Short Period Starvation in Rat: The Effect of Aloe Vera Gel Extract on Oxidative Stress Status Ion

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Abstract
The main aim of this experiment was to evaluate the effect of Aloe vera gel extract on oxidative stress status during starvation. For this purpose, twenty-four mature male albino Wistar rats were housed in standard cages. In this study starvation cycle (rats were starved for two days and then were fed for one day) was used. This study was performed during short period (20 days). Animals were divided into four experimental groups (six rats in each group): 1) normal control; 2) starved rats+water/ethanol; 3) starved rats+hydro-alcoholic Aloe vera gel extract (100 mg/kg); 4) starved rats+hydro-alcoholic Aloe vera gel extract (200 mg/kg). Blood samples were obtained using cardiac puncture. In blood samples, antioxidant enzymes including superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT), antioxidant trace elements including copper, zinc and manganese and antioxidant vitamins including vitamin A, vitamin E and vitamin C were measured. Plasma levels of antioxidant enzymes (SOD, GPx and catalase) significantly decreased in starved rats+water/ethanol group (P<0.05). Plasma levels of antioxidant enzymes after treatment with hydro-alcoholic Aloe vera gel extract at doses 100 and 200 mg/kg were significantly increased (P<0.05). Plasma levels of Cu, Zn and Mn in normal control group had no significant difference with starved rats+water/ethanol and starved rats+hydro-alcoholic Aloe vera gel extract in 100 and 200 mg/kg dose groups (P>0.05). Plasma levels of vitamin A and E in normal control group had no significant difference with starved rats+water/ethanol and starved rats+hydro-alcoholic Aloe vera gel extract in 100 and 200 mg/kg dose groups (P>0.05). Plasma level of vitamin C significantly decreased in starved rats+water/ethanol group (P<0.05). Plasma level of vitamin C after treatment with hydro-alcoholic Aloe vera gel extract at doses 100 and 200 mg/kg were significantly increased (P<0.05). Our results shown that short term starvation caused an increase in oxidative stress via impairing of antioxidant defense and Aloe vera treatment is able to improve antioxidative defense induced by starvation.

Introduction
Oxidative stress results in imbalance between reactive oxygen species (ROS) generation and cellular antioxidant defense. Oxidative stress contributes to pathogenesis of some diseases as well as ageing process (Valko et al., 2007). Various factors such as environmental, dietary factors and diseases affect stress oxidative level in body (Limón-Pacheco and Gonsebatt, 2009; Valko et al., 2007). Defense against oxidative stress, antioxidant defense, has different components involving enzymatic and non-enzymatic antioxidant defense. Enzymatic antioxidant defense includes superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT). Non-enzymatic antioxidants include ascorbic acid (vitamin C), tocopherol (Vitamin E), glutathione (GSH), carotenoids, flavonoids, and other antioxidants (Valko et al., 2007).

Starvation or food deprivation caused metabolic changes in body. Starvation decreased glucose plasma level. Lipid oxidation increased (Orellana et al., 1992; Wasselin et al., 2014) or was not changed (Marczuk-Krynicka et al., 2003) during starvation. Starvation has been reported to have pro-oxidative effect as a consequence of both elevated ROS generation and defeat in neutralization of ROS (Crescimanno et al., 1989; Domenicali et al., 2001; Robinson et al., 1997; Sorensen et al., 2006). However, one study has reported
starvation was not associated with increased oxidative stress in mallards (Geiger et al., 2012).

Recently, attention has been focused on herbal medicines which have antioxidant activities to prevent and protect oxidative damage caused by free radical species (Stavric, 1994). Aloe vera is a species of Aloe that belongs to Liliaceae family. This plant mainly grows in subtropical and tropical climates (Radha and Laxmipriya, 2015). Aloe vera contains different substances and is widely used for a variety of medicinal purposes. Many biological and medicinal properties of Aloe vera are associated with inner gel of the leaves. Several studies have focused on antioxidant effect of Aloe vera (Haritha et al., 2014; Mohamed, 2011; Nahar et al., 2013; Ramachandraiahgari et al., 2012; Trivedi et al., 2015). However, one study showed Aloe vera juice induced oxidative stress and significantly decreased antioxidant enzymes levels (Cock and Sirdaarta, 2011).

There is no information about the effect of Aloe vera on starvation. The present study was undertaken to evaluate the effect of Aloe vera gel extract on oxidative stress status during starvation. For this purpose, antioxidant enzyme activities including SOD, GPx and catalase, antioxidant trace elements including copper, zinc and manganese and antioxidant vitamins including vitamin A, vitamin E and vitamin C were evaluated.

Materials and Methods

Hydro-alcoholic Aloe Vera Gel Extract

Aloe vera gel powder was provided from Anamis Aloe vera Company. Then, the gel powder (100 g) was added to 500 ml ethanol 70%. The obtained solution was left in the percolator at room temperature for 72 h. Afterwards the solvent was completely removed from the hydroalcoholic extracts by Rota vapor at 40°C and dried in a vacuum desiccator.

Animal Ethics

This experiment was accomplished under the approval of the State Committee on Animal Ethics, Shiraz University, Shiraz, Iran. The recommendations of European Council Directive (86/609/EC) of November 24, 1986, regarding the standards in the protection of animals used for experimental purposes were also followed.

Experimental Design

Twenty-four mature male albino Wistar rats weighing 190-220 g were housed in standard cages. Animals were maintained under temperature control (23±1) with a 12:12 h light-dark cycle. In this study, starvation cycle (rats were starved for two days and then were fed for one day) was used. This study was performed during short period (20 days). The animals were divided into four experimental groups (six rats in each group): 1) normal control; 2) starved rats+water/ethanol; 3) starved rats+hydro-alcoholic Aloe vera gel extract (100 mg/kg); 4) starved rats+hydro-alcoholic Aloe vera gel extract (200 mg/kg). At the end of experiment rats were anesthetized with ketamin and acepromazine. Blood samples were obtained using cardiac puncture. 1.5 mL of blood was collected in heparinized tubes and used for hemolysate preparation. 2.5 mL of blood was collected in simple tubes and their serum separated by centrifuging at 750 g for 15 minutes and stored at -20°C for the subsequent assays.

Hemolysate Preparation

Whole blood (0.5 mL) was centrifuged at 700 g for 15 min. The plasma was aspirated off, and the erythrocyte pellet was washed three times with normal saline solution, and then distilled water was slowly added up to 2 mL. Hemoglobin (Hb) was measured using cyanmethemoglobin method (Jain, 1986). The biochemical tests were performed immediately after preparing the hemolysate.

Biochemical Assays

Antioxidant Enzyme Activities Measurement

The SOD activity was measured with commercial kit (RANSOD kit, Randox Com, UK). This method employs xanthine and xanthine oxidase to generate superoxide radicals, which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye. The enzyme activity was measured by the degree of inhibition of this reaction. GPx activity was measured calorimetrically by Commercial kit (RANSEL kit, Randox Com, UK) based on the method of Paglia and Valentine (1967). The activity of catalase (CAT) was determined with the commercial catalase assay kit (Oxford Biomedical Research, Inc., USA), based on the colorimetric method described by Slaughter and O’Brien (2000), which determines the power of enzyme to the disappearance of H2O2 at 520 nm. The activities of the enzymes were expressed as U/g of hemoglobin.

Trace Elements Measurement

The samples with hemolysis were discarded. Digestion of serum was performed by a mixture of perchloric and nitric acid (3:7 ratios respectively). Copper, zinc and manganese were measured using an atomic absorption spectrophotometer (Shimadzo AA-670, Kyoto, Japan). Argon was used as the purging gas. The background absorption was automatically corrected by the Zeeman Effect. One thousand micrograms per milliliter standard solution of each mineral was used in the measurements (Puls, 1994).
Antioxidant Vitamins

The concentrations of vitamins A, E and C were evaluated by application of the HPLC method using Ultraviolet detection. Vitamins A and E were measured based on the protocol described by Johnson-Davis et al. (2009) and vitamin C by a Commercial Kit (ALPCO Diagnostics, USA). The HPLC system used consisted of a solvent delivery pump (JASCO 980-PU, Tokyo, Japan), a reversed-phase column (Luna C18, 250×4.6 mm; Phenomenex, CA, USA), and a UV-Vis detector (Jasco, UV-975, Tokyo, Japan).

Statistical Analysis

All Statistical analyses were carried out using SPSS software (version 22). All data were presented as mean ±S.E. One-way ANOVA was used to test differences between various groups means followed by post hoc Tukey-HSD test. Significant level was less than 0.05.

Results

Blood levels of antioxidant enzymes are shown in Table 1. Plasma levels of antioxidant enzymes (SOD, GPx and catalase) significantly decreased in starved rats+water/ethanol group (P<0.05). Plasma levels of antioxidant enzymes after treatment with hydro-alcoholic Aloe vera gel extract at doses 100 and 200 mg/kg were significantly increased (P<0.05).

Plasma levels of Cu, Zn and Mn are shown in Table 2. Plasma levels of Cu, Zn and Mn in normal control group had no significant difference with starved rats+water/ethanol and starved rats+hydro-alcoholic Aloe vera gel extract at doses 100 and 200 mg/kg groups (P>0.05).

Table 1. Blood levels of antioxidant enzymes in different experimental groups during short period starvation of Wistar rats.

<table>
<thead>
<tr>
<th>Antioxidant enzymes</th>
<th>Normal control</th>
<th>Starved rats + water/ethanol</th>
<th>Starved rats + hydro-alcoholic Aloe vera gel extract (100 mg/kg)</th>
<th>Starved rats + hydro-alcoholic Aloe vera gel extract (200 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (U/g of hemoglobin)</td>
<td>182.6±21.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>56.31±32.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>134.42±21.11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>119.34±22.03&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>GSH-Px (U/g of hemoglobin)</td>
<td>102.87±4.68&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82.99±6.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>132.25±3.87&lt;sup&gt;c&lt;/sup&gt;</td>
<td>128.6±4.33&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Catalase (U/g of hemoglobin)</td>
<td>3.28±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.58±0.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.29±0.09&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.64±0.07&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Different letters indicate statistically significant differences (P<0.05)

Table 2. Plasma levels of Cu, Zn and Mn in different experimental groups during short period starvation of Wistar rats.

<table>
<thead>
<tr>
<th>Trace elements</th>
<th>Normal control</th>
<th>Starved rats + water/ethanol</th>
<th>Starved rats + hydro-alcoholic Aloe vera gel extract (100 mg/kg)</th>
<th>Starved rats + hydro-alcoholic Aloe vera gel extract (200 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu (μmol/L)</td>
<td>0.89±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.09±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.99±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.93±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Zn (μmol/L)</td>
<td>2.05±0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.90±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.06±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.93±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mn (μmol/L)</td>
<td>0.022±0.005&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.024±0.003&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.030±0.002&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.025±0.003&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Different letters indicate statistically significant differences (P<0.05)

Plasma levels of vitamins A, E and C are shown in Table 3. Plasma levels of vitamin A and E in normal control group had no significant difference with starved rats+water/ethanol and starved rats+hydro-alcoholic Aloe vera gel extract in 100 and 200 mg/kg dose groups (P>0.05). Plasma level of vitamin C significantly decreased in starved rats+water/ethanol group (P<0.05). Plasma level of vitamin C after treatment with hydro-alcoholic Aloe vera gel extract at doses 100 and 200 mg/kg was significantly increased (P<0.05).
Table 3. Plasma levels of vitamins A, E and C in different experimental groups during short period starvation of Wistar rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Vitamin A (μg/mL)</th>
<th>Vitamin E (μg/mL)</th>
<th>Vitamin C (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>0.35±0.05a</td>
<td>0.017±0.002a</td>
<td>111.63±22.91a</td>
</tr>
<tr>
<td>Starved rats + water/ethanol</td>
<td>0.33±0.02a</td>
<td>0.023±0.003a</td>
<td>24.01±4.84b</td>
</tr>
<tr>
<td>Starved rats + hydro-alcoholic Aloe vera gel extract (100 mg/kg)</td>
<td>0.32±0.01a</td>
<td>0.022±0.000a</td>
<td>58.34±9.81c</td>
</tr>
<tr>
<td>Starved rats + hydro-alcoholic Aloe vera gel extract (200 mg/kg)</td>
<td>0.26±0.03a</td>
<td>0.019±0.001a</td>
<td>49.80±3.07c</td>
</tr>
</tbody>
</table>

Different letters indicate statistically significant differences (P<0.05)

Discussion

Blood levels of antioxidant enzymes (SOD, GPx and catalase) significantly decreased in starved rats+water/ethanol group (P<0.05). Several studies have been done regarding generation of oxidative stress by starvation (Crescimanno et al., 1989; Domenicali et al., 2001; Robinson et al., 1997; Sorensen et al., 2006). Food deprivation or starvation results in reduction in critical metabolic substrate. In this situation decreased glucose utilization and parallel increase in fatty acids oxidation and keton bodies production takes place. From this sense starvation could be similar to diabetes (Godin and Wohaieb, 1988). Several studies have shown starvation stimulated peroxisomal fatty acid oxidation as well as increased H2O2 production (Godin and Wohaieb, 1988). Further studies have shown starvation stimulated mitochondrial fatty acid oxidation and subsequently increased NADH production and its influx to TCA cycle. It caused electron transport chain to be more active and finally enhanced ROS generation (Wasselin et al., 2014). Sorensen and co-workers have shown 72 h starvation increased free radical production and changed membrane composition in rat liver (Sorensen et al., 2006).

Antioxidant system components act against oxidative stress in body. Antioxidant enzymes could be affected through different factors such as environmental and dietary factors (Godin and Wohaieb, 1988; Limón-Pacheco and Gonsebatt, 2009). Effect of fasting on antioxidant enzymes is controversial. Godin and Wohaieb (1988) reviewed the effect of nutritional deficiency and starvation on tissue antioxidant enzymes. They have mentioned that alteration of antioxidant enzymes in different tissues was varied during starvation. Marczuk-Krynicka et al. (2003) have shown catalase and SOD activities decreased in fasted rat livers but they did not alter in GPx activity in fasted rat livers. Wasselin et al. (2014) evaluated gene expression and enzyme activities involved in the response to oxidative stress. They have shown a decrease in both catalase protein and its activity level in fasted rats. SOD gene expression showed no difference between experimental groups but GPx mRNA level was varied in fasting group depending on its isoforms.

It has been previously mentioned that starvation increased production of free radical oxygen. Superoxide radical inhibited catalase and this inhibition could be prevented by SOD. SOD and catalase act synergically. Also, SOD could be inactivated by H2O2 (Kono and Fridovich, 1982). Thus, increasing production of ROS and H2O2 during starvation could decrease SOD and catalase activities. Fasting caused body weight loss and hypoglycemia and hypoinsulinemia (Godin and Wohaieb, 1988; Marczuk-Krynicka et al., 2003; Wasselin et al., 2014). Hypoglycemia increased lipid and protein metabolism (Wasselin et al., 2014). Thus, starvation results in decreased protein production and enzyme activities as well as increasing protein degeneration. Antioxidant enzymes could be affected by these factors. Also, observed changes in antioxidant enzyme activities may have a relationship with hypoinsulinemia induced by food starvation. A previous study has shown insulin treatment normalized antioxidant enzyme abnormalities in diabetic animals (Godin and Wohaieb, 1988). Moreover, another study showed diabetic and fasted rats have an imbalance in antioxidant enzyme activities but insulin treatment in diabetic rats and refeeding in fasted rats could improve antioxidant enzyme abnormalities (Asayama et al., 1989).

Gîlcă et al. (2002) have shown SOD and GPx activities were lower in fasting group. Intracellular GSH content decreased during starvation and GPx express GSH for its reaction, so the decrease of intracellular GSH level may result in decreased GPx activity in fasted rats (Domenicali et al., 2001).

Blood levels of antioxidant enzymes (SOD, GPx and catalase) after treatment with hydro-alcoholic Aloe vera gel extract at 100 and 200 mg/kg doses were
significantly increased (P<0.05). Several studies have suggested antioxidant effects of Aloe vera (Haritha et al., 2014; Mohamed, 2011; Nahar et al., 2013; Ramachandraiahgari et al., 2012; Trivedi et al., 2015).

Our results have shown that Aloe vera treatment caused increased antioxidant enzyme activities in comparison with starved group. However, we observed the effect of Aloe vera gel extract on antioxidant enzyme activities was independent of dose. Studies have mainly focused on effects of Aloe vera on diabetes. Diabetes is similar to starvation in some aspects. Aloe vera treatment increased antioxidant enzyme levels in lens of diabetic group (Haritha et al., 2014). Mohamed (2011) has reported Aloe vera gel extract had antioxidant effects and significantly decreased serum MDA levels. Several studies have mentioned that Aloe vera has naturally antioxidant component including phenols, flavoids, vitamin C, vitamin E and some trace elements. These antioxidant component scavenge reactive oxidant and aid in endogenous antioxidant defense (Malik and Zarnigar, 2013; Radha and Laxmipriya, 2015).

Plasma levels of Cu, Zn and Mn in normal control group had no significant difference with starved rats+water/ethanol and starved rats+hydro-alcoholic Aloe vera gel extract in 100 and 200 mg/kg dose groups (P>0.05).

Regarding the unchanged levels of Cu, Zn and Mn, it can be suggested that despite the antioxidant activity of Cu, Zn and Mn, these trace elements could not indicate marked responses to oxidative injuries induced by the starvation. The unchanged levels of Cu, Zn and Mn in our work may be attributed to the occurrence of equilibrium between the levels of trace elements uptake and their levels in the serum and body tissues, particularly the liver storage which probably has a role in enhancing the decreased serum level of these trace elements in starved rats.

Plasma levels of vitamin A and E in normal control group had no significant difference with starved rats+water/ethanol and starved rats+hydro-alcoholic Aloe vera gel extract in 100 and 200 mg/kg dose groups (P>0.05). Plasma level of vitamin C significantly decreased in starved rats+water/ethanol group (P<0.05). Plasma level of vitamin C after treatment with hydro-alcoholic Aloe vera gel extract at doses 100 and 200 mg/kg was significantly increased (P<0.05).

Berggren Söderlund et al. (2003) have reported serum level of retinol decreased after 5 days fasting and significant correlation was observed between retinol and retinol binding protein and transthyretin plasma levels. Also, Hupert et al. (1989) showed increased hepatic retinol and decrease in plasma retinol during 72 h starvation. These results differed with our result, possibly because of longer starvation cycle.

Regarding the unchanged levels of antioxidant vitamins A and E on the one side and the significant decrease in serum level of vitamin C on the other, it can be suggested that despite the antioxidant activity of vitamins A and E, these vitamins could not indicate marked responses to oxidative injuries induced by the starvation. In contrast, vitamin C may play a significant role in protecting the cells from the invasion of free radicals. It can be proposed that despite cellular protective action of vitamins against oxidative stress as a result of starvation, the unchanged level of vitamins A and E in our work may be attributed to the occurrence of an equilibrium between the levels of vitamin uptake and their levels in the serum and body tissues, particularly the liver storage which probably has a role in enhancing the decreased serum level of these vitamins in starved rats.

Our results show vitamin C level significantly decreased in starved rats. Fukuwatari et al. (2010) have shown urinary excretion of ascorbic acid was not affected by fasting, these authors have also reported liver content of water soluble vitamins decreased during starvation but blood and muscle content of water soluble vitamins were not affected during starvation. Furthermore, ascorbic acid is synthesized from hexoses including glucose in many species. Previous study has shown synthesis of ascorbic acid is impaired during starvation (Stirpe and Comporti, 1965).

Aloe vera treatment increased antioxidant vitamin C levels in starved rats+hydro-alcoholic Aloe vera gel extract (100 mg/kg and 200 mg/kg) groups. Aloe vera gel extract had antioxidant effects and significantly increased serum vitamin C levels. Antioxidant activities have been increased in diabetic rats by Aloe vera treatment (Ramachandraiahgari et al., 2012). These antioxidant components scavenge reactive oxidant and aid endogenous antioxidants defense.

To summarize, plasma levels of antioxidant enzymes significantly decreased in starved rats (P<0.05). Plasma levels of antioxidant enzymes after treatment with hydro-alcoholic Aloe vera gel extract were significantly increased (P<0.05). Our results shown that short term starvation caused an increase in oxidative stress via impairing of antioxidant defense and Aloe vera treatment is capable of improving antioxidative defense induced by short term starvation.
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