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### Antihyperlipidemic potential of *tecoma stans* extracts in monosodium glutamate induced obesity in neonatal rats

Dr. Kavitha Rajesh M.Pharm, M.Phil, MBA, PhD<sup>1</sup>, G. Gnana Jebaslin M.Pharm<sup>2</sup>

<sup>1</sup>Coordinator Department of Wellness, Faculty of Health Sciences, Male', Maldives National University.

<sup>2</sup>Department of Pharmacology, Sree Krishna college of Pharmacy and Research center, Parasala, Trivandrum, India.

Corresponding author: Dr. Kavitha Rajesh

Email: akil.kavi@gmail.com

#### ABSTRACT

Objective of the present study to explore the antihyperlipidemic effect of *Tecoma stans* (*T.stans*) against monosodium glutamate (MSG) induced obesity in neonatal rats. The studies were carried out on newborn neonatal rats and were injected intraperitoneally with 2 mg/g of MSG on the 2<sup>nd</sup> and 4<sup>th</sup> postnatal days and 4 mg/g on 6<sup>th</sup>, 8<sup>th</sup> and 10<sup>th</sup> postnatal days. Ethanolic extract (EE) and acetone extract (AE) of *T.stans* was administered in a dose of 100 and 200 mg/kg, p.o./day at the age of 65 days. On day 60 of treatment, body weight, locomotor activity, body temperature, and various biochemical parameters like serum glucose, total cholesterol, triglyceride, and organs weights were recorded. There was a significant reduction in body weight, organs and increased body temperature, locomotor activity after treatment with extracts. *T.stans* decreased serum glucose, total cholesterol and triglyceride, low density lipoprotein (LDL) and very low density lipoprotein (VLDL) levels and increased high density lipoprotein (HDL) significantly has compared to MSG-control rats. *T.stans* extracts exhibited antihyperlipidemic effect and it possesses anti-obesity properties in MSG induced obese rats.

**Keywords:** Antihyperlipidemic, *Tecoma stans*, monosodium glutamate, neonatal, obesity

#### INTRODUCTION

Obesity is a worldwide epidemic characterized by excess adipose tissue that contributes to numerous chronic diseases and early mortality.<sup>1, 2</sup> Monosodium glutamate (MSG) treatments of neonatal rats produces various endocrine and behavioral abnormalities resulting from neurotoxicity to the arcuate nucleus in the hypothalamus.<sup>3</sup> MSG-treated rats develop obesity without hyperphagia as adults.<sup>4</sup>

*Tecoma stans* (common name yellow bell) also known as yellow trumpet bush belongs to the family Bignoniaceae. It is an ornamental plant. It is an erect, branched, sparingly hairy or nearly smooth shrub two to four meters in height. The leaves are opposite, odd-pinnate,

Up to 20 centimeters in length with 5 to 7 leaflets. The leaflets are lanceolate to oblong-lanceolate, 6 to 13 centimeters long, pointed at both ends and toothed on the margins. Trumpet shaped flowers are yellow faintly scented and borne in short, dense, terminal clusters. The calyx is green. 5 to 7 millimeters long and 5 toothed. Flowering can begin as early as April and continue in to fall. The flowers are followed by 6-inch-long, tan pods that are filled with small, papery winged seeds. Leaves of *Tecoma stans* contain the alkaloids tecomin and tecostamine are potent hypoglycaemic agent when given intravenously. Anthranilic acid is responsible for the anti-diabetic activity. Roots are powerful diuretic and vermifuge. *Tecoma* is not a toxic because this plant is used in latine America as a remedy for

diabetes and moreover for feeding cattle and goats in Mexico.<sup>5-10</sup>

## MATERIALS AND METHODS

### Collection and Authentication of Plant Material

Leaves of *T.stans* were collected in the month of May 2020 from Rasipuram (Namakkal District) Tamil Nadu. A herbarium specimen of the plant was deposited in the Department of Pharmacognosy. The plant was identified by Dr.G.V.S.Murthy, Joint Director of the Botanical Survey of India, Southern circle.

### Preparation of Extract

The leaves of *T.stans* were isolated, dried in the shade at room temperature, and powdered. The powder was defatted with petroleum ether and then extracted with 70% v/v ethanol and acetone for 24 h in a Soxhlet extractor. After extraction, solution obtained was evaporated at 45°C under reduced pressure till a viscous mass material was obtained. The yield of the ethanolic extract (EE) and acetone extract (AE) were found to be 33.45 and 28.76 (w/w), respectively. The dried EE and AE was stored in an airtight container and placed in a refrigerator. The EE and AE were used for the experimental study. The preliminary phytochemical screening was carried out on the EE and AE of the *T.stans* for qualitative identification by the standard method described by Khandelwal.<sup>11</sup>

### Animals

Female Albino Wistar rats (150-200 g) were purchased from the Central Animal House, Indian Institute of Science, Bangalore, and Karnataka. Animals were bred in specific-pathogen-free conditions at the Central Animal House, Indian Institute of Science, where mentioned under controlled standard animal house conditions with ad libitum access to food and water.

### Acute Toxicity Study

Acute toxicity study of EE and AE of the *T.stans* was carried out by adapting fixed dose method of CPCSEA, (OECD) guidelines no 401.<sup>12</sup> The female Swiss albino mice weighing between 20-25 g were used for the study. The animals were continuously observed 12 h to detect changes in autonomic or behavioral responses. Mortality was observed for 24 hours.

### Experimental Procedure

#### Induction of MSG-induced obesity

Newborn neonatal (on the day of delivery), pups were used and injected 2 mg/g i.p. of MSG on the 2<sup>nd</sup> and 4<sup>th</sup> postnatal days and 4 mg/g on 6<sup>th</sup>, 8<sup>th</sup>, and 10<sup>th</sup> postnatal days. Control rats received 10% NaCl (n = 08). The injection volume was 8 µl/g body weights. The Pups remained with their respective mothers until they were weaned at 4 weeks of age. At the age of 65 days, the rats' body weight was 80-100 g, rats were divided into different subgroups and later the treatment was given for 60 days orally for every day.<sup>13</sup> Group I: Vehicle control, Group II: MSG control, Group III: EE of *T.stans* 100 mg/kg, Group IV: EE of *T.stans* 200 mg/kg, Group V: AE of *T.stans* 100 mg/kg, Group VI: AE of *T.stans* 200 mg/kg.

The body weight and locomotor activity were recorded on 60<sup>th</sup> day in vehicle control MSG control and treated group. The locomotor activity (horizontal activity) was recorded using an actophotometer. The movement of the animal cuts off a beam of light falling on the photocell and a count was recorded and displayed digitally. Each rat was placed individually in the actophotometer for 10 min and basal activity score was obtained. The vehicle control, MSG control and extracts treated rats were placed in the actophotometer for recording the activity score as described earlier.<sup>14</sup> The body temperature was recorded on day 59 in, MSG obese rats using rectal telethermometer before and after extracts administration at 0, 30, 60, 90, 120- and 180-min time interval with a contact time of 1 min. On day 61, vehicle control, MSG control and treated rats were sacrificed by ether anesthesia and then organs like heart, spleen, liver, and kidney were removed rinsed with cold saline patted between papers and weighed.

### Biochemical Parameters

On day 61, blood was withdrawn from retroorbital sinus under ether inhalation anaesthesia and was subjected to centrifugation to obtain serum for estimating serum lipid profile. Glucose, total cholesterol, triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL), and very low-density lipoprotein (VLDL) levels were measured from serum sample by using the biochemical kits (Span diagnostic Ltd, Mumbai, India).

### Statistical Analysis

Statistical analysis was carried out using Graph Pad Prism version 3.0 (GraphPad Software Inc., San Diego, California, USA). Data were expressed as mean ± SD (n=6) and was analyzed using one-way ANOVA followed by Dunnett's test.  $P \leq 0.05$  was considered to be statistically significant.

## RESULTS

Preliminary phytochemical screening revealed that EE showed positive for carbohydrates, alkaloids, saponins, fixed oil, flavonoids, and tannins. The AE showed positive for the presence of glycosides, alkaloids, triterpenoids, saponins, and flavonoids. The acute toxicity studies of the EE and AE of *T.stans* was found to be non-lethal up to dose of 300 mg/kg body weight, but toxic symptoms occurs at dose at 2000 mg/kg, so the randomly 100 and 200 mg/kg doses were selected for anti-obese screening.

MSG induced obese rats showed increased in body weight and decreased the body temperature when compared to vehicle control group. Rats treated with EE and AE (200 mg/kg) showed a decrease in body weight and percentage of reduction in body weight (6.54% and 6.73%) when compared to MSG control are presented in [Table 1]. EE and AE of *T.stans* (200 mg/kg) caused significantly increased the body temperature in treated group. MSG control rats' significantly decreased locomotor activity when compared to vehicle control. Administration of EE and AE (100 and 200 mg/kg) caused significantly increased locomotor activity when compared to MSG control group [Table 1]. Administration of *T.stans* extracts at a dose of 200 mg/kg caused significantly reduction heart, liver, spleen, and kidney weights [Table 2].

**Table 1: Effect of ethanolic and acetone extract of *Tecomastans* leaves on average body weight, locomotor activity and body temperature in neonatal monosodium glutamate induced obese rats**

Treatment	Dose	Body weight (Difference between day 1 & day 60 of treatment)	Locomotor activity (10min)	Body temperature (°C)
Vehicle control	1ml/100g	43.75±1.91	654.00±33.57	33.00±0.195
MSG control	-	89.15±6.11	555.30±17.38	32.90±0.146
EE of <i>T.stans</i>	100	87.82±5.61 (-1.49)	598.81±13.30*	33.00±0.195 <sup>ns</sup>
EE of <i>T.stans</i>	200	83.32±1.73 (-6.54)	650.50±29.14**	33.40±0.171**
AE of <i>T.stans</i>	100	86.50±4.09 (-2.98)	623.50±26.22**	33.20±0.121
AE of <i>T.stans</i>	200	82.82±2.54 (-6.73)	695.80±19.34**	33.50±0.122**

The results are expressed mean±SD (n=6) \*P<0.05, \*\*P<0.01 as compared to MSG control group (ns=non-significant). The percentage of weight gain (+) compared to vehicle control group and percentage of weight reduction (-) compared to MSG control group are represented in parenthesis.

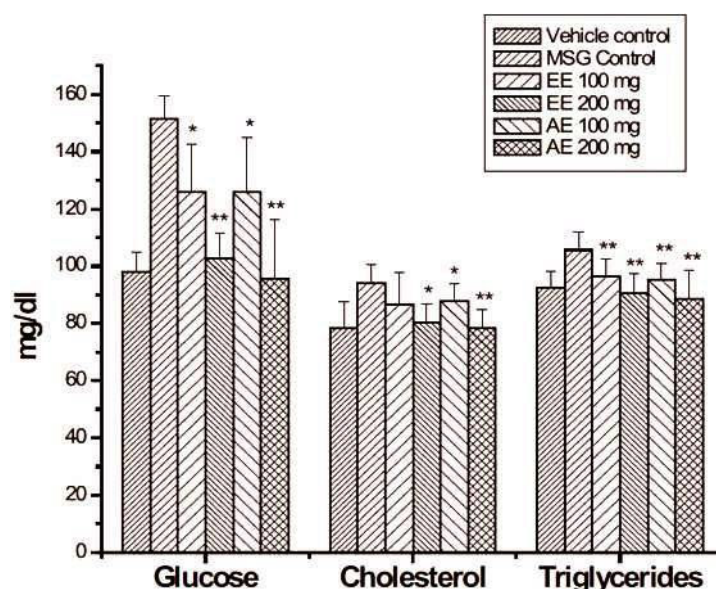
**Table 2: Effect of ethanolic and acetone extract of *Tecomastans* leaves on organ weight in neonatal monosodium glutamate induced obese rats.**

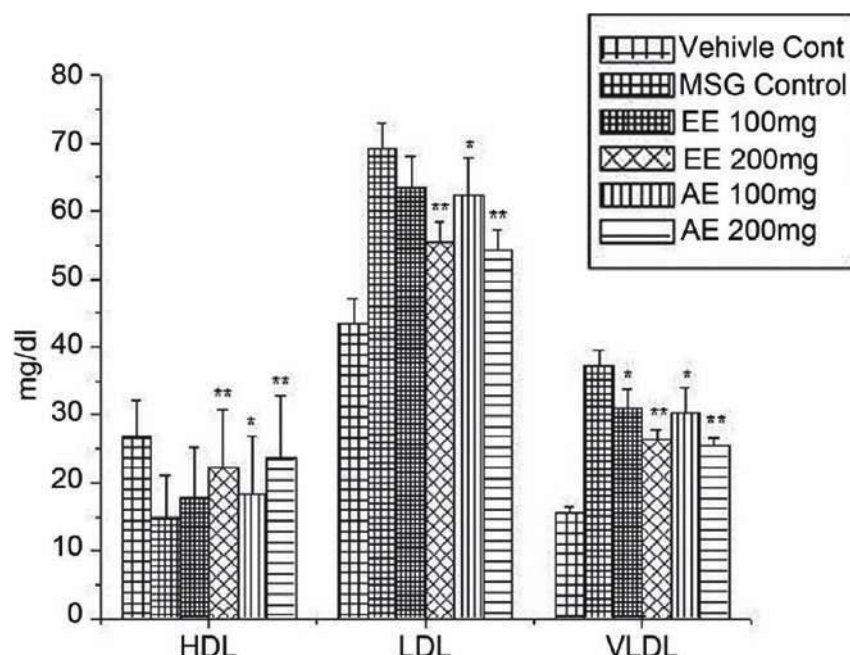
Treatment	Dose (mg/kg)	Heart	Liver	Spleen	Kidney (L)
Vehicle control	1ml/100g	0.647±0.01	7.086±0.01	0.675±0.006	0.614±0.006
MSG control	-	0.659±0.004	7.257±0.01	0.737±0.006	0.624±0.006
EE of <i>T.stans</i>	100	0.641±0.005**	7.037±0.03**	0.708±0.01**	0.619±0.006 <sup>ns</sup>
EE of <i>T.stans</i>	200	0.628±0.004**	6.719±0.03**	0.653±0.01**	0.586±0.007**
AE of <i>T.stans</i>	100	0.639±0.006**	7.026±0.03**	0.692±0.01**	0.615±0.01 <sup>ns</sup>
AE of <i>T.stans</i>	200	0.624±0.005**	6.689±0.03**	0.648±0.01**	0.561±0.005**

The results are expressed mean±SD (n=6) \*P<0.05, \*\*P<0.01 as compared to MSG control group (ns=non-significant).

Compared to vehicle control group, serum glucose, total cholesterol and triglyceride, LDL, and VLDL levels increased significantly in MSG control rats (P<0.01), where HDL level has been significantly (P<0.01) reduced in these group. These observations indicate that MSG increases the

lipid levels in experimental obese models. Treated group with EE and AE of *T.stans* decreased serum glucose, total cholesterol and triglyceride, LDL and VLDL levels, and increased HDL significantly has compared to MSG control rats. The results are showed in [Figure 1] and [Figure 2].

**Figure 1: Effect of ethanolic and acetone extract of *Tecomastans* on lipid profile in neonatal monosodium glutamate induced obese. The results are expressed mean ± SD (n = 6). \*P<0.05, \*\*P<0.01, as compared to MSG control group.**



**Figure 2: Effect of ethanolic and acetone extract of *Tecomastanson* lipid profile in neonatal monosodium glutamate induced obese. The results are expressed mean  $\pm$  SD (n = 6). \*P<0.05, \*\*P<0.01, as compared to MSG control group.**

## DISCUSSION

It is well known that the subcutaneously administration of MSG in newborn rodent produces adiposity when the animals reach adulthood. MSG obese animal resemble genetically obese animal in their greatly increased body lipid content, greatly decreased rates of hormone-stimulated lipolysis, and similar states of transient hyperglycemia. The present study demonstrated that the anti-obese activity of *T.stans* with respect to response to neonatal MSG treatment, although the degree of obesity was developed at 65 days of age. First, MSG developed obesity; increase in body weight and decrease in locomotor and body temperature ( $^{\circ}$ C) was attenuated in MSG control. Food restriction reduced body weight more in treated group than in MSG control and extract treatment abolished an accelerated reduction of locomotor activity and body temperature. Secondly, increase the weight of heart, liver, spleen, kidney in MSG control. Thirdly, hypertriglyceridemia, hypercholesteremia, and hyperglycemia were enhanced in MSG-control as compared with vehicle control rats throughout the experimental period.

Neonatal MSG treatment almost completely destroys neuronal cell bodies in the arcuate nucleus of the hypothalamus<sup>15</sup> and MSG-treated rats display a characteristic syndrome of obesity, blindness, stunted growth, hypogonadism, and short tail.<sup>3</sup> The occurrence of hypertriglyceridemia, hypercholesteremia was probably due to the increased hepatic VLDL synthesis induced by hyperinsulinemia. Olney<sup>3</sup> and Bunyan et al,<sup>4</sup> found that MSG treated mice were rather hypophagic and weighed less than the controls before the development of marked obesity. MSG treatment is likely suppressed energy expenditure due

to the altered hypothalamic-pituitary axis contributes to MSG induced obesity. In a study by Moss et al,<sup>16</sup> thermoregulatory thermogenesis was impaired in MSG-treated mice. It has been reported that blood flow in interscapular brown adipose tissue (BAT) is a major site for energy expenditure and thermogenesis.<sup>17</sup>

The efficacy of new obesity treatments should be assessed by their effects on body weight. As such, a treatment should be considered successful if it prevents further weight gain induces a 5-10% weight loss from the initial body weight and allows long-term maintenance of the weight loss once it is achieved. The present investigation shows that extracts of *T.stans* showed reduction in body weight in treated groups in a dose dependent manner. The major chemical constituents of *T.stans* are sterols, poly-phenols, and flavonoids such as taxifolin, quercetin and saponins.<sup>5, 18</sup> It is well established that saponins are useful in treatment of obesity<sup>19</sup> phytosterols have beneficial effects on hyperlipidemia<sup>20</sup> and poly-phenols and flavonoids have potential antioxidant properties. Therefore, it could be possible that presence of these compounds is responsible for observed glucose and lipid lowering activity.<sup>21, 22</sup> Treatment with both extracts of *T.stans* caused significant decrease in weights of different internal organs in MSG induced obese rats; it suggested that *T.stans* reduce adipose tissue formation in rats.<sup>23</sup>

## CONCLUSION

In conclusion, the present study suggests that EE and AE of *T.stans* showed significant antihyperlipidemic effect and it possess an anti-obesity activity against MSG induced obese rats.



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