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Research Article **Biochemistry**

Hypolipidemic effect of *Bryonopsis lacinosa* fruit in high fructose fed rats

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ABSTRACT

In the present study to investigate the hypolipidemic effect of *Bryonopsis lacinosa* fruit in high fructose fed rats. Animals 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 *Bryonopsis laciniosa* seed extract by oral gavage daily at a dose of 500 mg/kg body weight for 3 weeks. The observations made on different groups of experimental and control animals were compared. The results of the study concluded that reduction in body weight gain, serum lipids and lipid peroxidation levels suggests that *Bryonopsis laciniosa* possesses significant hypolipidemic potential. The hypolipidemic activity of *Bryonopsis laciniosa* may be due to the phytochemicals present in it.

Keywords: Lipid profile, Obesity, *Bryonopsis lacinosa*, Fructose, Hypolipidemic activity.

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INTRODUCTION

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 increased incidence of type 2 diabetes mellitus, hypertension, coronary heart disease, arthritis, sleep apnea, and certain forms of cancer (Ogden *et al.*, 2004).

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 increased 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 (Berghöfer *et al*., 2008).

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 Organization (WHO), approximately 80% of the world's population currently usesherbal medicines in healing different ailments. Among the estimated 400,000 plant species, only 6% have been studied for biological activity, and about 15% have been investigated phytochemically. This shows a need for planned activity guided phyto-pharmacological evaluation of herbal drugs (Ashis, 2003). In the present study, the chosen plant *Bryonopsis laciniosa* and evaluate their antiobesity 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±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 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 seeds of *Bryonopsis laciniosa* were collected in February 2015 from Thanjavur, Thanjavur district, Tamil Nadu, India.

Preparation of plant extract

The collected seeds of *Bryonopsis laciniosa* were cut into small pieces and shade dried at room temperature and makes a fine powder using grinder mixture. The powder material of *Bryonopsis laciniosa* was macerated with 50% methanol 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. 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.

Phytochemical analysis

Chemical tests were carried out on the alcoholic extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973, 1984).

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)

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

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 *Bryonopsis laciniosa* seed extract by oral gavage daily at a dose of 500 mg/kg body weight for 3 weeks.

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). Glucose was estimated by GOD/POD method (Trinder, 1969). Serum Cholesterol was estimated by Allain (1974). Serum triglyceride was determined by the method of Werner (1981). HDL cholesterol was estimated by the method of Allain *et al* (1974). Low Density lipoprotein

Cholesterol (LDL) and Very Low Density Lipoprotein (VLDL) were calculated as per Friedewald's (1972) equation

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 on the plant sample revealed the presence of medicinally active constituents. The phytochemical characters of the *Bryonopsis laciniosa* investigated a summarized in Table 1. The present study was carried out to evaluate the hypolipidemic activity of *Bryonopsis laciniosa* in rats. The observations made on different groups of experimental animals were compared as follows.

Table 1. Phytochemical screening of *Bryonopsis laciniosa*

Test	Result
Saponin	+
Flavonoids	+
Steroids	++
Alkaloids	+
Polyphenol	++

(+)Presence (-) Absence

Antiobesity activity

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

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 *Bryonopsis laciniosa* significantly decreased in the level of MDA when compared to group II.

Group II Fructose induced oxidative stress in rats showed a significant decreased in the level of GSH when compared to Group I rats. Group III Fructose induced oxidative stress in rats treated with *Bryonopsis laciniosa* significantly increased in the level of GSH as compared to groupII.

Table II Effect of *Bryonopsis laciniosa* on MDA and GSH in experimental rats

Parameters	Group I	Group II	Group III
MDA (nmol /L)	5.49±1.175	12.49±0.91*	5.45±0.77**
GSH (mg/dl)	2.27± 0.40	1.09 ±0.26*	2.29 ±0.42**

Values were expressed as mean ± SD for six rats in each group.

* Significantly different from Group I ($P < 0.001$)

** Significantly different from Group II ($P < 0.001$)

Table III 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 *Bryonopsis laciniosa* significantly decreased in the body weight when compared to group II.

Table III 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 *Bryonopsis laciniosa significantly* decreased in the levels of cholesterol and triglycerides when compared to group II.

Table IV represents the activity of HDL, VLDL and LDL cholesterol in serum of normal and experimental rats. Group II Fructose induced oxidative stress in rats showed a significant decreased in the content of HDL cholesterol when compared to Group I rats. Group III Fructose induced oxidative stress in rats treated with *Bryonopsis laciniosa* significantly increased in the content of HDL as compared to group II.

Table III Effect of *Bryonopsis laciniosa* on body weight, cholesterol and triglycerides in experimental rats

Parameters	Group I	Group II	Group III
Body weight (gm)	165 ± 10.24	242 ± 14.23*	192 ± 12.45**
Cholesterol (mg/dl)	137.33±9.02	400.74±23.94**	122.21±14.52**
Triglycerides (mg/dl)	88.52±1.23	114.75±27.83*	97.6±43.33**

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 ($P < 0.05$)

Group IV Fructose induced oxidative stress in rats showed a significant increased in the content of VLDL cholesterol when compared to Group I rats. Group III Fructose induced oxidative stress in rats treated with *Bryonopsis laciniosa* significantly decreased in the content of VLDL when compared to group II.

Group IV Fructose induced oxidative stress in rats showed a significant increased in the content of LDL cholesterol when compared to Group I rats. Group III Fructose induced oxidative stress in rats treated with *Bryonopsis laciniosa* significantly decreased in the content of LDL when compared to group II.

Table IVEffect of *Bryonopsis laciniosa* on Glutathione peroxidase, Catalase, and SOD in experimental rats

Parameters	Group I	Group II	Group III
HDL Cholesterol (mg/dl)	42.30 ± 11.21	17.94 ± 2.15*	66.02 ± 2.31**
VLDL Cholesterol (mg/dl)	87.83±0.26	342.3±23.94*	102.9±8.003**
LDL Cholesterol (mg/dl)	88.52±1.23	114.75±27.83*	97.6±43.33**

Values were expressed as mean ± SD for six rats in each group.
* Significantly different from Group I (P < 0.001)
** Significantly different from Group II (P < 0.001)

Group V Fructose induced oxidative stress in rats showed a significant increased in the content of Glucose cholesterol when compared to Group I rats. Group III Fructose induced oxidative stress in rats treated with *Bryonopsis laciniosa* significantly decreased in the content of Glucose when compared to group II.

Table V Effect of *Bryonopsis laciniosa* on Glutathione peroxidase, Catalase, and SOD in experimental rats

Parameters	Group I	Group II	Group III
Glucose (mg/dl)	87.09 ± 1.60	160.42 ± 1.52*	82.52 ± 1.44**

Values were expressed as mean ± SD for six rats in each group.
* Significantly different from Group I (P < 0.001)
** Significantly different from Group II (P < 0.001)

DISCUSSION

Administration of high fructose fed (HFD) diet induces the development of metabolic syndrome characterized by obesity, IR and liver steatosis. A body of evidence indicates that accumulation of fat in the serum increases the susceptibility to other insults such as oxidative stress and subsequent inflammation that results in the progression of steatosis to steatohepatitis, fibrosis and cirrhosis (Koteish and Diehl, 2002). Considering the recently recognized association between IR, oxidative stress and inflammation, the present experiment confirms that the combination of fructose and fat could result in oxidative liver injury. Induction of oxidative stress is evident from the increased peroxidation markers and inadequate antioxidant status in the blood and liver of rats fed HFD. The markers of oxidative injury (MDA, LHP and protein carbonyl) were significantly elevated. *Bryonopsis laciniosa* could effectively protect against the oxidative stress induced by HFD. These findings are concordant with those of other investigators (Oben *et al.*, 2006).

A mechanism for the hyperlipidemic effects of fructose has been suggested previously (Reiser and Hallfrisch, 1987). Once absorbed, fructose is primarily metabolized by the liver. Fructose metabolism is unique in that it enters glycolysis or gluconeogenesis at the triphosphate level, bypassing the need for insulin and the action of phosphofructokinase. After fructokinase catalyzes phosphorylation of fructose to fructose 1-phosphate, the resulting compound is split by hepatic aldolase B into glyceraldehyde and dihydroxyacetone phosphate. The activities of fructokinase and hepatic aldolase B are increased when the amount of fructose in the diet is increased, leading to enhanced hepatic lipogenesis. This process is likely to increase VLDL production and secretion, thus elevating both blood triglycerides and cholesterol. Furthermore, fructose does not appear to stimulate lipoprotein lipase (Rutledge and Adeli, 2007), which may result in reduced clearance of triglycerides from the plasma. It is important to note that chronic fructose feeding may result in adaptation by healthy animals without developing metabolic disturbances (Stark et al., 2000), and that shorter test periods as used in this study could produce adverse results that may be transient.

Levels of triglycerides and cholesterol were significantly elevated in serum. Evidence of lipid accumulation in liver exposed to HFD (Aragno *et al.*, 2009) and in rats drinking fructose- sweetened beverages (Jurgens *et al.*, 2005) has been reported. Fructose is highly lipogeneic and the HFFD diet used in this study may have resulted in the increased delivery of fatty acids through the portal circulation resulting in fatty liver. Treatment of HFD fed rats with *Bryonopsis laciniosa* 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). An evolving hypothesis is that metabolic disease, ROS formation and inflammation create a progressive cycle leading to disease progression and NAFLD (Raval *et al.*, 2006).

Hypertriglyceridemia may be due to a defect in removal of VLDL from plasma or increased secretion of VLDL. Lipoprotein lipase is an important enzyme responsible for the hydrolysis of triglyceride chylomicrons and VLDL. Significant reduction in the activity of LPL as seen in the present study, can cause hypertriglyceridemia and accumulation of VLDL in plasma of the fructose-fed rats. Hypertriglyceridemia found in fructose-fed rats was reversed when the rats were supplemented simultaneously with BABE. The triglyceride lowering effect of *Bryonopsis laciniosa* is attributed to both enhanced peripheral tissue clearance of plasma triglycerides and increased LPL activity.

Oxidative stress is evident from the increased peroxidation and inadequate antioxidant status in the heart tissue of high fat fed rats. Increased peroxidation in high fat fed rats could be due to elevation in blood glucose. A relationship between glucose concentration and oxidative stress has been shown in bovine endothelial cells (Giardino *et al.*, 1996). Hyperglycemia can generate free radicals by autooxidation and glycation of proteins (Ceriello, 2000). Prolonged hyperglycemia could result in an increased risk of fatal and non-fatal cardiovascular events. Hyperglycemia

can not only generate more ROS but can also attenuate the autooxidative mechanism through glycation of the scavenging enzymes (Jakus, 2000).

The action of insulin in lowering blood glucose levels results from the suppression of hepatic glucose production and the increased glucose uptake into muscle and adipose tissue via GLUT4. Muscle has long been considered the major site of insulin-stimulated glucose uptake in vivo, with adipose tissue contributing relatively little to total body glucose disposal. On the other hand, various transgenic studies have raised the possibility of a greater role for glucose uptake into fat in systemic glucose homeostasis. Over-expression of GLUT4 selectively in fat tissue enhances whole body insulin sensitivity and glucose tolerance (Shepherd and Kahn, 1999), and knocking out GLUT4 selectively from fat tissue results in a degree of insulin resistance similar to that seen with muscle-specific knockout of GLUT4. In all forms of obesity, there is downregulation of GLUT4, a major factor contributing to the impaired insulin-stimulated glucose transport in adipocytes (Shepherd *et al.,* 1993). However, in the skeletal muscle of obese humans, GLUT4 expression is normal. It has also been suggested that defective glucose transport may be due to impaired translocation, docking, or fusion of GLUT4- containing vesicles with the plasma membrane. With obesity there is reduced glucose disposal in adipose tissue. It has been suggested that obesity leads to the development of hyperglycemia, hyperlipemia hyperinsulinemia and insulin resistance. Molecules like FFA, leptin or TNF-α, all of which are released from adipose tissue, are known to affect glucose homeostasis indirectly (Colditz *et al*., 1990),

The results of the above study concluded that reduction in body weight gain, serum lipids, lipid peroxidation suggests that *Bryonopsis laciniosa* possesses significant hypolipidemic potential. The hypolipidemic activity of *Bryonopsis laciniosa* may be due to the phytochemicals present in it.

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