

The protective effect of chlorogenic acid on arsenic trioxide induced hepatotoxicity in mice

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ABSTRACT

Several studies have shown that chronic exposure to arsenic trioxide risk factor for many cancers such as lung, liver, kidney and bladder. Free radicals in various ways such as lipid peroxidation, protein oxidation and DNA damage that leads to many diseases. Chlorogenic acid, an antioxidant plant and prevent many diseases caused by oxidative stress such as cancer. In this study, the protective effect of chlorogenic acid on arsenic trioxide-induced liver toxicity were studied. Biochemical parameters including Alkaline phosphatase (ALP), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), and Glutathione (GSH) were assessed for liver damage. In compare to positive control group (arsenic trioxide, 10 mg/kg), the serum levels of ALP, ALT, and AST have significantly decreased ($p < 0.05$) and the level of GSH has significantly increased ($p < 0.05$) in the groups administered with chlorogenic acid (10, 50, and 100 mg/kg). The results showed that chlorogenic acid has significant protective effects against the hepatotoxicity caused by arsenic trioxide.

KEY WORDS: ALANINE AMINOTRANSFERASE, ALKALINE PHOSPHATASE, ARSENIC TRIOXIDE, ASPARTATE AMINOTRANSFERASE, GLUTATHIONE

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INTRODUCTION

Exposure to arsenic trioxide take place on variety ways, for example, fuel, drinking water, pesticides, air, and food that is a threat to human health (Holt *et al.*, 2009; Mittal *et al.*, 2012). Researches have shown that exposure to arsenic can cause acute and chronic effects on different organs of the body, like cardiovascular system, digestive system, respiratory system, and nervous system (Centeno *et al.*, 2002; Abernathy *et al.*, 2003). Kidney and liver are the most important organs for metals and have the highest level of metallothionein binding to metals (Sarvankumar *et al.*, 2011). Liver is an important site for the metabolism of arsenic trioxide and chronic exposure to arsenic trioxide cause liver disease (Flora *et al.*, 2007). Arsenic toxicity is due to its ability to react with sulfhydryl groups in proteins and enzymes and can be replaced phosphorus in many biochemical reactions (Stevens *et al.*, 2010, Šeruga and Tomac, 2014 and Aoyama and Nakaki, 2015).

Damages caused by arsenic in the liver are determined by changes in liver enzymes, including Alkaline phosphatase (ALP), Alanine aminotransferase (ALT), and Aspartate aminotransferase (AST) (De Azevedo *et al.*, 2008). Free radicals can lead to various injuries at cells, such as DNA damage, inhibition of mitochondrial respiratory chain enzymes, and membrane lipid peroxidation (Umamaheswari and Chatterjee, 2008). The free radicals by creating oxidative stress and toxic oxidative leads to various diseases (Halliwell and Whiteman, 2004). Antioxidants by neutralizing free radicals prevent damages caused by free radicals in the body organs (Gradecka *et al.*, 2001). Glutathione synthesis of glutamate, cysteine, and glycine has done with the help of two enzymes, including glutamylcysteine synthetase and glutathione synthetase. The liver is an important location for manufacturing and exporting of glutathione (Wu *et al.*, 2004). Glutathione is caused failure of reactive oxygen species such as lipid peroxy radical, peroxy nitrite, hydroxyl radical, and other free radicals (Fang *et al.*, 2002). Glutathione deficiency leads to oxidative stress. Oxidative stress plays an important role in various diseases such as cancer, diabetes, Alzheimer's disease, kwashiorkor, seizures, liver disease, and Parkinson's disease (Wu *et al.*, 2004).

Researches have shown that phenolic compounds as natural antioxidants play an important role in protecting the body's cells against the toxic oxidative (Bralley *et al.*, 2008). Chlorogenic acid as a polyphenol compound is an ester between caffeic acid and quinic acid, and there is in many foods like coffee and apple. Chlorogenic acid is a strong antioxidant that has pharmacological properties like anti cancer and antibacterial (Um *et al.*, 2006; Das *et al.*, 2012). The present study evaluates the

protective effects of chlorogenic acid on the liver toxicity caused by arsenic trioxide in vitro by assessing the biochemical parameters including Alkaline phosphatase (ALP), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), and Glutathione (GSH).

MATERIAL AND METHODS

42 male mice (27 ± 2 g) procured from the animal house of the Mazandaran University of Medical Sciences, Sari, Iran. They were maintained in a controlled environment (12 h light/dark cycles) and temperature ($28 \pm 1^\circ\text{C}$). The mice were fed with drinking water and standard diet.

Chlorogenic acid was obtained from Sigma-Aldrich Company (USA). Arsenic trioxide was purchased from Merck Company (Germany). Mice were divided in 7 groups and 6 mice in each group. In these experiments, the effects of intraperitoneal administration of different doses of chlorogenic acid on the biochemical parameters of the kidney were investigated. The first group was administered with normal saline (0.9%) (10 mg/kg) as control, the second group was administered with arsenic trioxide (10 mg/kg) as positive control, the third group was administered with chlorogenic acid (100 mg/kg) as negative control, and the fourth to the seventh groups were administered with different doses of chlorogenic acid (5, 10, 50, 100 mg/kg), then after 2 hours the fourth to the seventh groups were administered with arsenic trioxide (10 mg/kg) (Sabath and Robles-Osorio, 2012).

Blood samples were collected of heart with the syringe, then transferred to centrifuge tubes, and centrifuged at 4000 rpm for 15 minutes. After centrifuging, the serum was separated and stored in the refrigerator (Tapio and Grosche, 2006). The level of serum Alkaline phosphatase (ALP), Alanine aminotransferase (ALT), and Aspartate aminotransferase (AST) were measured on a chemistry auto analyzer (Tchounwou *et al.*, 2004). Homogenized 0.1 g of liver tissue sample with 1ml assay buffer (EDTA) was taken then, its contents were transferred to centrifuge tubes and 0.5 ml EDTA was added to those. In the next step, 1.5 ml TCA 10% was added to centrifuge tubes. Centrifuge samples for 15 min at 3000 rpm. Remove 1 ml of supernatants and place in new tubes. Then, was added 2.5 ml Tris buffer (0.4 M) and 0.5 DTNB. Absorbance was measured of solutions at 412 nm with spectrophotometry (Saeedi Saravi and Shokrzadeh, 2008; Shokrzadeh *et al.*, 2015). The data were analyzed with SPSS 16 software. Statistical analysis of data was carried out with one way analysis of variance and Tukey test. The differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

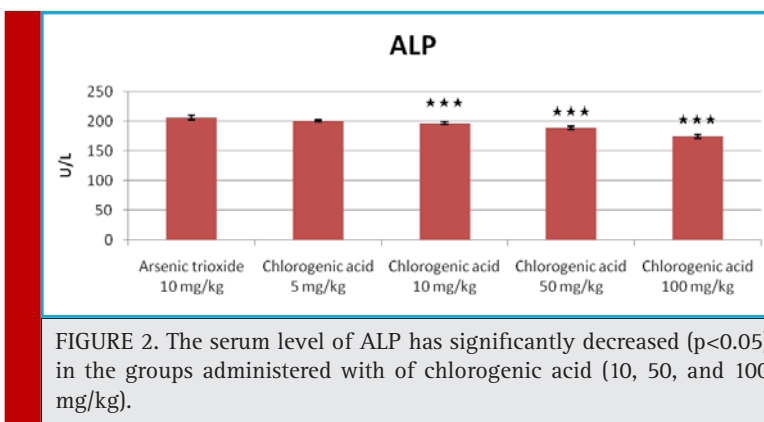
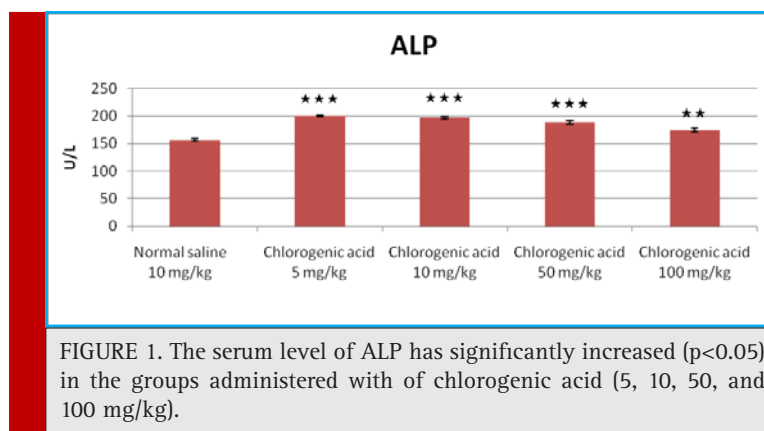
The serum level of ALP has significantly increased ($p < 0.05$) in the groups administered with of chlorogenic acid (5, 10, 50, and 100 mg/kg) when compared to the control group (normal saline, 10 mg/kg) (Figure 1). The serum level of ALP has significantly decreased ($p < 0.05$) in the groups administered with of chlorogenic acid (10, 50, and 100 mg/kg) when compared to the positive control group (arsenic trioxide, 10 mg/kg) but didn't show significant difference in dose of 5 mg/kg (Figure 2). The serum level of ALP has significantly increased ($p < 0.05$) in the groups administered with of chlorogenic acid (5, 10, 50, and 100 mg/kg) when compared to the negative control group (chlorogenic acid, 100 mg/kg) (Figure 3).

The serum level of ALT has significantly increased ($p < 0.05$) in the groups administered with of chlorogenic acid (5, 10, 50, and 100 mg/kg) when compared to the control group (normal saline, 10 mg/kg) (Figure 4). The serum level of ALT has significantly decreased ($p < 0.05$) in the groups administered with of chlorogenic acid (10, 50, and 100 mg/kg) when compared to the positive control group (arsenic trioxide, 10 mg/kg) but didn't show significant difference in dose of 5 mg/kg (Figure 5). The

serum level of ALT has significantly increased ($p < 0.05$) in the groups administered with of chlorogenic acid (5, 10, 50, and 100 mg/kg) when compared to the negative control group (chlorogenic acid, 100 mg/kg) (Figure 6).

The serum level of AST has significantly increased ($p < 0.05$) in the groups administered with of chlorogenic acid (5, 10, 50, and 100 mg/kg) when compared to the control group (normal saline, 10 mg/kg) (Figure 7). The serum level of AST has significantly decreased ($p < 0.05$) in the groups administered with of chlorogenic acid (10, 50, and 100 mg/kg) when compared to the positive control group (arsenic trioxide, 10 mg/kg) but didn't show significant difference in dose of 5 mg/kg (Figure 8). The serum level of AST has significantly increased ($p < 0.05$) in the groups administered with of chlorogenic acid (5, 10, 50, and 100 mg/kg) when compared to the negative control group (chlorogenic acid, 100 mg/kg) (Figure 9).

The level of GSH has significantly decreased ($p < 0.05$) in the groups administered with of chlorogenic acid (5, 10, 50, and 100 mg/kg) when compared to the control group (normal saline, 10 mg/kg) (Figure 10). The level of GSH has significantly increased ($p < 0.05$) in the groups administered with of chlorogenic acid (10, 50, and 100 mg/kg) when compared to the positive control group



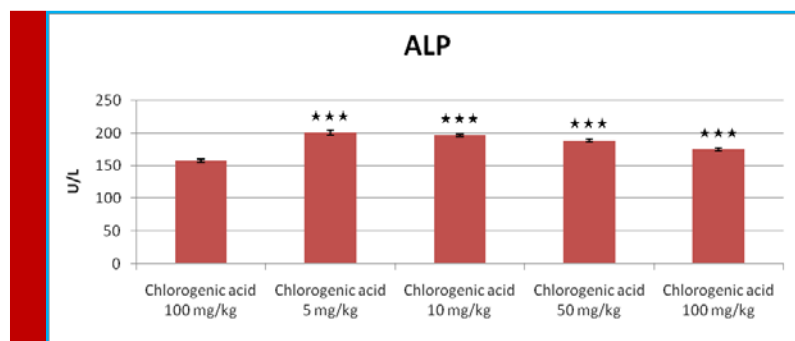


FIGURE 3. The serum level of ALP has significantly increased ($p < 0.05$) in the groups administered with of chlorogenic acid (5, 10, 50, and 100 mg/kg).

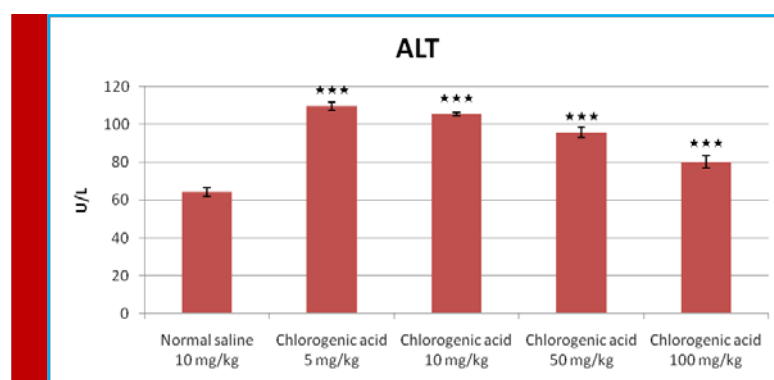


FIGURE 4. The serum level of ALT has significantly increased ($p < 0.05$) in the groups administered with of chlorogenic acid (5, 10, 50, and 100 mg/kg).

(arsenic trioxide, 10 mg/kg) but didn't show significant difference in dose of 5 mg/kg (Figure 11). The level of GSH has significantly decreased ($p < 0.05$) in the groups administered with of chlorogenic acid (5, 10, 50, and 100 mg/kg) when compared to the negative control group (chlorogenic acid, 100 mg/kg) (Figure 12).

Arsenic compounds are one of the pollutants of environment which are a serious threat to human health. Millions of people around the world through drinking water are exposed to arsenic compounds (Sabath and Robles-Osorio, 2012). Exposure to arsenic and its compounds can have dangerous effects on health (Tapio and

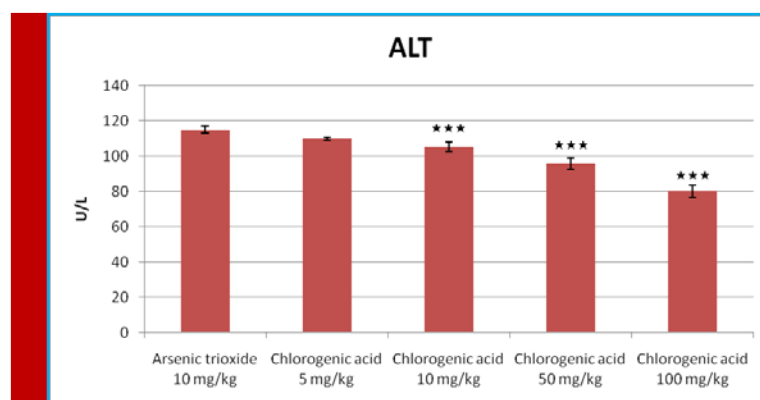
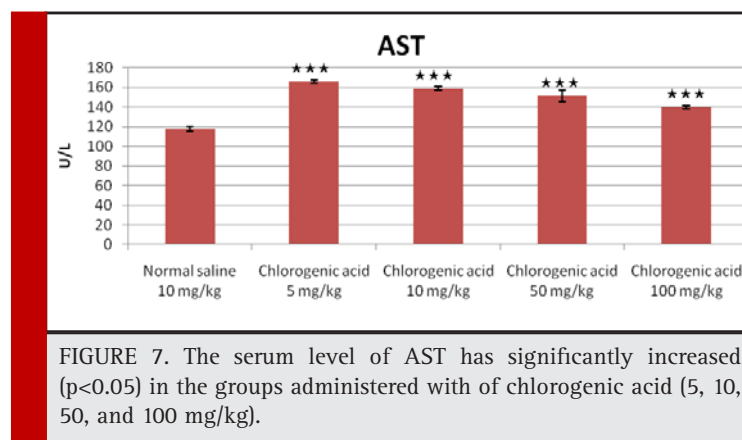
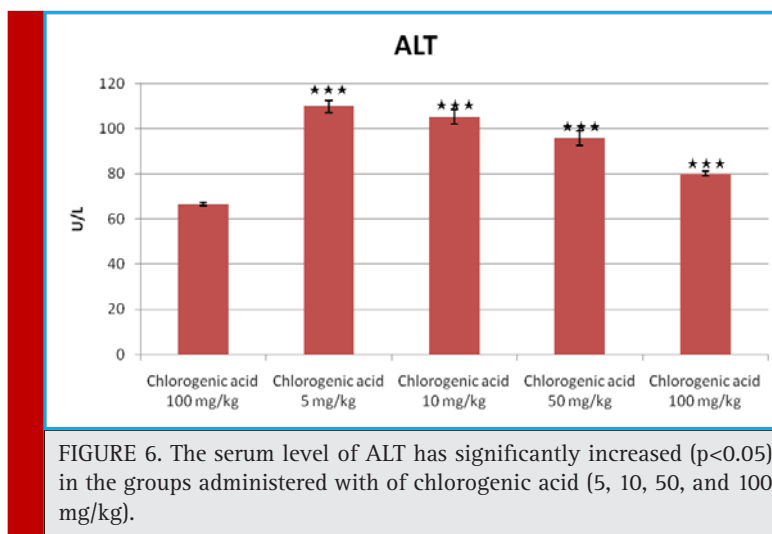
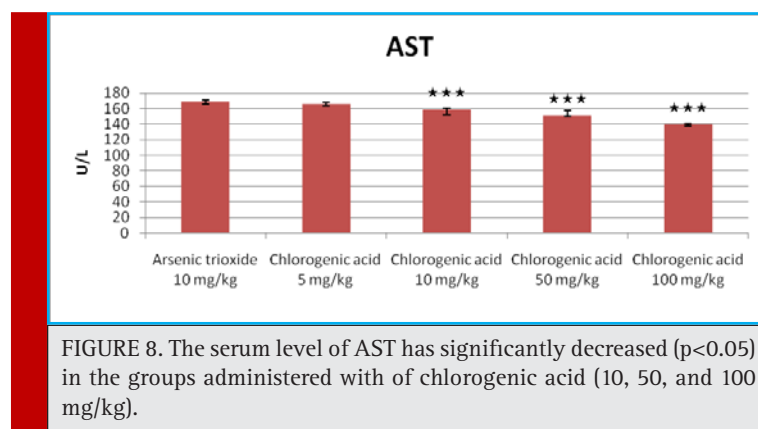


FIGURE 5. The serum level of ALT has significantly decreased ($p < 0.05$) in the groups administered with of chlorogenic acid (10, 50, and 100 mg/kg).



Grosche, 2006) and can lead to many types of cancers, such as skin, liver, kidney, lung, intestine, and bladder (Tchounwou *et al.*, 2004). Inorganic arsenic compounds as carcinogenic compounds are known. Epidemiological studies have shown that these compounds are related to the types of cancers like liver, kidney, and bladder

(Gradecka *et al.*, 2001). Researches have shown that consumption of drinking water containing inorganic arsenic compounds is related to liver diseases such as liver cancer (Islam *et al.*, 2011). In clinical studies of the liver, liver enzymes, including Alkaline phosphatase (ALP), Alanine aminotransferase (ALT), and Aspartate ami-



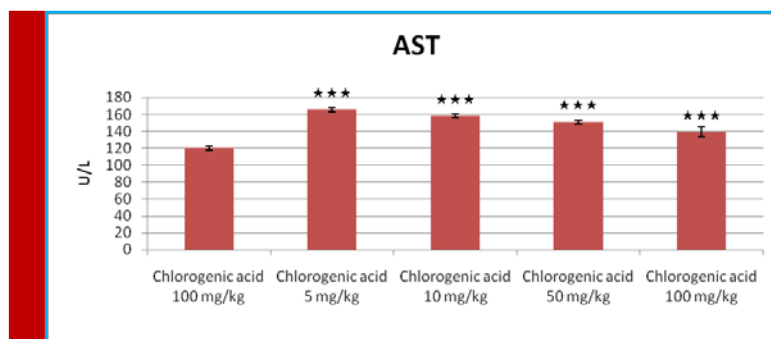


FIGURE 9. The serum level of AST has significantly increased ($p < 0.05$) in the groups administered with of chlorogenic acid (5, 10, 50, and 100 mg/kg).

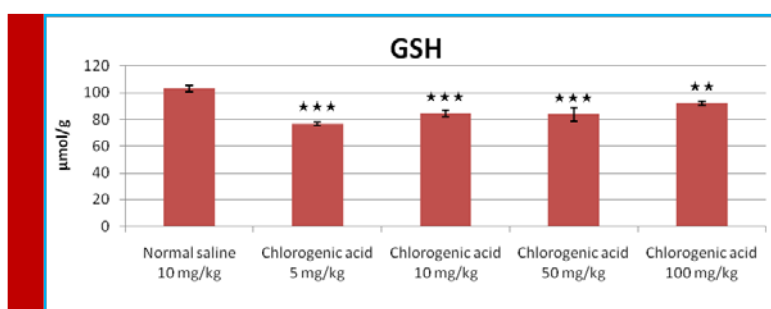


FIGURE 10. The level of GSH has significantly decreased ($p < 0.05$) in the groups administered with of chlorogenic acid (5, 10, 50, and 100 mg/kg).

notransferase (AST) are evaluated. The enzymes levels of ALT and AST are an indicator to assess the integrity of liver cells and the enzyme level of ALP represents the perfect synthesis of albumin and bile by the liver (Islam *et al.*, 2011). Our research has shown that the serum levels of ALP, ALT, and AST have significantly increased ($p < 0.05$) in all groups administered with of chlorogenic acid when compared to the control and negative control groups. Antioxidants are including two major categories, enzymatic and non enzymatic. Enzymatic antioxi-

dants like catalase, glutathione peroxidase, and superoxide dismutase, which are produced by the body and non enzymatic antioxidants like flavonoids, tannins, and carotenoids that obtained from plants (Lee *et al.*, 2004). Phenolic compounds as antioxidants in plants are caused by the elimination of free radicals. The antioxidant properties of phenolic compounds are related to the characteristic of the redox (oxidation and reduction) that reduce free radicals. Also, they are caused chelating of metals (Hsu, 2006). There is chlorogenic acid

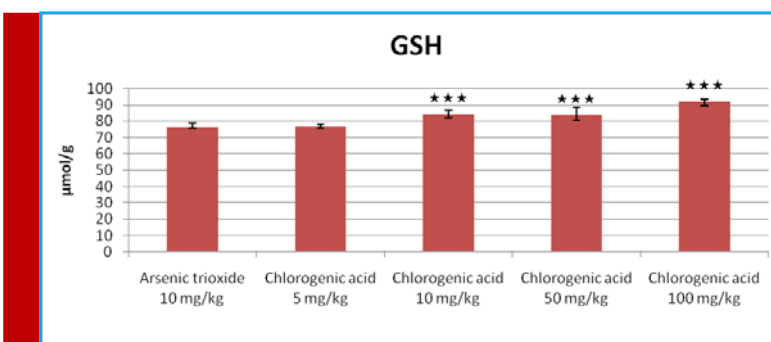


FIGURE 11. The level of GSH has significantly increased ($p < 0.05$) in the groups administered with of chlorogenic acid (10, 50, and 100 mg/kg).

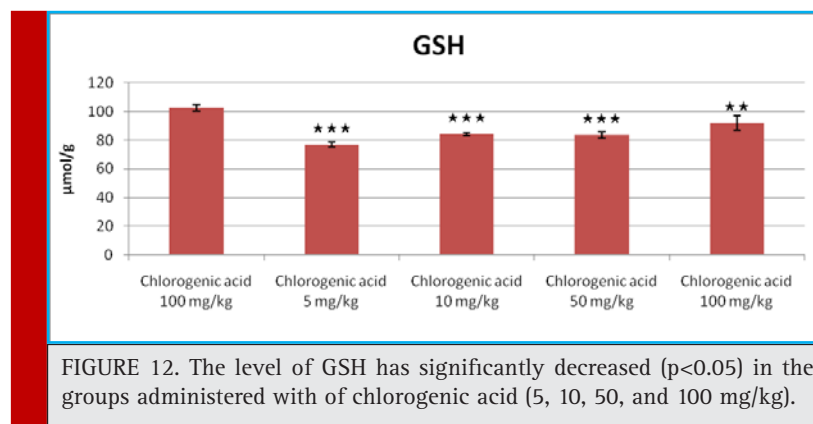


FIGURE 12. The level of GSH has significantly decreased ($p < 0.05$) in the groups administered with of chlorogenic acid (5, 10, 50, and 100 mg/kg).

as a polyphenolic compound widely in various plants (Šeruga and Tomac, 2014).

Studies, on the health benefits of consuming chlorogenic acid found in foods have shown that this antioxidant to reduce diseases such as cancer, stroke, obesity, diabetes, and Alzheimer's disease (Zhao *et al.*, 2012). The serum levels of ALP, ALT, and AST have significantly decreased ($p < 0.05$) in the groups administered with of chlorogenic acid (10, 50, and 100 mg/kg) when compared to the positive control group (arsenic trioxide, 10 mg/kg). It sounds that the antioxidant property of chlorogenic acid plays an important role in the protection of cells against free radicals caused by arsenic trioxide. The free radical is an atom or molecule that has an unpaired electron. The cells use of oxygen for produce energy that lead to the production of free radicals such as superoxide anion, hydroxyl, and peroxy that are known as reactive oxygen species (ROS) and mainly produced by mitochondria. Free radicals can lead to a wide range of toxic oxidative reactions like membrane lipid peroxidation, the failure of enzymes and proteins that could cause cell death (Umamaheswari and Chatterjee, 2008). Free radicals cause oxidative stress that plays an important role in the development of many diseases such as diabetes, cardiovascular, and cancer (Bandyopadhyay *et al.*, 1999). The studies show that many metals such as iron (Fe), lead (Pb), and copper (Cu) with modulating the redox (oxidation and reduction) in cell are causing the level change of thiols like glutathione in the cells (Tchounwou *et al.*, 2002). The glutathione (GSH) as a thiol compound and important in cells have antioxidant role and are caused destroy of free radicals. The glutathione (GSH) is in various body organs such as liver, kidney, brain, pancreas, and heart and highest levels of that found in the liver (Aoyama and Nakaki, 2015).

In this study, the level of GSH has significantly decreased ($p < 0.05$) in the groups administered with of chlorogenic acid (5, 10, 50, and 100 mg/kg) when compared to the control and negative control groups that

was specified arsenic trioxide reduced GSH in the liver but the level of GSH has significantly increased ($p < 0.05$) in the groups administered with of chlorogenic acid (10, 50, and 100 mg/kg) when compared to the positive control group that was identified chlorogenic acid as an antioxidant reduces free radicals and increases the glutathione level of the liver. The glutathione (GSH) with formation reversible disulfide bonds between thiols of protein can inhibit the oxidation of proteins during oxidative stress (Giustarini *et al.*, 2004). The glutathione (GSH) as an antioxidant have an important role in balancing of intracellular redox processes (Aoyama and Nakaki, 2015). Overall, our results showed that chlorogenic acid as a powerful and important antioxidant had significant protective effects against the toxicity of arsenic trioxide.

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