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Evaluation of the protective effect of vitamin C on hepatic damage caused by formaldehyde in rats

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ABSTRACT

Formaldehyde (methanol) is a chemical substance, with nasty smelling which is normally used to fix cadavers and histological process. The main use of formaldehyde is in production of synthetic resins, wood-plastic products and fiber industry. Formaldehyde has a negative impact on performance of organ system with oxidative stress in the body .specially on the hepatic. Some studies have demonstrated that antioxidants such as vitamin C can play an important role to protect from damage caused by harmful substance. The aim of this study was to evaluate the protective effect of vitamin C on hepatic damage caused by formaldehyde in rats.This study included 24 adult male rats with weighing 250-300 gr. rats were randomly divided into 3 groups each comprising 8 rats. 1- Control group: exposed to normal saline (1cc/kg per day for 10 days) that injected intraperitoneally, 2 -groups E1: exposed to formaldehyde 10mg/kg per day for 10 days, 3 - group E2: simultaneously exposed to vitamin C (200mg/kg) per day for 10 days& formaldehyde 10 mg/kg intraperitoneally 10 days. At the end of the exposure period, the mice were placed under anesthesia and surgery. The blood samples were taken for isolation of serum and measurement of AST and ALT in.In this study, AST and ALT levels of 24 adult rats in the all groups were measured and the results were compared. The amount of AST in the all groups was significant (P <0.05). The amount of AST in group E2 compared to controls was significant (P = 0<0.05) and the amount of AST in group E2 compared to E1 was not significant (P = P>0.05). Also, comparison of the results showed significant difference in serum ALT in third group. Serum ALT level in E1 compared to control group was significant (P <0.05) and ALT in group E2 compared to control group was significant (P <0.05). The amount of ALT in group E2 compared to E1 was not significant (P = 0.847).According to this study, intraperitoneal administration of formaldehyde at a dose of 10 mgr/kg per 10-day can increase AST and ALT and vitamin C at a dose of 200 (mg / kg) per day for 10-days can reduce amount of AST and ALT in adult rats exposed to formaldehyde

KEY WORDS: FORMALDEHYDE, AST, ALT, LIVER

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INTRODUCTION

Formaldehyde is a chemical material with a nasty smell, which is used in fixing cadavers, histological processes, synthetic resins, wooden and plastic productions and industrial fiber production. It has a negative effect on performance of body organs. It is metabolized to formic acid by dehydrogenase formaldehyde and dehydrogenase aldehyde enzymes in the liver and erythrocyte which is exorcised through urine, excretion and breathing with different macromolecules such as protein, acid nucleic or interact with the light molecules like amino acid. Formaldehyde can cause oxidative stress in the body and has a bad effect on respiratory system and blood circulation and liver (Golalipour et al., 2007 Mendis et al., 2007, Cheng et al., 2003, Collins and Lineker 2004 Kini et al., 2004). Formaldehyde was known as a harmful factor for liver cells in the different studies. Antioxidants are such components that help body to destroy free radicals oxidative stress is actually imbalance between oxidants and antioxidants. When the amount of oxidants increases, the cells are damaged. Antioxidants include Vitamin A, E, C, Zn and selenium, which play a crucial role on inhibition of free radicals and stability of cell membrane, (Kini et al., 2001, Gupta et al., 2004, Woolaqrd et a; 2002).

Vitamin C (Ascorbic Acid) is a white or yellow odorless solid substrate with the molecular formulation of C6H806. Ascorbic Acid is produced in the liver of plants and animals (except some special kind and human). Vitamin C operates as antioxidants in the body and cause acceleration of Fe, Cu and also revives of folic acid and collagen making. Vitamin C as a soluble antioxidant becomes active by moving oxygen and nitrogen elements. The role of vitamin E as a protective agent on liver damage caused by formaldehyde in the previous studies was approved. The role of A,E,C Vitamins as protective antioxidants on liver hepatotoxicity was also investigated. It is possible to evaluate liver function by checking the indicators in the blood, (Kum et al., 2011, Kini et al., m 2011, Djeffal et al., 2011, Ememghorashy et al., 2012).

With regard to the formaldehyde effects in making oxidative stress, free radicals and their effect on liver performance and in accordance with the performed studies related to the formaldehyde on the liver tissue changes as glomerular vascular congestion and also minor decline holes in the pipe cells and studies about the protection effect of Vitamin C on prevention of formaldehyde damage, in this study, we decided to investigate the protection effect of Vitamin C on liver performance of rats after exposing to formaldehyde.

METHODOLOGY

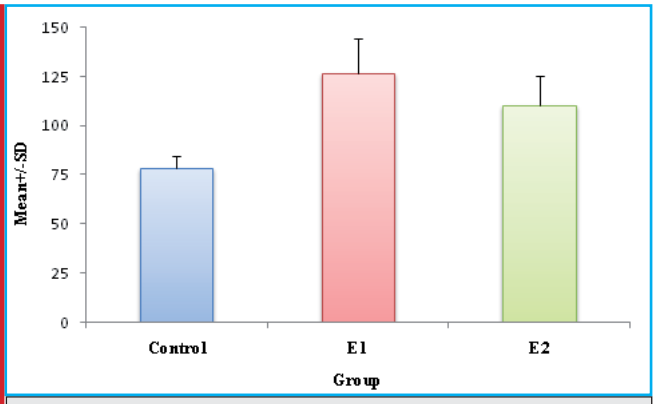
In this study 24 adult rat of Wistar bread were selected and divided into 3 equal groups. The first group (c) received 1cc/kg of normal saline and the second group (E2) received 10 mg/kg formaldehyde and the third group (E3) received both 10mg/kg formaldehyde and 200 mg/kg Vitamin C in 10 days by injection into peritoneal. During the study, the rats were under normal condition of shelter with 24±2 temperature and appropriate feeding. 3 weeks after finishing the injection phlebotomy, after anesthesia by sterile syringe was performed. After making flocculation by centrifuge, samples were separated with 1500 rpm during 10 minutes and they were kept in -20 c till testing for evaluation of ALT and AST. The amount of ALT and AST were measured by using Pars Azmoon kits and auto analyzer tool (BiotecticaInstrument BT 1000).

Data analysis was performed after entering the data to SPSS Ver.20program and to investigate normality of cantinas quantitative data distribution, Shapiro-wilk test was conducted. The explicit result of the groups was reported as average and deviation from standard deviation. The comparison between the investigated groups was performed by using ANOVA test. If the average differences of statistic results were meaningful the LSD test were used to compare them. The meaningful level was considered less than 0.05 ($P<0.05$).

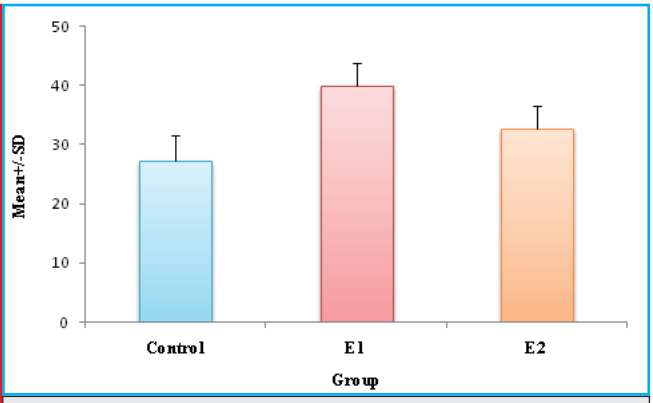
RESULTS AND DISCUSSION

In this study, 24 adult male rats of Wistar bread were involved. Among them 8 rats (33%) considered as control group, 8 rats (33%) considered as test group 2 (E2) and another 8 rats (33.3%) as test group 2 (E2). Descriptive factors and the comparison between the weights of rats among 3 groups of control E1, E2 was shown in the table 1. In order to compare AST level in 3 groups of control (c) E1,E2. Variance analysis test (ANOVA) was conducted and in order to compare 2 group's level of ALT, the LSD test was came out. Descriptive specifications and the comparison between ALT levels of three groups include group c, E1, E2 were listed in the table 2.

According to the comparison of AST level, it was suggested that there is a significant difference in 3 groups of control, E1, E2 ($p<0.05$). Also, with regard to the 2 groups comparison, The difference between the AST level of group c and group E1 was meaning ($p<0.05$) and the difference between AST level of control (c) and test group (E1) was also meaningful ($p<0.05$). However, there is not a meaningful difference between AST level of study group E1 and E2 ($p>0.05$) (plot 1). In order to compare, ALT level in 3 study groups of control (c), E1,



PLOT 1. AST level average plot for 3 study groups of control (c), Test 1 (E1), Test2 (E2)



PLOT 2. ALT level average plot of 3 study groups of control (c), Test 1 (E1), Test 2 (E2)

E2. The one way variance analysis (ANOVA) was conducted and in order to compare ALT level between 2 groups, the LSD test was used.

Descriptive specifications and the comparison of ALT level among 3 study groups of control (c), test 1 (E1) and test 2 (E2) was shown in the table 3. According to the comparison of three groups, the ALT level has a meaningful difference ($p<0.05$). It was also suggested that there is a significant difference between ALT level of control group (c) and test 1 group (E1). ($p<0.05$) and ALT

Table 1. Rats weight in 3 study groups of control (c), E1, E2

Specification	P-Value			
	Control	Test 1	Test 2	
Weight (gr) Standard deviation ± Mean Range	214,37 ± 11,01 202-235	212,12±2,79 208-217	215,5±3,96 211-222	0.62

Table 2. AST amount of 3 study groups of control (c), Test 1 (E1), Test 2 (E2)

specification	Group			Comparison among groups	
	Control	E1	E2	Statistic F	P-Value
AST Mean±SD Range	78.5±5.75 68-86	126.5±18.08 96-145	110.75±14.81 86-136	24.78	<0.001

Table 3. ALT among 3 study groups of control (c), Test 1 (E1), Test 2 (E2)

specification	Group			Comparison among groups	
	Control	E1	E2	Statistic F	P-Value
ALT Mean±SD Range	27.25±4.3 68-86	39.87±4.01 32-44	32.62±3.96 28-38	19.14	<0.001

level of C and E2 group ($p<0.05$) and also between E1,E2 groups ($p<0.05$).

Formaldehyde can affect the body organs due to creating free radicals. Formaldehyde can also affect the kidney performance such as excretion of waste substances. Antioxidant can prevent the harmful effect of formaldehyde . Vitamins are one of the most important antioxidants – especially Vitamin C – which play an effective role in protection against oxidative damage caused by free radicals . On the other hand, this Vitamin can enhance the activity of antioxidant enzymes in the liver tissue,(Claudia et al., 2003, HaiXia et al., 2012, Ukmali and Armutcuf 2011, Sajadi et al., 2008, Shang et al., 2014).

18-WILSON ET AL., 2014)

According to the above description, 24 rats of Wistar bread were involved in this study with regard to the previous studies and the ability and facility of laboratory and human resource present in the research center. This study was similar to the study of Ukmali et al. (2011). Histology of liver cells was just investigated and no treatment was suggested to prevent formaldehyde effect. In the study of Ukmali some evidences were shown about inflammation in the liver cells .

On the other hand this study was similar to that of Shang et al (2014), except that in this study, Vitamin C was fed to the rats and histology of kidney and liver cells was investigated. With regard to the performed studies and the side effect of formaldehyde which was approved by them and also concerning this fact that people and doctors are always exposed to this substrate without being able to perform histology investigations on human body, the liver enzymes was measured to evaluate ALT and AST indicators. It was shown that there were a significant difference between the control groups and the groups exposed to formaldehyde which can be related to the inflammatory response of liver cells to formaldehyde injection. Standardization was performed according to the weight, gender and bread. So, the meaningful difference statistically approved the harmful effect of exposing to this substrate ($p<0.0001$). On the other hand, ALT and AST level in the E2 group treated by vitamin C were decreased but not to the normal level of liver enzymes which is probably because of high toxicity effect of formaldehyde. More reduction may be reached by increasing the time of treatment or Vitamin C dose.

CONCLUSION

According to this study, intraperitoneal administration of formaldehyde at a dose of 10 mge/kg per 10-day can increase AST and ALT and vitamin C at a dose of 200 (mg / kg) per day for 10-days can reduce amount of AST and ALT in adult rats exposed to formaldehyde.

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