



ISSN (E): 2277- 7695  
ISSN (P): 2349-8242  
NAAS Rating 2017: 5.03  
TPI 2017; 6(3): 143-151  
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www.thepharmajournal.com  
Received: 21-01-2017  
Accepted: 22-02-2017

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## Preparation and use of *Cissus quadrangularis* and *Achyranthes aspera* formulation in the management of weight loss

**Tamanna Talreja, Mangesh Kumar, Parmendra Sirohi and Tribhuwan Sharma**

### Abstract

The present study was designed to test the efficacy of two medicinal plants—*Cissus quadrangularis* and *Achyranthes aspera* in the management of weight loss in humans. For this, frequency of obesity among the different age group people of Bikaner was surveyed and enlistment of volunteers for herbal treatment of obesity based on *Achyranthes aspera* and *Cissus quadrangularis* preparations was done. By using *Achyranthes aspera* and *Cissus quadrangularis* powder preparation of edible nutraceuticals their organoleptic test and evaluation of shelf life was done. Based on the result of these tests, formulations and the doses were decided. To clarify the anti-obesity efficacy of these plants experiment was designed on mice model. After getting satisfactory results, doses were decided for human and given to the patients enlisted as volunteers in the supervision of registered medical practitioner. These subjects were examined once a week during 10 weeks of study period and their Anthropometric as well as physiological measurements were recorded and Analytical and statistical analysis of data was done. Results showed that *Cissus* and *Achyranthes* brought about a significantly greater weight loss than placebo during the study period in obese individuals and a significant improvement in the lipid profiles and anthropomorphic profiles of study participants was seen.

**Keywords:** *Achyranthes aspera*, *Cissus quadrangularis*, organoleptic tests, shelf life

### Introduction

Obesity is a major public health burden in both developed and developing countries. Today, there are more than 250 million obese people worldwide, equivalent to seven percent of the adult population (WHO 1998). Pharmacologic treatment of obesity, despite short-term benefits, is often associated with rebound weight gain after the cessation of drug use, side effects from the medication, and the potential threats of drug abuse. Medicinal plants have offered an alternative over the past decade and herbal medicines have been accepted universally. Hence, medicinal plants continue to play an important role in the healthcare system of a large number of the world's population. These plants have been used for treating various diseases of human beings and animals since time immemorial. Herbal medicines have a strong base and potential to be useful as safe and effective drug for treating obesity. The use of natural remedies for weight loss has increased, based on reliability, safety, and cost compared with synthetic drugs or surgical procedures. Medicinal plant or their preparations may enhance satiety, boost metabolism, and speed up weight loss. Native people for their appetite suppressing effects have, also used certain herbs. This includes *Achyranthes aspera* (Apamarga) and *Cissus quadrangularis* (Hadjor). These plants have been used in traditional Indian medicine for thousands of years to treat various disorders. They can act as alternative medicine due to less side effects and low cost. Several reviews about anti-obesity properties on these important medicinal plants have been appeared in literature.

*Achyranthes aspera* (Amaranthaceae) is an important medicinal herb found as a weed throughout India. Though almost all of its parts are used in traditional systems of medicines, seeds, roots and shoots are the most important parts which are used medicinally. The special property of the seeds of *Achyranthes aspera*, as an anorexiant, bestows valuable effect, to cure the excessive hunger in obesity and stimulates the release of insulin from the beta-cells in human. Some studies have examined role of *Cissus quadrangularis* (Vitaceae) in fighting obesity and symptoms of metabolic syndrome has attracted interest in other parts of the world. A significant proportion of these plants have been observed to possess potent antioxidant activity, which may contribute to anti-obesity property in animals.

Considering, all the beneficial aspects and medicinal values of these plants, the present study, was designed to test the efficacy of these two extraordinary plants—*Cissus quadrangularis* and *Achyranthes aspera* in the management of obesity and obesity-related complications in humans.

**Material and Method**

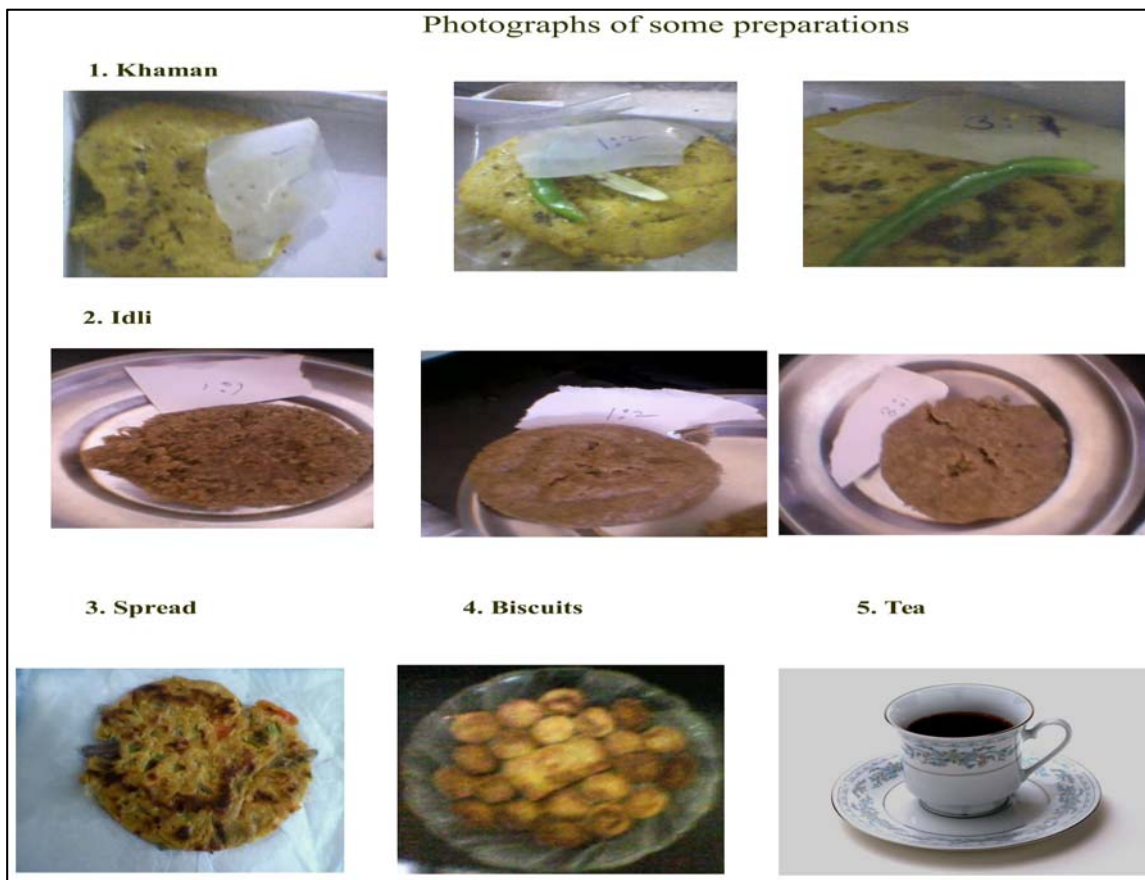
**(1) Survey:** Initially a survey was conducted to ascertain the frequency of obesity among the different age group people of Bikaner. Three Groups were made from selected schools, colleges, offices and in residential colonies. People visiting in antiobesity camps of Doctors of government hospitals were contacted and enlisted for this research. Group I: Teens (8-18years), Group II: Young adults (30-40 years), Group III: Senior adults (50-60 years). To access the frequency of obesity some information {(i.e.) Name of Patient, Age 3. Occupation, BMI, BMR, % Body Fat, W/H (waist to hip) ratio, Family history of disease, Do you want to join the herbal treatment programme, Contact address} was taken from the people contacted during survey. A consent form (Annexure III) has been developed to enlist volunteers for herbal treatment of obesity based on *Achyranthes aspera* and *Cissus quadrangularis* preparations.

**(2) Preparations of Edible products and Nutraceuticals of *Achyranthes aspera* and *Cissus quadrangularis* and their organoleptic test**

*Achyranthes aspera* seeds, its powder and the dried stem of *Cissus quadrangularis* have been used to prepare edible products. Seeds of *A. aspera* and dried stem of *Cissus quadrangularis* were grinded finely to prepare a powder which was added to the edible products in a definite ratio. Firstly, edible products namely Idli, Khaman, Biscuits, Spread, Tea

were prepared form *Achyranthes aspera* seed/ seed powder and *Cissus quadrangularis* stem powder to judge the acceptance of these products organoleptic tests were conducted, involving 20 people from each category. *Cissus quadrangularis* products were found not acceptable organoleptically due to bitter taste. After that only *Achyranthes aspera* seed powder has been used to prepare some nutraceuticals edible products like Biscuits, Ladoos, Porridge, Tikkia. Seeds of *A. aspera* were grinded finely to prepare a powder, which was added to the nutraceuticals edible products in a definite ratio. These nutraceuticals were made to provide an alternative means of dose to the patients who do not take tea or coffee. To judge the acceptance of these products same kind of organoleptic tests were conducted, involving 20 people from each category.

**(3) Evaluation of shelf life:** Here after, Evaluation of shelf life was done for the prepared nutraceuticals in 3:1. Shelf life is the length of time that a commodity may be stored without becoming unfit for use or consumption. It applies to foods, beverages, pharmaceutical drugs, chemicals, and many other perishable items. Shelf life testing describes how long a food will retain its quality during storage. Predictive modeling or challenge testing can be used to assess pathogen growth. However, food safety and product shelf life are inextricably linked. During the shelf life of a food it should: (1) Remain safe to eat (2) its appearance, odour, texture and flavour To test the shelf life of prepared products, these products were kept in airtight containers and were subjected to study the various factors of the shelf life study indicated were the most important for that product, e.g. loss of flavour, microbial growth, colour changes, browning and loss of nutrients , rancidity etc.



#### (4) Testing the Efficacy of Extract of *Cissus quadrangularis* and *Achyranthes aspera* on Weight Loss of Mice

**a. Extract preparation:** Methanol extracts of *Cissus quadrangularis* and *Achyranthes aspera* was prepared by Healthy stem of *Cissus* and seed samples of *Achyranthes* were collected from medicinal plant garden of the University. The fresh sample of *Cissus* stem and seeds of *Achyranthes* were dried separately at  $100 \pm 5^\circ\text{C}$ . These samples were grounded finely and the known quantity was filled in the thimble. These sample containing thimbles were placed in the middle section of soxhlet apparatus. The dried samples were separately soxhlet extracted in pure methanol (100% methanol) on a water bath for twenty four hours. Each of the extract was dried, weighed and the dark colored residue was collected in vials.

**b. Testing the efficacy of extract:** To clarify the anti-obesity efficacy of methanolic extract of *Achyranthes aspera* and *Cissus quadrangularis* experiment was designed on mice model.

- 20 mice were used in the preliminary studies. Animals were housed in clean cages with proper aeration and lightening. The animals had free access to a standard diet together with high fat diet and water. The experiment has been accomplished by following the rules of I.A.E.C. (Institutional Animal Ethics Committee).
- Mice were fed on standard diet and acclimatized to the lab conditions for one week before the commencement of the experiment.
- Pre experimental weight measurement was conducted in all selected animals.
- After that animals were made obese by administration of high fat diet (2:1 ghee: animal feed) and their weight was recorded.
- When they became obese their treatment was given to them. Plant extract was orally administered to high fat fed rats.

#### (5) Validation of anti-obesity properties of *Achyranthes aspera* and *Cissus quadrangularis* through Anti-obesity trials on humans in collaboration with Medical College in the region

As *Achyranthes aspera* mixed biscuits (3 part general ingredients and 1 part *A. aspera* seed powder) and Tea made from *Achyranthes aspera* seed powder in a ratio 3:1 (3 part general ingredients and 1 part *A. aspera* seed powder) were most acceptable in organoleptic test and have a good shelf life, were given to the patients for the validation of anti-obesity properties.

*Cissus quadrangularis* products were not acceptable organoleptically due to bitter taste in earlier study and were not used as edible nutraceuticals. To test the anti-obesity potential of *Cissus quadrangularis* powdered extract was filled in capsules. These capsules were given to the patients.

**a. Participants** Overweight and obese participants aged between 19 and 60 years of both sexes were selected from a group responding to survey. Diabetics as well as pregnant and lactating women were not included in the study. None of the participants took any specific diet or medication during trials. The purpose, nature, risks of the study was explained to the patients, and all subjects were given their written informed consent before participation.

#### b. Study

- A consent form has already been filled by the volunteers for herbal treatment of obesity based on *Achyranthes aspera* preparations and *Cissus quadrangularis* capsules.
- *Achyranthes aspera* mixed biscuits (3 part general ingredients and 1 part *A. aspera* seed powder) and Tea made from *Achyranthes aspera* seed powder in a ratio 3:1 (3 part general ingredients and 1 part *A. aspera* seed powder) were most acceptable in organoleptic test, were given to the enrolled volunteers.
- As *Cissus quadrangularis* was not found acceptable in organoleptic test due to its bitter taste, capsules were given to the patients.

#### c. Dose

- 1g/day/patient *Achyranthes aspera* were given to the patients. (ref. Anil mangal 2009)
- Powder of *Cissus quadrangularis* in capsule form was used in 500mg/day/patient. (ref. Shil C. Kothari *etal.*, 2011)
- These doses were instructed to administrate daily before meals in the morning.

#### d. Analysis of Obesity Parameters

These subjects were examined once a week during 10 weeks of study period and their Anthropometric as well as physiological measurements were recorded. These measurements were recorded by Omeron Body Fat Monitor (HBF 375), Inch tape and height scale.

#### Anthropometric Measurement

- Body weight
- Height
- Waist circumference
- W/H (waist /hip ratio)
- Fat %
- Visceral Fat %
- BMR (RM = Resting metabolism)
- BMI
- Body Age
- Area check
- Subcutaneous Fat (Whole body, Trunk, Arms, Legs)
- Skeletal Muscles (Whole body, Trunk, Arms, Legs)

#### Serological Test

- Blood samples were collected into heparinized tubes after 12 hours overnight fast at the beginning of the study and 4, 8 weeks and 10 weeks of treatment.
- The concentration of
  1. Serum cholesterol,
  2. HDL cholesterol (good cholesterol),
  3. LDL cholesterol (bad cholesterol),
  4. VLDL cholesterol,
  5. Serum Triglyceride
- CHO/HDL and LDL/HDL in plasma will be measured using lipid profile kit and auto analyzer.

Process of Validation of anti-obesity properties of the *Cissus quadrangularis* and *Achyranthes aspera* through Anti-obesity trials on humans was done in collaboration with Medical College in the region

**Result and Discussion**

**(1) Organoleptic test results of edible products and Nutraceuticals of *Achyranthes aspera* and *Cissus quadrangularis*:**

It is evident from table that *Cissus quadrangularis* products were not acceptable organoleptically due to bitter taste (Table

2, 4, 6). Biscuits, Spread, Tea made from *Achyranthes aspera* seed powder in a ratio 3:1 (3 part general ingredients and 1 part *A. aspera* seed powder) and *Achyranthes aspera* mixed biscuits and ladoos (in 3:1) were most expectable (Table 1, 3, 5) but porridge, tikkia was not acceptable due to bitter taste.

**Table 1:** 1. For Group I: Teens (8-18 years) organoleptic test of *Achyranthes aspera* mixed food products and nutraceuticals

S. N	Khaman			Idli			Biscuits			Spread			Tea			Ladoos			Porridge			Tikia		
	3:1	1:1	2:1	3:1	1:1	2:1	3:1	1:1	2:1	3:1	1:1	2:1	3:1	1:1	2:1	3:1	1:1	2:1	3:1	1:1	2:1	3:1	1:1	2:1
1	1	3	3	3	4	4	2	3	2	2	4	2	2	3	2	2	3	2	3	4	4	3	3	3
2	2	4	3	3	4	3	2	3	2	1	3	1	2	3	3	2	3	2	3	4	3	3	4	3
3	1	4	2	3	4	3	1	3	3	2	3	2	2	3	3	1	3	3	3	4	3	2	4	2
4	2	4	3	3	4	4	2	4	3	1	4	2	1	4	2	2	4	3	3	4	4	3	4	3
5	1	3	2	2	4	4	1	4	3	2	3	2	2	3	2	1	4	3	2	4	4	2	3	2
6	1	4	2	2	4	2	2	3	4	1	3	2	2	2	2	2	3	4	2	4	2	3	4	2
7	2	4	3	3	4	2	1	4	2	1	3	1	2	4	2	1	4	2	3	4	2	3	4	3
8	2	4	3	4	3	2	1	3	4	2	3	1	2	3	2	1	3	4	4	3	2	2	4	3
9	1	3	3	2	3	2	1	3	2	1	3	1	1	2	2	1	3	2	2	3	2	2	3	3
10	2	3	3	3	4	1	1	3	3	2	4	1	1	3	3	1	3	3	3	4	1	3	3	3
11	2	4	3	3	3	4	2	4	3	1	4	2	1	3	3	2	4	3	3	3	4	3	4	3
12	2	4	3	3	2	4	2	4	3	2	3	2	2	3	3	2	4	3	3	2	4	3	4	3
13	2	4	3	4	4	2	2	3	3	1	4	2	2	2	3	2	3	3	4	4	2	3	4	3
14	1	3	3	2	3	3	2	3	4	1	3	1	2	2	4	2	3	4	2	3	3	3	3	3
15	1	3	4	4	2	3	1	4	2	2	3	1	1	3	2	1	4	2	4	2	3	2	3	4
16	1	4	3	2	3	3	2	3	4	2	3	1	1	4	2	2	3	4	2	3	3	2	4	3
17	2	3	4	2	4	4	2	4	3	1	4	2	1	2	2	2	4	3	2	4	4	3	3	4
18	2	4	3	4	4	3	1	3	3	2	2	2	2	3	2	1	3	3	4	4	3	3	4	3
19	2	3	4	3	3	2	2	3	4	2	3	2	2	3	3	2	3	4	3	3	2	2	3	4
20	1	4	4	3	3	3	2	4	3	2	4	1	2	3	4	2	4	3	3	3	3	2	4	4

**Table 2:** 1. For Group I: Teens (8-18 years) organoleptic test of *Cissus quadrangularis* mixed food products

S. No	Khaman			Idli			Biscuits			Spread			Tea		
	3:1	1:1	2:1	3:1	1:1	2:1	3:1	1:1	2:1	3:1	1:1	2:1	3:1	1:1	2:1
1	2	3	3	4	4	3	3	3	3	3	4	3	4	4	4
2	3	3	3	4	3	4	4	4	3	3	3	3	4	3	3
3	3	4	3	3	3	3	3	3	3	3	3	3	3	3	3
4	4	2	4	4	4	3	3	3	4	4	4	4	4	4	4
5	3	3	3	3	3	3	3	4	3	3	3	3	3	3	3
6	3	3	3	3	3	4	4	3	3	3	3	3	3	3	3
7	3	4	3	3	3	3	2	3	3	3	3	3	3	3	3
8	3	2	4	4	3	4	3	3	4	4	3	4	4	3	3
9	3	3	2	2	3	3	4	3	2	2	3	2	2	3	3
10	4	4	3	3	4	3	3	3	3	3	4	3	3	4	4
11	4	3	4	4	4	3	3	3	4	4	3	4	4	4	4
12	3	3	3	2	3	4	4	4	3	3	3	3	2	3	3
13	4	4	3	3	4	2	3	2	3	3	3	3	3	4	4
14	3	3	3	3	3	3	3	3	3	3	4	3	3	3	3
15	3	3	3	4	3	4	3	4	3	3	2	3	4	3	3
16	3	4	3	2	3	3	4	2	2	3	4	3	2	3	3
17	4	3	3	3	4	4	2	3	3	3	3	3	3	4	4
18	2	3	3	2	2	2	3	3	4	3	3	3	2	2	2
19	3	3	3	3	3	3	4	4	3	3	4	3	3	3	3
20	4	3	3	3	4	4	3	3	2	3	3	3	3	4	4

\*1= Very Good; 2=Good; 3=Bitter; 4= Unacceptable

**Table 3:** 2. For Group II: Young adults (30-40 years) organoleptic test of *Achyranthes aspera* mixed food products and nutraceuticals

S.N	Khaman			Idli			Biscuits			Spread			Tea			Ladoos			Porridge			Tikia		
	3:1	1:1	2:1	3:1	1:1	2:1	3:1	1:1	2:1	3:1	1:1	2:1	3:1	1:1	2:1	3:1	1:1	2:1	3:1	1:1	2:1	3:1	1:1	2:1
1	3	4	4	2	4	2	2	4	2	1	3	3	2	3	2	2	4	2	2	4	2	3	4	4
2	3	4	3	3	4	4	1	3	1	2	4	3	2	3	3	1	3	1	3	4	4	3	4	3
3	3	4	3	3	4	3	2	3	2	1	4	2	2	3	3	2	3	2	3	4	3	3	4	3
4	3	4	4	3	4	4	1	4	2	2	4	3	1	4	2	1	4	2	3	4	4	3	4	4
5	2	4	4	2	4	4	2	3	2	1	3	2	2	3	2	2	3	2	2	4	4	2	4	4
6	2	4	2	2	4	2	1	3	2	1	4	2	2	2	2	1	3	2	2	4	2	2	4	2

7	3	4	2	3	4	2	1	3	1	2	4	3	2	4	2	1	3	1	3	4	2	3	4	2
8	4	3	2	4	3	2	2	3	1	2	4	3	2	3	2	2	3	1	4	3	2	4	3	2
9	2	3	2	2	3	2	1	3	1	1	3	3	1	2	2	1	3	1	2	3	2	2	3	2
10	3	4	1	3	4	1	2	4	1	2	3	3	1	3	3	2	4	1	3	4	1	3	4	1
11	3	3	4	3	3	4	1	4	2	2	4	3	1	3	3	1	4	2	3	3	4	3	3	4
12	3	2	4	3	4	4	2	3	2	2	4	3	2	3	3	2	3	2	3	4	4	3	2	4
13	4	4	2	3	4	4	1	4	2	2	4	3	2	2	3	1	4	2	3	4	4	4	4	2
14	2	3	3	3	4	3	1	3	1	1	3	3	2	2	4	1	3	1	3	4	3	2	3	3
15	4	2	3	3	4	4	2	3	1	1	3	4	1	3	2	2	3	1	3	4	4	4	2	3
16	2	3	3	2	4	4	2	3	1	1	4	3	1	4	2	2	3	1	2	4	4	2	3	3
17	2	4	4	2	4	2	1	4	2	2	3	4	1	2	2	1	4	2	2	4	2	2	4	4
18	4	4	3	3	4	2	2	2	2	2	4	3	2	3	2	2	2	2	3	4	2	4	4	3
19	3	3	2	4	3	2	2	3	2	2	3	4	2	3	3	2	3	2	4	3	2	3	3	2
20	3	3	3	2	3	2	2	4	1	1	4	4	2	3	4	2	4	1	2	3	2	3	3	3

\*1= Very Good; 2=Good; 3=Bitter; 4= Unacceptable

Table 4: 2. For Group II: Young adults (30-40 years) organoleptic test of Cissus quadrangularis mixed food products

S. No	Khaman			Idli			Biscuits			Spread			Tea		
	3:1	1:1	2:1	3:1	1:1	2:1	3:1	1:1	2:1	3:1	1:1	2:1	3:1	1:1	2:1
1	3	3	3	3	3	4	3	4	4	4	2	3	3	2	3
2	4	4	3	3	3	3	3	4	3	3	3	3	3	3	3
3	3	3	3	3	3	3	3	3	3	3	4	3	3	4	3
4	3	3	4	4	4	4	4	4	4	4	2	4	4	2	4
5	3	4	3	3	3	3	3	3	3	3	3	3	3	3	3
6	4	3	3	3	3	3	3	3	3	3	3	3	3	3	3
7	2	3	3	3	3	3	3	3	3	3	4	3	3	4	3
8	3	3	4	4	3	4	4	3	3	2	3	3	3	2	4
9	4	3	2	2	3	2	2	3	3	3	3	3	3	3	2
10	3	3	3	3	4	3	3	4	4	3	4	3	4	4	3
11	3	3	4	4	3	4	4	4	4	4	2	4	4	3	4
12	4	4	3	3	3	3	2	3	3	3	3	3	3	3	3
13	3	2	3	3	3	3	3	4	4	3	3	3	4	4	3
14	3	3	3	3	4	3	3	3	3	3	4	3	3	3	3
15	3	4	3	3	3	2	3	4	3	3	3	2	4	3	3
16	4	2	2	3	4	3	2	3	3	3	3	2	3	4	3
17	2	3	3	3	3	3	3	4	4	4	4	3	4	3	3
18	3	3	4	3	3	3	2	2	2	4	3	4	2	3	3
19	4	4	3	3	4	3	3	3	3	3	3	3	3	3	3
20	3	3	2	3	3	3	3	4	4	4	4	3	4	3	3

\*1= Very Good; 2=Good; 3=Bitter; 4= Unacceptable

Table 5: 3. For Group III: Senior adults (50-60 years) organoleptic test of Achyranthes aspera mixed food products and nutraceuticals

S.N	Khaman			Idli			Biscuits			Spread			Tea			Ladoos			Porridge			Tikia		
	3:1	1:1	2:1	3:1	1:1	2:1	3:1	1:1	2:1	3:1	1:1	2:1	3:1	1:1	2:1	3:1	1:1	2:1	3:1	1:1	2:1	3:1	1:1	2:1
1	2	4	2	3	4	4	2	4	2	2	4	2	2	3	2	2	4	4	3	4	3	4	4	2
2	3	4	4	3	4	3	1	3	1	1	3	1	2	3	3	2	4	3	3	3	3	3	4	4
3	3	4	3	3	4	3	2	3	2	2	3	2	2	3	3	2	4	3	3	3	3	3	4	3
4	3	4	4	3	4	4	1	4	2	1	4	1	1	4	2	2	4	4	4	4	4	3	4	4
5	2	4	4	2	4	4	2	3	2	2	3	2	2	3	2	2	4	4	3	3	3	3	4	4
6	2	4	2	2	4	2	1	3	2	1	3	1	2	2	2	2	4	2	3	3	3	3	4	2
7	3	4	2	3	4	2	1	3	1	1	3	1	2	4	2	3	4	2	3	3	3	3	4	2
8	4	3	2	4	3	2	2	3	1	2	3	2	2	3	2	1	3	2	4	3	4	4	3	2
9	2	3	2	2	3	2	1	3	1	1	3	1	1	2	2	2	3	2	2	3	2	2	3	2
10	3	4	1	3	4	1	2	4	1	2	4	2	1	3	3	3	4	1	3	4	3	3	4	1
11	3	3	4	3	3	4	1	4	2	1	4	1	1	3	3	3	3	4	4	3	4	3	3	4
12	3	4	4	3	2	4	2	3	2	2	3	2	2	3	3	3	2	4	3	3	3	3	4	4
13	3	4	4	4	4	2	1	4	2	1	4	1	2	2	3	3	4	2	3	3	3	3	4	4
14	3	4	3	2	3	3	1	3	1	1	3	1	2	2	4	2	3	3	4	3	3	4	3	3
15	3	4	4	4	2	3	2	3	1	2	3	2	1	3	2	1	2	3	3	2	3	3	4	4
16	2	4	4	2	3	3	2	3	1	2	3	2	1	4	2	2	3	3	3	4	3	3	4	4
17	2	4	2	3	4	4	1	4	2	1	4	1	1	2	2	2	4	4	3	3	3	3	4	2
18	3	4	2	3	4	3	2	2	2	2	2	2	2	3	2	2	4	3	3	3	3	3	4	2
19	4	3	2	3	4	3	2	3	2	2	3	2	2	3	3	2	4	3	3	4	3	4	3	2
20	2	3	2	3	4	4	2	4	1	2	4	2	2	3	4	2	4	3	3	3	3	3	3	2

\*1= Very Good; 2=Good; 3=Bitter; 4= Unacceptable

**Table 6:** 3. For Group III: Senior adults (50-60 years) organoleptic test of *Cissus quadrangularis* mixed food products

S No	Khaman			Idli			Biscuits			Spread			Tea		
	3:1	1:1	2:1	3:1	1:1	2:1	3:1	1:1	2:1	3:1	1:1	2:1	3:1	1:1	2:1
1	3	4	3	4	4	4	3	4	3	4	4	4	3	4	3
2	3	3	3	4	3	3	3	3	3	4	3	3	3	3	3
3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
5	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
6	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
7	4	3	4	4	2	3	3	2	3	3	2	3	3	2	3
8	2	3	3	2	3	3	3	3	3	3	3	3	3	3	3
9	3	4	3	3	3	4	3	3	4	3	3	4	3	3	4
10	4	4	3	4	4	2	4	4	2	4	4	2	4	4	2
11	2	3	4	2	3	3	3	3	3	3	3	3	3	3	3
12	3	4	2	3	3	3	3	3	3	3	3	3	3	3	3
13	3	3	3	3	3	4	3	3	4	3	3	4	3	3	4
14	4	3	4	4	3	2	4	3	2	4	3	2	4	3	2
15	2	3	3	2	3	3	2	3	3	2	3	3	2	3	3
16	3	4	4	3	4	4	3	4	4	3	4	4	3	4	4
17	2	2	2	2	4	3	4	4	3	4	4	3	4	4	3
18	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
19	3	4	4	3	4	4	3	4	4	3	4	4	3	4	4
20	4	3	4	4	3	3	3	3	3	3	3	3	3	3	3

\*1= Very Good; 2=Good; 3=Bitter; 4= Unacceptable

**(2) Evaluation of shelf life**

It is evident from table that in Nutraceuticals, *Achyranthes aspera* mixed biscuits (3 part general ingredients and 1 part A.

*aspera* seed powder) shown maximum organoleptic acceptability as well as shelf life.

**Table 7:** Evaluation of shelf life

S. No.	Food Product	Time (hours or days)	Main limiting factor				Typical shelf life
			loss of flavour	Visible microbial growth	colour changes	browning and loss of nutrients	
1.	Porridge	2 hour	x	x	X	x	About 6-8 hours
		4hour	x	x	X	x	
		6hour	x	x	X	x	
		8 hour	✓	x	X	x	
		10 hour	✓	x	✓	x	
		12 hour	✓	✓	✓	✓	
2.	Ladoos	Day 1	x	x	X	x	About 3-4 days
		Day2	x	x	X	x	
		Day3	x	x	X	x	
		Day4	✓	x	X	✓	
		Day5	✓	x	✓	✓	
		Day6	✓	✓	✓	✓	
3.	Tikia	Day1	x	x	X	x	About 6-7 days
		Day2	x	x	X	x	
		Day3	x	x	X	x	
		Day4	x	x	X	x	
		Day5	x	x	X	x	
		Day6	x	x	X	x	
		Day7	x	x	✓	✓	
		Day8	✓	x	✓	x	
		Day9	✓	✓	✓	✓	
4.	Biscuits	Day1	x	x	X	x	About 10-12 days
		Day2	x	x	X	x	
		Day3	x	x	X	x	
		Day4	x	x	X	x	
		Day5	x	x	X	x	
		Day6	x	x	X	x	
		Day7	x	x	X	x	
		Day8	x	x	X	x	
		Day9	x	x	X	x	
		Day 10	x	x	X	x	
		Day11	x	x	X	x	
		Day12	x	x	X	x	
		Day13	x	x	✓	✓	
		Day14	✓	x	✓	✓	
		Day15	✓	✓	✓	✓	

### (3) Effect of *Cissus quadrangularis* and *Achyranthes aspera* on Weight Loss of Mice

Results show that intake of high fat diet led to an approximate increase of 9.17 % in body weight. Significant difference in body weights of different groups were detected particularly between HF diet control and Extract + HF treated group. The results indicate that the Group C (treatment with mixture of both *Cissus quadrangularis* and *Achyranthes aspera* extract) shows significant reduction (3.02 gm) in body weight which is

a clear sign of anti-obesity effect. It is further clarified that there is reduction in the body weight for both groups treated with *Cissus* (1.44gm) and *Achyranthes* (1.26gm) respectively. But when the mixture of extracts of both *Cissus* and *Achyranthes* were given to the group C, there is a remarkable decrease (3.02 gm) in the body weight. Moreover, no significant adverse effects compared to controls were observed and no mortality was reported during after treatment.

**Table 8:** Effect of the extract of *Cissus* and *Achyranthes* on different groups' in relation to the body weights (g) before and after treatment

Group	Pre-experimental body weight in grams	Weight after high fat (HF) diet	Weight after Treatment
Group A	30.2 ± 02.94	33.38 ± 03.19	31.94 ± 03.23
Group B	31.1 ± 03.36	35.4 ± 01.78	34.14 ± 02.11
Group C	32.56 ± 03.93	34.68 ± 03.85	31.66 ± 03.80
Group D	33.7 ± 0.90	35.8 ± 01.15	35.98 ± 0.72

Values represent mean ± S.D of 5 mice.

- ❖ Group A means the group which has been given the treatment of *Cissus quadrangularis* extract
- ❖ Group B means the group which has been given the treatment of *Achyranthes aspera* extract
- ❖ Group C means the group which has been given the treatment of mixture of both *Cissus quadrangularis* and *Achyranthes aspera* extract
- ❖ Group D means the group which has been treated as control (Placebo)

### 4) Results of Anti-obesity trials of *Achyranthes aspera* and *Cissus quadrangularis* on humans

#### Anthropomorphic characteristics

Waist circumference and waist hip ratio is an extremely important determinant in the diagnosis of obesity and metabolic syndrome. As shown in Table 12, there was significant reduction in these variable across both treatment groups was paralleled by significant reductions in body weight, BMR and BMI for the both *Cissus* and *Achyranthes* supplemented groups. There was highly significant effect ( $P<0.01$ ) on subcutaneous fat of trunk and legs, significant effect ( $P<0.05$ ) on subcutaneous fat of whole body and non-significant effect on subcutaneous fat of arms whereas there was significant effect ( $P<0.05$ ) on skeletal muscle of arms and legs and non-significant effect on skeletal muscle of whole body and trunk by supplementation of *Cissus* and *Achyranthes*. To explain the loss over 10 weeks into actual measurements, the mean change in weight (kg) for the *Cissus*, *Achyranthes* supplemented group and placebo was 3.11±0.49 kg, 2.86±0.41 kg and 1.59±0.19 kg respectively. The mean reduction in weight was comparable with each other in the both *Cissus* and *Achyranthes* supplemented groups which was significantly higher ( $P<0.05$ ) than placebo group. The mean change in waist circumference and waist hip ratio was 2.16±0.4419 and 0.016±0.0076 in *Cissus* and 2.125±0.311 and 0.029±0.008 in *Achyranthes* supplemented group which was significantly higher ( $P<0.01$ ) than change in waist circumference and waist hip ratio of placebo group 0.59±0.146 and 0.026 ±0.018. On comparison of mean for BMR reduction it was observed that both *Cissus* (33.31±6.92) and *Achyranthes* (32.81±9.37) supplemented group was at par with each other but significantly higher than placebo (2.86±1.37). BMI for the *Cissus*, *Achyranthes* supplemented group and placebo was 1.24±0.21, 1.23±0.18 and 0.68±0.13. Thus, over a period of 10 weeks, there was reduction in BMI for both *Cissus* and

*Achyranthes* supplemented group which was significantly higher than placebo.

#### Serological characteristics

As shown in Table 13, there was a significant effect on serum cholesterol, HDL and triglyceride similarly there was highly significant effect on LDL, CHO/HDL and LDL/HDL for the *Cissus* and *Achyranthes* supplementation. Serum total cholesterol was also significantly ( $P<0.05$ ) reduced by use of the *Cissus* (13.46±5.91) and *Achyranthes* (18.33±1.80) similarly triglyceride was also significantly ( $P<0.05$ ) reduced in *Cissus* (15.39±5.86) and *Achyranthes* (11.92±1.39) supplemented group whereas in placebo triglyceride was increased by 4.01±6.99 in Eight-week. On comparison of mean for HDL ratio it was observed that highest increase was recorded in *Cissus* supplemented group (5.76±1.92) which was significantly higher than *Achyranthes* supplemented group (1.74±0.6), however in placebo (0.21±1.31) there was decrease in HDL ratio. An increase in the concentration of circulating HDL-cholesterol in both *Cissus* and *Achyranthes* supplemented group shows a large reduction in the ratio of total cholesterol to HDL-cholesterol ratios as well as LDL-cholesterol to HDL cholesterol ratios.

On comparison of mean for LDL cholesterol it was observed that the reduction in it was comparable with each other in both *cissus* (15.12±2.41) and *Achyranthes* (18.84±3.40) supplemented group which was significantly higher than placebo (1.97±0.48) similarly LDL/HDL ratio was at par in both *cissus* (0.418±0.0072) and *Achyranthes* (0.44±0.07) supplemented group which was significantly higher than placebo (0.15±0.040) however highest decrease in CHO/HDL ratio was recorded in *Achyranthes* (0.38±0.11) supplemented group. The effect of supplementation of *Achyranthes* (0.42±0.07) and *Cissus* (0.44±0.07) on LDL/HDL ratio was comparable with each other but significantly higher than Placebo (0.15±0.04). The increase in the concentration of HDL-cholesterol and a decrease in the concentration of LDL-cholesterol could lead to a lowering of the atherogenicity and therefore a significant reduction in the potential incidence of coronary heart disease.

The *Cissus* and *Achyranthes* brought about a significantly greater weight loss than placebo during the study period in obese individuals. This was accompanied by a significant improvement in the lipid profiles and anthropomorphic profiles of study participants.

**Table 9:** Distribution of participants into treatment groups. The number given in parentheses represents the participants whose complete measurements were done over the study period.

Group No	Participants	Treatment	No. of Participants
1.	Males/Females BMI>25	Placebo 10 weeks	15
2.	Males/Females BMI>25	Cissus capsule 10 weeks	15
3.	Males/Females BMI>25	Achyranthes Tea/ biscuits 10 weeks	15

**Table 10:** Effect of the Cissus and Achyranthes treatment on Anthropometric Measurement

		Group 1 (Placebo 10 weeks)		Group 2 (Cissus capsule 10 weeks)		Group 3 (Achyranthes Tea/ biscuits 10 weeks)	
Time (weeks)		T=0	T= 10	T=0	T= 10	T=0	T= 10
Body weight(Kg)		87.63±11.24	86.03±11.27	81.18 ± 11.31	78.06 ± 11.42	91.01±14.22	88.14±14.11
Waist circumference (inch)		42.63±3.83	42.03±3.72	37.41±4.99	35.25±5.05	42.93±3.64	40.81± 3.25
W/H (waist /hip ratio)		0.92±0.06	0.93±0.09	0.866±0.08	0.85±0.07	0.94±0.07	0.91±0.06
Fat %		37.54±5.42	37.01±5.77	32.61±4.94	31.04± 5.72	36.5±5.75	35.24±5.46
Visceral Fat %		17.81±5.70	17.06±5.76	15.68±5.65	14.18±5.51	18.81±6.95	16.48±6.51
BMR (RM = Resting metabolism)		1732.19±168.15	1729.31±170.02	1725.313±176.43	1692±175.27	1787.13± 213.11	1754.31±224.69
BMI		32.77±4.07	32.09±3.99	29.77±3.12	28.53± 3.16	33.46±6.80	32.23±6.55
Body Age		59.31±9.95	58.75±10.36	52±12.14	48.94±12.43	59.63±9.56	55.5±10.60
Sub-Cutaneous Fat	Whole body	31.25±8.24	30.69±8.39	23.11±4.02	21.59±3.40	30.47±8.82	29.52±8.61
	Trunk	28.33±7.31	27.90±7.46	21.18±3.66	19.64±3.40	27.79±7.96	26.78±7.80
	Arms	44.38±10.55	43.79±10.54	34.25±7.92	31.17±5.90	41.83±11.49	38.80± 12.65
	Legs	42.88±9.76	43.35±10.42	33.99±7.75	31.59±6.02	40.89±10.17	40.28± 11.03
Skeletal Muscles	Whole body	24.99±3.34	25.21±3.88	28.14±2.69	19.54±3.39	25.41±3.52	25.33±3.83
	Trunk	17.73±4.64	18.61±5.17	19.54±3.39	20.50±3.30	18.36±4.59	17.23±3.72
	Arms	26.63±9.32	26.88±9.94	34.84±2.26	35.43±2.39	26.6±9.33	26.35±9.59
	Legs	41.00±4.60	41.15±5.30	45.64±2.65	46.19±2.45	41.44±4.89	40.98±5.22

These physiological measurements were recorded by Omeron Body Fat Monitor (HBF 375), Inch tape and height scale.

\* These data are shown as means with standard deviations (± SD)

**Table 11:** Effectiveness of the Cissus and Achyranthes treatment on Serological Characteristics (\*unit: mg/dl)

		Group 1 (Placebo 6 weeks)		Group 2 (Cissus capsule 10 weeks)		Group 3 (Achyranthes Tea/ biscuits 10 weeks)	
Time (weeks)		T=0	T= 10	T=0	T= 10	T=0	T= 10
Serum cholesterol		205.56±13.23	202.82±17.38	198.03 ± 14.42	184.57±28.78	196.4±20.14	178.07±17.34
HDL cholesterol		43.48±2.65	43.28±5.35	41.13± 2.6	46.89±6.72	41.38±2.46	43.12±2.76
LDL cholesterol		139.95±14.26	137.95±13.75	129.78± 11.76	114.66±13.62	137.56±21.81	118.72±12.49
VLDL cholesterol		26.29±6.63	26.45±9.42	23.49± 5.84	23.02±9.83	24.68±6.38	22.57±6.53
Serum Triglyceride		131.46±33.14	135.46±43.30	117.41± 29.23	113.58±48.7	118.75±27.93	109.97±30.23
CHO/HDL		4.77±0.18	4.66±0.25	4.48± 0.43	4.09±0.51	4.6±0.27	3.83±0.47
LDL/HDL		3.16±0.16	3.01±0.18	2.99± 0.25	2.58±0.39	3.09±0.22	2.65±0.49

These data are shown as means with standard deviations (± SD)

**Table 12:** Effectiveness of *Cissus quadrangularis* and *Achyranthes aspera* formulation on Anthropometric characteristics: difference in means from Week 0 to week 10 Week 8

Anthropometric Characteristics		Group 1 (Placebo) (10 weeks)	Group 2 (Cissus capsule) (10 weeks)	Group 3 (Achyranthes Tea/ biscuits) (10 weeks)
Time (weeks)		T=0 to T=10	T=0 to T= 10	T=0 to T= 10
Body weight(Kg)*		-1.59±0.19 <sup>a</sup>	-3.11±0.49 <sup>b</sup>	-2.86±0.41 <sup>b</sup>
Waist circumference**		-0.59±0.15 <sup>a</sup>	-2.16±0.44 <sup>b</sup>	-2.12±0.31 <sup>b</sup>
W/H (waist /hip ratio)**		0.026±0.018 <sup>a</sup>	-0.016±0.008 <sup>b</sup>	-0.029±0.008 <sup>b</sup>
Fat %		-0.52±0.42	-1.57±0.59	-1.26±0.21
Visceral Fat %		-0.75±0.09	-1.51±0.31	-2.34±1.06
BMR (RM = Resting metabolism) **		-2.87±1.37 <sup>a</sup>	-33.31±6.92 <sup>b</sup>	-32.81±9.36 <sup>b</sup>
BMI*		-0.67±0.13 <sup>a</sup>	-1.24±0.21	-1.23±0.18 <sup>b</sup>
Body Age		-0.56±0.20	-3.06±0.46	-4.12±2.01
Sub-Cutaneous Fat	Whole body*	-0.56±0.14 <sup>a</sup>	-0.95±0.21 <sup>ab</sup>	-1.52±0.27 <sup>b</sup>
	Trunk **	-0.43±0.10 <sup>a</sup>	-1.01±0.14 <sup>b</sup>	-1.53±0.24 <sup>c</sup>
	Arms	-0.59375±0.21	-3.02±1.88	-3.08±0.95
	Legs**	0.49±0.86 <sup>a</sup>	-1.80±0.35 <sup>b</sup>	-2.41±0.64 <sup>b</sup>
Skeletal Muscles	Whole body	0.22±0.252	-0.087±0.21	0.056±0.68
	Trunk	0.88±0.28	-1.13±1.11	0.96±0.27
	Arms*	0.25±0.23 <sup>ab</sup>	-0.26±0.23 <sup>b</sup>	0.59±0.11 <sup>a</sup>
	Legs*	0.15±0.30 <sup>ab</sup>	-0.46±0.24 <sup>b</sup>	0.56±0.21 <sup>a</sup>

Values are means ± SE, comparing starting point to end point, significant differences were at \*p<0.05=Significant difference,

\*\*p<0.01 Highly Significant difference for the same treatment

a, b, c= means superscripted with different letter with in a column differ significantly from each other



**Table 13:** Effectiveness of *Cissus quadrangularis* and *Achyranthes aspera* formulation on serological characteristics: difference in means from Week 0 to week 10

Serological characteristics	Group 1 (Placebo) (10 weeks)	Group 2 (Cissus capsule) (10 weeks)	Group 3 (Achyranthes Tea/ biscuits) (10 weeks)
Time (weeks)	T=0 to T= 10	T=0 to T= 10	T=0 to T= 10
Serum cholesterol*	-2.74±2.90 <sup>a</sup>	-13.46±5.91 <sup>b</sup>	-18.34±1.80 <sup>b</sup>
HDL cholesterol*	-0.21±1.31 <sup>a</sup>	5.76±1.92 <sup>a</sup>	1.73±0.6 <sup>a</sup>
LDL cholesterol**	-1.97±0.48 <sup>a</sup>	-15.12±2.41 <sup>b</sup>	-18.84±3.40 <sup>b</sup>
VLDL cholesterol	0.16±1.63	-0.48±1.79	-2.11±0.27
Serum Triglyceride*	4.01±6.99 <sup>a</sup>	-15.39±5.86 <sup>b</sup>	-11.92±1.39 <sup>b</sup>
CHO/HDL**	-0.11±0.07 <sup>a</sup>	-0.38±0.11 <sup>a</sup>	-0.77±0.14 <sup>b</sup>
LDL/HDL**	-0.15±0.04 <sup>a</sup>	-0.42±0.07 <sup>b</sup>	-0.44±0.07 <sup>b</sup>

Values are means ± SE, comparing starting point to end point, significant differences were at \* $p < 0.05$  = Significant difference, \*\* $p < 0.01$  Highly Significant difference for the same treatment a, b, c = means superscripted with different letter with in a column differ significantly from each other

### Conclusion

*Cissus quadrangularis* and *Achyranthes aspera* brought about significant reductions in weight, while decreasing serum cholesterol thus improving cardiovascular risk factors thereby supporting the clinical data for weight loss and improving cardiovascular health. These results support the hypothesis that the use of a *Cissus quadrangularis* and *Achyranthes aspera* has efficacy in the management of weight loss.

### Acknowledgement

Authors are thankful to Dr. Jagdish Vaishnav and Dr. Mangesh for Analytical and statistical analysis of data and to UGC, New Delhi to provide the fund to pursue this research work.

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