrifuge 4000 rpm for 20 min then stored at -20 ^oC prior to biochemical analysis. Determination of GSH in liver tissue homogenate was performed according to (Beutler et al 1963).Determination of GST in liver tissue homogenate was performed according to Habig *et al.,* (1974). Determination of MDA in liver tissue homogenate was performed according to Ohkawa et al., (1979).

2.7. Histopathological examination

Autopsy samples were taken from liver and kidney in different group of rat. Samples fixed in formalin solution 20%. Washing was done under tape water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene embedded in paraffin at 56 degree in hot air oven for twenty four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by slidge microtome the obtained tissue sections were collected on glass slides, deparaffined and stained by hematoxyline and eosin stains for histopathological examinations using light microscope **(**Banchroft et al., 1996)

2.8. Statistical analysis:

The data were analyzed for obtaining mean, standard deviation (SD) and statistical comparisons between means of different

groups. The statistical analyses were done by one way ANOVA and DUNCAN test using SPSS program version 16. (Kirk 1982). *P* value < 0.05 was assumed for statistical significance.

3. RESULTS

3.1. Effect of administrated copper sulfate and cobalt chloride on the body weight (gm) and weight gain (percentage) of white albino rat for 6 months:

There was non significant decrease of mean body weight at first eight weeks, while from 9th week to 24th week there was significant decrease of mean body weight of rats that received (1/10 LD_{50}) copper sulfate and high significant decrease at group received copper sulfate (1/5LD_{50}) compared with control group. While in cobalt chloride there were non-significant decrease of mean body weight at first eight weeks, while after 9th week to 24th week there were significant decrease of mean body weight in rats received cobalt chloride (1/10 LD_{50}) and high significant decrease at group received cobalt chloride (1/5 LD_{50}) compared with control group. The weight gain of different treated groups was lower than the control group. Table (1).

3.2. Effect of copper sulphate and cobalt chloride on serum Alanine aminotransferase (ALT), Aspartat aminotransferase (AST), alkaline phosphatase (AP) and Gamma Glutamyle transferase (GGT):

Aspartat aminotransferase (AST) level at 2nd 1st month was significantly increase in treated groups in comparison to control group, while at 4th and 6th month was more significant increase in treated groups in comparison to control group. ALT level at 2nd month was non-significant increase in treated groups in comparison to control group, while at 4th and 6th month was more significant increase in treated groups in comparison to control group. These results were more clear at group receive large dose of copper sulphate and cobalt chloride compared with control group. [Table (2)].

3.3. Effect of administration of copper and cobalt on oxidative cascade in liver tissue [glutathione reduced (GSH), malondialdehyde (MDA) and glutathione-s-transferase (GST)] of white albino rats.

Concerning to reduced glutathione (GSH) and glutathione-s-transferase (GST) detected in liver tissue of rats in groups treated with copper and cobalt (low and high dose) there was significant decrease compared to control group at the end of the experiment. Regarding to malondialdehyde (MDA) detected in liver tissue of rats in groups treated with copper and cobalt (low and high dose) there was significant increase compared to control group at at 2nd**,** 4th and 6th month of experiment. [Table (3)].

4. DISCUSSION:

Copper is one of the key trace minerals required for an effective immune response. The biological functions of copper include electron- transfer catalysis by means of its two accessible oxidation states [Georgopoulos *et al* ., 2001]. Cobalt is an essential metal for human and is part of the enzyme cyanocobalamin (vit B12) but high exposure levels has serious effect.

Body weight: significant decrease on body weight were recorded, this reduction was clear in groups treated by high dose (1/5 LD_{50}) of copper sulphate. These similar to result obtained by Paumen et al., (2008). These result attributed to improper assimilation or metabolism of feed due to enteritis, which shown as diarrhea WHO (1998) and Oldenquist and Salem (1999). Or due to tissue burden of copper, which in turn could cause reduction in the consumption rate, poor food conversion efficiency and increased oxygen consumption rate Lett et al., (1976**)**. Or may attributed to increased metabolic costs and reduced food consumption James et al ., (2004). In cobalt groups may be attributed to improper assimilation or metabolism of

Table (1): Effect of administrated copper sulfate and cobalt chloride on the body weight (gm) and weight gain (percentage) of white albino rats for 6 months (mean± SD): -

Group Date	GroupI control G I	Copper sulphate groups		Cobalt chloride groups	
		G II 1/10 LD50	GIII 1/5 LD50	GIV 1/10 LD50	GV 1/5 LD50
Initial body weight	175.7 ± 0.33[b]	181.25 ±0.32 [a]	181.3 ± 0.67 [a]	178.4 ± 142.5 [a]	182.6 ± 3.5 [a]
Final body weight	341.8 ± 1.14 [a]	326.0 ± 1.26 [b]	264.3 ± 2.65 [c]	330.1 ± 1.89 [b]	266.8 ± 4.4 [b]
Weight gain	166.1 ± 2.4 [a]	144.75 ± 3.6 [c]	83.0 ± 4.3 [d]	151.7 ± 3.4 [b]	84.2 ± 3.6 [d]

Mean with different letters at the same row differ significant ($P < 0.05$).

feed due to enteritis Dieter et al., (1988) which shown as diarrhea and confirmed by hepatic lesions, livers of the treated rats showed marked congestion and dilatation of blood sinusoids (Fig. 1) with activation of Von Kupffer's cells. Multifocally, fibrous tissue proliferation was also observed in portal areas around blood vessel wall and bile ducts. Moreover, hyperplasia of the biliary epithelium with formation of newly formed bile ductules was markedly seen.Or may be due to decrease in food consumption and absorption or may be due to increase degredation of lipids and proteins as a result of the direct effect of cobalt Galbraith and Kappas (1989).

Concerning to the effect of copper sulphate on the ALT, AST, AP and GGT levels, it revealed significant increase on ALT, AST and GGT level at 2nd month and highly significant increase at 4th and 6th month of experiment compared to control group, our result similar to result obtained by (Abou El- Naga et al., 2005, Galhardi et al., 2004). The significant increase on ALT and AST activities throughout the experimental period is directly related to that chronic copper toxicity primarily affects the liver because this is the first site of copper deposition after it enters the blood, copper toxicity is typically manifested by the development of liver cirrhosis with episodes of hemolysis and progressive liver damage and necrosis which leading to liberation of these enzymes or due to extensive break down of body tissue (Sokol et al*.,* 1993, Linder and Hazegh-Azam 1996). Or may also attributed to liver mitochondrial dysfunction due to oxidized state (Ashish et al., 2012). This result disagreed with (Heath 1987) which reported low level of ALT and AST due to its sharing in transforming protein to glycogen. Assessment of serum (AP) level of treated rats by copper sulphate Table (2) revealed highly significant increase in treated groups compared with control group, our result supported by (Figueirdo-Fernandes et al., 2007). The significant elevation of (AP) could be attributes to the effect of copper on the liver, kidney and heart which consequently liberating their intracellular enzyme in to blood stream (Geyvan 1991). The significant elevated ALT, AST, AP and GGT activities are in agreement with the results obtained by (Novelli, and Borbosa 1998).

Table (2) level of serum (ALT), (AST), (AP) and (GGT) per (U/ L) on rats received 1/10&1/5 LD_{50} of $CuSo_4$ and $CoCl_2$ (Mean ± SD):

Items		2nd month				4th month				6th month			
Groups		AST U/L	ALT U/L	AP U/L	GGT U/L	AST U/L	ALT U/L	AP U/L	GGT U/L	AST U/L	ALT U/L	AP U/L	GGT U/L
control	Group I	10.78 ± 0.52 c	5.68 ± 2.0 c	32.1 ±2.9 d	7..13 ±0.09 c	10.88 ± 0.82 c	5.75 ± 3.11 c	31.8 ±5.9 c	6.98 ±1.2 c	10.91 ±0.64 c	6.01 ±2.19 c	31.78 ±0.72 c	6.91 ±.45 c
	Group II 1\10 LD50	27.33 ± 2.89 b	7.93 ± 2.4 b	53.2 ±1.36 c	9.75 ±0.83 b	34.28 ±4.92 b	21.56 ± 1.67 b	58.4 ±2.08 b	18.24 ±2.77 b	50.24 ±0.81 b	32.37 ±0.87 b	68.42 ±2.46 b	34.89 ±3.87 b
	Group III 1\5 LD50	36.81 ±0.87 a	18.09 ±3.01 a	89.87 ±0.4 a	13.51 ±2.17 a	47..91 ±2.27 a	38..14 ±2.82 a	109..3 ±2.81 a	22..21 ±0.83 a	59..19 ±0.75 a	45..92 ±0.89 a	115..1 ±2.19 a	49.41 ±2.28 a
	Group IV 1\10 LD50	25.11 ±2.19 b	6.81 ±3.08 b	41..8 ±2.4 c	8.92 ±1.29 b	32.10 ±2.27 b	19.16 ±1.59 b	55.21 ± 1.3 b	16.98 ±0.13 b	47.38 ±2.67 b	30.87 ±3.01 b	64.71 ±1.83 b	32.76 ±1.61 b
	Group V1\5 LD50	34..12 ±3.9 a	15.14 ±4.7 a	68.83 ±5.9 b	12.79 ±1.02 a	45.20 ±1.36 a	35.64 ±2.2 a	98.47 ±0.69 a	21..53 ±1.29 a	55.7 ±2.84 a	42.85 ±2.18 a	106.8 ±2.19 a	43..34 ±1.81 a

Mean with different letters at the same column differ significant ($P < 0.05$).

Table (3): Effect of administrated copper sulphate and cobalt chloride on oxidative cascade in liver tissue of white albino rats for 6 months (mean± SD):

Items / Groups	2nd month GSH (µmol/g wet tissue)	2nd month GST (nmol/min/mg protein)	2nd month MDA (nmol/g wet tissue)	4th month GSH (µmol/g wet tissue)	4th month GST (nmol/min/mg protein)	4th month MDA (nmol/g wet tissue)	6th month GSH (µmol/g wet tissue)	6th month GST (nmol/min/mg protein)	6th month MDA (nmol/g wet tissue)
Group I control	0.220 ±0.31 [a]	0.370 ±0.21 [a]	10.73 ±0.73 [c]	0.243 ±0.04 [a]	0.390 ±0.82 [a]	10.83 ±0.43 [c]	0.261 ±0.01 [a]	0.410 ±0.4 [a]	10.96 ±0.3 [c]
Group II 1\10 LD50	0.200 ±0.15 [a]	0.320 ±0.27 [a]	14.62 ±1.34 [b]	0.197 ±0.12 [b]	0.270 ±0.58 [b]	17.31 ±1.52 [b]	0.190 ±0.02 [b]	0.200 ±0.7 [b]	21.30 ±0.1 [b]
Group III 1\5 LD50	0.183 ±0.71 [b]	0.220 ±0.63 [b]	16.85 ±0.93 [a]	0.152 ±0.52 [c]	0.130 ±0.36 [c]	19.74 ±0.41 [a]	0.110 ±0.03 [c]	0.080 ±0.48 [c]	24.70 ±0.03 [a]
Group IV 1\10 LD50	0.210 ±0.42 [a]	0.340 ±0.01 [a]	14.12 ±0.81 [b]	0.199 ±0.35 [b]	0.280 ±0.26 [b]	16.82 ±0.53 [b]	0.200 ±0.4 [b]	0.250 ±0.45 [b]	20.80 ±0.02 [b]
Group V 1\5 LD50	0.191 ±0.83 [b]	0.240 ±0.36 [b]	16.31 ±0.34 [a]	0.169 ±0.24 [c]	0.150 ±0.86 [c]	19.23 ±0.76 [a]	0.140 ±0.6 [c]	0.100 ±0.09 [c]	24.10 ±0.2 [a]

GSH: glutathione reduced. GST: glutathione-s-transferase. MDA: malondialdehyde .

Mean with different letters at the same column differ significant ($p<0.05$).

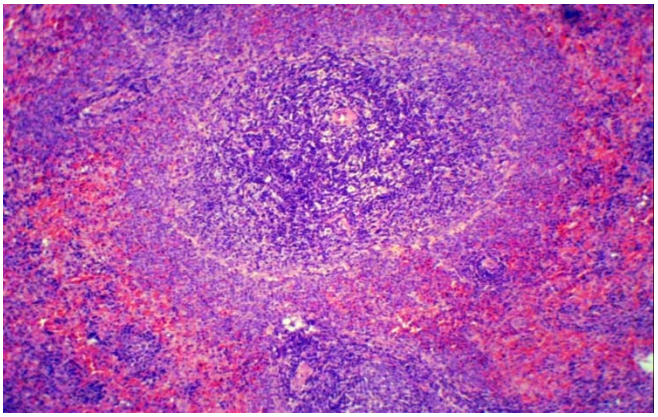

Fig (3): Spleen of rat administered 1/10LD_{50} copper for 4 months showing hyperplasia of lymphoid elements of white pulp with focal areas of hemorrhages at the margin of the white pulp and in the red pulp. H&E stain x 200.

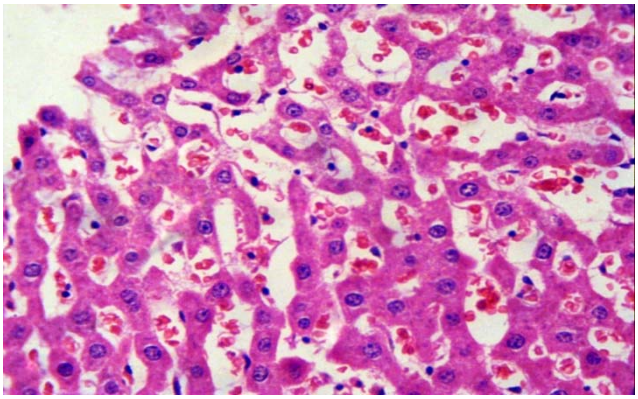

Fig (1): Liver of rat administered 1/5 LD_{50} cobalt chloride for 6 months showing multinucleated hepatocyte with increased cytoplasmic basophilia. H&E stain x 600.

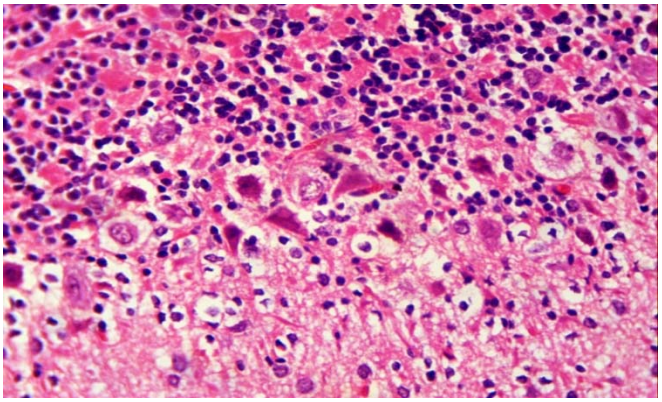

Fig (2): Liver of rat administered 1/5 LD_{50} copper for 6 months showing hypereosinophilic necrotic tubular epithelial cells with shrunken, pyknotic nuclei, loss of cellular detail, and karyolysis. H&E stain x 400.

Regarding to the effect of copper sulphate on the oxidative cascade in liver tissue showed in significant decrease in antioxidant including (GSH) and (GST) in treated groups compared to control group. While (MDA) showed significant increase in treated groups compared to control group. These results were agreed (Engle et al., 2000). These results may be attributed to copper was found to be quite effective in forming toxic oxygen types and starting the process of lipid peroxidation (Weckx. and Clijsters 1996). Or may be due to copper increase lipoxygenase activity, catalyzing lipid peroxidation, especially of unsaturated fatty acids, as a result of these reactions, various radicals were formed and these lead to increasing concentration of MDA which is a product of peroxidation , which are an indicator of oxidative stress after heavy metals dosing and the increase level correlates with the increase of metal concentrations (Wu et al., 2003).

Concerning to the effect of cobalt chloride on oxidative cascade. There were significant decrease in GSH, GST and significant increase in MDA. These results agreed with (Christova et al*.*, 2002). These results may be attributed to that cobalt has ability to form complexes with critical SH groups (including lipoic acid), which results in the inhibition of key process such as oxidative phosphorylation and hence cell energy production. Also cobalt make induction of oxidative stress in vitro and in vivo. Cobalt catalyses the generation of hydroxyl radicals (OH) from hydrogen peroxide (Lloyd et al., 1997) causing reduced glutathione (GSH) level. In addition an increase in lipid peroxidation products such as malondialdehyde and other thiobarbituric reaction substances (Gonzales et al*.*, 2005). Or may be due to reduced antioxidant production was due to the increased oxygen metabolites and the elevated free radicals, which cause a decrease in the activity of the antioxidant defense system (Kusal et al., 2001).

The conclusion we draw from the results is that, copper sulphate and cobalt chloride most likely have an oxidative stress. For this reason, it is necessary to be careful when using it in feed as feed additives.

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التأثير السمي الكبدي لكبريتات النحاس وكلوريد الكوبلت كإضافات اعلاف على الفئران البيضاء

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قسم الطب الشرعي والسموم-كلية الطب البيطري-جامعة بنها

الملخص العربي

في هذه الدراسة تم تقييم التأثير السمي لكبريتات النحاس و كلوريد الكوبلت على التغيرات في انزيمات الكبد (انزيمات الالانين امينو ترانس فيريز و الاسبرتيت امينو ترانس فيريز) ، الانزيمات المضادة للأكسدة ، الجلوتاثيون المختزل و الجلوتاثيون اس ترانس فيريز و المالونداياالديهيد في انسجة ذكور الفئران البيضاء . هذا وقد استخدم لإجراء هذه الدراسة عدد 100 من ذكور الفئران البيضاء اعمارها حوالي اربعة اسابيع واوزانها من 175-182 جرام وقد قسمت الى خمس مجموعات متساوية اشتملت كل مجموعة على عدد عشرون فأر وتم توزيعها كالاتي: المجموعة الاولى: (المجموعة الضابطة): اشتملت على عشرون فأر واستخدمت كمجموعة ضابطة للمجموعات الاخرى. - المجموعة الثانية: تكونت من عشرون فأر تم اعطاؤها 10/1 من الجرعة نصف المميتة لكبريتات النحاس لمدة ستة أشهر. المجموعة الثالثة: تكونت من عشرون فأر تم اعطاؤها 5/1 من الجرعة نصف المميتة لكبريتات النحاس لمدة ستة أشهر. المجموعة الرابعة: تكونت من عشرون فأر تم اعطاؤها 10/1 من الجرعة نصف المميتة لكلوريد الكوبلت لمدة ستة أشهر. المجموعة الخامسة تكونت من عشرون فأر تم اعطاؤها 5/1 من الجرعة نصف المميتة لكلوريد الكوبلت لمدة ستة أشهر عن طريق الفم. وقد تم تجميع عينات الدم والانسجة في الشهر الثاني والرابع والسادس من بداية التجربة. وقد اسفرت نتائج التحليل البيوكيميائى عن وجود زيادة في انزيمات الكبد (انزيمات الالانين امينو ترانس فيريز والاسبرتيت امينو ترانس فيريز). كما تم قياس مضادات الاكسدة في كبد الفئران والتي تضمنت قياس الجلوتاثيون المختزل والجلوتاثيون اس ترانس فيريز اوضحت النتائج انخفاض معنوي في المجموعات التي عولجت بكبريتات النحاس وكلوريد الكوبلت بينما قياس المالونداياالديهيد اوضح ارتفاع معنوي في تلك المجموعات مقارنة بالمجموعة الضابطة.

(مجلة بنها للعلوم الطبية البيطرية: عدد 27(1):146- 156, سبتمبر 2014)