Efficacy and Safety Evaluation of a Novel Weight Management Herbal Formulation LOWAT

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ABSTRACT

Overweight and obesity have become a global health concern. Therefore, it is essential to develop effective and safe therapeutics for weight management. To achieve this, 480 herbal extracts were screened for their adipogenesis inhibitory activities. Extracts from *Piper betle* and *Dolichos biflorus* exhibiting potent anti-adipogenic potentials were chosen for further analysis. The synergistic anti-adipogenic effects of the two extracts were assessed by combining the individual extracts at various ratios to create a proprietary formulation LOWAT which was significantly better than the individual extracts in terms of adipogenic inhibition. *In vitro* studies showed that LOWAT inhibited pre-adipocyte differentiation and potentiated lipid breakdown in mature adipocytes. *In vivo* studies indicated that LOWAT significantly reduced body weight gain while increased serum adiponectin level in rats on a high fat diet as compared to the control rats. Adiponectin is secreted from adipose tissue into the bloodstream and the level is inversely correlated with body fat percentage. Toxicological studies on LOWAT demonstrated that acute oral and acute dermal LD_{50} were greater than 5.0 g/kg and 2.0 g/kg, respectively. Primary eye irritation study showed that the LOWAT was mildly irritating to the eye. No toxicity was observed in primary skin irritation test. Repeated dose 28-day subchronic oral toxicity test revealed no toxic effects on biochemical or clinical parameters. The no-observable-adverse-effect-level (NOAEL) for LOWAT in male and female Sprague-Dawley rats was concluded to be at least 2.5 g/kg body weight. Results suggest that this formulation may be an effective weight management agent.

INTRODUCTION

Piper betle is a vine belonging to the Piperaceae family, which includes pepper and Kava. *Piper betle* is an Ayurvedic shrub indigenous throughout India and Malay region as treatment for diabetes, cough, indigestion and other ailments. Betel leaf is aromatic, carminative and stimulant. In previous studies, significant reduction in the blood glucose levels on oral administration of both hot water and cold ethanol extracts was observed. Oral administration of leaf suspension for 30 days resulted in significant reduction in blood glucose and improved other markers of diabetes in rats. *Piper betle* extract showed significant hypolipidemic effect in comparison to control on chronic oral administration. *Dolichos biflorus* is a small twining herb, popularly known as horse gram. It is used as a home remedy for treating urolithiasis, dysuria and bleeding piles. In dysuria, its action is known to be due to its diuretic property. It is also used to reduce crystalluria and to dissolve stones. The powdered seeds are used as a poultice to induce sweating. In previous studies, administration of *Dolichos biflorus* to experimental rabbits manifested protection against high fat diet (HFD) induced oxidative stress in different tissues. It also showed lipid lowering effect in experimental rats. In India, the powdered seed of *Dolichos biflorus* is described as an anti-diabetic therapeutic product.

SPECIFIC AIM

To provide a safe and effective dietary weight management supplement comprising of extract from *Piper betle* leaves and *Dolichos biflorus* seeds for overweight and obese human subjects.

MATERIALS & METHODS

LOWAT is a novel herbal composition comprising of Betel leaves (*Piper betle*) extract and Horse gram (*Dolichos biflorus*) extract in 2:3 ratio. All toxicological studies were done as per OECD guidelines.

TOXICITY STUDIES

Acute Oral Toxicity Study: Up to 5,000 mg LOWAT/kg body weight was administered to healthy female (n=5) Sprague-Dawley rats by oral gavage. All animals were observed for 25 days for signs of mortality, signs of intoxication and body weights recorded.

Acute Dermal Toxicity Study: Up to 2,000 mg/kg body weight of LOWAT was applied to skin of healthy male (n=5) and female (n=5) Sprague-Dawley rats. The animals were observed for mortality, signs of gross toxicity, and behavioral changes. Body weights were recorded prior to application and at the end of study duration. Necropsies were performed on all animals at termination.

Primary Dermal Irritation Study: LOWAT (0.5 g) was applied to skin of 3 healthy male and female New Zealand albino rabbits for 4 hours. Following exposure, dermal irritation was evaluated by the method of Draize *et al* (1944).

Primary Eye Irritation Study: LOWAT (0.07 g) was instilled to the right eye of 3 male & female New Zealand albino rabbits. The left eye served as a control. Ocular irritation evaluated by the method of Draize *et al* (1944).

Sub-acute Oral Toxicity (28 day) Study: A dose-dependent 28 day repeat dose sub-acute toxicity study was conducted on male and female Sprague-Dawley rats. The animals in the treatment group were supplemented with a feed containing 0.1% or 0.5% or 2.5% of LOWAT corresponding to 0.8 g or 4.0 g or 20.0 g of human equivalence dose (HED) respectively, for 28 days. All groups of animals were sacrificed after 28 days of treatment and target organs such as adrenal glands, brain, epididymis, esophagus, eyes, heart, intestine, kidney, liver, lymph nodes, lungs, mammary glands, ovaries or testes, pancreas, pituitary, prostate, salivary glands, seminal vesicles, skin, spleen, stomach, thymus gland, thyroid gland, trachea, uterus and urinary bladder were extracted and evaluated for toxic symptoms.

IN VITRO AND *IN VIVO* STUDIES

LOWAT in 3T3-L1 Adipocytes: LOWAT (25 µg/ml and 50 µg/ml) was assessed in mature 3T3-L1 adipocytes as per the procedure of Chemicon International, USA, by measuring free glycerol secreted into the culture medium.

LOWAT in Diet Induced Obese Sprague Dawley Rats: LOWAT was tested against high fat diet induced obesity (DIO) in Sprague Dawley rats. Healthy young adult male SD rats were provided high fat diet and concomitantly supplemented daily with allocated doses (100 and 250 mg/kg body weight) of LOWAT or vehicle or sibutramine for 8 weeks. Various parameters including body weight, serum biochemistry and adiponectin were tested in control and LOWAT supplemented animals.

RESULTS

TOXICITY STUDIES

A broad spectrum safety evaluation of LOWAT in rats and rabbits indicated no adverse effects. No toxicity was observed in female Sprague-Dawley (SD) rats supplemented with LOWAT at a dose of 5 g/kg body weight, a dose level considered to be several fold higher than the recommended daily human dose. No acute dermal toxicity was observed for LOWAT at a topical dose of 2 g/kg body weight in SD Rats. The acute dermal LD50 for LOWAT is greater than 2 g/kg body weight. LOWAT was classified as non-irritating to skin upon topical application to clipped skin in primary dermal irritation test conducted on New Zealand albino rabbits. LOWAT showed minimal irritation to eye in primary eye irritation test performed also on New Zealand albino rabbits. Hematology, serum chemistry, and histopathological evaluations did not show any adverse effects in any of the organs tested.

Toxicological Data

Acute Oral Toxicity in rat: LD_{50} > 5.0 g/Kg
Acute Dermal Toxicity in rat: LD_{50} > 2.0 g/Kg
Primary Skin Irritation in rabbit: Non-irritating to skin
Primary Eye Irritation in rabbit: Mildly irritating to eye
Sub-acute Oral Toxicity (28 Day) in rat: LD_{50} > 2.5 g/Kg

LOWAT in 3T3-L1 Adipocytes

Series of independent assays showed that LOWAT decreases adiposity in 3T3-L1 adipocytes by inhibiting adipogenesis in a dose-dependent manner (**Figure 2**). LOWAT at 50 µg/ml concentration exhibited 44.74% increase in glycerol release when compared with the vehicle treated 3T3-L1 cells. This indicates that LOWAT significantly (p=0.0134) increased lipolysis in 3T3-L1 adipocytes (**Figure 3**).

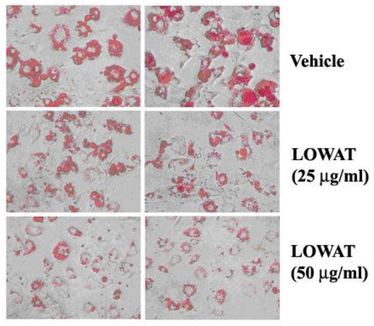

Figure 2. LOWAT inhibits fat accumulation in adipocytes in a dose dependent a manner.

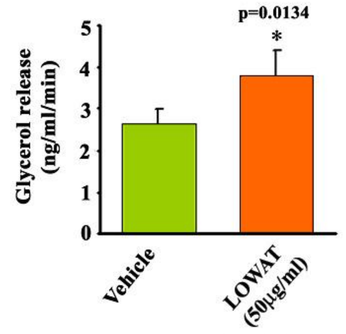

Figure 3. LOWAT potentiates break down of stored fat (lipolysis) in mature adipocytes.

LOWAT in Diet Induced Obese Sprague Dawley Rats

Simultaneous supplementation of LOWAT with high fat diet for eight weeks resulted in reduction of body weight gain as depicted in **Figure 4**. A 16% reduction in body weight was observed in LOWAT 250 mg/kg treated group. Animals were well tolerated for chronic treatment (8 week duration) with LOWAT. *In vivo* study also suggests that LOWAT supplementation can improve the circulatory adiponectin level in diet-induced obese rats as summarized in **Figure 5**. compared with the control the serum adiponectin concentration increased by 13.96% in diet induced obese rats supplemented with 250 mg/kg body weight of LOWAT.

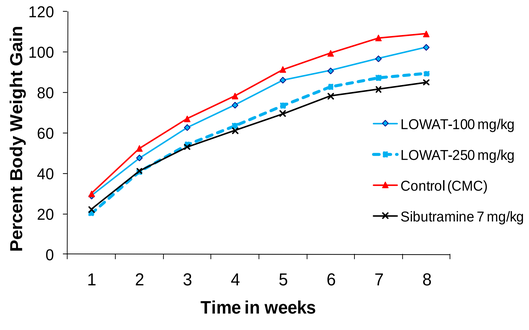

Figure 4. LOWAT with high fat diet for eight weeks resulted in reduction of body weight gain.

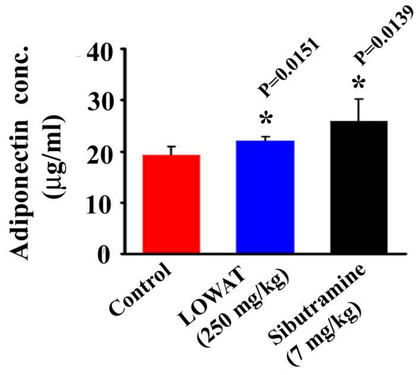

Figure 5. Improvement of serum adiponectin level in diet induced obese rats supplemented with LOWAT.

CONCLUSION

LOWAT is a weight management composition that inhibits preadipocyte differentiation process thereby; it slows down formation of fat cells in the body and inhibits the accumulation of lipid droplets in mature adipocytes. In addition, it also potentiates lipid breakdown in mature adipocytes. In diet induced obese rats, LOWAT inhibits body weight gain. In addition, it increases serum adiponectin level and thereby suggesting it's potential application in weight management, blood sugar control and improvement of cardiovascular health. A comprehensive perusal of the safety data indicated that the no observed adverse effect level (NOAEL) for male and female SD rats supplemented with LOWAT is presumed to be at least 20.0g per day HED.