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

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ABSTRACT	Article Info:
<p>The present study was carried out to evaluate the Antioxidant activity of <i>Aerva lanata</i> flower extract on Fructose induced oxidative stress in rats. Supplementation of <i>Aerva lanata</i> flower extract to high fructose diet fed rats exerts the following results, Decreased the body weight, Normalized the cholesterol and triglycerides, Declined the oxidative stress marker as Malondialdehyde (MDA), Restored the enzymatic antioxidants SOD, GPx and CAT, Improved the non enzymatic antioxidant GSH, Vitamin E and C. The results of the above study demonstrated that reduction in body weight gain, serum lipids, lipid peroxidation, and improvement in antioxidant levels suggests that <i>Aerva lanata</i> flower extract possesses significant anti-obesity and antioxidant potential. The anti-obesity activity of <i>Aerva lanata</i> flower extract may be due to the chrysin present in it.</p> <p>Citation: M. Kowsalya and T. Malarvili. (2015) Effect of chrysin on lipid peroxidation and antioxidant status in high fructose fed rats. World Journal of Science and Research. 1(1): 19-23.</p>	<p>Received on 14 April 2015</p> <p>Accepted on 18 May 2015</p> <p>Keywords:</p> <p><i>Aerva lanata</i>, Antioxidant, Obesity, Fructose, Oxidative stress</p>
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INTRODUCTION

According to the World Health Organization (WHO), obesity is classified as chronic and severe disease in developed and developing countries, affecting both adults and children. Recent research data suggest that the global incidence of obesity has increase more than 75% since 1980, while the last twenty years has tripled in developing countries and particularly, in low-income countries. More than 1.1 billion adults are overweight, of which 312 million are obese. According to estimates of the International Obesity Task Force, 1.7 billion people are exposed to health risks related to body weight, while the increase in Body Mass Index (BMI) is responsible for more than 2.5 million deaths annually, which is expected to double by 2030 (Berghofer *et al.*, 2008).

Obesity is a chronic disease which has spread all over the world and threatens public global health. The phenomenon of obesity has drawn the attention of the scientific community, organizations and governments worldwide because it affects people's lives negatively and imposes excessive financial implications in every health system. In addition, obesity has been the major interest in health sciences and many research studies have focused not only on the prevalence and the risk factors of obesity but also on the significant consequences on the quality of patients' life. Furthermore, is associated with increase incidence of type 2 diabetes mellitus, hypertension, coronary heart disease, arthritis, sleep apnea, and certain forms of cancer (Ogden *et al.*, 2004). Medicinal plants are believed to be much safer and proved elixir in the treatment of various ailments including obesity. The

medicinal value of chosen plant *Aerva lanata* flower was evaluated for anti-obesity and antioxidant activity.

MATERIALS AND METHODS

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±2°C 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 acclimatization 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 of Experiments on Animals (CPCSEA), New Delhi, India.

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.

Plant material:

The mature flower of *Aerva lanata* was collected in January 2014 from Thanjavur, Thanjavur district, Tamil Nadu, India.

Preparation of plant extract

The collected flower of chrysin was shade dried at room temperature and made a fine powder using grinder mixture. The powder material of *Aerva lanata* flower extract 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 45°C. 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.

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.

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 *Aerva lanata* flower extract by oral gavage daily at a dose of 500 mg/kg body weight for 3 weeks.

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

Ingredients	Control diet	High-fructose (HF) diet
Casein	200	200
Corn starch	530	----
Sucrose	100	----
Fructose	---	630
Soybean oil	70	70
Mineral mixture	35	35
Vitamin mixture	10	10
Cellulose powder	50	50
L-Cystine	3	3

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.

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 mitochondrial 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). Serum Cholesterol was estimated by Allain (1974). Serum triglyceride was determined by the method of Werner (1981).

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.

RESULTS

The present study was carried out to evaluate the Antioxidant activity of *Aerva lanata* flower extract 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 increase in the body weight when compared to Group I rats. Group III Fructose

induced oxidative stress in rats treated with *Aerva lanata* flower extract significantly decreased the body weight when compared to group II.

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

Table II Effect of *Aerva lanata* flower extract on body weight, cholesterol and triglycerides in experimental rats

Parameters	Group I	Group II	Group III
Body weight (gm)	190± 11.11	255±16.3*	198 ± 10.44
Cholesterol (mg/dl)	81.81±0.84	0.27±11.11*	106.03±9.43*
Triglycerides (mg/dl)	110.73±6.50	218±12.39*	116.66±4.97*

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)

Table III 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 increase in the level of MDA when compared to Group I rats. Group III Fructose induced oxidative stress in rats treated with *Aerva lanata* flower extract significantly decreased the level of MDA when compared to group II.

Group II Fructose induced oxidative stress in rats showed a significant decrease in the level of GSH when compared to Group I rats. Group III Fructose induced oxidative stress in rats treated with *Aerva lanata* flower extract significantly increased in the level of GSH as compared to group II.

Table III Effect of *Aerva lanata* flower extract on MDA and GSH in experimental rats

Parameters	Group I	Group II	Group III
MDA (nmol /L)	18.02±3.17	32.87±1.92*	20.14±0.97**
GSH (mg/dl)	41.29±0.60	25.35±8.97*	27.16±0.45**

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)

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 decrease in the activity of SOD when compared to Group I rats. Group III Fructose induced oxidative stress in rats treated with *Aerva*

lanata flower extract significantly increase the activity of SOD as compared to group II.

Group II Fructose induced oxidative stress in rats showed a significant decrease in the activity of Catalase when compared to Group I rats. Group III Fructose induced oxidative stress in rats treated with *Aerva lanata* flower extract significantly increased the activity of Catalase when compared to group II.

Group II Fructose induced oxidative stress in rats showed a significant decrease in the activity of GPx when compared to Group I rats. Group III Fructose induced oxidative stress in rats treated with *Aerva lanata* flower extract significantly increased the activity of GPx as compared to group II.

Table IV Effect of *Aerva lanata* flower extract on (GPx), (CAT), and SOD in experimental rats

Parameters	Group I	Group II	Group III
SOD(U/ml)	4.31±1.03	2.16±0.85*	4.97±0.65**
CAT (U/ml)	5.27±0.03	1.41±0.38*	5.81±0.52**
GPx (U/ml)	2.95±0.25	0.81±0.15*	2.17±0.18**

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)

Table IV represents the levels of vitamin C and vitamin E in serum experimental rats. Group II Fructose induced oxidative stress in rats showed a significant decrease in the level of vitamin C when compared to Group I rats. Group III Fructose induced oxidative stress in rats treated with *Aerva lanata* flower extract significantly increase the level of vitamin C when compared to group II.

Group II Fructose induced oxidative stress in rats showed a significant decrease the level of vitamin E when compared to Group I rats. Group III Fructose induced oxidative stress in rats treated with *Aerva lanata* flower extract significantly increased the level of vitamin E as compared to group II.

Table IV Effect of *Aerva lanata* flower extract on Vitamin C and Vitamin E in experimental rats

Parameters	Group I	Group II	Group III
Vitamin C (mg/dl)	11.42±38.87	8.97±0.38*	1.073±7.51**
Vitamin E (mg/dl)	3.96±0.16	1.50±0.08*	3.51±0.14**

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)

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 (Diemen *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; Elliott *et al.*, 1992). 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 increase triacylglycerol, content due to increase 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 increase 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). Increase 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 *Aerva lanata* flower extract 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 (Amirkhizi *et al.*, 2007). The data presented in this study showed that obesity increase lipid peroxidation in serum as expressed by increase tissue levels of MDA. Our results are in basic agreement with the results of Vincent, *et al.*, (2001), Olusi *et al.*, (2002), and Amirkhizi *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 increase 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 (Lachietner *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. *Aerva lanata* flower extract 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 series of events deregulates the cellular functions leading various pathological conditions (Blokina *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 increase lipid peroxidation lead to inactivation of the enzymes by crosses linking with MDA; this will cause an increase accumulation of superoxide, H₂O₂ 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 catalase, 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. *Aerva lanata* flower extract supplementation improved the antioxidant defense mechanisms and suppressed oxidative damage in HFD-fed rats.

Ascorbate (vitamin C) plays an important role with the lipophilic antioxidant α -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 increase free radicals accumulation in Supplementations 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 α -tocopherol through reduction of the α -tocopheroxyl radical (Bors *et al.*, 1990). *Aerva lanata* treatment to flower extract 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, anticarcinogenic as well as ability to modify the gene expression (Cao *et al.*, 1997). The potential activity of anti-obesity and antioxidant activity of *Aerva lanata* flower due to the presence of chrysin (Liu *et al.*, 2014).

CONCLUSION

Supplementation of *Aerva lanata* flower extract to high fructose diet fed rats exerts the following results, Decreased the body weight, Normalized the cholesterol and triglycerides, Declined the oxidative stress marker as Malondialdehyde (MDA), Restored the enzymatic antioxidants SOD, GPx and CAT, Improved the non enzymatic antioxidant GSH, Vitamin E and C. The results of the above study demonstrated that reduction in body weight gain, serum lipids, lipid peroxidation, and improvement in antioxidant levels suggests that *Aerva lanata* flower extract possesses significant anti-obesity and antioxidant potential. The anti-obesity activity of *Aerva lanata* flower extract may be due to the chrysin present in it.

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