Histological Study on the Effect of Nicotine administration in the Bone of Adult Male Albino Rat and the Possible Protective Role of Vitamin E

Thesis

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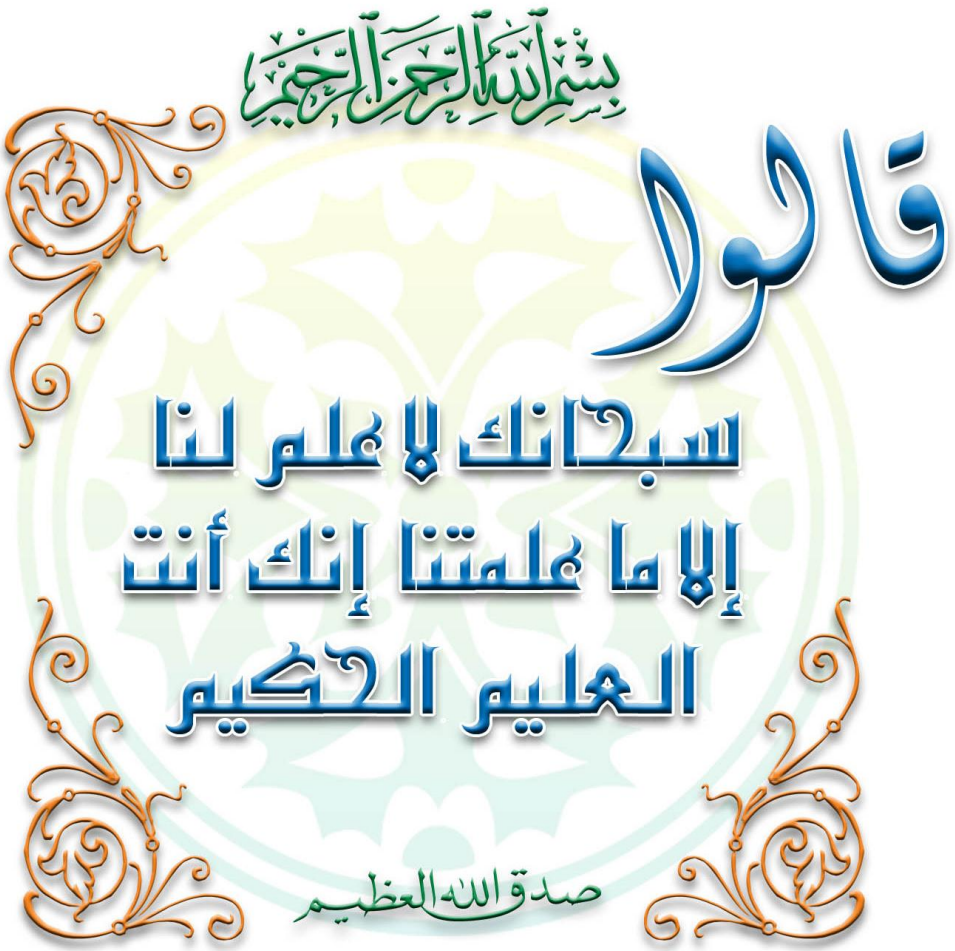

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Dedication

Words can not express my thanks, gratefulness, respect and love to my parents, my wife and all members of my family. Without their help, support, patience and encouragement, I would have never achieved any success.

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List of Abbreviations

ALP	Alkaline phosphatase
ANOVA	Analysis of variant
BMD	Bone mineral density
Ca	Calcium
CFU-O	Colony forming unit osteoprogenitor
EDTA	Ethylene-diamine-tetra acetic acid
EDX	Energy dispersive X-ray composion analysis
GSH	Glutathione
GTT	Gamma-tocotrienol
IL	Interleukin
NaDOC	Sodium deoxycholate
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B
NIH	National institutes of health
P value	Probability of significance value
PYD	Urinary total pyridinoline
SD	Standard deviation
SEM	Scanning electron microscope
TEF	Tocotrienol-enhanced fraction
TNF	Tumer necrosis factor

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Abstract

Introduction Nicotine is an organic alkaloid compound found naturally throughout the tobacoo plant with high concentration in the leaves. Thousands of researches had reported the deleterious effects of smoking but few researches studied the hazardous effects of nicotine on bone. α-tocopherol is the most important lipid-soluble antioxidant. Its anabolic effect on bone had been reported through many researches. **Aim of the work** was to evaluate the hazardous effects of nicotine on bone and the possible protective effect of vitamin E through different histological techniques. **Materials and methods** Forty five adult male Albino rats were used for this study and were divided into three groups fifteen for each. Group I served as control. Group II received nicotine alone at a dose of 7mg\kg\d for three months. Group III received nicotine at the same dose of group II with concomitant administration of alpha tocopherol form of vitamin E at a dose 60mg\kg\d for three months. At the end of the experiment, animals were sacrificed then the proximal end of the femur was dissected and processed for light and scanning electron microscopic examination, histomorphometrical analysis was done on the metaphysis region. Bone mineral density was also estimated and the results were statistically analysed. **Results** The L\M examination of the nicotine treated group revealed significant decrease in both

outer cortical bone and inner cancellous bone trabeculae. The trabecular bone was formed of few, thin and disconnected struts. Multiple fractures, bone necrosis were also seen in this group. Fatty change and hemorrhage were observed in bone marrow spaces. The scanning electron microscopic examination results confirmed the light microscopic results. Also measuring of the bone mineral density revealed highly significant decrease of the bone calcium content in nicotine treated rats compared to that of control group. Vitamin E reversed most of the previous osteoporotic signs. The L\M examination of the vitamin E treated group (group III) showed significant increase in the outer cortical bone thickness and inner cancellous bone thickness and volume when compared to that of nicotine treated group. The scanning electron microscopic examination results supported the L\M results. Moreover significant increase in the bone ca content of this group as compared to that of nicotine group was noticed. **Conclusion** concomitant administration of vitamin E with nicotine could protect the bone from the hazardous effects of nicotine.

Keywords: nicotine, vitamin E, histomorphometry, bone mineral density, rats.

Introduction

Nicotine was an organic alkaloid compound found naturally throughout the tobacco plant with high concentration in the leaves ***(Norzaline et al., 2007)*.**

Scollo et al. (2003) mentioned that cigarettes smoking had killed up to half of their users nearly six millions people each year. Moreover more than 600 000, nonsmokers exposed to second-hand smoke were also killed. They added that unless urgent action was taken, the annual death could rise to more than eight millions by 2030**.**

Smoking was associated with decrease bone mass and was considered a risk factor for osteoporosis in human. Osteoporosis was a progressive systemic skeletal disease characterized by low bone mass and micro-architectural deterioration of bone tissue. The decrease of the bone mineral density (BMD) was the most important risk factor for fracture ***(Rodriguez-Martinez and Garcia-Cohen, 2002).***

Nicotine had a direct action on bone metabolism by influencing the bone remodeling process ***(Hermizi et al., 2009)***.

Vitamin E was a lipid soluble natural antioxidant that was found to improve bone metabolism especially in the laboratory animals that had a high metabolic rate due to small surface area ***(Ima-Nirwana et al., 1999).***

Review of Literature

Nicotine and its hazardous effects:

Schmeltz and Hoffmann (1977) stated that nicotine constituted 0.3 to 5% of the tobacoo plant by dry weight, with biosynthesis taking place in the roots, and accumulated in the leaves. In addition to the tobacco plant, nicotine was also found in lower quantities in other members of the nightshade family which included tomato, potato, and green pepper. Nicotine alkaloids were also found in the leaves of the coca plant. Nicotine salts & derivatives generally used in pharmaceutical application, were nicotine sulphate, nicotine salcylate, nicotine hydrochloride, nicotine tartrate, nicotinic acid, and b-nicotyrine.

Svensson (1987) stated that absorption from the oral mucosa was the principal site of nicotine absorption in the individuals who chew tobacco. Nicotine was also readily absorbed from the nasal mucosa and respiratory tissues. He added that nicotine distributed extensively into body tissues, transferred across the placenta and into breast milk in humans and was excreted unchanged in urine (the predominant route of nicotine elimination was hepatic metabolism). Its half-life being only 2hours.

Many studies and researches investigated the hazardous effects of nicotine on the bone, one of them was made by ***Fang et al. (1991)*** who studied the effect of nicotine on the cellular metabolism. They examined the effects of nicotine on cellular proliferation, by [^{3}H] thymidine incorporation and cell count in the rat osteoblastic osteosarcoma cells. These cells were cultured with varying concentrations of nicotine. They found that nicotine produced a dose-dependent suppression of thymidine incorporation and cellular proliferation in osteoblast-like cells. These results might be of significance in the development of osteoporosis and alveolar bone loss associated with the use of nicotine.

Nicotine could stimulate angiogenesis and atherosclerosis. It was a risk factor for the development and progression of kidney disease in patients with both insulin-dependent and non insulin-dependent diabetes mellitus. Nicotine increased the risk of protinuria and accelerated renal failure ***(Heeshen et al., 2001; Gabbai and Khang, 2001).***

Moreover, ***Brody et al. (2004)*** studied the effect of nicotine on CNS. Nicotine caused brain atrophy and impairment of cognitive function**.** Moreover ***Allam et al. (2004)*** stated that former and current smokers had a lower incidence of

Parkinsonism disease compared to people who had never smoked.

Seddigheh et al. (2004) stated that nicotine suppressed the migration of leukocytes to the inflammation/infection site. It increased the influenza titer in the rat lung and decreased inflammation response correlated with lower chemotaxis/ chemokinesis of peripheral blood mononuclear cells. They added that, because nicotine suppressed leukocyte migration, it might contribute to the delayed wound healing and increased incidence of respiratory infections among smokers.

Moreover ***Devereux (2006)*** reported that smoking produced chronic obstructive pulmonary disease known as tobacco disease. This disease caused incurable reduction of pulmonary capacity. He added that emphysema and chronic bronchitis were also associated with smoking.

Moreover ***Wu et al. (2006)*** stated that nicotine directly increased cellular mutagenic events by being metabolized into highly carcinogenic nitrosamines. They added that nicotine by itself stimulated cancer cells proliferation through multiple mutagenic signaling pathways. Nicotinic stimulation provided pro-survival signals to cancer cells so they become resistant to apoptosis induced by chemotherapeutic agents or ionizing