Toxicity of Margarine on liver Enzymes (Aspartate amino transferas and Alanine amino transferase) in Rats

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ABSTRACT

Background: Margarine contain trans fatty acids as major sources of dietary free radicals, and oxidative stress. Effect of oral administration of margarine on hepatic enzyme was investigated in Wistar rats.

Methods: Thirty six (36) adult Wistar rats were uniformly divided into three groups of control , margarine 15% and 20%, twelve rats in each groups (6males & 6 females). Margarine was added to the second and third groups chow as 15% and 20% (w/w) respectively. After four months, their blood samples were obtained and the serum levels of Aspartate Amino Transferase (AST) and Alanine amino transferase (ALT) were measured.

Results: Serum levels of AST and ALT in margarine groups were higher than control groups, but it was not significant. Treatment of rats with margarine were not significantly alter the serum levels of liver enzymes in all groups.

Conclusion: This result suggests that consumption of margarine may not be toxic at the doses investigated.

Keywords: Alanine Aminotransferase, Margarine, Aspartate Aminotransferase.

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INTRODUCTION

Fats are important constituents of our diet and, beside their functions as sources of energy and carriers for fat-soluble vitamins, they supply essential fatty acids that are vital components of cell membrane (1). Widespread use of partially hydrogenated vegetable oils containing large amounts of trans fatty acid has raised questions concerning the biological effects of consumption of significant amounts of these isomers (2). It is well known that saturated fats are the strongest method of raising serum cholesterol. In order to avoid butter which is very high in saturated fat, we naturally turn to margarines which are made of vegetable oils which have no significant amounts of saturated fat or cholesterol. The problem with margarine is that the wrong ones can have the same effect as saturated fat, depending on how hard they are. In order to make the vegetable oils hard enough to spread on bread, hydrogen must be added. The more hydrogenation, the more saturated and the more atherogenic they are. The stick margarines are the hardest. Unfortunately, the more hydrogenation, the more it produces atherogenic trans fatty acids (3). Trans fatty acids, or trans fats are unsaturated fats produced through partial hydrogenation, when vegetable oils are heated in the presence of metal catalysts and hydrogen (4). Hydrogenation increases the shelf life and stability of fatty acids and foods containing them. During partial hydrogenation, some double bonds between carbon atoms remain but may shift to a different position along a chain and alter their configuration from cis to trans fats (5). Intake of trans fats has been shown to increase the risk of heart disease by raising low density lipoprotein cholesterol levels and lowering high density lipoprotein cholesterol levels (6). The effect on triglyceride and LP(a) lipoprotein levels is also greater than that of saturated fats. High blood levels of LP(a) lipoprotein have been independently

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linked with an increased risk of coronary artery disease (CAD): trans fats may also have other adverse effects on thrombogenesis through altered prostaglandin balance (7). A recent observational study for short-term randomized trial indicated that trans fatty acid intake increases systemic inflammation in generally healthy persons (8,9). Biomarkers of trans fatty acid consumption have been associated with increased risks of heart and colon cancer, although no studies have examined their association with liver disease. Little is known about the effects of using stick margarine on liver, although they have been associated with increased risks of breast cancer (10,11) and cardiovascular disease (12,13). This study examined relationship between oral administration of margarine on hepatic enzymes in Wistar rat liver.

MATERIALS AND METHODS

Animals

Thirty six (36) adult wistar rats (both sex) weighing 250-300 g born and bred in the experimental animal center of Arak University of Medical Sciences were divided into three groups of control, margarine 15% and margarine 20%. Twelve rats in each group, six males and six females. Rats were housed in groups of three animals of the same sex in cages, under standard condition of temperature (24±2°C) and relative humidity (46±5%) with a 12 hour light-dark cycle and adequate ventilation. Body weight of each rat was recorded in the first and last day of experiment. Care of laboratory animals was in full compliance with the Guide for the care and use of laboratory animals (National Research Council, 1996), and protocols were approved by the ethical committee at Arak University of Medical Sciences.

Diet

The control group was fed on a commercially available pelleted diet (Pars animal feed company), which contained carbohydrate 60% (w/w), fat 2% (w/w), protein 17.5% (w/w) and fiber 8% (w/w) about 3.28 Kcal/g, while the second and third groups were fed on the same diet supplemented with stick margarine 15% and 20% (w/w) respectively for four months. Margarine was added to diet daily and excess pellet were removed after 24 hours. All groups had free access to drinking water.

Collection Of Blood And Analysis

After 12 hours fasting, the animals were anaesthsized by intrapretoian injection of ketamin (20mg/100g). Blood was obtained directly from right ventric of heart into sample tubes, allowed to stand for one hour and then centrifuged at 2000 g for 10 minutes to separate serum from the blood cells. The blood serums activities of liver enzymes (AST &ALT) were assayed using laboratory kits obtained from Randox laboratory Ltd., United Kingdom and absorbance were read using a Uv -Vis spectrophotometer.

Statistical Analysis

Data are presented as mean±SD and analysed for normality of data by one sample K-S, statistical significance among groups means by one way analysis of variance (ANOVA) and among male and female in each group by T-Test, with the help of a software SPSS 16.0 for windows. A p-value less than 0.05 was considered statistically significant.

RESULTS

In the present study, effect of margarine consumption on liver enzyme activities in serum of young rats was compared between control and margarine treated rats (15% & 20%).

Our result was shown no significant change in terms of change in mean body weight, food and water intake was observed between rats treated with margarine and control rats (data not shown). The activities of ALT and AST in control group and margarine treated animals(15% and 20%) is shown in Table1.

Table 1: Activities Of Alt And Ast In Experimental Animals

Parameter	Sex	control	Margarine 15%	Margarine 20%	P value
ALT (IU/L)	Male	24.25±2.50	26.10±2.05	27.10±2.69	0.15
	Female	24.24±3.84	24.50±3.08	25.96±2.50	0.62
	Total	24.26±3.09	25.30±2.63	26.53±2.55	0.15
AST (IU/L)	Male	29.46±5.77	31.56±1.74	33.26±2.62	0.25
	Female	24.26±4.64	24.30±1.00	24.68±0.95	0.96
	Total	26.86±5.68	27.93±4.03	28.97±4.86	0.53

Table 2: Comparison Of Alt & Ast Activities Between Male And Female In Each Group

Parameter	Groups	Male	Female	Pvalue
ALT (IU/L)	Control	24.25±2.50	24.24±3.84	0.9
	Margarine15%	26.10±2.05	24.50±3.08	0.3
	Margarine20%	27.10±2.69	25.96±2.52	0.4
AST (IU/L)	Control	29.46±5.77	24.26±4.64	0.1
	Margarine15%	31.56±1.74	24.30±1.00	0.001
	Margarine20%	33.26±2.60	24.68±0.95	0.001

The data were shown, oral administration of margarine (15% and 20%) has been increased the activity of these enzymes, and serum activities of ALT and AST in all margarine treated groups, were higher than control, but differences were not significant (P>0.05), apparantly margarine consumption, did not cause any abnormal changes and significant liver damage. Although none of the higher levels were statistically significant (P>0.05), but increases recorded for each parameter were dose dependent.

In Table 2. we compared activity of ALT and AST between male and female rats, in control and margarine treated rats. ALT activities in control group, approximately was the same in male and female rats. Activity of this enzyme has been increased in both sexes as long as margarine consumption but the difference between them were not significant.

AST activities in male rats were higher than female rats in control group (not significant), with margarine consumption (15% and 20%) AST activities has been increased in both groups, but level of AST activities in male rats, significantly were higher than female rats in the same group (P-value<0.05).

DISCUSSION

We have recently examined the effect of margarine on oxidative stress markers in young male rats, and reported that margarine intake has a positive effect on the markers of oxidative stress (14). A number of tissue enzymes are valuable tools as diagnostic agent. The application of blood serum or plasma enzymes as markers to measure organ damage, cell damage, enzyme indication, activation or inhibition of enzymes becoming very common in toxicity studies. A variety of blood biochemical measurements could be used to evaluate the severity of tissue damage, possible target organs and measure impaired organ functions. With the combination of these tests, we can evaluate a broad range of information on their physiological and their metabolic functions. The aim of this study was to evaluate the effect of margarine on the activity of serum AST and ALT. Occurence of a number of biochemical reactions and continuation of life is all

supported by enzymes. For this reason, changes that occur in enzyme activities are considered to be as an indicator of the health of an organism. Liver is the terminal controlling organ of the metabolism. By measuring the metabolic activity of liver, determination of functional events could be estimated.

Because hepatic injury is often associated with alterations in the serum and liver levels of some enzymes notably ALT, AST and ALP (15). ALT and AST are two of the most reliable markers of hepatocellular injury or necrosis. Their levels can be elevated in a variety of hepatic disorders. Of the two, ALT is present mainly in the cytosol of the liver and in low concentrations elsewhere and it is a more specific indicator of liver inflammation than the AST, as the AST may also be elevated in diseases affecting other organs (16). AST can be identified to some extent in the heart, skeletal muscles, kidneys, brain, pancreas and blood cells, it is found in both mitochondria and cytoplasm (17,18). The cause of this rise in ALT and AST remains unclear and may be multifactorial.

ALT and AST were demonstrated to be constituents of liver tissue. Any damage to this tissue as a result of lipid accumulation or fatty infilteration could result in a significant increase in serum values becoming a valuable diagnostic tool for cardiovascular disease. Hepatic leakage enzymes are usually cytosolic enzymes. Altered permeability of hepatocellular membrane caused by injury on liver results in the release of soluble cytosolic enzymes into blood (19). They are generally escaped through basal-lateral side of hepatocytes facing the sinusoids which causing the elevation in blood (20). Those enzymes are release into blood from the cytosol and subcellular organelles of hepatocytes once liver is injured or damaged. An induction of certain hepatic enzymes is commonly associated with the development of liver damage. In this finding, there was no significant change in serum ALT and AST and liver functions are not deranged from normal range at these dose levels, therfore, margarine at these dose can be given safely to patients suffering from hepatitis, but it seems margarine consumption higher than these, can be harmful for hepatic patients and can cause liver damage.

CONCLUSION

Margarine was not significantly altered serum ALT& AST levels at lower doses and as the liver functions were not deranged, margarine can be safely given to patient suffering from liver disease, but it may be toxic in higher dose.

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