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The Role of Melatonin in alleviating Hyperlipedemia in the Rats

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ABSTRACT

The protective action of melatonin can be manifested in our study following the induction of hyperlipidemia in rodent animals or rats to counteract the harmful effects of excess fat. 30 wistermale rats, divided into 3 groups; 10 of them were subjected to normal diet (control group) group A with melatonin, group B had a diet rich in lipid(atherogenic diet)for three weeks without melatonin injection, while group C taking atherogenic diet but with melatonin injection (intraperitoneal) with serial estimation of serum cholesterol ,triglyceride ,LDL and VLDL through blood sample of trunk blood (cardiac puncture of the rat) for a period from 25 Feb. 2013 to 25 April 2013. Enhanced catabolism of cholesterol to bile acids is likely studied by an increase in fecal bile acid excretion following melatonin treatment as will be seen in general stool examination (G.S.E.) of the rats. In group B marked increase in serum cholesterol, VLDL and triglyceride level following dietary regime (followed by) slight reduction in serum cholesterol and triglyceride level after melatonin injection as seen in group C , associated with high bile salts level in stool examination ,matching with no response as seen in group A .These findings suggest that the hypolipidemic effect of melatonin may work through the augmentation of endogenous cholesterol clearance mechanisms especially in cases with induced or secondary hyperlipidemia, this is accompanied by the lowering of the triglyceride fraction associated with low density lipoproteins.

Keyword: Melatonin, Rats, Serum Cholesterol, Triglyceride, LDL, VLDL, General stool examination, Diet rich in cholesterol(atherogenic) , Centrifuge and Bioanalyzer.

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INTRODUCTION

Although the rat is frequently used for experimental studies of the metabolism of the serum lipoproteins, adequate characterization of these lipoproteins has not been reported¹⁻⁶. In previous studies, rat serum lipoproteins have been isolated using the conditions established for separation of the lipoproteins of human serum^{7,8}; recent studies^{1-3,9} have suggested Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins. That different densities may be more suitable for isolation of the various classes of serum lipoproteins in the rat. It is the purpose of this paper(electrophoresis) to define the conditions for separation of lipoproteins from normal and hyperlipidemic rat serum, nowadays ,the characterization of the isolated lipoproteins by new techniques done by the use of bioanalyzer methods for rat serum.

Constant exposure to light is one important step for induction of hyperlipidemia along with diet, since constant light exposure also(promoted) an increase in lipid levels.⁵

Melatonin is a powerful antioxidant ,¹¹it easily crosses cell membranes and the blood–brain barrier¹¹ Unlike other antioxidants, melatonin does not undergo redox cycling, which is the ability of a molecule to undergo repeated reduction and oxidation. . Melatonin, once oxidized, cannot be reduced to its former state because it forms several stable end-products upon reacting with free radicals. Therefore, it has been referred to as a terminal (or suicidal) antioxidant. Antioxidants are used as food additives to help guard against food deterioration. Exposure to oxygen and sunlight are the two main factors in the oxidation of food, so food is preserved by keeping in the dark and sealing it in containers or even coating it in wax, as with cucumbers. However, as oxygen is also important for plant respiration, storing plant materials in anaerobic conditions produces unpleasant flavors and unappealing colors consequently¹², packaging of fresh fruits and vegetables contains a ~8% oxygen atmosphere. Antioxidants are an especially important class of preservatives as, unlike bacterial or fungal spoilage, oxidation reactions still occur relatively rapidly in frozen or refrigerated food.¹¹ These preservatives include natural antioxidants such as ascorbic acid and tocopherols, as well as synthetic antioxidants such as propyl gallate, tertiary butyl hydroquinone, butylatedhydroxyanisole and butylatedhydroxytoluene^{11,12} The most common molecules attacked by oxidation are unsaturated fats; oxidation causes them to turn rancid.⁽¹²⁾Since oxidized lipids are often discolored and usually have unpleasant tastes such as metallic or sulfurous flavors.

MATERIALS AND METHODS

In Our study 30 wister male rats weighing 400-500 g were obtained from Veterinary College –

Baghdad (Abu Ghreb), divided into three groups each group had 10 rats:

Group A: received normal diet with melatonin - control group.

Group B: received atherogenic diet without melatonin injection.

Group C: fed with a food containing soya bean, cow milk, rural cheese and olive oil rich in cholesterol (atherogenic diet) and were maintained on this diet for at least three weeks to produce hyperlipidemia, they allowed for free access to food, with constant exposure to light, since constant light exposure also prompted an increase in lipid levels. Melatonin injection intraperitoneally i.p (50 micrograms/kg/day) for group A & C done 1 week before (1st week) and after food intake (5th week). Blood was drawn by cardiac puncture at 10 am (trunk blood was drawn) two times weekly for 5 weeks, with the help of veterinary doctors in veterinary hospital in Alkarkh Directorate/Baghdad. After centrifuge, pooled serum samples from (rats were) analyzed for triglyceride and cholesterol content of the lipoprotein in the top of the tube and results were obtained with the aid of bioanalyzer was carried out, and the heights of the peaks were measured. The cholesterol, triglyceride, LDL and VLDL contents of the standard were determined by protein measurements on the fractions isolated from the standard; these values were plotted against the appropriate time (5 weeks) before and after food intake and melatonin injection respectively. Stool examination for measuring bile acids carried out simultaneously for all rats. two times weekly for 5 weeks.

RESULTS AND DISCUSSION

Figure: 1 reveal changes in control group with normal values in mg/dl for triglycerides, cholesterol and lipoproteins, in spite of the use of melatonin in the 1st week and 5th week, it can be seen that the normal amount of triglyceride in the top fraction remained constant. This indicated for normal dietary intake of lipid under these conditions, with normal stool examination (no increase in level of bile acids in stool neither any changes within the colors).

Figure: 2 reveals marked increase in triglyceride, cholesterol and lipoprotein fraction following food intake especially in the 4th week after food intake with no changes in stool examination (normal values for bile acids levels and normal colors).

Figure: 3 showed reduction in cumulative effects of lipid in the lipoprotein fractions which was plotted against the time especially after 4th week following food intake, the concentration of lipids was much lower than in normal rats. Up to two times decrease in the lipoprotein fractions isolated contained mainly triglycerides, while the fractions appearing from 2nd to 4th week contained a greatly increased proportion of cholesterol. More than one-third decrease of the total

serum cholesterol was found in these rats of the lipoprotein distribution in the serum. General stool examination showed increased level of bile acids especially during the 3rd, 4th week and 5th week revealing a state of a change in stool color (dark brown).

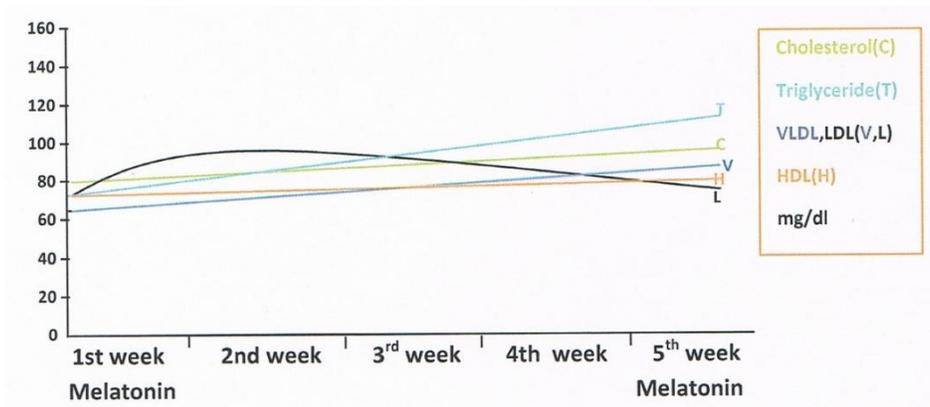


Figure 1: Reveal changes in mg/dl of lipid profile in group A – control group- within five weeks, with melatonin injection.

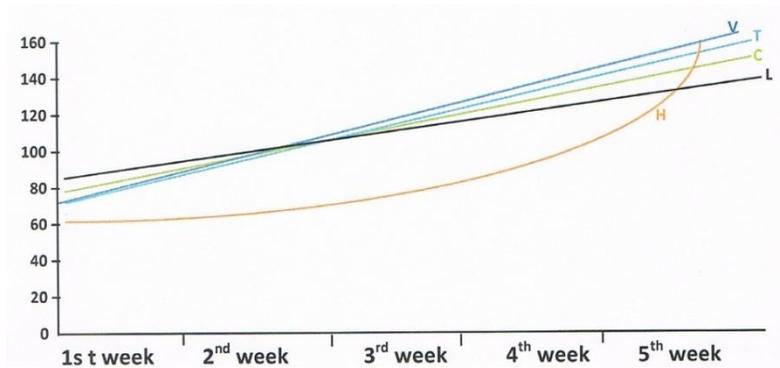


Figure 2: Reveal changes in lipid profile in group B without melatonin injection.

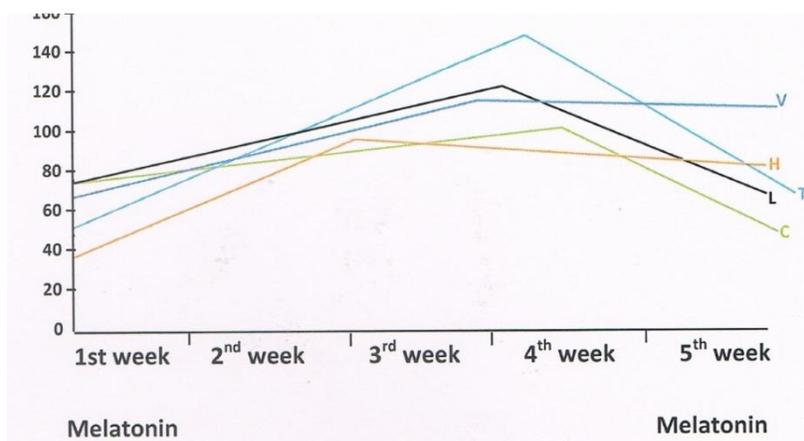


Figure 3: Changes in lipid profile in group C with melatonin injection.

Melatonin, which is synthesized in the pineal gland and other tissues, has a variety of physiological, immunological, and biochemical functions. It is a direct scavenger of free radicals and has indirect antioxidant effects due to its stimulation of the expression and activity of

antioxidative enzymes such as glutathione peroxidase, superoxide dismutase and catalase, and NO synthase, in mammalian cells.^(13,14) Melatonin also reduces serum lipid levels in mammalian species, and helps to prevent oxidative stress in diabetic subjects. Long-term melatonin administration to diabetic rats (secondary hyperlipidemia) reduced their hyperlipidemia and hyperinsulinemia, and restored their altered ratios of polyunsaturated fatty acid in serum and tissues. It was recently reported that melatonin enhanced insulin-receptor kinase and IRS-1 phosphorylation, suggesting the potential existence of signaling pathway cross-talk between melatonin and insulin. Because TNF-alpha has been shown to impair insulin action by suppressing insulin receptor-tyrosine kinase activity and its IRS-1 tyrosine phosphorylation in peripheral tissues such as skeletal muscle cells,¹⁵ it was speculated that melatonin might counteract TNF-alpha-associated insulin resistance in type 2 diabetes. This review will focus on the physiological and metabolic effects of melatonin and highlight its potential use for the treatment of carbohydrate and cholesterol/lipid disorder through enhancement of cholesterol clearance to bile salts in feces radicals, both endogenous and exogenous, in this organ¹⁶.

CONCLUSION:

These findings suggest that the hypolipidemic effect of melatonin may work through the augmentation of endogenous cholesterol clearance mechanisms in cases with induced hyperlipidemia while it had no beneficial effects in normal cases who had normal diet. This is accompanied by the lowering of the cholesterol fraction associated with low density lipoproteins, The protective action of melatonin was manifested only following the induction of hyperlipidemia in such animals, enhanced catabolism of cholesterol to bile acids is likely involved as shown by an increase in fecal bile acid excretion (G.S.E.) following melatonin treatment., additional studies are required to establish accurately the role of this pineal indole in the regulation and dynamics of the antioxidative defense enzyme system, which neutralizes the damaging effect of free

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