EFFECT OF SAPONIN ON LIPID PEROXIDATION AND ANTIOXIDANT STATUS IN HIGH FRUCTOSE FED RATS

V. Prabakaran1 and T Malarvili2

1P.G. and Research Department of Biochemistry, Rajah Serfoji Govt. College (Autonomous), Thanjavur-613 005, Tamil Nadu, South India.
2P.G. and Research Department of Biochemistry, Rajah Serfoji Govt. College (Autonomous), Thanjavur-613 005, Tamil Nadu, South India.

Corresponding author: malarvili96@gmail.com

Received 21 November 2014; Accepted 16 December 2014

Abstract
Majority of the diseases/disorders are mainly linked to oxidative stress due to free radicals. Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism. The most common reactive oxygen species (ROS) ROS have been implicated in over a hundreds of diseases states which range from arthritis and connective tissue disorders to carcinogenesis, aging, physical injury, infection and acquired immunodeficiency syndrome. In treatment of these diseases, antioxidant therapy has gained an immense importance. Many synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) are very effective and possess potential health risk and toxic properties to human health. Natural products/ dietary photochemicals have aroused considerable interest in recent years as potential therapeutic agents to counteract oxidative stress. The medicinal value of chosen plant Achyranthes aspera seeds and investigate the effect of saponin on lipid peroxidation and antioxidant status in high fructose fed rats. Supplementation of Achyranthes aspera to high fructose diet fed rats showed a reduction in body weight gain, serum lipids, lipid peroxidation, and improvement in antioxidant levels suggests that Achyranthes aspera possesses significant antioxidant potential. The antioxidant activity of Achyranthes aspera may be due to the phytochemicals present in it.

Key words: Antioxidant, Lipid peroxidation, Achyranthes aspera, Fructose, Oxidative stress

1. INTRODUCTION
Obesity is set to be the world’s major cause of morbidity and mortality in the 21st Century. In 2012, more than 1.4 billion adults were overweight in the world, and at least 200 million men and nearly 300 million women from them were obese. Obesity significantly increases the risk of developing various life threatening diseases, including type II diabetes, hypertension, coronary heart disease, stroke and certain cancers. Many factors affect the onset of obesity including satiety control, reduced levels of physical exercise, and hormonal and genetic parameters which influence the metabolic pathways leading to increase in stored fat. Oxidative stress is highly correlated with a wide variety of inflammatory and metabolic disease states, including obesity. It is highly correlated with cumulative damage in the body done by free radicals inadequately neutralized by antioxidants. (Ogden et al., 2004).

The prevalence of obesity has been increasing worldwide, which has a great impact on lifestyle-related disorders such as coronary heart disease, atherosclerosis, and diabetes. Excess visceral abdominal fat accumulation appears to be a key feature of abdominal obesity contributing to the development of the metabolic syndrome. Therefore, preventing abdominal fat accumulation is an ideal option for the treatment of obesity and related diseases. Although most of the available drugs, such as orlistat, sibutramine, and rimonabant, have modest clinical efficacy, their use is often associated with gastrointestinal or cardiovascular and central nervous system side effects. The rich potential of nature to combat obesity has not been fully explored yet, and many newer leads can be obtained from natural sources. Medicinal plants are believed to be much safer and proved elixir in the treatment of various ailments including obesity.

Medicinal plants are assuming greater importance in the primary health care of individuals and communities in many developing countries. There has been an increase of demand in international trade because of very effective, cheaply available, supposedly have no side effects and used as alternative to allopathic medicines. Medicinal plants are believed to be much safer and proved elixir in the treatment of various ailments. According to the World Health
2. MATERIALS AND METHODS

2.1 Animals

Male albino rats of Wistar strain approximately weighing 180-190g were used in this study. They were healthy animals procured from Sri Venkateswara enterprises, Bangalore, India. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27±2ºC and 12 hours light / dark cycle) throughout the experimental period. All the animals were fed with experimental diet and water *ad libitum*. Diets were freshly mixed in small amounts every 2–3 days. They were acclimatized to the environment for 1 week prior to experimental use. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), New Delhi, India.

2.2 Chemicals

Fructose, Ethylene diamine tetra acetic acid (EDTA), Starch, Cellulose powder, casein, Trichloro acetic acid (TCA) was purchased from Sisco Research Laboratories Pvt. Ltd., India. All other chemicals and solvents used were of analytical grade available commercially.

2.3 Plant material:

The mature seeds of *Achyranthes aspera* were collected in January 2014 from Thanjavur, Thanjavur district, Tamil Nadu, India.

2.4 Preparation of plant extract

The collected seeds of *Achyranthes aspera* were cut into small pieces and shade dried at room temperature and makes a fine powder using grinder mixture. The powder material of *Achyranthes aspera* was macerated with 70% ethanol at room temperature for 3 days. After 3 days, the supernatant was transferred into china dish. The supernatant was completely removed by keeping the china dish over a boiling water bath at 45ºC. The ethanol extract of the plant contained saponins reported by Kumar *et al.*, (1990). A semi solid extract was obtained after complete elimination of alcohol. The obtained residue was kept in the refrigerator for further use. The extract was made up to a known volume in distilled water just before oral administration.

2.5 Preparation of control and high fructose diet

The control and high fructose diet were prepared by the method of Suwannaphet *et al.*, (2010). Table 1 represents the composition of the experimental rats.

Table 1 shows the composition of the experimental diets (g/kg diet)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Control diet</th>
<th>High-fructose (HF) diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Corn starch</td>
<td>530</td>
<td>---</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100</td>
<td>---</td>
</tr>
<tr>
<td>Fructose</td>
<td>---</td>
<td>630</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Vitamin mixture</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Cellulose powder</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

2.6 Experimental design

Body weights of the animals were recorded and they were divided into 3 groups of 6 animals each as follows.

**Group 1:** Normal control rats fed with control diet and served as a control.

**Group 2:** Fructose-fed animals received fructose-enriched diet for a period of 3 weeks

**Group 3:** Fructose-fed animals treated with *Achyranthes aspera* seed extract by oral gavage daily at a dose of 500 mg/kg body weight for 3 weeks.

2.7 Collection of blood and preparation of serum sample

At the end of the experimental period, the animals were killed cervical dislocation after an overnight fasting. The blood sample was collected. The blood was allowed to clot by standing at room temperature for 30 minutes and then refrigerated for another 30 minute. The resultant clear part was centrifuged at 3000 rpm for 10minutes and then the serum (supernatant) was isolated and stored at refrigerated until required for biochemical analysis.

2.8 Biochemical analysis

Reduced glutathione was estimated by method of Moron (1979). Malondialdehyde was estimated by the thiobarbituric acid assay method of Beuge and Aust (1978). Superoxide dismutase activity was determined by the procedure of Kakkar *et al.*, (1984). The activity of glutathione peroxidase was assayed by the method of Rotruck *et al.* (1973). The activity of catalase was assayed by the method of Beers and Sizer (1952). The level of ascorbic acid was estimated by the method of Omaye *et al.* (1979). α-tocopherol was estimated by the method of Baker *et al.* (1980).

Cholesterol was estimated by Allain (1974). Triglyceride was determined by the method of Werner (1981).

2.9 Statistical Analysis

The results were presented as mean ± SD. Data was statistically analyzed using student ‘t’ test. P values set as lower than 0.05 was considered as statistically significant.
Table I Effect of *Achyranthes aspera* on body weight, cholesterol and triglycerides in experimental rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (gm)</td>
<td>185 ± 10.24</td>
<td>242 ± 14.23*</td>
<td>192 ± 12.45**</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>65.4±24.22</td>
<td>266±75.02*</td>
<td>78.7±13.06**</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>75.45±5.19</td>
<td>106.44±1.81*</td>
<td>77.7±7.71**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for six rats in each group.
* Significantly different from Group I (P < 0.05)
** Significantly different from Group II (P < 0.05)

Table II Effect of *Achyranthes aspera* on MDA and GSH in experimental rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol /L)</td>
<td>9.84±1.48</td>
<td>22.11±2.5*</td>
<td>13.02±0.98**</td>
</tr>
<tr>
<td>GSH (mg/dl)</td>
<td>8.77±0.63</td>
<td>5.18±0.07*</td>
<td>8.44±0.12**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for six rats in each group.
* Significantly different from Group I (P < 0.05)
** Significantly different from Group II (P < 0.05)

Table IV Effect of *Achyranthes aspera* on Glutathione peroxidase, Catalase, and SOD in experimental rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD(U/ml)</td>
<td>1.93±0.34</td>
<td>1.84±0.13*</td>
<td>2.69±0.09**</td>
</tr>
<tr>
<td>Catalase (U/ml)</td>
<td>5.88±0.71</td>
<td>2.5±1.05*</td>
<td>6.28±1.16**</td>
</tr>
<tr>
<td>GPx (U/ml)</td>
<td>2.81±0.45</td>
<td>1.67±0.21</td>
<td>2.38±0.37**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for six rats in each group.
* Significantly different from Group I (P < 0.05)
** Significantly different from Group II (P < 0.05)

Table III Effect of *Achyranthes aspera* on Vitamin C and Vitamin E in experimental rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin C (mg/dl)</td>
<td>9.06±1.03</td>
<td>7.76±1.55 *</td>
<td>9.03±0.18   **</td>
</tr>
<tr>
<td>Vitamin E (mg/dl)</td>
<td>1.38±2.66</td>
<td>9.83±1.94 *</td>
<td>16.91±3.46**</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± SD for six rats in each group.
* Significantly different from Group I (P < 0.05)
** Significantly different from Group II (P < 0.05)

3. RESULTS

The present study was carried out to evaluate the Antioxidant activity of *Achyranthes aspera* on Fructose induced oxidative stress in rats. The observations made on different groups of experimental and control animals were compared as follows.

Table I represents the body weight of normal and experimental rats. Group II Fructose induced oxidative stress in rats showed a significant increased in the body weight when compared to Group I rats. Group III Fructose induced oxidative stress in rats treated with *Achyranthes aspera* significantly decreased in the body weight when compared to group II.

Table I represents the levels of cholesterol and triglycerides in normal and experimental rats. Group II Fructose induced oxidative stress in rats showed a significant increased in the levels of cholesterol and triglycerides when compared to Group I rats. Group III Fructose induced oxidative stress in rats treated with *Achyranthes aspera* significantly decreased in the levels of cholesterol and triglycerides when compared to group II.

Table II represents the levels of MDA and GSH in serum of normal and experimental rats. Group II Fructose induced oxidative stress in rats showed a significant increased in the level of MDA when compared to Group I rats. Group III Fructose induced oxidative stress in rats treated with *Achyranthes aspera* significantly decreased in the level of MDA when compared to group II.

Table II represents the levels of MDA and GSH in serum of normal and experimental rats. Group II Fructose induced oxidative stress in rats showed a significant increased in the level of GSH when compared to Group I rats. Group III Fructose induced oxidative stress in rats treated with *Achyranthes aspera* significantly decreased in the level of GSH when compared to group II.
compared to Group I rats. Group III Fructose induced oxidative stress in rats treated with Achyranthes aspera significantly increased in the level of GSH as compared to group II.

Table IV represents the activity of SOD, Catalase and Glutathione peroxidase (GPx) in serum of normal and experimental rats. Group II Fructose induced oxidative stress in rats showed a significant decreased in the activity of SOD when compared to Group I rats. Group III Fructose induced oxidative stress in rats treated with Achyranthes aspera significantly increased in the activity of SOD as compared to group II.

Group II Fructose induced oxidative stress in rats showed a significant decreased in the activity of Catalase when compared to Group I rats. Group III Fructose induced oxidative stress in rats treated with Achyranthes aspera significantly increased in the activity of Catalase when compared to group II.

Group II Fructose induced oxidative stress in rats showed a significant decreased in the activity of GPx when compared to Group I rats. Group III Fructose induced oxidative stress in rats treated with Achyranthes aspera significantly increased in the level of vitamin C when compared to group II.

Group II Fructose induced oxidative stress in rats showed a significant decreased in the level of vitamin E when compared to Group I rats. Group III Fructose induced oxidative stress in rats treated with Achyranthes aspera significantly increased in the level of vitamin C when compared to group II.

Group II Fructose induced oxidative stress in rats showed a significant decreased in the activity of GPx as compared to group II.

Table III represents the levels of vitamin C and vitamin E in serum experimental rats. Group II Fructose induced oxidative stress in rats showed a significant decreased in the level of vitamin C when compared to Group I rats. Group III Fructose induced oxidative stress in rats treated with Achyranthes aspera significantly increased in the level of vitamin C when compared to group II.

Group II Fructose induced oxidative stress in rats showed a significant decreased in the activity of GPx when compared to Group I rats. Group III Fructose induced oxidative stress in rats treated with Achyranthes aspera significantly increased in the level of vitamin C when compared to group II.

4. DISCUSSION

Feeding of high fructose diet (HFD) to rats was proved to be a useful model of putative effects of dietary fat in humans (Lopez et al., 2003). Rat models are therefore useful tools for inducing obesity as they will readily gain weight when fed high fructose diets (Dienm et al., 2006).

In the present study, obesity was induced in white albino rats by using a high fructose diet formula. Obesity was induced in 3 weeks. The weight gained by rats fed HFD formula, was significantly more than that gained by those fed the normal diet. Many workers were able to induce obesity in rats using different formulas of high fructose diets (Suwannaphet et al., 2010). The response of animals to the HFD is a subtle but cumulative effect, because it took over a 10 weeks period. The difference in weight gain in all above studies may be due to age, genetic makeup of the different strains and composition of different formulas. HFD resulted in dyslipidemic changes as illustrated by increasing serum levels of triacylglycerol and total cholesterol as compared with control; a finding in accordance with that of Woo et al (2008). Dyslipidemic changes occurs in obesity may be due to the increased triacylglycerol, content due to increased influx of excess Non etherified fatty acids (NEFAs) into the liver (Grundy, 2004). It has been revealed that altered lipid concentrations and qualitative changes of the lipoprotein fractions in obesity are associated with an increased risk of various adverse effects of obesity (Despres et al., 2008). Additionally, lipid alterations have been considered as contributory factors to oxidative stress in obesity (Leopold et al., 2008). Increased production of reactive oxygen species as well as reduced antioxidant defense mechanisms have been suggested to play a role in both humans and animal models of obesity (Keaney et al., 2003). Treatment of HFD fed rats with Achyranthes aspera showed considerable restoration of lipid levels to that of control. Lipid dysregulation in fructose-fed rat model has been associated to the activation of oxidative stress and inflammatory pathways in the liver which favours the progression to Nonalcoholic fatty liver disease (NAFLD) (Basciano et al., 2005).

Lipid peroxidation is thought to be a component of obesity-induced pathology (Amirkhizī et al., 2007). The data presented in this study showed that obesity increased lipid peroxidation in serum as expressed by increased tissue levels of MDA. Our results are in basic agreement with the results of Vincent, et al., (2001), Olusi et al., (2002), and Amirkhizī et al., (2007) who showed that, obesity is an independent risk factor for increasing lipid peroxidation and decreased activity of cytoprotective enzymes. Obesity can cause increased lipid peroxidation by progressive and cumulative cell injury resulting from pressure of the large body mass. Cell injury causes the release of cytokines, especially tumor necrosis factor alpha (TNF-α) which generates ROS from the tissues which in turn cause lipid peroxidation (Lachitieiner et al., 2000). The hypertriglyceridemia seen in obese rats may contribute to the alteration in the oxidant antioxidant balance, suggesting that an increase in the bioavailability of free fatty acids can increase lipid peroxidation (Vincent, et al., 2001). The markers of oxidative injury (MDA, LHP and protein carbonyl) were significantly elevated. Achyranthes aspera could effectively protect against the oxidative stress induced by HFD. These findings are concordant with those of other investigators (Oben et al., 2006).

It has been shown that animal body had an effective mechanism to prevent the free radical induced tissue cell damage, this accomplished by a set of endogenous antioxidant enzymes and protein such as SOD, CAT, GPX, GRD and non-enzymatic antioxidants GSH, Vitamin C and E. When the balance between ROS production and antioxidant defense is lost oxidative stress results; which through a serious of events deregulates the cellular functions leading various pathological conditions (Blokhina et al., 2002). GST, CAT and GPX constituted a mutually supportive team of defense against reactive oxygen species. In the present study GST, CAT and GPX enzymes activity and GSH, Vitamin C and E were measured in serum and the data showed clearly a significant decrease in the activities of SOD, CAT and GPX enzymes in obese rats as compared to the control group. GSH, Vitamin C and E levels showed significant decrease in obese rats. Our results were in agreement with many
authors (Lannaud et al., 1999). There are several mechanisms explaining the reduction of antioxidant enzymes in obese rats;

The increased lipid peroxidation lead to inactivation of the enzymes by cross-links with MDA; this will cause an increased accumulation of superoxide, \( \text{H}_2\text{O}_2 \), and hydroxyl radicals which could further stimulate lipid peroxidation. This mechanism has a clue from work of Demori et al., (2006) and Moya et al., (2008) who showed that the catalese, glutathione peroxidase, and superoxide dismutase were reduced in response to the cafeteria-diet feeding in obese rats. Furthermore our correlation study indicated that there is negative correlation between MDA and enzymes activities of SOD, CAT and GPx in the serum and supported the concept of inactivation of antioxidant enzymes and proteins by high level of lipid peroxidation in obesity. Decrease of antioxidant enzyme may be due to rapid consumption and exhaustion of storage of this enzyme in fighting free radicals generated during development of obesity. ). Our results show that HFD caused significant decreases in SOD, CAT and GPx activities. *Achyranthes aspera* supplementation improved the antioxidant defense mechanisms and suppressed oxidative damage in HFFD-fed rats.

Ascorbate (vitamin C) plays an important role with the lipophilic antioxidant \( \alpha \) – tocopherol in protecting the membrane from oxidative stress. Recycling of ascorbic acid requires GSH, which reduces dehydroascorbate to ascorbate (Winkler, 1992). Ascorbate in turn is essential for the recycling of tocopherol radical to tocopherol (Packer et al., 1997). The observed decline in glutathione level may contribute to the decrease in ascorbate as well tocopherol concentration in aged rats. In the present study the decreased level of plasma vitamin C and Vitamin E were observed in C.S.E., rats, demonstrating the increased free radicals accumulation in Supplementation of plant to obese rats improved the vitamin C and Vitamin E level as compared to control rats, which may be due to the presence of Vitamin C and polyphenolic component in plant. Earlier reports suggest that polyphenols may regenerate \( \alpha \)-tocopherol through reduction of the \( \alpha \)-tocopheroxy radical (Bors et al., 1990) *Cinnamomum cassia* treatment to obese induced oxidative stress in rats maintained the normal level of Vitamin C and E.

Plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free-radical terminators. They possess a wide spectrum of biochemical activities such as antioxidant, anti-obesity, antimutagenic, antitumor, and enzymes activities of SOD, CAT and GPx in the serum and supported the concept of inactivation of antioxidant enzymes and proteins by high level of lipid peroxidation in obesity. Decrease of antioxidant enzyme may be due to rapid consumption and exhaustion of storage of this enzyme in fighting free radicals generated during development of obesity. ). Our results show that HFD caused significant decreases in SOD, CAT and GPx activities. *Achyranthes aspera* supplementation improved the antioxidant defense mechanisms and suppressed oxidative damage in HFFD-fed rats.

Ascorbate (vitamin C) plays an important role with the lipophilic antioxidant \( \alpha \) – tocopherol in protecting the membrane from oxidative stress. Recycling of ascorbic acid requires GSH, which reduces dehydroascorbate to ascorbate (Winkler, 1992). Ascorbate in turn is essential for the recycling of tocopherol radical to tocopherol (Packer et al., 1997). The observed decline in glutathione level may contribute to the decrease in ascorbate as well tocopherol concentration in aged rats. In the present study the decreased level of plasma vitamin C and Vitamin E were observed in C.S.E., rats, demonstrating the increased free radicals accumulation in Supplementation of plant to obese rats improved the vitamin C and Vitamin E level as compared to control rats, which may be due to the presence of Vitamin C and polyphenolic component in plant. Earlier reports suggest that polyphenols may regenerate \( \alpha \)-tocopherol through reduction of the \( \alpha \)-tocopheroxy radical (Bors et al., 1990) *Cinnamomum cassia* treatment to obese induced oxidative stress in rats maintained the normal level of Vitamin C and E.  

The results of the above data demonstrated that reduction in body weight gain, serum lipids, lipid peroxidation, and improvement in antioxidant levels suggests that *Achyranthes aspera* possesses significant antioxidant and anti-obesity potential. The anti-oxidant activity of *Achyranthes aspera* may be due to the phytochemicals present in it.

5. **Acknowledgment**

The authors are thankful to Dr. S. Velavan, Director, Harman Institute of Science Education and Research for providing necessary support to complete the work.

6. **References**


25. Source of support: Nil; Conflict of interest: None declared