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Research Article

Effect of Aqueous Leaf Extract of Copper Leaf (*Acalypha wilkesian*: Euphorbiaceae) on Weight in Monosodium Glutamat (MSG)-Induced Obesity in Albino Wistar Rats

Lamidi Tajudeen Babatunde*, Imohiosen Ojeaga, Umar Aisha Muhammad
Department of Science Laboratory Technology, Federal Polytechnic Bali, Bali, Nigeria

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ABSTRACT

Obesity is a condition characterized by excess weight or body fat that can adversely affect health. This study investigated the potential effects of the aqueous extract of *Acalypha wilkesiana* leaves on Monosodium Glutamate (MSG) induced obesity in albino wistar rats. Induction of overweight was achieved using MSG at a dose of 400 mg/kg.

In vivo investigations reveale that an oral daily dose of 200 mg/kg of the aqueous extract resulted in a significant loss of body weight after fourteen days, while a dose of 400 mg/kg showed significant weight loss after just seven days. Phytochemical screening indicated the presence of alkaloids, flavonoids, phenols, tannins and phlobatannins in the extract.

Consequently, *Acalypha wilkesiana* could serve as a vital source of supplementary diets containing several bioproducts essential for biochemical functions in humans and may provide health benefits. This study demonstrates that *Acalypha wilkesiana* exhibits anti-obesity effects.

Keywords: Albino rats, Monosodium glutamate, Medicinal plants, Obesity, *Acalypha wilkesiana*

*Correspondence: Lamidi Tajudeen Babatunde, Department of Science Laboratory Technology, Federal Polytechnic Bali, Bali, Nigeria, E-mail: tajudeenlamidi68@gmail.com

INTRODUCTION

Excess body weight is a serious problem, particularly in North America, Europe and certain parts of Africa. It has been referred to as a pandemic due to its gradual increase over the past few decades. Obesity occurs when a person has excess weight or body fat that may adversely affect their health. A doctor typically diagnoses obesity based on a high Body Mass Index (BMI).

Furthermore, excess body weight significantly raises the risk of several diseases and clinical conditions, including all-cause mortality, coronary and cerebrovascular diseases, various cancers, type 2 diabetes mellitus, hypertension, liver disease, asthma and psychopathology. Unfortunately, overweight and obesity have become increasingly common among young children and adolescents (Freedman DS, *et al*., 2013; Sasaki Y, *et al*., 2009).

While the causes of excess body weight are multifactorial, the most significant contributors are excessive caloric intake coupled with limited energy expenditure. Therefore, lifestyle modifications and the use of medicinal plants can significantly reduce the risk of morbidity and mortality, thereby increasing longevity and improving quality of life (Knight JA, 2011).

In recent years, the use of medicinal plants for healing purposes has been an integral part of human culture and is becoming increasingly popular in Nigeria (Okonkwo EE, 2012). *Acalypha* is the fourth largest genus in the Euphorbiaceae family, comprising approximately 450-570 species. Several *Acalypha* species, including *Acalypha wilkesiana*, are utilized as medicinal plants in Africa, particularly in Nigeria. Almost every part of the plant leaves, stems and roots is used as traditional remedies to treat and manage various ailments (Seebaluck R, *et al*., 2015).

Traditionally used *Acalypha* species have been reported to possess at least one of the following biological activities: Antimicrobial, anti-diabetic, antioxidant, anti-inflammatory, larvicidal, pupicidal, hepatoprotective, anticancer, leishmanicidal, antihyperglycemic, antihypertensive, anti-venom, analgesic, anthelmintic, antiemetic, laxative, expectorant, diuretic, post-coital antifertility effects and wound healing. A total of 167 compounds have been identified from 19 species of *Acalypha*; among these, 16 compounds from eight species have been reported to exhibit bioactivity.

MSG is a flavor enhancer that increases sweetness and saltiness while reducing sour and bitter tastes in food products. L-glutamate binds to receptors in the taste buds to stimulate the distinctive umami taste provided by MSG. The use of MSG in food has increased due to the popularity of fast food. The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) have stated that MSG is a food flavor enhancer with an acceptable daily intake ranging from 0-120 mg/kg (Kurtanty D, *et al*., 2018).

However, excessive and continuous consumption of MSG can increase the activity of nitric oxide synthase and protein kinase C through elevated α-ketoglutarate levels, leading to increased lipid peroxidation. This condition heightens the risk of obesity (Onyema OO, *et al*., 2006).

MSG, commonly known as AJI-NO-MOTO (the origin of flavor), is the sodium salt of glutamic acid, one of the most abundant naturally occurring non-essential amino acids.

MSG consists of 78% glutamic acid, 22% sodium and water. Glutamic acid is a major component of many proteins found in foods such as meat, fish, milk and some vegetables, playing an essential role in human metabolism (Shrestha S, *et al*., 2018).

Description of Acalypha wilkesiana

Acalypha wilkesiana belongs to the family Euphorbiaceae and is propagated by stem cuttings at any time of the year. Under ideal conditions, it grows as a spreading evergreen shrub with upright branches that can reach heights of up to 3.1 m and a similar spread. The leaves are alternate, elliptic to oval, serrate and meas-

ure between 12.7-20.3 cm long. The plant also produces small inconspicuous flowers 10.2-20.3 cm that hang in catkin-like racemes beneath the foliage (*Figure 1*) (Ganong WF, 1995).

Figure 1: Picture of *Acalypha wilkesiana* during fieldwork in 2022

Acalypha wilkesiana is characterized as an evergreen shrub that typically grows to 3 m in height and spreads about 2 m across. Its erect stem features many branches covered with fine hairs and has a closely arranged crown. The leaves are coppery green with red splashes, giving them a mottled appearance. They are large and broad, measuring 10-20 cm long and 15 cm wide, with finely hairy surfaces that can be flat or crinkled. The flowers are reddish spikes located at the ends of branches and consist of separate male and female flowers on the same plant; male flowers form long spikes that hang downward while female flowers are in shorter spikes, often hidden among the leaves. The flower stalks measure 10-20 cm long.

This tropical and subtropical plant is native to Vanuatu and occurs in the Pacific Islands. It prefers light, well-drained soil and thrives in a protected shady position but is susceptible to damage from drought and frost. *Acalypha wilkesiana* requires a minimum temperature above 10°C and is suited for hardiness zones 9-12.

Effect of MSG on rats

Administration of MSG to neonatal rats causes damage to the ventromedial hypothalamic nucleus and arcuate nucleus, leading to obesity due to impaired energy metabolism and decreased sympathetic and thermogenic activity of Brown Adipose Tissue (BAT) (Gomathi N, *et al*., 2008). Additionally, damage occurs to the cholinergic infundibular system in the hypothalamus. The hypo activity of this system results in choline accumulation, leading to metabolic disorders such as increased phospholipid synthesis/transport in obese mice (Matysková R, *et al*., 2008).

MSG induces alterations in glucose utilization rates and decreases antioxidant defenses. The generation of reactive oxygen species in various body cells can damage Deoxyribonucleic Acid (DNA), lipids and proteins while causing lipid peroxidation in cellular membranes due to damage to polyunsaturated fatty acids. This may ultimately lead to cellular death by apoptosis (Dolnikoff M, *et al*., 2001; Singh K and Ahluwalia P, 2003). The mechanisms underlying MSG-induced damage include free radical production that alters mitochondrial activity and genetic information. MSG is metabolized in the liver and eliminated through the kidneys (Henry T, 2013; Shrestha S, *et al*., 2018).

Significance of the study

Obesity is a chronic condition that is often mistakenly viewed as a temporary problem that can be resolved with a short-term, strenuous diet. However, as many overweight individuals understand, weight control requires a lifelong commitment. For any weight-loss program to be safe and effective, it must adopt a long-term approach; otherwise, the effort is largely a waste of time, money and energy.

Aim and objectives of the study

The aim of this study is to determine the potential effect of the aqueous extract of the leaves of *Acalypha wilkesiana* on MSG induced obesity in albino wistar rats.

The specific objectives of the study are as follows:

- To identify the phytochemicals, present in the leaves of *Acalypha wilkesiana*.
- To assess the effect of the aqueous extract of *Acalypha wilkesiana* leaves on the weight of induced albino wistar rats.
- To examine the relationship between different aqueous concentrations of the extract and the weight of induced rats.

The scope and limitation of the study

The scope of this research includes identifying the chemical components in the leaves of *Acalypha wilkesiana* and determining the relationship between different aqueous concentrations of the extract and their effects on the weight of induced albino wistar rats (Chukwu CA, 2022).

MATERIALS AND METHODS

Preparation of laboratory reagents for phytochemical analysis

Wagner's reagent: 2 g of iodine crystals and 3 g of potassium iodide were dissolved in 100 cm^3 of distilled water and stirred thoroughly to create a homogeneous solution.

Ferric chloride solution: A solution of ferric chloride was prepared by dissolving 5 g of ferric chloride (Iron III chloride) in 50 cm^3 of distilled water, resulting in a homogeneous solution.

Dilute solution of hydrochloric Acid: To create a dilute solution of hydrochloric acid, 2 cm^3 of hydrochloric acid was measured using a measuring cylinder and poured into a 250 cm^3 beaker containing distilled water. The mixture was stirred to form a homogeneous dilute solution of Hydro Chloric Acid (HCl).

Collection and identification of Acalypha wilkesian leaves

Fresh samples of *Acalypha wilkesiana* leaves were collected in August, 2021 from the rest house residence in Bali, Taraba State, Nigeria. The plant was identified using relevant taxonomic literature (Kalwij JM, 2012).

The collected leaves were dried on the laboratory table at the Science Laboratory Technology Department, Federal Polytechnic Bali. After drying at room temperature, the leaves were milled using a pestle and mortar and the resulting powder was used for extraction.

Aqueous extraction preparation of Acalypha wilkesian leaves

100 g of fine powdered *Acalypha wilkesiana* leaves were weighed and mixed with 500 mL of distilled water in a round-bottom flask. A reflux condenser was attached to the flask, which was then placed on a heating mantle.

The mixture was refluxed for one hour and subsequently filtered using whatman filter paper No. 1. This reflux process was repeated twice with fresh distilled water at each stage. The filtrate was then heated to dryness by evaporation. Part of the dry filtrate (extract) was used for phytochemical screening, while the remainder was stored for treatment (*Figure 2*).

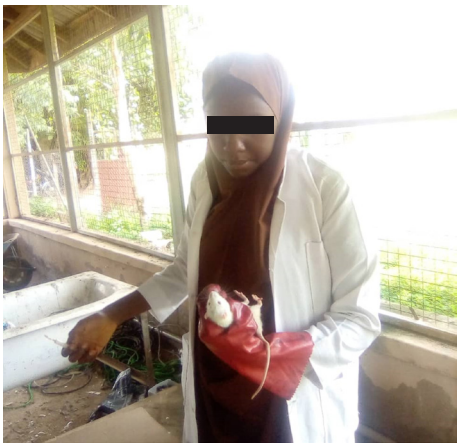

Figure 2: A researcher handling the albino rat in the animal house at polytechnic Bali

Procedures for phytochemical analysis

The phytochemical screening of the plant constituents was conducted using qualitative methods as described by (Trease GE and Evans WC, 1989; Sheet T, 1968).

Alkaloid test: Equal volumes of the solvent extract 5 cm and wagner's reagent were mixed in a clean test tube and observed for several minutes. The presence of alkaloids was indicated by the formation of a brown precipitate.

Phenol test: Two milliliters of extract were added to two milliliters of Ferric Chloride ($FeCl_3$) solution. The formation of a deep bluish-green solution indicated the presence of phenols.

Phlobatannins test: One milliliter of hydrochloric acid and one milliliter of solvent extract were placed in a clean test tube and heated for about 10 minutes. A reddish-green coloration indicated the presence of phlobatannins.

Flavonoids test: Five milliliters of the solvent extract were placed in a test tube, followed by the addition of a few pieces of magnesium chips and concentrated hydrochloric acid dropwise, then in excess. The appearance of a reddish coloration indicated the presence of flavonoids.

Glycosides test: Twenty-five milliliters of dilute sulfuric acid were added to five milliliters of the extract in a test tube, boiled for 15 minutes, cooled and neutralized with 10% Sodium Hydroxide (NaOH). Five milliliters each of fehling solution A and B were then added. The formation of a brick-red precipitate indicated the presence of glycosides.

Saponins (Froth test): One gram of the sample was weighed into a conical flask and 10 mL of sterile distilled water was added before boiling for 5 minutes. The mixture was filtered and 2.5 mL of the filtrate was added to 10 mL of sterile distilled water in a test tube, which was then stopped and shaken vigorously for about 30 seconds. After allowing it to stand for half an hour, honeycomb froth indicated the presence of saponins.

Volatile oils test: Two milliliters of extract solution were shaken with 0.1 mL dilute sodium hydroxide and a small quantity of dilute HCl. The formation of a white precipitate indicated the presence of volatile oils.

Hydrolysable tannins test: Four milliliters of the extract were shaken in a test tube, after which 4 mL of 10% ammonia solution was added. The formation of an emulsion upon shaking indicated the presence of hydrolysable tannins.

Tannin test: Three grams of powdered sample were boiled in 50 mL distilled water for 3 minutes on a hot plate. The mixture was filtered and a portion of the filtrate was diluted with sterile distilled water in a ratio of 1:4 before adding three drops of 10% ferric chloride solution. A blue or green color indicated the presence of tannins.

Protein test: One milliliter of extract was shaken with about 2 mL of Millon's reagent; a brick-red coloration indicated the presence of protein.

Collection of MSG, AJI-NO-MOTO

Monosodium glutamate (AJI-NO-MOTO), containing 99% MSG, was obtained from alhaji danladi's shop at sabon layi market in Bali, Taraba State, Nigeria.

Experimental animals

Fifteen apparently healthy albino wistar rats *Rattus norvegicus* of different sexes, weighing between 13.09 g (0.13 kg) and 33.00 g (0.33 kg), were obtained from the National Veterinary Research Institute (NVRI) in Vom, Jos, Nigeria. The rats were housed in cages at the Department of Science Laboratory Technology at Federal Polytechnic Bali, Taraba State, Nigeria, under standard environmental conditions with controlled temperature and relative humidity, having free access to water and standard food (Onaolapo OJ and Onaolapo AY, 2011).

Administration of the MSG

Monosodium glutamate was administered orally to the rats using a cannula and syringe every morning for fourteen days (Walker R and Lupien JR, 2000).

Experimental design

After an acclimatization period of two weeks (14 days), the rats were randomly assigned into four groups: A, B, C and D (three rats per group). Their weights were measured using a Mettler Toledo digital/sensitive weighing balance and recorded according to their respective groups. Group A served as the control group receiving normal saline, while Group B served as the overweight group receiving 400 mg/kg body weight MSG for 14 days without treatment. Groups C and D served as dose groups receiving 400 mg/kg body weight MSG for 14 days (Erhirhie EO *et al*., 2014; Ogbuagu EO, *et al*., 2019). The weights were recorded again after 14 days.

Treatment

Treatment commenced on day fifteen, one day after the administration of the last dose of aqueous MSG, which was considered the first day of treatment with *Acalypha wilkesiana* extracts. This treatment continued for an additional fourteen days. The rats from groups A, B, C and D were re-weighed after this fourteen-day period.

The rats were divided into four groups comprising three animals each as follows:

Group A: Control group given only saline solution.

Group B: Overweight controls.

Group C: Overweight rats treated with *Acalypha wilkesiana* leaf extract at 200 mg/kg body weight (low dose) daily for fourteen days.

Group D: Overweight rats treated with *Acalypha wilkesiana* leaf extract at 400 mg/kg body weight (high dose) daily for fourteen days.

RESULTS AND DISCUSSION

The increase in body weight of rats following the oral administration of MSG at a dose of 400 mg/kg, as shown in *Table 1*, indicates the development of overweight. Previous studies have reported that obesity can be induced with this agent MSG, resulting in overweight after 14 days of administration. This finding aligns with reports that observed significant weight gain in animals fed doses of 500, 750 and 1000 mg/kg body weight. The Lethal Dose 50 (LD50) for MSG was determined to be 500 mg/kg, which is the median between 200 mg/kg (which did not cause mortality) and 800 mg/kg (which resulted in the death of all treated animals) (Nakamura H, *et al*., 2013).

Table 1: Effect of oral daily dosage (200 mg/kg and 400 mg/kg body weight) of the aqueous leaf extract of *Acalypha wilkesian* on mean body weight (kg) of rats

Mean body weight (Kg)				
Experiment days	**Group A**	**Group B**	**Group C**	**Group D**
0	0.126 ± 0.01	0.119 ± 0.02	0.103 ± 0.01	0.114 ± 0.02
7	0.136 ± 0.01	0.128 ± 0.03	0.125 ± 0.01	0.130 ± 0.01
14	0.146 ± 0.02	0.144 ± 0.03	0.124 ± 0.01	0.140 ± 0.00
21	0.137 ± 0.00	0.149 ± 0.01	0.125 ± 0.02	0.141 ± 0.00
28	0.154 ± 0.01	0.153 ± 0.01	0.134 ± 0.01	0.137 ± 0.00

Note: Mean ± standard deviation based on 3 observations. Group A: Control group; Group B: Overweight group; Group C: Treatment with 200 mg/kg extract; Group D: Treatment with 400 mg/kg extract

The observed weight gain may be attributed to lesions in the arcuate nucleus of the hypothalamus, leading to increased caloric intake that exceeds utilization. This is consistent with findings from (Diniz YS, *et al*., 2004). The study revealed that consumption of MSG at 750 mg/kg body weight for approximately eight weeks significantly increases body weight and may induce obesity in the long run, corroborating the observations made by Matysková R, *et al*., 2008. Reports on the effects of MSG consumption on body weight vary widely; some studies indicate an increase in body weight, while others report a decrease.

Additionally, some research has shown no effect on body weight at all, as noted by Tordoff MG, *et al*., 2012; Madziga HA, *et al*., 2010).

Table 2 presents the presence of bioactive components in the aqueous leaf extract of *Acalypha wilkesiana*. Alkaloids were highly detected, followed by moderate levels of flavonoids. Tannins, phlobatannins, phenols and saponins were present in low amounts, while volatile oils were not detected (Seebaluck R, *et al*., 2015).

Table 2: The result of chemical components of aqueous leaf extract of *Acalypha Wilkesian*

Phytochemicals	Test	Aqueous extract
Alkaloids	Wagner's	+++
Tannins	Ferric chloride	+
Phlobatannins	Hydrochloric acid	+
Phenol	Ferric chloride	+
Flavonoids	Magnesium chips	++
Saponins	Frothing	+
Volatile oils	Sodium hydroxide	-

Note: (+++): Highly detected; (++): Moderately detected; (+): Lowly detected (-): Not detected

CONCLUSION

The administration of 200 mg of *Acalypha wilkesiana* leaf extract to rats in group C demonstrated no significant weight loss after the first seven days; however, slight weight loss was observed by the end of the fourteen-day period. In contrast, all rats in group D receiving a dosage of 400 mg began to show drastic and significant weight loss.

This study indicates that the leaf extract of *Acalypha wilkesiana* contains important bioactive components or phytochemicals such as alkaloids, flavonoids, phenols, tannins, phlobatannins and saponins; however, volatile oils were not detected. Thus, this plant could serve as a vital source of supplementary diets to reduce weight in animals and potentially humans, thereby mitigating the risk of obesity.

ETHICAL COMMITTEE CONSENT

This study has been approved by the Nigerian Institute for Medical Research Health Research Ethics Committee (NHREC/11/02/2009a).

RECOMMENDATIONS

Based on this research work, the following recommendations are made:

- Similar research should be conducted on different plants to investigate their anti-obesity potential.
- The chemical compounds detected should be analyzed further to identify active components for possible isolation and characterization.

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