

Evaluation of Apple Hydro Extracts on Liver Enzyme ALT, AST and ALK in Female Rats Receiving Glyphosate Toxins

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doi: <http://dx.doi.org/10.13005/bbra/1382>

(Received: 02 August 2014; accepted: 04 September 2014)

Due to agricultural prosperity and in order to improve production, the herbicide glyphosate toxin, one of the organophosphates toxins is used. Apple has a therapeutic effect on liver diseases. The aim of this experiment is to evaluate the effect of apple hydro extracts on liver enzymes changes in female rats which receive glyphosate toxins. In this study 64 female rats were divided into 8 groups of 8 rats. The first group (control group), the second, third and fourth groups respectively received 200 mg / kg, 400mg / kg and 800 mg / kg of apple hydro extracts as gavage feeding for 14 days. The fifth group received 200 mg/kg glyphosate toxins as gavage feeding. Sixth, seventh and eighth groups received glyphosate toxins with apple hydro extracts. At the end of the study, the amount of ALT, AST, and ALK were measured. To analyze the obtained data, one-way ANOVA was used. Statistical analysis was performed by SPSS software. The serum levels of AST, ALT, and ALK of control group and group 4 which received the maximum dose of apple hydro extracts were reduced. In group 5 and group 8 which received the maximum dose of apple hydro extracts in addition to glyphosate toxins, the percentage reduction was increased more than group glyphosate. Serum levels of AST, ALT and ALK in the groups that received apple hydro extracts in addition to glyphosate toxins compared to the group which only received apple hydro extracts and control group have higher levels. In this research, apple hydro extracts reduced the serum levels of liver enzymes and maximum dose showed the greatest effect. It could be due to the antioxidant, phenol and poly phenol compounds' feature and high percentage of potassium and phytoestrogens of apple.

Key words: Glyphosate, Apple, Liver enzymes.

Due to the agricultural prosperity, farmers and agricultural engineers use glyphosate herbicide to control weeds and improve the products. Glyphosate was presented in the past with the brand Randap 21% and today glyphosate is presented as glyphosate but with 41% concentration. This toxin is in the form of white crystals. Its solubility in 20°C water is about 1/1 g in 100 ml and is slightly soluble in organic soluble¹. Glyphosate

toxins are a kind of organophosphate toxin. Effects of organophosphorus compounds are acute and chronic². The major problem of organophosphates is thesever effects that raise at least some sign of illness and stimulate the parasympathetic nervous system peak. If exposure to these compounds is short, the amount of cholinesterase is lowered without the appearance of any specified symptoms with only occasional headaches and flu-like symptoms which are due to the accumulation of acetylcholine in the brain receptors². More exposure to organophosphates causes fatigue, shivering and dizziness. In addition to the above moods due to the occupation of nicotinic receptors by long acetylcholine, it also causes muscle contraction and

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secretion of adrenaline from the adrenal gland in addition to restlessness³.

In investigating the biochemical and histopathological effects of glyphosate on Carp it was revealed that high doses of toxins might have adverse effects on the liver⁴. Also, the glyphosate toxin was observed to cause toxicity when injected into the peritoneum of rats with increased rectal temperature and seizures⁵. Moreover, in this study the glyphosate herbicide effect on liver cells and Biotrans formation activity in rats has been reported in which glyphosate reduces the cytochrome enzymes P-450 and also intestinal activity⁶. Glyphosate toxins effect on the endocrine tissue has been studied and observed and this toxin has a damaging effect on cells⁷. It has been observed that if the glyphosate herbicide is used widely, it will inhibit the synthesis of aromatic amino acids via the shikimate acid way⁸. In studying the damage of DNA cytogenetic in erythrocytes of fish exposed to this herbicide it has been reported that these herbicides affect the structure of nucleus and cause nuclear abnormality and destroy the DNA⁹. Apple with the scientific name of *Malus domestica* borkh from the Rosaceae has a wide farm variety according to smell and color of the fruit¹⁰. The fruit of this tree is known as an oral treatment with multiple treatment effects¹⁰. In traditional medicine, Ibn Sina considered apple as a heart tonic and as a treatment for the stomach ailments¹¹. Based on Seydanieh of Aboureihaan, Alabniyeh Heravi and Makhzan Aladviiyeh of Aghili Khorasani which are considered as the most important traditional medicine sources after The Canon of Medicine by Avicenna, apple is considered according to traditional medicine as the heart, brain and liver tonic and therefore strengthens the reproduction ability^{12,13}. Tonic effects, narcotic, laxative, glucose decrease, antioxidants, heart-protective, anti-bacterial, anti-inflammatory and anti-androgens effects have been reported for apple¹⁴. Apple can reduce blood cholesterol up to 16% and helps regulate blood glucose¹⁰. Anti-cancer effects of pug that have significant amounts of phenol compounds have been investigated in vitro about colon cancers¹⁵. Phytoestrogens of apple have been shown to contain antiviral effects¹⁶. Phytoestrogens are divided in two categories: flavonoids and non- flavonoids (such as lignan)¹⁶. The amount of phytoestrogen quercetin of apple is 4.7 mg in 100 g

of fruit¹⁶. Bioactive compounds of apple contain the flavonoids quercetin and kaempferol, epinephrine catechin, chlorogenic acid, para-coumaric acid and phloridzin^{16,17}. Flavonoids such as quercetin and its derivatives have been considered as the anti-free radical factors of this fruit¹⁸. Apple is used as adjunctive therapy in arteriosclerosis, liver disease and even diabetes due to the high percentage of poly phenol and potassium in apple¹⁴.

The liver has a major role in many essential physiological processes such as glucose homeostasis, forming the essential proteins of plasma, forming lipoproteins and lipid, production and secretion of bile acids and vitamins' storing¹⁹. Many reasons of liver disease are unknown, but undoubtedly oxidizing agents play an important role in pathological changes of liver, especially in toxic and alcoholic liver. Thus, these compounds through peroxidation of unsaturated fatty acids, biological membranes disrupt cell membrane structure and cause pathological changes²⁰. The liver is a large organ with many lobes in the abdominal cavity and its function is closely associated with alimentary canal and it works as the first location for a process of more absorption of nutrients and formation of bile acids which plays a major role in the absorption of fat. In addition, liver is an important metabolic plant for conversion of a variety of metabolic waste to the disposal form. Also, the liver stores or generates a number of the body's required substances such as glucose, amino acids and plasma protein. The liver plays a vital role in the proteins' metabolism²¹⁻²³. GOT is a microsomal enzyme that is found in large amounts in the liver and in addition to damaging liver tissue is released in large amounts in the blood²⁴. GPT is a liver-specific enzyme that exists in the cytosol and acts as an indicator of liver cells and is more sensitive and more specific criteria to determine the amount of liver cell damage²⁵. Alkaline phosphatase exists in most tissues and increases due to liver diseases. However, the increased amount of these enzymes is different depending on the type of liver diseases¹². GOT and GPT are specific enzymes of the liver and in addition to more sensitivity to liver toxicity and histopathology changes than other enzymes are measured in a shorter time period²⁶. This study was done due to the availability of apple, although apple hydro extracts affects the liver by glyphosate

toxins have not been studied due to various features of apple, especially red apple with more antioxidant and phenol compounds features than green and yellow apple and also having poly phenol and a high percentage of potassium phytoestrogens, and no side effects of chemical medication.

Previous studies on the liver

Regarding the antioxidant effects of apple skin it has been stated that: consumption of fruits and vegetables is effective in the prevention of chronic diseases. These benefits are often dependent on the high antioxidant content of some of these plants, the high content of phenol compounds, antioxidant activity and anticancer activity of apple suggests that the health benefits at the time of eating the apple should be considered as a valuable source of antioxidants²⁷. In a study in the past about the effect of poly phenol in apple in liver injury which is induced by CCL4 in mouse stated: apple hydro extracts considerably inhibited the elevation of serum level of ALT and AST in acute liver injury induced by CCL4 and considerable improvements were observed in liver tissue, it has also been shown to inhibit free radicals in vitro²⁸.

Studies have shown that rats that received apple skin have further inhibited lipid peroxidations²⁹. The effect of apple hydro extracts

on NF-Kb in human endothelial cells vein of funiculus stated: mechanisms that reduce the risk of chronic disease are still unclear and this hypothesis stated: the flavonoids in apple may help reduce oxidation of LDL in vitro and reduce signaling base of NF-Kb and show antioxidant effects of flavonoids of apple hydro extracts³⁰.

Method of research

For conducting the study, 64 female Wistar rats with an average weight of 180-200g, 10 weeks old, and bred at the Animal Laboratory of Jahrom University were used. After weighing, 4 rats each were placed in a cage. Rats were given one week to adjust to the environment consisting of 12h light/12h dark, humidity around 50 to 55% and they were fed with standard food and water ad libitum. The rats were then weighed again and randomly weighed again and then randomly divided into 8 groups of 8 rats. The first group was the control group, the rats were fed with standard water and food in order for the level of liver enzymes of observer and test group to be measured in this group. Second, third and fourth groups were positive observer group in which the second rats group received 200 mg / kg and the third group received 400mg / kg. The fourth group received 800 mg / kg of apple hydro extracts which

Table 1. Comparison of mean and standard deviations of study parameters of blood serum of group 8 of rats compared with each other at the end of the fourteenth day (end of treatment) (N = 8)

Parameter Group	AST ¹	ALT ²	ALK ³
Group1	94.63 ±3.21	108.25±2.74	298.38±7.12
Group2	78.50±1.91* ▲	92.88±2.62✦	273.25±7.17
Group3	74.00±2.71★ ▲	81.75±2.19✦	247.88±4.24
Group4	65.13± 3.82★*	63.88 ±2.16	206.50±4.43
Group5	225.75±3.90	291.38±3.38	649.38±4.81
Group6	197.25±3.04	244.75±4.40	596.75±3.32
Group7	177.75±4.96	222.50±4.25	507.38±6.18
Group8	128.88±3.19	195.88±3.87	409.63±5.49

Oxaloasetate transaminase = AST¹ (SGOT), pirvate transaminase = ALT² (SGPT), alkaline phosphatase = ALK³ and the unit of each is IU / L.

Only the group that has the common mark of ✦ or ▲ or * or ★ are not significantly different and the remaining groups are significantly different with each other at P≤ 05/0. Group 1 (without receiving extract and toxins), Groups 2,3 and 4 (received red apple extract at doses of 200, 400 and 800 mg per kg body weight, which was dissolved in 20 cc distilled water.), group 5 (received 200 mg/kg of glyphosate toxins of body weight dissolved in 20 ml of distilled water) and groups 6,7 and 8 (received red apple extract at doses of 200, 400 and 800 mg per kg of body weight plus 200 mg/kg glyphosate toxins of body weight dissolved in 20 ml of distilled water.

dissolved in 20 CC of distilled water as gavage feeding for 14 days and fed each day at 10 am²⁸. Fifth group were considered as negative observer group that received glyphosate toxins with a dose of 200 mg/kg and dissolved in 20 CC of distilled water as gavage feeding for 14 days and fed each day at 10 am. (Lethal dose was 800mg/kg which was obtained by the researcher *et al* in a previous study³¹). Sixth, seventh and eighth groups were the experimental groups in which the sixth group received 200 mg /kg, seventh group received 400mg /kg and the eighth group received 800 mg/kg of apple hydro extracts with 200mg / kg dose of glyphosate toxins and 20 CC of distilled water as gavage feeding for 14 days and were fed each day at 10 am. At the end of the study (fifteenth day), after weighing and anesthetizing the rats with ethyl ether, blood was taken directly from the heart into a 5 ml syringe and blood serum by a centrifuge (for 15 min and 3000 rpm) and was kept at -20°C in freezer until the experiment. For measuring biochemical factors of blood which include alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALK), Iranian biochemistry measurement kits, colorimetric method³² and Netherlands Selectera XL auto analyzer model were used.

Methods of data analysis

To analyze the data and obtained data, one way ANOVA variance analysis was used. Statistical analysis was performed using SPSS software.

Inclusion and exclusion criteria

Inclusion criteria were healthy female Wistar rats weighing 180 to 200 g at ten weeks of age and exclusion criterion was the death of the animal.

Findings

The findings of the enzymes study in the blood

Comparison of mean and standard deviations of studied parameters of serum obtained the following results of the 8 rats' groups relative to each other at the end of the fourteenth day (end of treatment), including Group 1 (without receiving extract and toxins), Groups 2,3 and 4 (received red apple extract with doses of 200, 400 and 800 mg per kg of body weight in 20 ml distilled water), Group 5 (receive glyphosate toxins of 200 mg per kg of body weight dissolved in 20 ml distilled water) and Groups 6,7 and 8 (received red apple extract at a dose of 200, 400 and 800 mg per kg of body weight plus 200 mg glyphosate toxins per kg of body weight; every substance was dissolved in 20 ml of distilled water). (Table 1 and Figure 1).

Changes in serum level of AST

Mean changes in serum levels of AST of group 3 compared to group 2 by $P=0/983$ and group 4 compared to group 2 by $P=0/132$ and group 4 compared to group3 by $P=0/610$ have no significant differences. In group 2 compared to the control group mean changes in serum levels of AST was $P=0/033$ and in group 3 compared to the control group was $P=0/002$ and group 7 compared to group 6 was $P=0/004$ and the difference between these groups weresignificant, and the othergroups toward each other has significant difference by $P=0/000$.

- 1) Levels of serum AST were reduced incontrol group (1) to group 4 that received maximum dose of apple hydro extracts, so that the serum levels of AST in group 2 were reduced about 17%, in group 3, 22%, and in group 4 about 32% compared to group 1.
- 2) Levels of serum AST were reduced in the

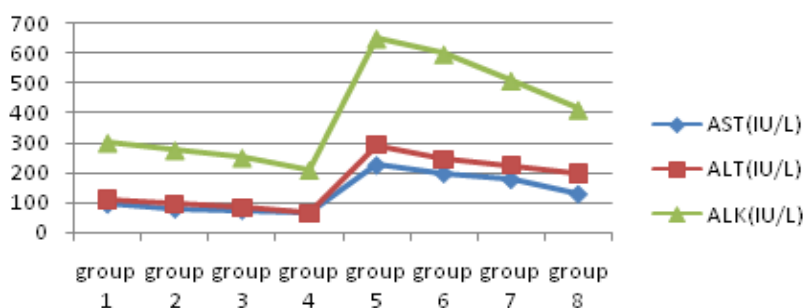


Fig. 1. Graph comparing the mean of study parameters of blood serum of the 8 groups of rats at the end of the fourteenth day

- toxins receiving group alone (5) for the groups 8 that received maximum dose of apple extract plus glyphosate toxins so the serum levels of AST are reduced about 13% in group 6, about 23% in group 7, and 43% in group 8 compared to group 5.
- 3) The serum levels of AST in the groups that received apple extract plus glyphosate toxins compared to groups receiving apple extract alone and control, have greater amounts.

Changes in serum level of ALT

Mean changes in serum levels of ALT of group 3 compared to group 2 at $P=0/272$ have no significant differences and group 2 compared to control group 2 at $P=0/035$ and group 4 compared to group 3 at $P=0/008$. The difference between the two groups was significant and the remaining groups have significant differences at $P=0/000$.

- 1) Levels of serum ALT were reduced in control group (1) compared to group 4 that received maximum dose of apple hydro extracts, so that the serum levels of ALT in group 2 are reduced about 14 %, in group 3 about 24%, and in group 4 about 41% compared to group 1.
- 2) Levels of serum ALT were reduced in the toxins receiving group alone (5) for for the 8 groups that received maximum dose of apple extract plus glyphosate toxins so the serum levels of ALT reduced about 16% in group 6, about 22% in group 7, and in group 8 is reduced about 33% compared to group 5.
- 3) Serum levels of AST in the groups that received apple extract plus glyphosate toxins compared to groups receiving apple extract alone and control, have greater amounts.

Changes in serum level of ALK

Mean changes in serum levels of ALK of group 3 compared to group 2 at $P=0/037$ and group 2 compared to control group 2 at $P=0/041$ have significant difference and the remaining groups have significant differences at $P=0/000$.

- 1) - Levels of serum ALK were reduced in control group (1) to group 4 that received maximum dose of apple hydro extracts, so that the serum levels of ALK in group 2 were reduced, in group 3 about 17%, and

- in group 4 about 31% compared to group 1.
- 2) Levels of serum ALK were reduced in the toxins receiving group alone (5) for groups 8 that received maximum dose of apple extract plus glyphosate toxins so the serum levels of ALK in group 6 reduced about 8%, in group 7 about 22%, and in group 8 about 37% compared to group 5.
- 3) The serum levels of ALK in the groups who received apple extract plus glyphosate toxins compared to groups receiving apple extract alone and control have greater amounts.

DISCUSSION

The serum levels of AST, ALT and ALK were reduced in the control group (group 1) In order to group 4 which received maximum apple extract. Also group 5 (receiving toxins alone) In order to group 8 which received maximum apple extract maximum dose plus glyphosate toxins and the reduction percentage was also show increase. In addition, the serum levels of AST, ALT, and ALK in the groups which received apple extract plus glyphosate compared to the group receiving apple extract alone and control group have higher values.. Since apple according to the Duke JA report in 2002 apple is rich in antioxidants and poly phenols, and the high percentage of potassium can be used as adjunctive therapy in arteriosclerosis, liver disease and even diabetes¹⁴. Moreover, according to Martin JHJ's report in 2007, apple has phytoestrogens and bioactive compounds including the flavonoids quercetin and kaempferol, epinephrine catechin, chlorogenic acid, para-coumaric acid and phloridzin¹⁶. Based on Cefarelli G in 2006, flavonoids such as quercetin and its derivatives are the anti-free radical of this fruit¹⁸ so decreasing the liver enzymes may be due to the antioxidant feature and phenol compounds and also the high percentage of potassium and phytoestrogens of apple and, with increasing doses of apple extracts its effect on lowering serum levels of liver enzymes increased. As previously noted in investigating apple poly phenol effect on liver injury induced by CCL4 in mouse it has been indicated that apple extracts significantly inhibited the elevation of serum ALT and AST in acute liver injury induced by CCL4 and wide

improvement of liver tissue was also observed. It has also been shown to inhibit free radicals in vitro²⁸. Studies have shown that rats receiving apple skin have further inhibited lipid peroxidations²⁹. In studying the effect of apple extract on NF-Kb human endothelial cells vein of funiculus showed that there are mechanisms that can reduce the risk of chronic disease, however, it is still unclear and the hypothesis that maybe flavonoids in apple extract reduce oxidation of LDL in vitro and reduce signaling base of NF-Kb showing antioxidant effects of flavonoids of apple extract is presented³⁰. But the groups which received glyphosate toxins in addition to apple extract, due to the damaging effects on the liver tissue, cells' death and excessive increase of liver enzymes³¹, the given doses of apple extract in this study significantly reduced the rate of liver enzymes levels, but the decline of liver enzymes was not observed to a normal extent. However, apple extract was partly able to decrease liver enzymes caused by glyphosate toxicity.

CONCLUSION

Apple extract consumption reduces the serum levels of liver enzymes and based on the present study, the maximum dose has the most effect.

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