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INVESTIGATION ON THE TISSUE AND ENZYMATIC CHANGES IN LIVER AND SERUM LEVELS OF CALCIUM AND PHOSPHOR IN RATS, FEEDING WITH GERMAN SAUSAGES

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ABSTRACT

Background: The existence of chemical additives in foodstuff is particularly important. The nitrite used in processing meat products is related to nitrosamine formation. Most nitrosamines cause liver cancer. The purpose of this study is an evaluation of the effects of eating German sausages on the liver, and also on the ALT, AST, Alkaline phosphatase enzyme levels, calcium, phosphorus serum levels in rats' blood.

Materials and methods: 20 Wistar rats were randomly selected and divided into two groups that each group consisted of 10 rats. One group was considered as the control group. After 40 days, the rats in the two groups were examined macroscopically, in terms of serum levels of liver enzymes and blood factors. The findings showed that there was no significant change in ALT or AST enzyme levels or in the serum levels of calcium, phosphorus ($p>0.05$). Only the serum levels of Alkaline phosphatase enzyme increased significantly ($p<0.05$). Macroscopically, the liver enlarged ($p<0.05$). So, the macroscopic examination didn't show a significant change in the studied tissues ($p>0.05$).

*Conclusion :*Based on the findings of this study, it seems that, except for increasing the liver weight and volume, feeding on German sausages did not have a significant effect on ALT, AST enzyme levels, calcium or phosphorus, in the short run, i.e. 40 days and there was no sign of tissue destruction despite the increase in Alkaline phosphatase enzyme level.

Keywords: Rat, German sausages, Chemical additives, Nitrosamine, Tissue changes, Enzymatic changes

INTRODUCTION

The liver is the largest internal organ and gland. Its weigh is about 1.5 Kg and is brownish red in the human body. The foodstuff absorbed into the gastrointestinal system are processed and stored for further use by other parts of the body in the liver. The liver is also the key organ for metabolism and secretion continuously detoxifying foreign compounds such as environmental and chemical pollutants [6].

Aspartate aminotransferase is also called glutamate oxaloacetate transaminase (SGOT). This enzyme connects the amine agent of glutamic acid onto oxaloacetate and produces alfacetoglutaric acid and aspartic acid or reverse order which is seen in all animals' serums and tissues [22]. In very high concentrations, it is distributed in heart, liver, muscle, kidney tissues and red cells, and the damages suffered by each of those tissues will lead to the liberation of enzyme into the plasma. This enzyme exists in cells' cytoplasm and mitochondria [1,18]. Alanin aminotransferase is also called glutamate pyruvate transaminase (SGPT). The amine agent transfers glutamic acid onto pyruvic acid and makes alanin and alpha glutamic acid or reverse order. It exists in high amounts in the liver and in smaller amounts in muscles. It is also useful in diagnosing liver cellular destruction. This enzyme enters the blood as a result of liver pathologies; hence, it is called the special liver indicator [2,9]. Serum ALT levels can be higher than those of AST in some cases of severe hepatitis and liver damages from drugs and chemicals [13].

ALP (alkaline phosphatase) includes isoenzymes derived from liver, bones, intestines and placenta that perform phosphatase hydrolysis in alkaline pH usually 9 to 10.5 [2,9] and cause phosphomonoesthers' hydrolysis to alcohol and mineral phosphate [12]. These enzymes are distributed vastly in different tissues. The enzyme itself and related isoenzymes can be measured through different methods. Isoenzymes are found in various tissues, such as bones, liver, kidneys, intestine walls, milk glands and placenta. [2,18].

2. MATERIALS AND METHODS

In this study, 20 similar albino rats has been selected that the weight of each of them was 150±5 g. The rats were randomly divided into two 10 member groups. A special ration was considered for each group. One group (experimental group) was only given German sausages manufactured by Buffalo Co, and the other group (control group) was given standard rat food as ready pellets for 40 days.

The rats were kept in individual cages at 22±2^{o}C for 12 hour in light and dark cycle with free access to food and water in the laboratory animals section of University of Isfahan. On 40th day, in order to prepare the rats for surgical operation, anesthesia was carried out by injecting regular Xylazine (Alphasun, Netherlands) and Ketamine (RotexMedica, Germany). For pre-anesthesia, Xylazine was injected at 5 mg per kg body weight into the thigh using an insulin-size syringe. To induce anesthesia, Ketamine was injected at 40 mg per kg body weight based on the pre-anesthesia substance [11]. Then, blood was directly taken from the rats' hearts, placed in special tubes containing EDTA (ethylene diamine tetra acetic acid) and transferred to the lab for hematological tests. After making sure of the their death, the rats were dissected and their livers were extracted, weighed, placed in formalin 10% and sent to the pathology lab for sectioning and microscopic examination. Tissue samples of lesions were prepared and placed in buffer formalin 10% in special containers bearing numbers and sent to the lab. After coloring by Hematoxylin-Eosin method, the samples were examined by optical microscope. In this experiment, 6 rats were dead.

Data Analysis

After examining colored samples, the data from Nikon LM study were evaluated through statistic analysis. All the tests were done with 95% confidence. The independent sample t test was used for testing the hypotheses.

3. RESULTS

The findings showed that there is no significant change in ALT and AST enzyme levels or calcium, phosphorus serum levels ($p>0.05$) but only Alkaline phosphatase enzyme level increased significantly($p<0.05$). The microscopic examination did not show a significant change in the studied tissue. ($p>0.05$). Microscopically, the liver enlarged ($P<0.05$) (Table 1)(Table 2).

As seen in figures 1 and 2, feeding on sausages significantly changed the average weight of liver and Alkaline phosphatase level.

Table 1.Group Statistics

Group Statistics

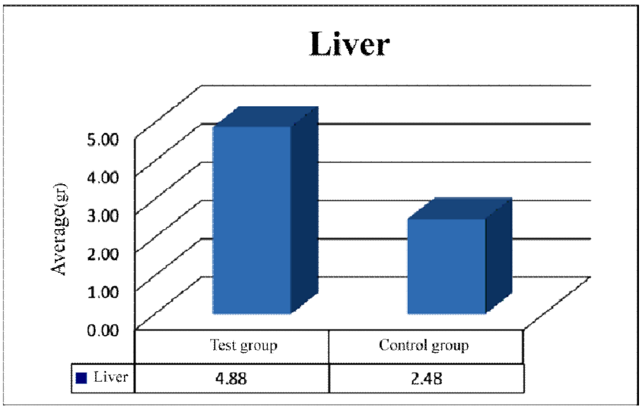
Variables	N	Mean $_{T*}$	Mean $_{C*}$
Liver weight	7	4.8829 (gr)	2.4786(gr)
AST	7	125.4285(U/L)	112.2857(U/L)
ALT	7	26.7143(U/L)	11.5714(U/L)
Alkaline phosphatase	7	6.5886E_2(U/L)	2.7171E_2(U/L)
Phosphorus	7	10.2571(mg/dl)	10.6286(mg/dl)
Calcium	7	9.3714(mg/dl)	9.6000(mg/dl)

T=Test C=Control

Table 2: Independent t test to examine tissue and serum effects after feeding on sausages

Variables	T	Df	Sig. (2-tailed)
Liver weight	8.100	12	0.000*
AST	-0.247	12	0.809
ALT	1.617	7.765	0.146
Alkaline phosphatase	3.678	12	0.003*
Phosphorus	-0.149	12	0.884
Calcium	-0.195	12	0.849

*Sig=Significant



Fgure 1. Mean of liver weight in control and experimental groups

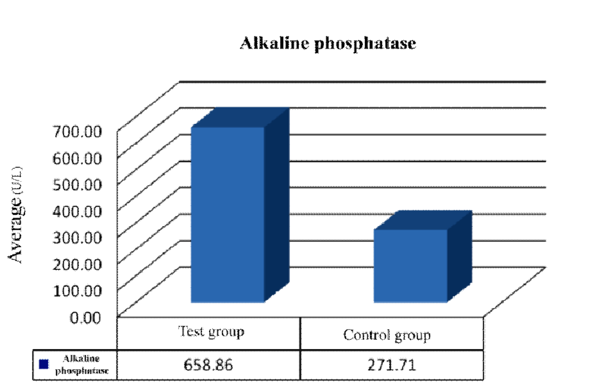


Figure 2: Mean of Alkaline phosphatase in control and experimental groups

3.1. DISCUSSION

Additives are used to keep foodstuff for a long time without changing of color, taste, smell, nutritional value, etc. Among additives are nitrate and nitrite which are usually used to fix color in lean meat tissues, contribute to the taste of processed meat, prevent the growth of spoilage microorganisms or food poisoning and postpone pungent tastes resulting from fat oxidation [10, 14, 15, 16]. One of the most important issues of additives (especially nitrate and nitrite) is their carcinogenicity [7,8,15]. A study on the effects of additives and pasteurization on nitrosamine standard levels showed that sodium ascorbate and sodium chloride reduce volatile nitrosamine amounts and also sodium ascorbate is more effective in doing so in addition to the fact that polyphosphates act as catalysts on nitrosamine formation. Pasteurization increases nitrosamine analysis rates. According to standard definitions, meat products are those products with meat that are constituting at least half their raw materials and fat, water, ice, sodium chloride, alkaline phosphates, sodium nitrite and other additives constituting the remaining half [5,17]. Self-induced oxidation of fat in meat products is an important cause of taste and quality change of these products [19]. This type of oxidation is due to free fatty acids or triglycerides containing such acids [3]. Oxidative stress was first introduced by Sies et al. in 1985 who suggested oxidative stress was due to imbalance in prooxidant/antioxidant system [20]. Barbuio et al. (2007) showed that oxidative stress contributes to steatosis-to-steatohepatitis transformation [4]. Artificial antioxidant compounds such as BHA (butylated hydroxyl anisole), TBHQ (tertiary butyl hydro quinine), BHT (butylated hydroxyl toluene) can produce low energy free radicals through resonance hybrids due to their phenol structure which will lead to more stability of products [21].

4. CONCLUSION

All aforementioned discussion about carcinogenicity of additives especially nitrosamines and artificial antioxidant compounds and their criticism by experts, despite significant increases in Alkaline phosphatase serum level and enlargement of weight gain in liver that was due to feeding on sausages for 40 days. Also ,the results of this study showed no trace of tissue destruction. There wasn't a significant change in Calcium, Phosphorus, ALT or AST enzymes.

REFERENCES

1. Andrewly, Loscalzo, M. Arjmand, 2004, Digestive diseases, liver and biliary ducts. Tehran.

2. Mohammady, A. , 2006. Survey of effects of PycnocycalSpinosa on enzymes of rat' s liver. Tehran university, page 12

3. Allen, J. C. and R.J. Hamilton. 1989. Rancidity in Foods. 2nd ed., Elsevier Applied Science, New York.

4. Barbuio R, Milanski M, Bertolo MB, Saad MJ, Vellosa LA. 2007, Infliximab reverses steatosis and improves insulin signal transduction in liver of rats fed a high-fat diet. *J Endocrinol*; 194(3): 539- 50.

5. Carascosa, A., etal.1999. Application of factorial analysis to Spanish dry – cared ham processes. P.868, trial. *Journal of Clinical Nutrition*.,84(6): 1543-1548.

6. Fook-Hong, N., Kai-Chu, CH., Chi-Sing, CH., Carolyn, K., Wing-Fung, N., and Chun-

YU, W., 1999. High a fetoprotein level in HCV-related nodular liver cell dysplasia.The American Journal of Gastroenterology. 94. 2269-2298.

7. Harold, B.H; Wood, G., Jay, D., Deoevr, P ,1994,. Principles of meat science, third edition, Kendall/Hunt publishing company, pp: 133-162.

8. Hauser, E; Yam,I.,Yerman,P ,1980. Nitrosamine in lebensmitteln, Swiss Food,2, pp: 13-22.

9. Jemery, kn., 1996, clinical Diagnosis and management by laboratory methods (19^{th} edition), WB sounders co, page 276-280.

10. Jen-Kun-Lin; Jay,K.,1994,. Hepatotoxic actions of dietary amines. Toxicology and Ecotoxicology News; 1(3) 82-86.

11. Khorasgani A, Karimi AH, Nazem MR. 2010, A comparision of healing effects of propolis and silver sulfadiaZine on full thickness skin wounds in Rats. Pak vet J. 30(2): pp:72-740.

12. Kolder. G., 1983, methods in laboratory medicine, clin enzyme.

13. Marc, G., Sturgill and George, H., 1997, Xeno-biotic-induced hepato toxicity: mechanisms of Liver Injury and methods of monitoring hepatic function, clinchem, page 1512-1526.

14. Muller, D.W;1991,. Curing and smoking, are the healthier processes today than they used to be.Flerschwirtsch, 71(1), 61-63.

15. Pearson A. M. and Tauber F.W. 1984; Processed Meat,2nd ed. AVI Publishing Company.London, 46-67, 69-81, 352-354.

16. Polic, M; 1994,.Technological and health aspects of using nitrite in meat industry, Technology.J. Mesa, 35(1/2) 25-28.

17. Potthast , K. 2001 . Chemie der fette and fettbegleitstoff . P.292

18. Ramaiah, SH. K., 2007. A toxicologist guide to the diagnostic interpretation of hepatic biochemical parameters.Food and Chemical Toxicology. 45, 1551-1557.

19. Saleemi, Z.Q.,P.K. Juanita, P.D. Wanasundara and F. Shahidi. 1993. Effects of Low- Pungency ground mustard seed on oxidative stability, cooking yield and odor characteristics of comminuted pork. J. Agric. Food Chem. 41,641-643.

20. Sies H. 1985, Oxidative stress introductory remarks oxidative stress. New York Academic Journal, 5: 1– 8.

21. Stiebing , A and Roedel ,W. 2003 . KontinuierlichesMessenderoberflaechen – wasseraktivitaet von Rohwurst . P.432

22. Tietz, NW. Burtis, CH, Edward, R, 1994, clinchem (2^{nd}ed), w. B. s, company Philadelphia, page 788-791.