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ORIGINAL ARTICLE

PROTECTIVE EFFECT OF VITAMIN E IN PREVENTION OF NON-ALCOHOLIC FATTY LIVER SYNDROME IN Rats

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ABSTRACT: Vitamin E is the best-researched fat soluble antioxidant known for its protective effects on lipid membranes and unsaturated fatty acids. Fatty liver, also known as fatty liver disease (FLD), is a reversible condition where large vacuoles of triglyceride fat accumulate in liver cells via the process of steatosis (i.e. abnormal retention of lipids within a cell). The aim of present study was to evaluate the protective effect of vitamin E in prevention of non-alcoholic fatty liver syndrome in rats. In this study, 90 male Wistar rats (220–250 g and 2-3 month age) were selected for the study and randomly divided into five equal groups: group1; received high fat diet; Group 2 received high fat diet plus Clofubrate as positive control group at a dose of 320 mgkg-1/day as emulsion in methylcellulose 0.5% (2ml/kg) for 4 weeks through gastric gavage; Group 3 till 5 received high fat diet plus vitamin E at a dose of 100, 200 and 300 mg/day, respectively. At the end of the period, blood samples were obtained from retrobulbar sinus for measurement of some biochemical factors. Data obtained from measurement and analysis of parameters is given in tables. Based on data showed in tables it comes that the numerical value of parameters with exception of LDL has been affected by vitamin E different doses. Comparative diagrams also are given which indicate a significant protective effect of vitamin E. In conclusion can state that vitamin E has protective effect against fatty liver disease in rats fed high fat diet.

KEYWORDS: Fatty Liver Syndrome, Vitamin E, Protection, Liver, Rats.

INTRODUCTION

Vitamin E is the best-researched fat soluble antioxidant known for its protective effects on lipid membranes and unsaturated fatty acids. Vitamin E is well documented to prevent atherosclerosis and may also help prevent Alzheimer's disease. Its protective effects include the heart, brain, skin, eyes, liver, breasts, and prostate. It stabilizes blood fats so the blood vessels and heart are protected from free-radical induced injury. Selenium benefits treat or prevent some health conditions like, heart diseases, HIV and AIDS, miscarriages, arthritis, muscular degeneration, strokes, gray hair and different types of cancer. Our body requires a very little amount of selenium. It also promotes antioxidant activity in the body. Selenium has also proved effective in fighting viruses that cause cold sores and shingles. Some studies have shown that consumption of selenium is helpful in making the blood "less sticky" which prevents heart strokes (Dursun *et al.*, 1998; Griffith, 2000). Vitamin E is necessary for the optimum function and metabolism of the nervous, muscular, circulatory and immune systems, and the latter highlights its importance in maintaining the health (Schwenke, 2002). Its function is basically to prevent the breakdown of oxygen at a cellular level (oxidation) when toxic products including hydrogen peroxide and hydroxyl radicals are produced. These oxidizing agents are powerful tissue poisons (Bendich and Machlin, 1988). Supplementation with vitamin E and selenium had a preventive effect on the elevation of the hepatic, thiobarbituric acid reactive substances (TBARS) and improved the diminished activities of the antioxidative enzymes and the levels of GSH. So the effectiveness of vitamin E and selenium in reducing hepatic damage (Beytut and Aksakal, 2003). Antioxidative nutrients such as vitamin E and selenium can prevent liver fibrosis induced by carbon tetrachloride (Parola *et al.*, 1992).

Fatty liver, also known as fatty liver disease (FLD), is a reversible condition where large vacuoles of triglyceride fat accumulate in liver cells via the process of steatosis (i.e. abnormal retention of lipids within a cell). Despite having multiple causes, fatty liver can be considered a single disease that occurs worldwide in those with excessive alcohol intake and those who are obese (with or without effects of insulin resistance). The condition is also associated with other diseases that influence fat metabolism

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(Reddy and Rao, 2006). Morphologically, it is difficult to distinguish alcoholic FLD from nonalcoholic FLD, and both show microvesicular and macrovesicular fatty changes at different stages.

Accumulation of fat may also be accompanied by a progressive inflammation of the liver (hepatitis), called steatohepatitis. By considering the contribution by alcohol, fatty liver may be termed alcoholic steatosis or nonalcoholic fatty liver disease (NAFLD), and the more severe forms as alcoholic steatohepatitis (part of alcoholic liver disease) and Non-alcoholic steatohepatitis (NASH).

Fatty liver (FL) is commonly associated with alcohol or metabolic syndrome (diabetes, hypertension, obesity and dyslipidemia), but can also be due to any one of many causes such as Metabolic (Abetalipoproteinemia, glycogen storage diseases, Weber-Christian disease, acute fatty liver of pregnancy, lipodystrophy), Nutritional (Malnutrition, total parenteral nutrition, severe weight loss, refeeding syndrome, jejunoileal bypass, gastric bypass, jejunal diverticulosis with bacterial overgrowth), Drugs and toxins (Amiodarone, methotrexate, diltiazem, expired tetracycline, highly active antiretroviral therapy, glucocorticoids, tamoxifen, environmental hepatotoxins e.g., phosphorus, mushroom poisoning) and Other (Inflammatory bowel disease, HIV, hepatitis C (especially genotype 3), and alpha 1-antitrypsin deficiency) (Angulo, 2002; Bayard *et al.*, 2006; Valenti *et al.*, 2006).

The aim of present study was to evaluate the protective effect of vitamin E in prevention of non-alcoholic fatty liver syndrome in rats.

MATERIALS AND METHODS

Present study is experimental intervention types of study. In this study, 90 male Wistar rats (220–250 g and 2-3 month age) were selected for the study and were purchased from Animal House, Islamic Azad University and randomly divided into five equal groups: group1; received high fat diet; Group 2 received high fat diet plus Clofubrate as positive control group at a dose of 320 mgkg-1/day as emulsion in methylcellulose 0.5% (2ml/kg) for 4 weeks through gastric gavage; Group 3 till 5 received high fat diet plus vitamin E at a dose of 100, 200 and 300 mg/day, respectively. Animal care and experiments confirmed with the Guide for the Care and Use of Laboratory Animals of China and approval of the ethics committee of Islamic Azad University was obtained before the commencement of the study. The animals were housed under standard environmental conditions (23±1°C, with 55±5% humidity and a 12 h light/12 h dark cycle) and maintained with free access to water and a standard laboratory diet ad libitum. For induction of NAFLD, the routine method presented by Liang *et al.*, (2006) was used. In this method, animals were received high fat emulsion at a dose of 10 ml/kg daily for 4 weeks through gastric gavage. Also, animals of control group were received methylcellulose 0.5% at a dose of 2ml/kg.

At the end of the period, blood samples were obtained from retrobulbar sinus for measurement of some biochemical factors such as SGOT, SGPT (Reitman and Frankel, 1957), ALP (Kind and King, 1954), NEFA, TAG, Albumin, Glucose and Total proteins (Lowry *et al.*, 1951). Blood samples were centrifuged at 2500rpm for 15 min at 30°C then serum was separated.

At the end, the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA), version 13.0, was used for statistical analysis. All data are presented as mean ± SEM. Before statistical analysis, all variables were checked for normality and homogeneity of variance by using the Kolmogorov-Smirnoff and Levene tests, respectively. The data obtained were tested by ANOVA followed by Tukey's post-hoc multiple comparison test. $P<0.05$ was considered statistically significant.

RESULTS

Data obtained from measurement and analysis of parameters is given in tables. Based on data showed in tables it comes that the numerical value of parameters with exception of LDL has been affected by vitamin E different doses. Comparative diagrams also are given which indicate a significant protective effect of vitamin E (Figure 1-6).

Table 1: The Mean±SD of parameters at the time 0 of study

Parameter / Group	NEFA (µEq/L)	Glucose (mg/dl)	Albumin (g/dl)	VLDL (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
1 (high fat diet)	880.75±30.32	37.25±3.8	3.49±0.6	21.75±3.3	79.75±7.6	65.25±14.6
2 (HFD+Clofubrate)	759.75±32.52	44.75±2.5	3.57±0.2	21.75±1.7	83.00±5.5	44.00±4.5
3 (HFD+Vit E 100)	747.50±13.52	36.25±2.5	3.55±0.5	15.25±3.3	82.00±4.0	45.50±4.7
4 (HFD+Vit E 200)	725.00±12.02	35.75±1.7	3.55±0.4	19.50±2.6	74.00±3.3	41.00±4.0
5 (HFD+Vit E 300)	704.75±7.41	36.00±2.1	3.30±0.3	14.75±3.5	66.00±14	45.50±9.9

Table 2: The Mean±SD of parameters after 30 days

Group \ Parameter	NEFA (µEq/L)	Glucose (mg/dl)	Albumin (g/dl)	VLDL (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
1 (high fat diet)	961.33±110.4	32.83±5.4	1.94±1.5	15.16±3.1	57.16±34	100.83±47.3
2 (HFD+Clofubrate)	756.16±20.17	46.33±21	3.36±0.4	15.50±3.5	79.00±6.1	48.66±5.9
3 (HFD+Vit E 100)	737.00±14.15	39.66±1.8	3.42±0.2	17.33±6.0	80.83±5.7	46.33±7.5
4 (HFD+Vit E 200)	728.83±17.38	38.50±2.5	3.82±0.2	21.16±4.7	78.83±4.9	49.66±6.2
5 (HFD+Vit E 300)	702.83±15.58	35.66±2.1	4.08±0.3	17.66±0.8	72.33±5.4	52.50±5.7

Table 3: results obtained from comparison day 0 and 30 of study using ANOVA

Parameter	F	Significance
NEFA	20.523	0.000
Glucose	1.496	0.183
Albumin	4.383	0.001
VLDL	2.776	0.013
LDL	1.852	0.088
HDL	5.381	0.000

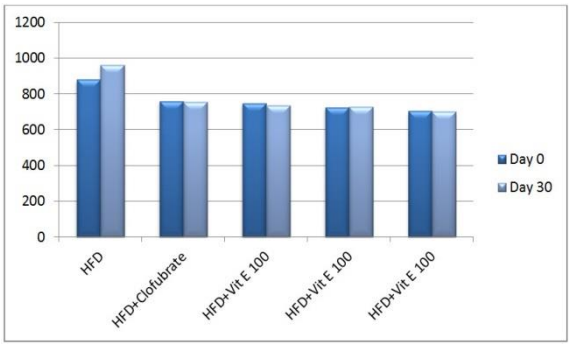

Figure 1: Comparison diagram of day 0 and 30 in term of NEFA

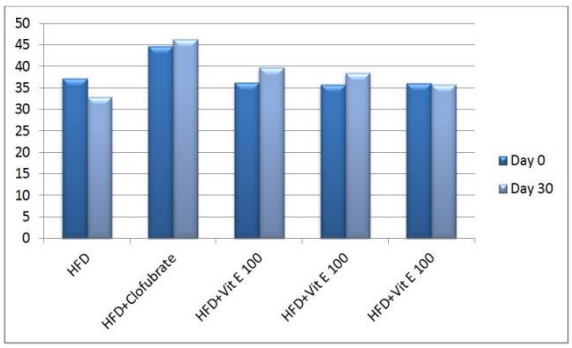

Figure 2: Comparison diagram of day 0 and 30 in term of Glucose

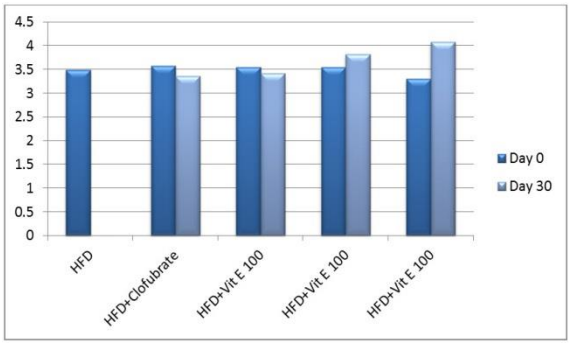

Figure 3: Comparison diagram of day 0 and 30 in term of Albumin

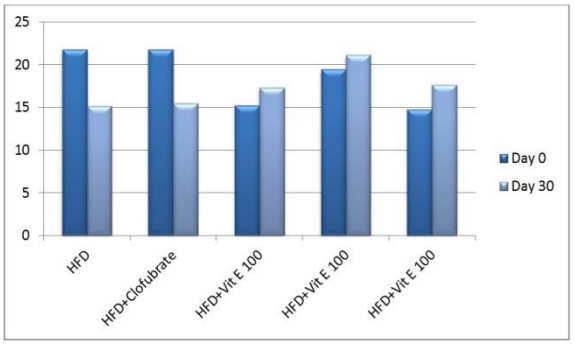

Figure 4: Comparison diagram of day 0 and 30 in term of VLDL

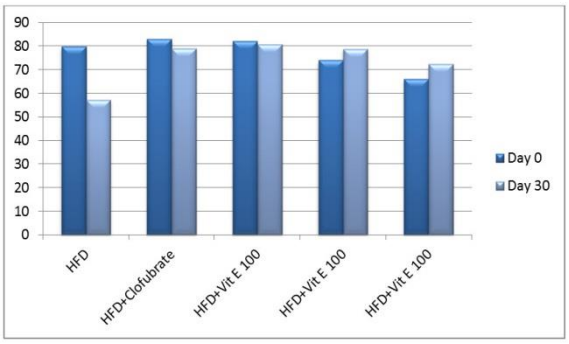

Figure 5: Comparison diagram of day 0 and 30 in term of LDL

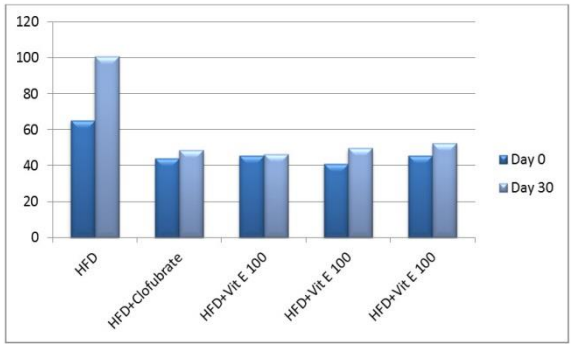

Figure 6: Comparison diagram of day 0 and 30 in term of HDL

DISCUSSION AND CONCLUSION

The liver is the major organ responsible for metabolism, detoxification and secretory functions in the body. Hence, it regulates various important metabolic functions in mammalian systems. Hepatic damage is associated with the distortion of these metabolic functions. The liver tissue is reported to be one of the tissues with a high regenerative capacity (Khan and Mudan,

2007). According to Rabelo et al. (2006) hepatocytes exhibit a very good regenerative response to several stimuli, including massive destruction of hepatic tissue by toxins, viral agents, or surgical extraction. Regeneration of the liver tissues is a result of an organized and controlled response of the liver toward tissue damage induced by toxic agents, chemical agents, trauma, infections, or post-surgery resection which could induce oxidative stress in the liver. Oxidative stress can be defined as an increase in oxidants and/or a decrease in antioxidant capacity. Our data showed a significant effect of vitamin E against FLD in rats. Similar findings were reported by Beytut and Aksakal (2003), where the effectiveness of vitamin E and selenium in reducing hepatic damage in glucocorticoid-treated Wistar rats was confirmed. On the same direction, (Naziroğlu and Çay, 2000), showed that intraperitoneally administered vitamin E and selenium have significant protective effects on the blood, liver, and muscle against oxidative damage of diabetes.

Drug and chemical substances ingested by humans and animals are known to have adverse effects. Among these reported effects is hepatotoxicity. This could not be far from the involvement of the liver in drug and chemical metabolism. Continually chemical agents are being screened for hepatoprotective properties. Vitamin E is one of the agents that have received wide attention due to it reported hepatoprotective effect. Researches with animals showed that vitamin E has hepatoprotective property. It was reported that administration of vitamin E (35 mg/kg body weight) attenuated Lipopolysaccharide (10 mg/kg body) induced oxidative stress (liver damage) by reducing levels of MDA, restoring the levels of glutathione superoxide dismutase and catalase. The modulation of these biochemical parameters led to the amelioration of hepatic architecture (Bharrhan *et al*., 2010).

The ability of vitamin E to reverse or prevent chemical agents induced hepatoxicity was demonstrated by Khalifa *et al*., (2009), he and co-workers showed that 0.2g/kg/ day of vitamin E normalized aspartate aminotransferase and alanine aminotransferase levels elevated by carbon tetrachloride in rats. Liu *et al*., (1995) proved that pretreatment of mice with water soluble emulsion of vitamin E significantly inhibited acute hepatic injury induced by carbon tetrachloride in mice. Fariss, (1991) also reported the hepatoprotective effect of vitamin E on cadmium induced toxicity. Administration of vitamin E with carbon tetrachloride caused marked amelioration of the severity of hepatic alterations in rats induced by carbon tetrachloride. These reports agreed with the findings of Fariss *et al*., (1993). Fariss and coreseachers revealed that administration of 100 mg/kg body weight of hemisuccinate esters of tocopherol to carbon tetrachloride intoxicated rats showed a powerful protective effect against carbon tetrachloride induced hepatotoxicity. Investigation showed that vitamin E at concentrations of 50, 100 and 225 mm produced significant hepatoprotective effect against carbon tetrachloride induced toxicity in liver cells from BRL3A cell line by lowering the leakage of intracellular enzymes, reducing the oxidation of proteins and decreasing incidence of apoptosis (Kamel *et al*., 2010). In conclusion can state that vitamin E has protective effect against fatty liver disease in rats fed high fat diet.

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