Physiological Study on the Antioxidant Activities of Some Dietary Supplements in Male Albino Rats

BY

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ABSTRACT

The protective and treatment effects of vitamin E, melatonin, curcumin and coenzyme Q_{10} against oxidative stress. We studied in the present experiment, 120 male albino rats were divided into 6 equal groups, 1 st group was negative control group, The rats of the other five groups were subjected to stress daily induced by intraprotenial puncture once daily for nine successive weeks. Stress was stopped during the 10th and 11th week for recovery. The rats of the 2nd group were considered as a stressed controls. Starting from the 4th week and to the rest of the experimental period. The rats of the 3rd, 4th, 5th and 6th groups received Vit. E (200 mg/kg b.w.), curcumin (80 mg/kg b.w.), melatonin (20 mg/kg b.w.) or CoQ_{10} (150 mg/kg b.w.), respectively every other day using stomach tube. Stress caused significant increase in liver and kidney superoxide dismutase activity and malondialdehyde (MDA) levels, Vitamin E and melatonin produced a significant decrease in SOD and MDA levels in the liver and kidney. Curcumin and Coenzyme Q_{10} produced significant decrease in SOD and MDA levels in the liver and kidney. It could be concluded that, stress caused induction of free radicals and induced marked and significant effects on antioxidant status. The different feed supplements used succeeded to protect the animal against the harmful effect of oxidative stress and lead to the improvement of the endogenous antioxidant status as well as liver and kidney functions.

Introduction

Free radical production occurs consciously in all cells as a part of normal cellular function. Oxidation is a normal process that takes place in the body. It causes damage to our cells. It's believed that this cumulative damage is what causes aging, atherosclerosis, cancer, immune system decline, brain dysfunction and eventually death. Antioxidants are chemicals that generously offer up their own electrons to the free radicals, thus preventing cellular damage. There are many chemicals that perform as antioxidants, such as vitamins, nuts, fruits, vegetables and meats.Antioxidants prevent free radical induced tissue damage by: a) preventing the formation of radicals, b) scavenging them, or c) promoting their decomposition (oxidant-antioxidant balance). Antioxidants are either endogenous, present normally in biological system or exogenous which can be administrated exogenously. The endogenous antioxidants may be hormones as melatonin or enzymes such as superoxide dismutase,

glutathione peroxidase and catalase, while the exogenous antioxidants may be divided into: 1) vitamins such as ascorbic acid (vitamin C), vitamin E and vitamin A. 2) minerals such as selenium and zinc and 3) plant extracts such as curcumin, grape seed extract and green tea.Nutrition researchers suggest that consumption of antioxidant-rich food reduces damage to cells from free radicals. This may slow down, prevent or even reverse certain diseases that result from cellular damage, and perhaps even slow down the natural aging process.

Material and Methods

Animal The present work was carried out on 120 albino male rats of Wistar strain. Their body weights ranged from 100 to 120 g and their ages ranged from 55-60 days. All animals were subjected to acclimatization for two weeks

Chemicals:-

-Antioxidants used: i) Vitamin E (di-alpha tocopherol acetate). It was obtained in an oily form (96%) from Pharmasuid Company. It was further diluted to the required dose using corn oil. ii) Melatonin (N-acetyl-5-methoxy tryptamine). It was a gift from Memphis Company for Pharmacy and Chemical Industry. iii)Curcumin, from Algomhoria Company for Chemical Industry . iiii) CoenzymeQ10 (ubiqnone) from Global Nappi for Chemical Industry.

Experimental Design:

-This study was carried out for 11 successive weeks. The rats were classified into six groups of 20 rats each as follow:-

Group –I (control negative group): The rats were kept unstressed as a control negative group and received normal rat ration without any feed additives for 3 successive weeks. At the 4th week of the experiment, each rat received corn oil (250 ul/kg b.w) and ethanol 8% (500ul/kg b.w) (the vehicles of the drugs used) every other day using stomach tube until the end of the 11 weeks experimental period. Rats of the other 5 groups were subjected to sham stress during the first 9 weeks of the experiment by false intraperitonial injection .Sham stress was stopped in the 10th and 11th week for recovery. Group –II (control positive, sham stressed group): Starting from the the 4th week of experiment, the sham stressed rats received corn oil (250ul/kg b.w.) and ethanol 8% (500ul/kg b.w.) using stomach tube every other day up to the end of the experimental period and sereved as control +ve group. Group –III (sham stress and vitamin E): Starting from the 4th week of the experiment and up to the end of the experimental period sham stressed rats received vitamin E (in corn oil 250ul/kg b.w.) every other day at a dose of 200 mg / kg b.w. (Halim et al., 1997) together with ethanol 8% (500ul/kg b.w.) using stomach tube. Group –IV (sham stresse and curcumin): At the 4th week and up to the end of the experimental period, the rats received curcumin (in corn oil 250ul/kg b.w.) every other day at a dose of 80 mg / kg b.w. (Kalpana and

Menon, 2004) together with ethanol 8% (500ul/kg b.w.) using stomach tube. Group – V (sham stressed and melatonin group): At the 4th week and up to the end of the experiment, the sham stressed rats received melatonin dissolved in ethanol 95% and diluted by normal saline untile 8% (500ul/kg b.w.) at a dose of 20mg/kg b.w. (Othman et al., 2004) every other day together with corn oil (250ul/kg b.w.) using stomach tube. Group VI (sham stressed and coenzyme Q_{10} group): Starting from the 4th week the rats received coenzyme Q_{10} (in corn oil 250ul/kg b.w.) at a dose of 150mg/kg b.w. (kwong et al., 2002) every other day together with ethanol 8 % (500ul/kg b.w.) using stomach tube.

Collection of Samples:

Every 2 weeks 5 rats were sacrified from each group. The liver and kidney of each rat were dissected, homogenized with phosphate buffer (pH 7.4) and stored at - 20 ^{0}C until used for the determination of antioxidant enzymes activities and malondialdehyde (MDA) levels.

Liver and kidney malondialdehyde (MDA) were determined according to the method described by Yoshkochi and Masters (1979). Reduced glutathione was estimated according to Chanarin (1989). Superoxide dismutase (SOD) was estimated according to Giannopolitis and Ries (1977).

All data presented as mean ± standard error (SE) and were subjected to analysis of variance (ANOVA) test according to Snedecor and Cochran (1980). Treatment means were compared by the least significant difference test (LSD) at 0.05 and 0.01 levels of probability.

RESULTS

As shown in figure (1) Sham stress produced a significant increase ($p<0.05$) in SOD of liver vs. non stressed control at the 5th and 7th week of treatment. This increase became non significant at the 9th and 11th week of treatment (even after stoppage of shame stress). Vitamin E and melatonin produced a non significant decrease in liver SOD vs. sham stressed control during most of the sampling periods (except at the 7th and 5th week of treatment, respectively). Curcumin induced a significant decrease in SOD of liver versus sham stressed control at the 7th and 9th week of treatment. CoQ_{10} induced a significant decrease in SOD of liver versus sham stressed control during most of sampling periods (except at the 5th week of treatment). The over all means of liver SOD were significantly lower in rats treated with the different feed supplements vs. sham stressed control.

As shown in figure (2) Sham stress produced a significant increase ($p<0.05$) in SOD of the kidney vs. non stressed control at the 5th and 9th week of treatment. Administration of vitamin E, melatonin, curcumin and CoQ_{10} to sham stressed rats produced a significant decrease in SOD of the kidney vs. sham stressed control during most of sampling periods (except at the 7th week of treatment). The over all means of SOD of the kidney were significantly lower ($p< 0.05$) in rats treated with different feed supplements vs. sham stressed control.

As shown in figure (3). Sham stress induced a significant decrease in liver GSH vs. non stressed control at most sampling periods. This

recorded a decrease in MDA levels in the liver of curcumin treated rats.

In addition, Reddy and lokesh (1994) showed that curcuminoids inhibited lipid peroxidation in rat brain homogenates and rat liver microsomes.

In addition Soudamini et al. (1992); Unnikrishnan and Rao (1992) and Sreejayan and Rao (1994) attributed the antioxidant mechanism of curcumin to a) scavenging or neutralizing of free radicals, b) interacting with oxidative cascade and preventing its outcome, c) quenching oxygen and making it less available for oxidative reaction, d) inhibiting oxidative enzymes like cytochrome P450 and chelating and disarming oxidative properties of metal ions such as iron. Moreover Huang et al. (1997) attributed the potent inhibitors of curcumin on ecosanoid generation and lipid peroxidation to inhibition of lipoxygenase and cyclo-oxygenase pathways of archidonate metabolism.

Furthermore, administration of CoQ_{10} to sham stressed rats in the current study, significantly decreased the sham stress-induced increase in the overall mean values of MDA in liver and kidney These result matches with those reported by Abd-El Gawad et al. (2004) who found that CoQ_{10} treatment decreased the levels of MDA in rats. The CoQ10-induced decrease in MDA levels was thought to be due to its stabilizing effect on cell membranes (lipid-containing structures essential to maintaining cell integrity) and its ability to prevent free radical damage to other important cellular components (Crane et al., 1993; Overvad et al. 1999 and Pepping 1999).

The reduced glutathione is the most abundant thiol in mammalian tissues involved in a variety of intracellular functions as the protection of the cell against damage from electrophiles, free radical and ROS formed during xenobiotic metabolism (Meister, 1991).

The authers attributed that effect to the central role played by GSH in coordination of the body's antioxidant defense mechanism, as it was largely consumed by glutathione related enzymes under oxidative stress conditions. Moreover, Xu et al. (2002) added that colorimetric analysis of extracts of ventricular tissue from rats showed that the level of reduced glutathione (GSH) was significantly less in rats with experimental diabetes, as a result of oxidative stress condition.The obtained results revealed that vitamin E caused a significant increase in glutathione (GSH) concentration in liver and kidney homogenates of rat. This findings are in agreement with the results reported by Shakun and Koval'Chuk (1987) who found that administration of α-tocopherol acetate not only decreased lipid peroxidation and prevented depletion of the reduced glutathione pool but also retained a functional liver disturbance in case of liver damage. In addition, Burton (1994) announced that vitamin E was well known as a traditional antioxidant, it was the most lipophilic antioxidant in biological tissue, so it protected

the unsaturated fatty acids of membrane phospholipids from oxidative degeneration.The results of the present study also revealed that melatonin significantly increased the overall mean values of liver and kidney GSH in sham stressed rats. These findings are in agreement with the results reported by Meki and Hussein (2001) and Sener et al. (2003).

Many investigators attributed the melatonin induced increase in the concentration of GSH to: a) the stimulation of gene expression for antioxidant enzymes (Rodriguez et al., 1998; Galberg and Wiesenberg, 1995; Steinhilber, et al., 1995 and Kotler et al., 1998), b) the increase in the mRNA level of antioxidant enzymes (Antolin et al., 1996) and c) the enhancement of the production of enzymes that are involved in the synthesis of glutathione (Reiter et al., 1999).

Administration of curcumin to sham stressed rats in the current study revealed that curcumin could counteract significantly the sham stress-induced decrease in the over all means of the liver and kidney GSH. This effect might be explained either by lowering lipid peroxidation by maintaining the activities of antioxidant enzymes at higher levels (Reddy and lokesh, 1994), or by increasing the level of glutathione to preventing thiol depletion occurring typically during apoptosis (Jaruga et al., 1998).Meanwhile administration of CoQ_{10} to sham stressed rats in the current study significantly increased the overall mean values of GSH in the liver and kidney. These results matche with those reported by Genova et al. (2003) how reported that exogenous Co Q_{10} protects cells from oxidative stress by conversion into its reduced antioxidant form by cellular reductases.

The present investigation revealed that sham stress significantly increased the overall mean values of SOD in the liver and kidney of sham stressed rats. These results are in agreement with the findings of Tavazzi et al. (2000) who documented that protective enzymes such SOD are activated under stress conditions that stimulate production of oxygen free radicals. Moreover

Shaheen et al., (2000) showed that oxidative stress resulted in a significant increase in SOD indicating an initial compensatory response to oxidative stress. In another word, this might be due to a stimulatory effect of free radicals on liver and kidney to increase the SOD levels. Gouda et al. (2002) attributed the increase in SOD and GST activities to the elevated levels of O_2 in liver and brain microsomes of stressed rats. The Superoxide disproportionate spontaneously to produce H_2O_2 but the reaction was several times more effective if catalyzed by SOD.

The present study revealed that vitamin E, curcumin, melatonin and CoQ10 significantly decreased the SOD in the liver and kidney of rats vs. stressed control group. The finding concerning the effect of CoQ10 on the SOD of sham stressed rats are in agreement with Al-Thakafy et al. (2004) who found that Daily supplementation with CoQ_{10} to rats after induction of diabetes resulted in a significant decrease in the SOD activity and lipid peroxidation end products. However SOD activity was found to be increased in rats after administration of vit E. (El Demerdash et al., 2004 and Anil et al.,

2005), curcumin (Park et al., 2000), melatonin (Kaya et al., 1999 and Meki and Hussein, 2001) and CoQ10 (Abde-El Gawad et al., 2004)

The reasons for these contradictory results may be attributed to the role of the used antioxidants in scavenging the free radicals formed as a result of oxidative stress resulting in absence of stimulatory effect of free radicals on SOD activity. However, this point needs further investigation.It is concluded that: Stress is considered as an oxidative stress and causes induction of free radical. Exposure to stress induced marked and significant effects on hematological and antioxidant status. The different feed supplements used succeeded to protect the animal against the harmful effect of oxidative stress and lead to the improvement of the endogenous antioxidant status as well as liver and kidney functions. Vitamin E, melatonin, coenzymeQ_{10} and curcumin respectively considered the best feed supplements used as antioxidants.

Referances

Abd-El Gawad, Hanan M.; Abdallah, Dalaal M. and El-Abhar, Hanan S. (2004): Rotenone-induced Parkinson's Like Disease: Modulating Role of Coenzyme Q10. Journal of Biological Sciences, 4 (4): 568-574.

Acuna-castroviejo, D.; Macias, M.; Escames, G.; Leon, J.; Khaldy, H. and Reiter R. J. (2001): Melatonin, mitochondria, and cellular bioenergetics. J. pineal Res., 30: 302-310.

Ahmed, H. H.; Essawy, G. S.; Salem, H. A. and Abd El-Daim, M. A. (2005): Melatonin has a strong antioxidant activity and improves liver and kidney functions in broiler chicks. Egypt. J. Basic and Appl. Physiol., 4(1): 77-92.

Al-Thakafy, H. S.; Khoja, S. M.; Al-Marzouki, Z. M.;, Zailaie, M. Z.; Al-Marzouki, K. M. (2004): Alterations of erythrocyte free radical defense system, heart tissue lipid peroxidation, and lipid concentration in streptozotocin-induced diabetic rats under coenzyme Q10 supplementation. Saudi Med. J., 25(12):1824-1830.

Anil, K.; Bansal, M.; Soni, G. and Bhatnagar, D. (2005): Protective role of Vitamin E pre-treatment on N-nitrosodiethylamine induced oxidative stress in rat liver. Chemico-Biological Interactions, 156, (2-3): 101-111.

Antolin, I.; Rodriguez, C.; Sainz, R. M.; Mayo J. C. and Uria H. (1996): Neurohormone melatonin prevents cell damage: Effect on gene expression for antioxidant enzymes, FASAB J. 10: 882–890.

Baydas, G.; Ercel, E.; Ganatan, H.; Donder, E. and Akyol, A. (2001): Effect of melatonin on oxidative status of rat brain, liver and kidney tissues under constant light exposure. Cell Biochem. Funct., 19 (1): 37 – 41.

Burton, G. W. (1994): Vitamin E molecular and biological function. Prac. Nutr. Soc., 53 (2): 251-62.

Chanarin, I. (1989): Textbook of laboratory Haematology: An Account of Laboratory Techniques Churchill Livingstone New York, 107-109.

Crane, F. L.; Sun, I. L. and Sun, E. E. (1993): The essential functions of coenzyme Q. J. of Clinical Investigation, 71 (suppl 8): 55-59.

Dieber-Rotheneder, M.; Puhl, H.; Waeg, G.; Striegl, G. and Esterbauer H. (1991): Effect of oral supplementation with α-tocopherol on the vitamin E content of human low density lipoproteins and resistance to oxidation. J. Lipid Res., 32: 1325–1332.

Dinkova-Kostova, A. T. and Talalay, P. (1999): Relation of structure of curcumin analogs to their potencies as inducers of phase 2 detoxification enzymes. Carcinogenesis (Lond.), 20: 911–914.

Ebelt, H.; Peschke, D.; Bromme, H. J.; Morke, W.; Blume, R. and Peschke, E. (2000): Influence of melatonin on free radical induced changes in pancreatic beta – cell in vitro. J. Pineal Res., 28 (2): 65 – 72.

El-Demerdash, F. M.; Yousef, M. I.; Kedwany, F. S. and Baghdadi, H. H. (2004): Role of α-tocopherol and β-carotene in ameliorating the fenvalerate-induced changes in oxidative stress, hemato-biochemical parameters and semen quality of male rats. Journal of Environmental Science and Health B, 39: 443–459.

El-Sokkary, G. H.; Kamel, E. S. and Reiter, R. J. (2003): Prophylactic effect of melatonin in reducing lead-induced neurotoxicity in the rat, Cell. Mol. Biol. Lett., 8: 461–470.

Galberg, C. and Wiesenberg, I. (1995): The orphan receptor family RZR/ROR, melatonin and 5-lipooxygenase an unexpected relationship. J. Pineal Res., 18: 171 – 178.

Genova, Maria L.; Pich, Milena M.; Biondi, E.; Bernacchia, A.; Falasca, A.; Bovina, C.; Formiggini, G.; Castelli, G. P. and Lenaz, G. (2003): Mitochondrial production of oxygen radical species and the role of coenzyme Q10 as an antioxidant. Expermintal Biology and Medicine, 228: 506-513.

Giannopolitis, C. N. and Ries, S. K. (1977): superoxide dismutases occurance in higher plants. Plant Physiol., 59: 309-314.

Gouda, Eman M.; Abd Alazeem, Eman M.; Ibrahim, E.A.; Mabrouk, N.A. (2002): effects of meprobamate on lipid peroxidation and antioxidant status in rats under stress. Proceeding of the 5th National Conference of Biochemistry and Molecular Biology, 12-14 May 2005, Cairo, Egypt, pp 221-233.

Halim, A. B.; El-Ahmdy, O.; Hassab-Allah, S.; Abdel-Galil, F.; Hafez, Y. and Darwish, A. (1997): Biochemical effect of antioxidants on lipids and liver function in experimentally induced liver damage. Ann. Clin. Biochem., 34 (6): 656-663.

Halliwell, B. and Gutteridge, J. M. C. (1989): Free radicals biology and medicine, 2nd ed.; Japan Scientific Societies Press: Tokyo, Japan, 67-80.

Huang, M. T.; Ma, W.; Yen, P.; Xie, J. G.; Han, J.; Frenkel, K.; Grunberger, D. and Conney, A. H. (1997): Inhibitory effects of topical application of low doses of curcumin on 12-O-tetradecanoylphorbol-13-acetate induced tumor promotion and oxidized carcinogenesis (Lond.)., 18: 83-88.

Jaruga, E.; Salvioli, S.; Dobrucki, J.; Chrul, S.; Bandorowicz-pikula, J.; Sickora, E.; Franceschi, C.; Cossarizza, A. and Bartosz, G.

(1998): Apoptosis-like, reversible changes in plasma membrane asymmetry and permeability, and transient modifications in mitochondrial membrane potential induced by curcumin in rat thymocytes. FEBS Lett., 433: 287-293.

Kalpana, C. and Menon, V. P. (2004): Curcumin ameliorates oxidative stress during nicotine-induced lung toxicity in wister rats. Ital. J. Biochem., 53 (2):82-86.

Kotler, M.; Rodriguez, C.; Sainz, R. M.; Antolin I. and Menendez-Pelaez, A. (1998): Melatonin increases gene expression for antioxidant enzymes in rat brain cortex, J. Pineal. Res., 24: 83–89.

Kumar, K. V.; Naidu, M. V.; Shifow, A. A.; Prayag, A. and Ratnakar, K. S. (1999): Melatonin: antioxidant protects against cyclosporine induce nephrotpxicity. Transplant., 67 (7): 1065 – 1068.

Kwong, L. K.; Kanzalov, S.; Rebrin, I.; Anne-Cecile, V. B.; chandan, K. J.; Paul, M.; Michael, J. F. and Rajindar, S. S. (2002): Effect of Coenzyme Q10 administration on its tissue concentrations, mitochondrial oxidant generation, and oxidative stress in the rat. Free Radical Biology and Medicine; 33 (5): 627-638.

Maellaro, E.; Casini, A. F.; Del Bello, B. and Comporti, M. (1990): Lipid peroxidation and antioxidant systems in the liver injury produced by glutathione depleting agents. Biochem. Pharmacol., 39: 1513-1521.

Meister, A. (1991): Glutathione deficiency produced by inhibition of its synthesis, and its reversal: application in research and therapy. Pharmacol. Therap. 51: 155-194.

Meki, A. R. and Hussein, A. A. (2001): Melatonin reduces oxidative stress induced by Ochratoxin A in rat liver and Kidney. Comp. Biochem. Physiol. C. Toxicol. Pharmacol., 130 (3): 305 – 313.

Montilla, P.; Tunez, I.; Munoz, M. C.; Lopez, A. and Soria, J. V. (1997): Hyperlipidemic nephropathy induced by adriamycin: Effect of melatonin administration. Nephron, 76: 345 – 350.

Nagar, (2004): Protective effects of Curcuma longa (turmeric) on ischemia-reperfusion induced myocardial injuries and their mechanisms. Life Sci., 75(14):1701-1711.

Othman, A. I.; Al sharawy, S. and EL missiry, M. A. (2004): Role of melatonin in ameliorating lead induced hematotoxicity. Pharmacological Research, (50): 301-307.

Overvad, K.; Diamant, B. and Holm, L. (1999): Coenzyme Q10 in health and disease. European Journal of Clinical Nutrition, 53(10): 764-770.

Ozbek, M. (2000): Melatonin administration prevent nephrotoxicity induced by gentamicin. B. J. U., 85 (6): 742 – 746.

Ozturk-Urek, R.; Bozkaya, L. A. and Tarhan, L. (2001): The effect of some antioxidant vitamin and trace element supplemented diets on activities of SOD, CAT, GSH- PX and LPO levels in chicken tissues. Cell Biochem. Funct., 19 (2): 125- 132.

Pablos, M. I.; Agapito, M. T.; Gutierrez, R.; Recio; Reiter. R. J. Barlow-Walden, L.; Acuna-castroviejo, O. and Menedez-Peplaez, A. (1995): Melatonin stimulates the activity of the detoxifying

enzyme glutathione peroxidase in several tissues in chicks. J. Pineal Res., 19 (3): 111 – 115.

Park, E. J.; Jeon, C. H.; Ko, G.; Kim, J. and Sohn, D. H. (2000): Protective effect of curcumin in rat liver injury induced by carbon tetrachloride. J. Pharm. Pharmacol., 52: 437–440.

Pepping, J. (1999): Coenzyme Q. American Journal of Health-System Pharmacy; 56: 519-521.

Pieri, C.; Merra, M.; Moroni, F. and Marcheselli, F. (1994): Melatonin is a hydroxyl radical scavenger more effective than vitamin E. Pergamon, 55 (15): 271 – 276.

Pierrefiche, G.; Topall, G.; Courboyn, G.; Henryet, I. and Laborit, H. (1993): Antioxidant activity of melatonin in mice, Res. Commun. Chem. Pathol. Pharmacol., 80: 211–223.

Poeggeler, B.; Saarela, S.; Reiter, R. J.; Tan, D. X.; Chen, L. D.; Manchester, C. and Barlow-Walden, L. R. (1994): Melatonin- a highly potent endogenous radical scavenger and electron donor: New aspects of the oxidation chemistry of the indole accessed in vitro. Ann. N. Y. Acad. Sci., 738: 419 – 420.

Reddy, A. C. P. and Lokesh, B. R. (1994): Studies on the inhibitory effects of curcumin and eugenol on the formation of reactive oxygen species and the oxidation of ferrous iron. Mol. Cell. Biochem., 37: 1–8.

Reiter, R. J.; Carnerio, R. C. and Oh, C. S. (1997): Melatonin in relation to cellular antioxidative defense mechanisms. Horm. Metab. Res., 29: 363-372.

Reiter, R. J.; Mechiarri, D.; Sewerynek, E.; Poeggler, B.; Barlow Walder, L. R.; Chuang, J. I.; Ortiz, G. G. and Acuna-Gastroviezo, D. (1995): A review of the evidence Supporting melatonin role as antioxidant. J. Pineal Res., 18: 1 – 11.

Reiter, R. J.; Tan, D. X.; Manchester, W.; Qi, L. C.; Karbownik M. and Calvo, J. R. (2000): Pharmacology and physiology of melatonin in the reduction of oxidative stress in vivo, Biol. Signals Recept., 9: 160–171.

Reiter, R. J.; Tan, D. X.; Cabrera, J.; D'Aropa, D.; Sainz, R. M.; Mayo J. C. and Ramos, S. (1999): The oxidant/antioxidant network: role of melatonin. Biol. Signals Recept., 8: 56–63.

Reiter, R. J.; Tan, D. X.; Manchester, L. C. and Qi, W. (2001): Biochemical reactivity of melatonin with reactive oxygen and nitrogen species, Cell. Biochem. Biophys., 34: 237–256.

Rodriguez, A. B.; Nogales, G.; Ortega, E. and Barriga, C. (1998): Melatonin controls superoxide anion level modulation of superoxide dismutase activity in ring dove heterophils. J. Pineal Res., 24 (1): 9 – 14.

Sener, G.; Tosun, O.; Sehirli, A. O.; Kacmaz, A.; Arbak, S.; Ersoy Y. and Ayanoglu-Dulger, G. (2003): Melatonin and N-acetylcysteine have beneficial effects during hepatic ischemia and reperfusion. Life Sci., 72: 2707–2718.

Shaheen, Amira A.; Adel, A.; Kheir-Eldin; Amal, A.; Abdel-fattah and Darwish, Hebatalla A. (2000): Effect of some food additive on

oxygen scavenger system in young male rats, The Egyptian J. of Biochem., 18 (2):100-107.

Shakun, N. P. and koval'Chuk, S. F. (1987): Effectiveness of antioxidants incombined carbon tetrachloride and ethanol lesion of liver. Farmakol. Toksikol., 50 (3): 97.

Snedecor, G. W. and Cochran, W. G. (1980): Statistical methods. 7th ed., Ames: Iowa State University Press.

Somasundaram, S.; Edmund, N. A.; Moore, D. T.; Small, G. W.; Shi, Y. Y. and Orlowski, R. Z. (2002): Dietary curcumin Inhibits Chemotherapy induced Apoptosis in Models of Human Breast Cancer[1] *Cancer Research* 62: 3868-3875.

Soudamini, K. K.; Unnikrishnan, M. C.; Soni, K. B. and Kuttan, R. (1992): Inhibition of lipid peroxidation and cholesterol levels in mice by curcumin. Ind. J. Physiol. Pharmacol., 36: 239-243.

Sreejayan, N. and Rao, M. N. (1994): Curcuminoids as potent inhibitors of lipid peroxidation. J. Pharm. Pharmacol., 46: 1013-1016.

Sreejayan, R. and Rao, M. N. (1997): Nitric oxide scavenging by curcuminoids. J. Pharm Pharmacol., 49: 105-107.

Steinhilber, D.; Brungs, M.; Werz, O.; Wiesenberg, I.; Danielsson, C.; Kahlen, J. P.; Nayeri, S.; Schrader, M. and Calberg, C. (1995): The nuclear receptor for melatonin represses 5-lipoxygenase gene expression in human B lymphocytes. J. Biol. Chem., 270: 7037 – 7040.

Tavazzi, B.; Di-Pierro, D.; Amorini, A. M.; Fazzina, G.; Tuttobene, M.; Giardina, B. and Lazzarino, g. (2000): Energy metabolism and lipid peroxidation of human erythrocytes as a function of increased oxidative stress. Eu. J. Biochem., 267: 684-689.

Unnikrishnan, M. K. and Rao, M. N. A. (1992): Curcumin inhibits nitrite induced methemoglobin formation. FEBS, 301: 195-196.

Xu, Z.; Patel, K. P.; Lou, M. F. and Rozanski, G. J. (2002): Up-regulation of K(+) channels in diabetic rat ventricular myocytes by insulin and glutathione. Cardiovasc. Res., 53(1): 80-88.

Yashkochi, Y. and Masters, B. S. S. (1979): Some properties of a detergent. Solubilizied NADPA cytochromic (cytochrome P. 450) reductase purified by biospecific affenity chromatography. J. Biol. Chem., 251 (17): 5337 – 5344.

Yousef, M. I.; Abdallah, G. A. and Kamel, K. I. (2003): Effect of ascorbic acid and vitamin E supplementation on semen quality and biochemical parameters of male rabbits. Animal Reproduction Science, 2352: 1–13.

Figures:

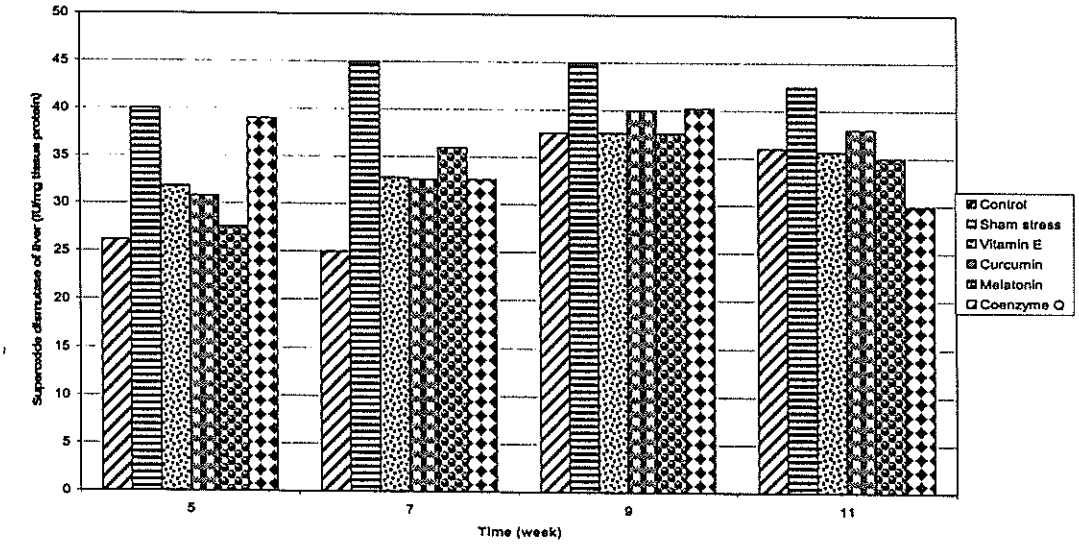

Figure (1): Superoxide dismutase (IU/gm protein) of liver in rats treated with different feed supplements.

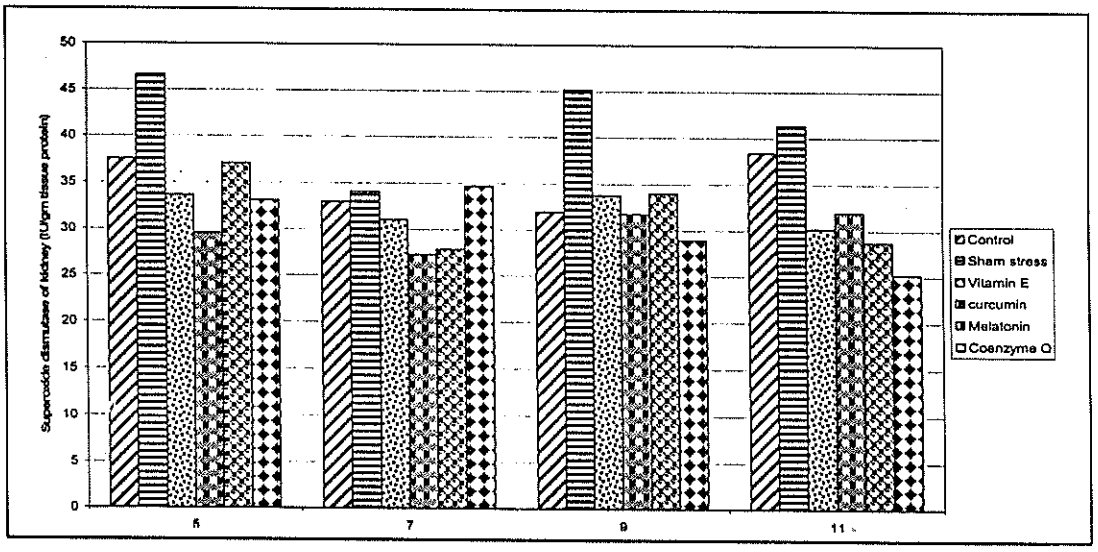

Figure (2): Superoxide dismutase (IU/gm protein) of kidney in rats treated with different feed supplements.

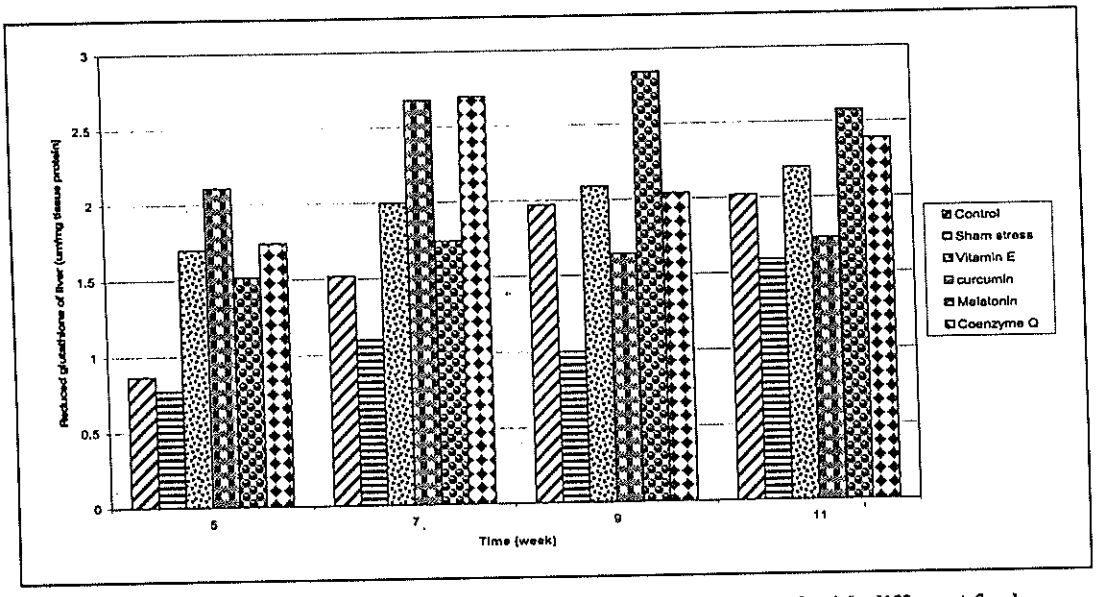

Figure (٣): reduced glutathione (um/mg protein) of Liver in rats treated with different feed supplements.

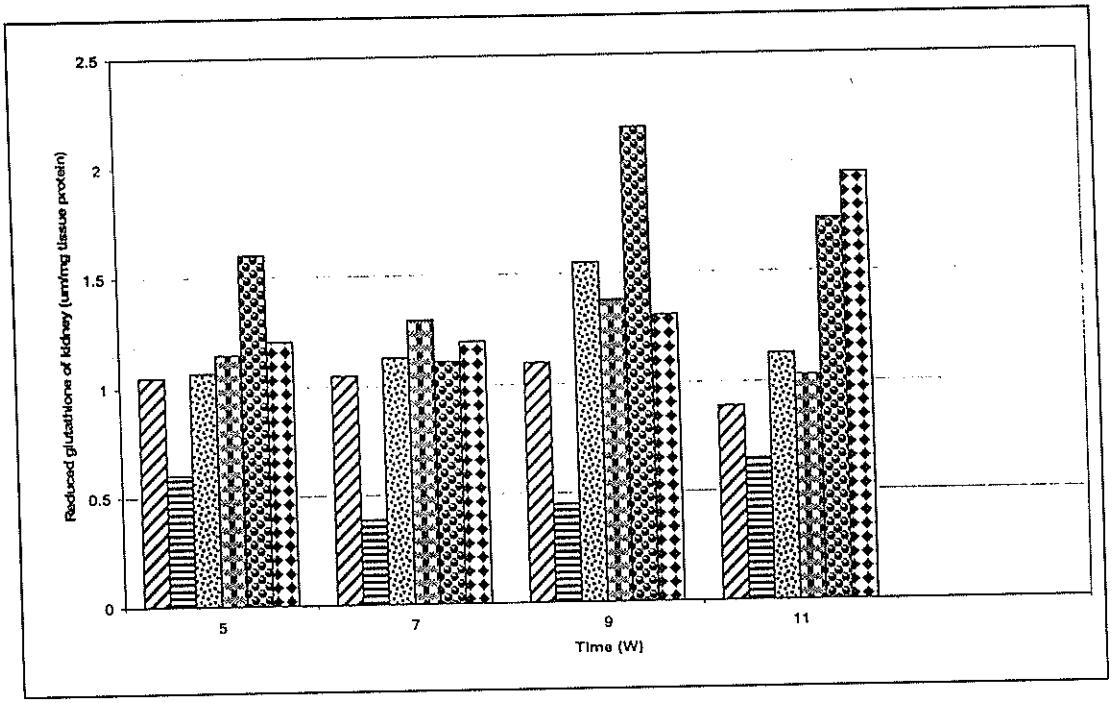

Figure (4): Reduced glutathione in kidney (um/mg protein) of rats treated with feed supplements.

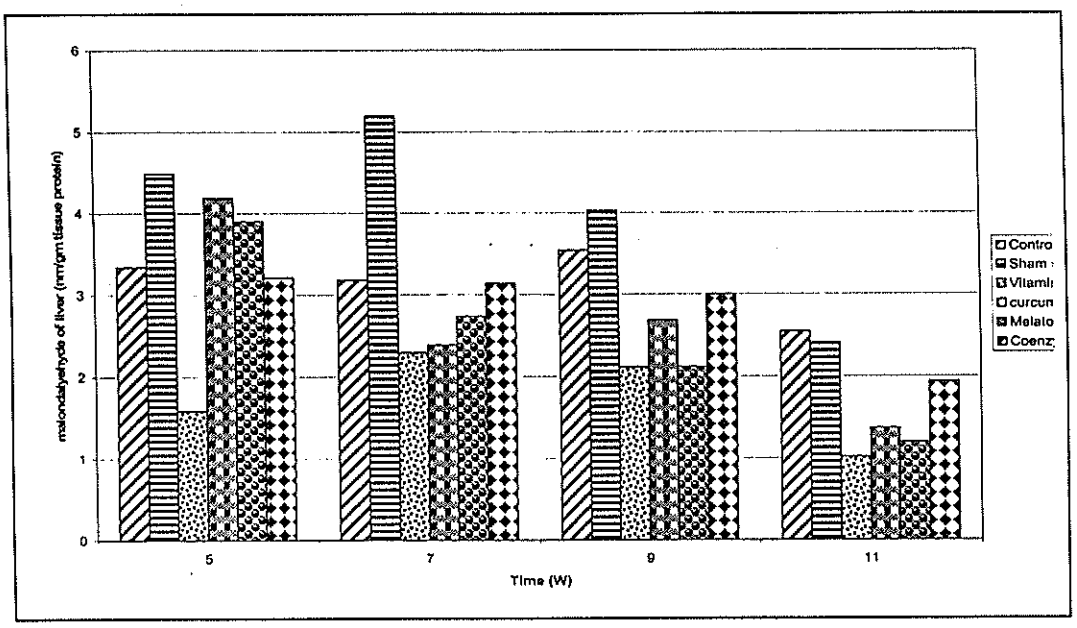

Figure (5): Malondaldehyde levels (nm/gm tissue protein) of Liver in rats treated with feed supplements

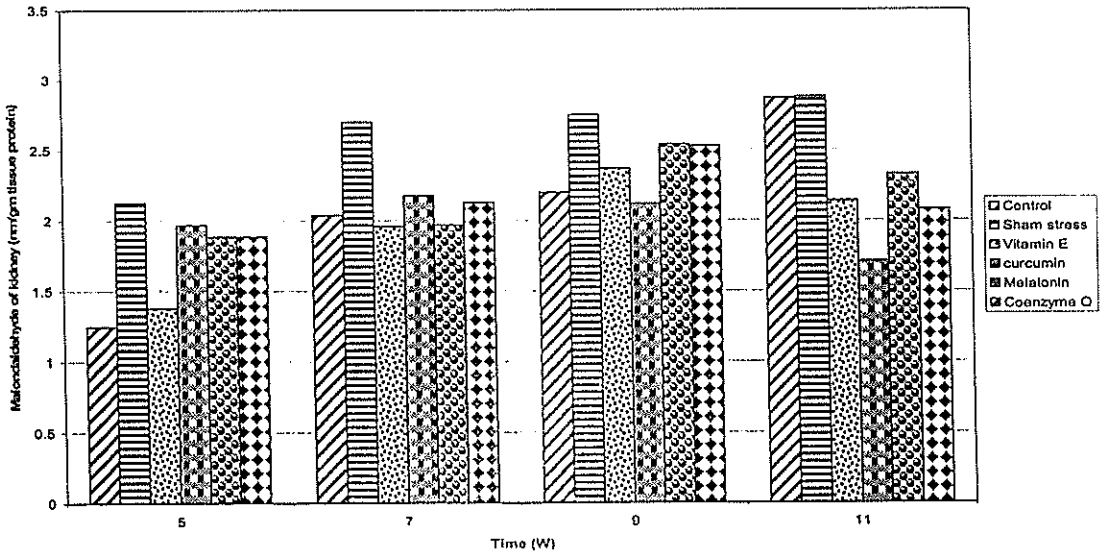

Figure (6): Malondialdehyde levels (nm/gm tissue protein) of kidney in rats