

**LIPIDS LOWERING EFFECT OF *HIBISCUS ROSA-SINENSIS* FLOWER PETALS ON MONOSODIUM GLUTAMATE (MSG) INDUCED OBESE RATS**

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**Summary**

Monosodium glutamate (MSG) regularly used as a flavoring agent in cooking. The consumption of MSG has been shown to cause endothelial dysfunction long before development of any significant obesity. The present study was aimed to evaluate the lipids lowering effect of *Hibiscus rosa-sinensis* flower petals on MSG induced obesity in female albino rats. The feed intake and body weight gain was increased in MSG rats. The levels of free fatty acids, triglycerides (TG), phospholipids, total cholesterol (TC), low-density lipoprotein (LDL) cholesterol and very low-density lipoprotein (VLDL) cholesterol were increased and high-density lipoprotein (HDL) cholesterol level was decreased in MSG obese rats. After administration of *H. rosa-sinensis*, the feed intake and bodyweight gain were normalized and also the levels of free fatty acids, TG, phospholipids, TC, VLDL cholesterol, LDL cholesterol and HDL cholesterol were reverted to near normal. The results are also compared with those of the silymarin, the standard drug treatment to MSG induced obese rats.

**Keywords:** *Hibiscus rosa-sinensis*; Lipids; Lipoproteins; Monosodium glutamate; Obesity

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## Introduction

Monosodium glutamate (MSG) regularly used as a flavoring agent in cooking to increase palatability of food [1,2]. Therefore, the consumption of MSG has increased throughout the world in recent years. However, consumption of a highly palatable diet has been shown to cause endothelial dysfunction long before development of any significant obesity [3], and overfeeding has been associated with cancer [4], cardiovascular disease [5], and various age-related degenerative disease processes [6]. Obesity is considered to be the most striking clinical manifestation observed in adults after MSG administration during the neonatal / infant period [7]. Hyperlipidemia is related to obesity, which in turn may be due to neuronal necrosis in the hypothalamus after injection of MSG [8]. MSG has been shown to induce obesity, insulin resistance and dyslipidemia associated with chemical ablation of the arcuate nucleus [9].

*Hibiscus rosa-sinensis* Linn (Family: Malvaceae) is an evergreen woody glabrous showy shrub [10]. The major constituents of *H. rosa-sinensis* flowers are reported to contain quercetin [11], anthocyanins, flavanoids, cyclopeptide alkaloid [12] and vitamins [13]. Previous studies showed that the flowers of *H. rosa-sinensis* possess anti-tumor [14], anti-fertility effect [15,16], analgesic, anti-pyretic and anti-inflammatory [17], anti-viral [18], anti-diabetic [19], hypoglycemic activities [20]. The use of these flowers to treat heart disorders have also been described [21].

Therefore, in the present study, an investigation was made on the regulation of lipid factors by *H. rosa-sinensis* were determining in MSG induced obese rats. The results are also compared with those of the silymarin, the standard drug treatment to MSG induced obese rats.

## Methods

### Animals

Healthy female adult albino rats (Wistar strain) 6-7 weeks old, weighing  $100 \pm 20$  g were housed in clean sterile polypropylene cages with proper aeration and lighting ( $12 \pm 1$  hr day/night rhythm) throughout the experimental period. During the course of the experiments, the temperature was maintained between  $27^{\circ}\text{C} \pm 2^{\circ}\text{C}$ . The animals were fed with commercially available pelleted rat feed (Gold-Mohur, M/S Hindustan Lever Ltd, Mumbai, India) during the acclimatization period and water *ad libitum*. The usage and handling of experimental rats followed the rules and regulations given by the Institutional Ethics Committee.

### Collection and identification of plant material

The flower petals of *H. rosa-sinensis* (HRF) were collected from local farms in Pudukkottai, during the months of September-November 2003. The flowers were identified and authenticated by Dr. M. Jegadessan, Professor and Head, Department of

Environmental and Herbal Sciences, Faculty of Science, Tamil University, Vakaiyur, Thanjavur, Tamilnadu. The voucher specimen of *H. rosa-sinensis* (TUH-268) has been deposited in the Department of Siddha Medicine, Faculty of Science, Tamil University, Thanjavur, Tamil Nadu, South India.

#### **Preparation of the crude powders of *H. rosa-sinensis***

Shade dried flowers of *H. rosa-sinensis* were coarsely powdered and stored in airtight containers until use. The crude powders of *H. rosa-sinensis* was dissolved in normal saline at the time of use and given orally through gastric intubation to the experimental rats.

#### **Experimental design**

The rats were divided into four groups, each group consisting of six animals.

- Group I : Control rats received saline solution as vehicle
- Group II : MSG administered (1gm/kg body weight) orally as bidose for 10 weeks
- Group III : MSG administered rats treated with the crude powder of *H. rosa-sinensis* flowers (1000mg/kg body weight) twice daily for a period of 3 weeks
- Group IV : MSG administered rats treated with the standard drug silymarin (100mg/kg body weight) for a period of 3 weeks.

At the end of the experiments the rats were fasted overnight and killed by cervical decapitation under mild ether anesthesia. Blood was collected in heparin rinsed tubes to separate the plasma. Blood collected in another set of test tubes without anticoagulant was used to separate the serum. Brain, liver, kidney and heart were perfused *in situ* with cold 0.15M NaCl at 37°C and homogenized with a motor-driven Teflon-coated homogenizer in ice-cold 0.1M Tris-HCl buffer (pH 7.4) to obtain a 10% homogenate.

#### **Biochemical estimations**

The level of serotonin was measured by the method of Sharma and Dey [22,23]. Free fatty acids were estimated by the method of Falholt et al. [24]. TG was estimated by the method of Werner et al. [25]. Phospholipid content was estimated by the method of Zilversmit and Davis [26] and liberated phosphorus was estimated by using Fiske and Subbarow method [27]. The TC was estimated by the method of Allain et al. [28]. HDL cholesterol was separated by adding phosphotungstic acid and magnesium chloride to the fresh samples to precipitate other lipoproteins and the HDL cholesterol was estimated by the method of Allain et al. [28]. The concentration of LDL cholesterol and VLDL cholesterol were calculated by using the Friedwald formula [29].

#### **Statistical analysis**

Values were expressed as mean±standard deviation for six rats in the each group and statistical significant differences between mean values were determined by one way analysis of variance (ANOVA) followed by the Tukey's test for post-hoc multiple comparison tests. Graphpad Instat Software Package was used and p<0.05 was considered to be significant.

## Results

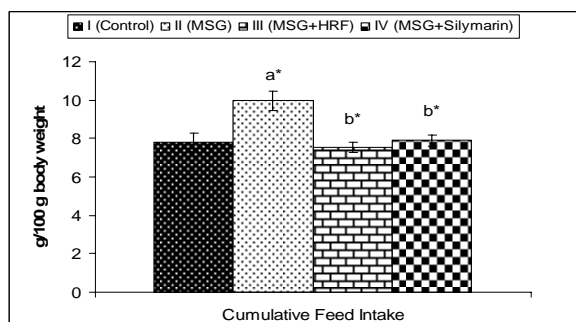


Figure 1. Effect of HRF on feed intake in experimental rats. Values are expressed as mean  $\pm$ SD for six animals in each group. Superscript letters represent  $p < 0.05$  (Tukey's Test). <sup>a</sup>As compared with Group I, <sup>b</sup>As compared with Group II. \* $p < 0.001$ .

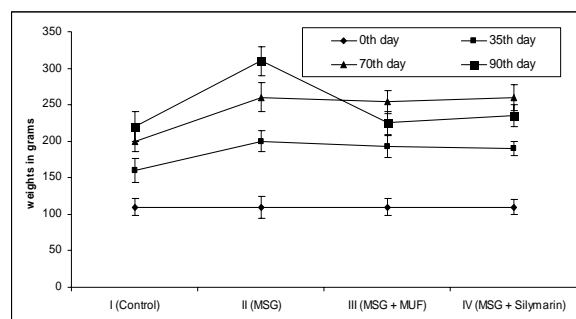


Figure 2. Effect of HRF on bodyweight in experimental rats. Values are expressed as mean  $\pm$ SD for six animals in each group.

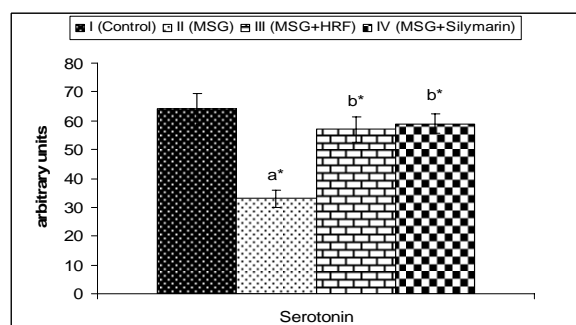


Figure 3. Effect of HRF on serotonin in experimental rats. Values are expressed as mean  $\pm$ SD for six animals in each group. Superscript letters represent  $p < 0.05$  (Tukey's Test). <sup>a</sup>As compared with Group I, <sup>b</sup>As compared with Group II. \* $p < 0.001$ .

There was a significant increase ( $p < 0.001$ ) in the feed intake (21.49%) by the animals among MSG rats than normal rats. Rats administered with MSG+HRF (group III) and MSG+silymarin (group IV) showed a significant decrease ( $p < 0.001$ ) in the feed intake (20.78%, 24.10%, 22.19%) when compared with the MSG (group II) rats (figure 1).

The bodyweight of MSG (group II) administered rats shown a significant increase ( $p < 0.001$ ) than control (group I) rats. Rats in MSG+HRF (group III) and MSG+silymarin (group IV) showed a significant ( $p < 0.001$ ) decrease in the body weight than MSG (group II) rats (figure 2).

The level of serotonin in brain homogenate of MSG (group II) induced rats showed a significant ( $p < 0.001$ ) decrease (48.44%) than that of control (group I) rats. The oral administration of HRF and silymarin to the group III and group IV rats showed a significant ( $p < 0.001$ ) increase in the level of serotonin (45.90%, and 44.07%) in brain than MSG induced obese rats.

Table 1 shows the level of free fatty acids, TG, phospholipids and TC in serum, liver, kidney and heart of control and experimental rats. The significant increased levels of free fatty acids (44.90% in serum, 39.78% in liver, 49.62% in kidney and 49.97% in heart), TG (45.44% in serum, 53.41% in liver, 46.09% in kidney and 56.15% in heart), phospholipids (39.03% in serum, 40.04% in liver, 42.89% in kidney and 40.24% in heart) and TC (23.17% in serum, 56.58% in liver, 32.32% in kidney and 36.76% in heart) were found in MSG (group II) induced rats when compared with group I control rats. After administration of HRF and silymarin a significant decrease in the level of free fatty acids, TG, phospholipids and TC ( $p < 0.001$ ) in serum, liver, kidney and heart of the MSG+HRF (group III) and MSG+silymarin (group IV) rats was observed when compared with MSG (group II) induced rats.

Table 1. Levels of free fatty acids, triglycerides, phospholipids and total cholesterol in serum, liver, kidney and heart of experimental rats.

Groups	Serum (mg/dL)	Liver (mg/g tissue)	Kidney (mg/g tissue)	Heart (mg/g tissue)
<b>Free fatty acids</b>				
Control	121.96±11.40	11.96±1.01	9.36±0.91	7.50±0.65
MSG	221.36±18.56 <sup>a*</sup>	19.86±1.84 <sup>a*</sup>	18.58±1.52 <sup>a*</sup>	14.99±1.31 <sup>a*</sup>
MSG+HRF	132.59±11.22 <sup>b*</sup>	11.54±0.95 <sup>b*</sup>	11.25±1.38 <sup>b*</sup>	9.06±0.71 <sup>b*</sup>
MSG+Silymarin	135.42±11.74 <sup>b*</sup>	12.19±1.06 <sup>b*</sup>	10.69±0.91 <sup>b*</sup>	8.91±0.71 <sup>b*</sup>
<b>Triglycerides</b>				
Control	78.36±6.11	8.06±0.52	5.38±0.43	2.78±0.19
MSG	143.62±12.53 <sup>a*</sup>	17.30±1.51 <sup>a*</sup>	9.98±0.61 <sup>a*</sup>	6.34±0.49 <sup>a*</sup>
MSG+HRF	98.92±9.54 <sup>b*</sup>	12.36±0.82 <sup>b*</sup>	7.27±0.37 <sup>b*</sup>	3.27±0.31 <sup>b*</sup>
MSG+Silymarin	96.18±9.06 <sup>b*</sup>	10.08±0.83 <sup>b*</sup>	6.24±0.61 <sup>b*</sup>	3.01±0.27 <sup>b*</sup>
<b>Phospholipids</b>				
Control	70.92±5.44	9.36±0.82	8.35±0.43	8.02±0.45
MSG	116.31±9.34 <sup>a*</sup>	15.61±1.31 <sup>a*</sup>	14.62±0.93 <sup>a*</sup>	13.42±1.12 <sup>a*</sup>
MSG+HRF	73.91±5.42 <sup>b*</sup>	12.01±0.86 <sup>b*</sup>	9.75±0.68 <sup>b*</sup>	10.34±0.91 <sup>b*</sup>
MSG+Silymarin	72.34±6.81 <sup>b*</sup>	10.99±0.73 <sup>b*</sup>	8.91±0.72 <sup>b*</sup>	9.56±0.86 <sup>b*</sup>
<b>Total Cholesterol</b>				
Control	74.36±3.44	5.48±0.42	6.03±0.33	3.87±0.29
MSG	96.78±8.31 <sup>a*</sup>	12.62±0.92 <sup>a*</sup>	8.91±0.48 <sup>a*</sup>	6.12±0.51 <sup>a*</sup>
MSG+HRF	78.31±4.56 <sup>b*</sup>	7.91±0.61 <sup>b*</sup>	7.16±0.59 <sup>b*</sup>	4.34±0.37 <sup>b*</sup>
MSG+Silymarin	75.06±4.31 <sup>b*</sup>	6.61±0.50 <sup>b*</sup>	6.84±0.60 <sup>b*</sup>	4.36±0.29 <sup>b*</sup>

Values are expressed as mean  $\pm$ SD for six animals in each group. Superscript letters represent  $p < 0.05$  (Tukey's Test). <sup>a</sup>As compared with Group I, <sup>b</sup>As compared with Group II. \*  $p < 0.001$ .

Table 2. Levels of serum VLDL, LDL and HDL of experimental rats.

Groups	VLDL (mg/dL)	LDL (mg/dL)	HDL (mg/dL)
Control	15.67±1.48	20.09±1.88	38.60±2.16
MSG	28.72±2.08 <sup>a*</sup>	46.75±2.46 <sup>a*</sup>	21.31±1.64 <sup>a*</sup>
MSG+HRF	19.78±1.33 <sup>b*</sup>	22.92±2.08 <sup>b*</sup>	35.61±2.72 <sup>b*</sup>
MSG+Silymarin	19.24±1.73 <sup>b*</sup>	22.59±2.06 <sup>b*</sup>	33.23±3.01 <sup>b*</sup>

Values are expressed as mean  $\pm$ SD for six animals in each group. Superscript letters represent  $p < 0.05$  (Tukey's Test). <sup>a</sup>As compared with Group I, <sup>b</sup>As compared with Group II. \*  $p < 0.001$ .

The results tabulated in table 2 showed a significant ( $p < 0.001$ ) increase in the levels of VLDL cholesterol (45.44%) and LDL cholesterol (57.03%) with a significant ( $P < 0.001$ ) decrease in the level of HDL (44.79%) in serum of MSG (group II) induced rats when compared with group I control rats. On comparison of the MSG+HRF (group III) and MSG+silymarin (group IV) rats with the MSG (group II) induced rats, a significant ( $p < 0.001$ ) decrease in the levels of VLDL cholesterol (31.13% for HRF and 33.01% for silymarin) and LDL cholesterol (50.97% for HRF and 51.68% for silymarin) with an increase in the level of HDL (40.16% for HRF and 35.87% for silymarin) was observed.

### **Discussion**

In the present results reveals that the decreased concentration of serotonin and increased feed intake and body weight gain were observed in MSG rats. The decrease in the level of serotonin is also well correlated with the increased feed intake in MSG treated rats. MSG administration is reported to induce hyperphagia and increase the energy intake [30], MSG treatment might also induce hepatic metabolic shifting which results in further brain injury. The regulation of energy balance in rats is generally considered to result from a precise control of energy intake, which serves to maintain body energy stores constant, despite fluctuation in energy expenditure [31]. There is much evidence to show that an important stimulus for the control of food intake and energy balance is activated by the circulatory energy pool that consists mainly of glucose and lipids [32]. On administration of HRF to the MSG treated rats, a significant increase in the level of plasma serotonin a decreased feed intake and loss of body weight were observed. The increased level of serotonin decreased feed intake and loss of body weight observed in the herbal flower administered rats is well correlated with the inhibition of the activities of the lipogenic enzymes and increasing the synthesis of serotonin [33].

Rats treated with MSG for a period of 10 weeks showed a significant elevation in the level of free fatty acids. Free fatty acid concentrations are one of the metabolic consequences of a chronic positive energy balance and increased body adiposity [34]. The elevated free fatty acid concentrations are one of the metabolic consequences of chronic energy balance on increased adiposity. Obesity is also known to be accompanied by decreased sensitivity of adipose tissue cells to insulin. This could lead to an increase in activity of hormone sensitive lipase in adipose tissue, which would contribute to increased levels of plasma free fatty acid. Decreased adipose tissue activity [35], increased adipocytes and liver lipogenesis rate [36] have been demonstrated in MSG induced obese animals. A significant decrease in the level of free fatty acids was observed in the MSG induced obese rats when treated with HRF. The possible mechanism may be due to alkaloids, flavonoids in HRF, which may exhibit a regulatory role. Increased free fatty acid concentrations are typically associated not only with insulin resistance and type 2 diabetes, but also lipid dystrophy, because of alterations of the fat between the adipocyte and muscle or liver. This change leads to the intracellular accumulation of TG and intracellular fatty acid metabolites (fatty acyl CoA, diacylglycerol, ceramides among others) in insulin-responsive tissues, which leads to acquired insulin signaling defects and insulin resistance.

The significant increase in TG observed among in the MSG rats is in consistent with the earlier report of Lorden and Caudle [37] who reported that consumption of MSG was associated with an increasing plasma TG level. Nakia et al. [38] also reported that subcutaneous administration of high doses of MSG in rats induced hyper-triglyceridemia and resulted in significant increase in the serum concentrations of phospholipids and free fatty acids. Decreased adipose tissue lipolytic activity [35], increased the rate of adipocytes and liver lipogenesis [36] and decreased lipase activity [39] demonstrated in the MSG induced obese animals (or) rats is well corroborated by the results of the present findings, which show an increased rate of lipogenesis as evidenced through increased TG level and decreased lipolysis through total lipase activity. Some of the earlier reports showed that an increase in de novo fatty acids might stimulate hepatic TG synthesis and plasma TG concentration [40,41]. Administration of HRF to MSG rats had significantly decreased the TG level. The plant and plant products have been proved for their potency against hypertriglyceridemia. The active constituents such as alkaloids and flavonoids present in the HRF might enhance the rate of TG clearance in liver.

In the present study the phospholipids were found to be elevated in MSG induced obese rats. Moreover, high dose levels of MSG (4 and 8 mg/g b.w.) might be causing the destruction of the cholinergic infundibular system in the hypothalamus [42], hypo-activity of this system could lead to accumulation of choline and hence can divert metabolism towards increased synthesis/transport of phospholipids and may be responsible for significant elevation of phospholipids. A significant decrease in the level of phospholipids was observed in MSG induced obese rats when treated with the crude powders of HRF. This demonstrates that the potential role of HRF in regulating the phospholipids which were found to be elevated in the MSG obese rats. The effective regulation caused by these herbal flowers proves the potency of the phyto-active constituents such as alkaloids and flavonoids which are found in HRF [43].

Endogenous cholesterol biosynthesis and absorption of dietary cholesterol contributes to needs for cholesterol to provide membrane lipid, steroid hormones, and bile acids [44] and to establish serum and tissue cholesterol levels. The relative contribution of each source varies among individuals. In the present study, the TC level was found to be elevated in MSG obese rats. It was proved that the MSG consumption favours lipogenesis in experimental rats. Administration of HRF to MSG rats had significantly reduced the cholesterol level. This may be due to the presence of phyto-active constituents such as alkaloids, flavonoids and proteins present HRF. The observed hypolipidemic effect may be due to decreased cholesterologenesis and fatty acid synthesis [45].

Lipid abnormalities associated with obesity include increased overall production of lipids with elevated concentrations of fatty acids, triacylglycerols, and LDL cholesterol, as well as VLDL cholesterol. In the present study an increase in VLDL cholesterol, LDL cholesterol and decrease in HDL-cholesterol was observed in MSG treated rats. Obesity in humans has consistently been associated with low plasma HDL cholesterol levels, with the degree of adiposity and levels of plasma HDL cholesterol inversely correlated [46].

The increases in VLDL-TG secretion and plasma TG concentration seen in association with chronic, endogenous hyperinsulinemia were due to increase in the use of free fatty acid, by the liver for TG synthesis. The results observed in the present study well correlated with Taghibiglou et al. [47] who reported that the potential reason for the over production of VLDL cholesterol in the insulin-resistant state is the due enhanced lipoprotein assembly, reduced intracellular apolipoprotein-B degradation, and increased expression of microsomal triglycerol transfer protein in animal models. Hepatic overproduction of VLDL cholesterol in the state of insulin resistance may result from direct hepatic effect of insulin as well as indirect metabolic effect, such as increased availability of free fatty acids for TG secretion [48]. On administration of HRF to MSG rats showed a significant increase in HDL and decrease in the levels of LDL cholesterol and VLDL cholesterol. This may be due to the hypolipidemic activity of the HRF.

From the results of the present study, it is observed that HRF had effectively controlled the increase in body weight gain and induced anorexia through the regulation on the anorexic compounds such as serotonin. Alterations observed in the free fatty acids, TG and phospholipids in MSG treated rats were potentially regulated by administration of HRF, which were the determinant factor for obesity. Also, the deviations observed in the levels of LDL cholesterol, VLDL cholesterol, HDL cholesterol, TC were effectively controlled. Regulation of these factors evidences the anti-obesity and anti-atherogenic potentials of *H. rosa-sinensis*.

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