

# Comparative Study of Aqueous and Alcoholic Extracts of Roots of *Bauhinia variegata* Linn. on Cafeteria Diet Induced Obesity

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## ABSTRACT

**Background:** Obesity is typically characterized by excess deposition of adipose tissue. Along with dietary management, increase in physical activity, lifestyle modification, surgical intervention, drug treatment is also available which aid in weight loss. However, most of these drugs exhibit side effects like insomnia, irritability, epigastric discomfort, constipation, some are even habit-forming drugs. Hence, the present study was designed to screen, evaluate and discover newer safer anti-obesity drugs from plant sources. **Materials and Methods:** In this study, the aqueous and 90% alcoholic extracts of roots of *Bauhinia variegata* Linn. were prepared and evaluated for their anti-obesity activity. Obesity was induced using the cafeteria diet in female Albino Wistar rats weighing 150-200 g. The parameters evaluated were body temperature, body weight, serum biochemical parameters, internal organ weight and histology of liver tissue. **Results:** The results of the study show that the extracts were capable of reducing body weight, inducing thermogenesis and maintaining serum biochemical parameters. Among the extracts the aqueous extract of roots of *Bauhinia variegata* Linn. has shown better pharmacological activity than the alcoholic extract. Therefore, this group of animals were subjected to histological studies which showed only mild fatty liver change when compared to the positive control group. **Conclusion:** Further studies are required for evaluating the mechanism of action, isolating the compound/s responsible for the activity, structurally elucidating the same to then formulate it into anti-obesity drug with enhanced efficacy.

**Keywords:** *Bauhinia variegata* Linn., Cafeteria diet, Anti-obesity activity, Serum biochemical parameters, Histopathological studies.

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**Received:** 29-06-2023;

**Revised:** 30-10-2023;

**Accepted:** 29-11-2024.

## INTRODUCTION

World statistics presented by the WHO show that more than a million people in the world are obese this includes around 650 million adults, 340 million adolescents and 39 million children. It further states that by 2025, 167 million people which include adults and children will become obese.<sup>1</sup>

The term obesity refers to having an abnormally high proportion of fat and adipose tissue in comparison to lean muscle mass. The prevalence of this lifestyle disorder has reached to epidemic levels especially in the industrialized countries making it a serious health problem. The causative factors ranges from being genetic, environmental, nutritional, physiological, psychological,

social and cultural. In the past few decades, the societal changes, paradigm transition in nutritional values, rise in family economic conditions, rise of population consuming diets loaded with sugars and fats and at the same shift of nature of work to be less physically demanding, increase in work automation, technological facilities available at finger tips all of these has fueled and blown up the situation of obesity out of proportions worldwide. The most common tool to assess and declare obesity is the calculation of the Body Mass Index (BMI). BMI is defined as the weight in kilograms divided by the square of height in meters, (kg/m<sup>2</sup>).

- A BMI over 25 kg/m<sup>2</sup> is defined as overweight.
- A BMI over 30 kg/m<sup>2</sup> is defined as obese.<sup>2</sup>

Life style disorder obesity is associated with several serious chronic diseases. The unprevalent increase in the cases of hypertension and type-2 diabetes is a direct result of the obesity epidemic. Abnormal obesity promotes development of insulin resistance, leading to hyper-insulinemia, hyperglycaemia along



DOI: 10.5530/ijper.20254509

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with hyper-triglyceridemia, low HDL-cholesterol, it promotes development of atherosclerosis and all these are strongly associated with hypertension and coronary heart diseases. Similarly, disorders associated with the gastrointestinal tract like GERD reflux, gallbladder and liver disorders are all related to and result of obesity. The global epidemic of obesity is responsible for the rise in NAFLD cases in adults and children worldwide. Along with the pulmonary system of obese people is also seen to be affected obesity hypoventilation syndrome, obstructive sleep apnea and other functional abnormalities. Similarly, hyperuricemia, gout, osteoarthritis are also the precipitants of obesity. The risk of development of cancer and malignancy in several organs like liver, esophagus, gall bladder, pancreas, breast, uterine, cervical, prostate is also increased.<sup>3</sup>

Controlling obesity and brining about weight loss involves the implementation of calorie deficit diet, behavioral modification and physical activity and exercise. In extreme cases to treat obesity aid weight loss surgical intervention and medication may also be required. The anti-obesity drugs available for treatment include those which inhibit non-systemic fat absorption, appetite suppressors and lipolytic agents. An ideal anti-obesity drug should effectively reduce weight and its dependent disease, it should not have any additive properties, should be pocket friendly, should have long term efficacy, its mechanism of action should be known and its side effects should be tolerable. Based on their mechanism of action the anti-obesity drugs are classified as:

- Drugs which minimize food intake.
- Drugs which reduce absorption of nutrients.
- Drugs which increase energy expenditure.<sup>4,5</sup>

However, most of these drugs have safety concerns; termination of their use has seen to be resulting in the return of the weight lost and some being CNS stimulants are even habit-forming drugs thus considered unsafe for use.<sup>6,7</sup> Herbal preparations, plant extracts and isolated compounds from plant sources have always been an alternative for synthetic drugs for the treatment of several diseases from several years. In this experiment the aqueous and alcoholic extract of roots of *Bauhinia variegata* Linn. was screened for its anti-obesity activity by inducing Cafeteria diet and compared with standard drug Orlistat- a lipase inhibitor.<sup>8-11</sup>

## MATERIALS AND METHODS

Drugs and Chemicals;

Orlistat-Cipla Ltd., Mumbai.

Glucose diagnostic kit-Beacon Diagnostics Pvt. Ltd., Navasari.

Cholesterol Diagnostic Kit - Beacon Diagnostics Pvt. Ltd., Navasari.

Triglycerides Diagnostic Kit - Lab Care Diagnostics Pvt. Ltd., Sarigam.

Regular Laboratory Reagents and Chemicals;

Condensed milk;

Bread;

Chocolate;

Biscuits;

Dried coconut;

Cheese;

Boiled potatoes.

## Plant material and extract

The roots of *Bauhinia variegata* were collected from forest of Dandeli, District-Karwar and from local areas of Belgaum. The plant was authenticated from botanist Dr. R. S. Goudar, Department of Botany, R.L.S. Institute, Belgaum, Karnataka, India. Roots were cut into small pieces, cleaned and shade dried, subjected to size reduction to get coarse powder and then passed through sieve no.40 to get uniform powder. To store the powder, it was transferred in an airtight polyethylene bag. For the preparation of the extract 200 g of powder was subjected to hot continuous extraction (Soxhlet) initially with petroleum ether (40-60°C) to remove fats and oils for 18 hr and then with 90% pure alcohol for 18 hr. After the effective extraction, alcohol was concentrated at room temperature in reduced pressure using a rotary evaporator (Gucchi Rota vaporator). The extract obtained was semi solid brown viscous mass and the total percentage yield of the extract was found to be 9.16% w/w.

## Experimental Animals

For the study female Albino rats (150-200 g) of Wistar strain were used, which were obtained from Animal House, of KLE College of Pharmacy, Hubli. All experimental procedures were approved by the Institutional Ethics Committee (IAEC). Project Code: KLEU's011/IAEC.HBL/31<sup>st</sup> Aug 2013.

## Acute Toxicity Studies

For carrying out the acute toxicity studies OECD guidelines 423 were followed and to execute the toxicity studies healthy young albino rats of either sex weighing 150-220 g (8 to 12 weeks old) were used. For determining the acute toxicity and LD50 values of both the alcoholic extract and the aqueous extract the animals were acclimatised after keeping them fasting for 18 hr being provided only with water. In total there were three groups, in which one received the aqueous extract, other group received alcoholic extract, one group received the standard drug and another control group received normal saline. To administer a suspension of the extracts was prepared with water and 2% gum

**Table 1: Effect of aqueous and alcoholic extracts of roots of *Bahunia variegata* Linn. on body weight in Cafeteria diet fed rats.**

Group	Treatment	Average body weight difference between day 1 and day 40 in grams
I	Normal diet	20.83±1.53
II	Cafeteria diet	86.67±7.92 <sup>++</sup>
III	Cafeteria diet+Aqueous extract	37.5±1.70 <sup>**</sup>
IV	Cafeteria diet+Alcoholic extract	54.17±4.54 <sup>**</sup>
V	Cafeteria diet+Orlistat	35.83±2.71 <sup>**</sup>

Values are MeanSEM for group of 6 rats.<sup>\*\*</sup> $p < 0.01$  as compared to Cafeteria diet.<sup>++</sup> $p < 0.01$  as compared to Normal diet.

acacia. A dose of 5000 mg/kg of body weight was administered orally to the animals. Further they were observed for 24 hr for behavioural changes, locomotion, muscle spasms, tremors, convulsions and mortality and further were observed for a period of 14 days for occurrence of any toxic symptoms. At the end of the toxicity studies since both the extracts showed no mortality at the dose of 5000 mg/kg b.w. for further experimental studies and evaluation of its anti-obesity activity 1/10<sup>th</sup> and 1/20<sup>th</sup> of the aforesaid dose of both the alcoholic and aqueous was used.<sup>11</sup>

### Experimental Design and Drug Treatment

Anti-Obesity activity: For the main study female albino Wistar rats (150-200 g) were obtained from the animal house of KLES's College of Pharmacy Belgaum. They were kept in standard laboratory conditions at 22°C with light (12 hr):dark (12 hr) cycle in polypropylene cages and they were provided with normal pellet chow and water *ad libitum* for a period of 1 week for the purpose of acclimatization. The animals were divided into FIVE groups and each group comprised of SIX animals each. The treatment and Cafeteria Diet (CD) were continued for 40 days.<sup>12</sup>

- Group I comprised of animals which were provided with normal pellet chow and water *ad libitum*.
- Group II animals were provided with Cafeteria Diet (CD) and normal pellet chow and
- water.
- Group III animals were provided with Cafeteria Diet (CD)+normal pellet chow+water+treatment with Aqueous extract at the dose of 500 mg/kg/day p.o.
- Group IV animals were provided with Cafeteria Diet (CD)+normal pellet chow+water+treatment with Alcoholic extract at the dose of 500 mg/kg/day p.o.
- Group V animals were provided with Cafeteria Diet (CD)+normal pellet chow+water+treatment with Orlistat 32.4 mg/kg/day p.o.

**Table 2: Effect of aqueous and alcoholic extracts of roots of *Bahunia variegata* Linn. on body temperature in Cafeteria diet fed rats.**

Group	Treatment	Average body temperature recorded on day 39 <sup>th</sup> in C
I	Normal diet	33.0±0.06
II	Cafeteria diet	31.55±0.28 <sup>++</sup>
III	Cafeteria diet+Aqueous extract	33.07±0.06 <sup>**</sup>
IV	Cafeteria diet+Alcoholic extract	32.82±0.44 <sup>**</sup>
V	Cafeteria diet+Orlistat	33.82±0.14 <sup>**</sup>

Values are MeanSEM for group of 6 rats.<sup>\*\*</sup> $p < 0.01$  as compared to Cafeteria diet.<sup>++</sup> $p < 0.01$  as compared to Normal diet.

### Composition of Cafeteria diet

The cafeteria diet consisted of 3 diets (condensed milk 48 g+bread 48 g), (chocolate 18 g+biscuits 36 g+dried coconut 36 g) and (cheese 48 g+boiled potatoes 60 g). The three diets were given to each group of 6 rats on day 1, 2 and 3 respectively and then repeated in same succession for 40 days in addition to normal pellet chow.

### Parameters Studied

#### Body weight

At the beginning of the study the weight was recorded in (g) and then on alternate days for the period of 40 days.

#### Body Temperature

On the day 39 of the study the body temperature of each of the animal was recorded using a digital thermometer with a contact time of 1 min after the drug was administered and the average reading of 6 animals in each group was calculated.

### Biochemical Parameters

On completing the study at day 41 the animals were anesthetized using light ether and blood was collected using retro orbital route using a capillary tube. The collected blood samples were then subjected to centrifugation and serum was obtained. These serum samples were then used for estimating glucose levels, total cholesterol, HDL-cholesterol, LDL and VLDL cholesterol and triglycerides using biochemical kits.<sup>13-16</sup>

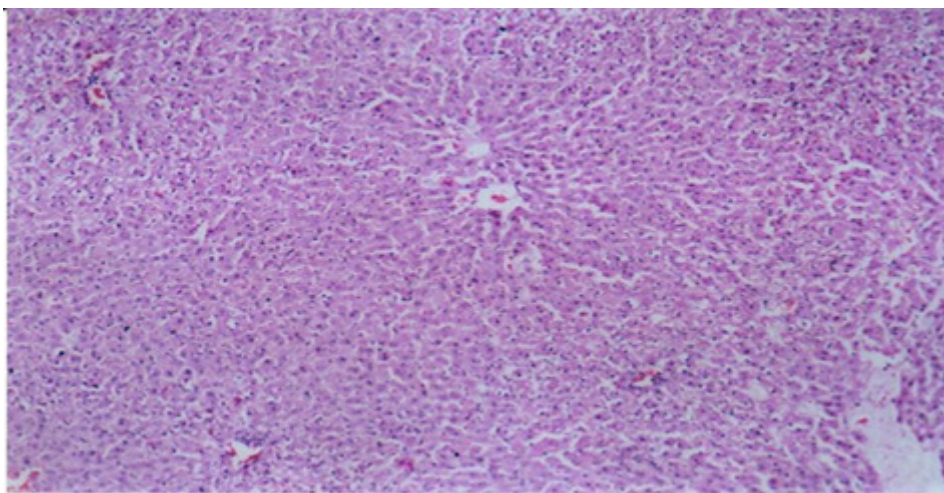
### Histopathological Studies

For histological studies the animals were sacrificed, liver organ isolated and a 5  $\mu$  section of the tissue was isolated using a standard microtome, fixed using 10% formalin, immersed in paraffin and studied at 100x. For staining purpose of the tissue haematoxylin and eosin was used.

**Table 3:** Effect of aqueous and alcoholic extracts of roots of *Bauhinia variegata* Linn. on serum biochemical parameters at day 41 on Cafeteria diet fed rats.

Group	Treatment	Glucose (mg%)	Cholesterol (mg%)	HDL (mg%)	LDL (mg%)	VLDL (mg%)	TGs (mg%)
I	Normal	66 4.7	131.5 2.91	33.17 1.24	14.77 1.0	83.5 4.2	73.83 5.22
II	Cafeteria diet	97.33** 4.92	155.2**4.4	17.67**2.4	24.57**1.12	113.4** 6.56	122.8**5.61
III	Cafeteria diet+Aqueous extract	63** 3.83	106**3.51	35.5** 5.74	13.33**1.07	67.0**6.21	66.67**5.37
IV	Cafeteria diet+Alcoholic extract	73.83** 3.016	120.7**3.7	30.83**0.9	17.43**1.07	72.4** 3.16	87.17**5.38
V	Cafeteria diet+Orlistat	80** 3.864	98.67**3.8	30.67** 1.96	12.77**0.86	55.17**5.06	64** 4.28

Values are MeanSEM for group of 6 rats.\*\* $p < 0.01$  as compared to Cafeteria diet.++ $p < 0.01$  as compared to Normal diet.



**Figure 1:** T.S. of Liver Tissue from animal belonging to Normal Group (H&E, 100X).

### Statistical Analysis

The results and outcomes of the experimental values was expressed as mean  $\pm$  standard deviation and evaluated using statistical one-way ANOVA followed by *post-hoc* Dunnett's 't' test or Bonferroni's test using GraphPad Prism 5 software. The  $p$  value of  $< 0.05$  was measured and considered as statistically significant.

### RESULTS

Results show that the aqueous and alcoholic extracts of roots of *Bauhinia variegata* Linn. have significant anti-obesity profile, further analysis of the results high light that the aqueous extract activity is more significant than the alcoholic extract of the plant.

During the period of the study the body weight of the animals was observed keenly and at the end of the study it was noticed that the animals which were fed on cafeteria diet which was rich in fat and carbohydrate showed significant increase in body weight when compared to the group of animals which were fed on normal diet.

The group of animals which received the treatment of aqueous extract of roots of *Bauhinia variegata* Linn. demonstrated significantly lesser weight gain than compared to the group of animals treated with the alcoholic extract of roots of *Bauhinia variegata* Linn. which showed moderate decrease in weight. The group of animals which received standard drug Orlistat which is a NE and 5-HT reuptake inhibitor has also shown significant decrease in weight (Table 1).

Similarly, both the extracts were evaluated for its effect on the body temperature and it was observed from the study that the body temperature of group of animals who were fed on cafeteria diet without any treatment was lower than those group of animals belonging to the normal group as well as the group of animals which received aqueous and alcoholic extracts and the standard drug Orlistat as treatment (Table 2).

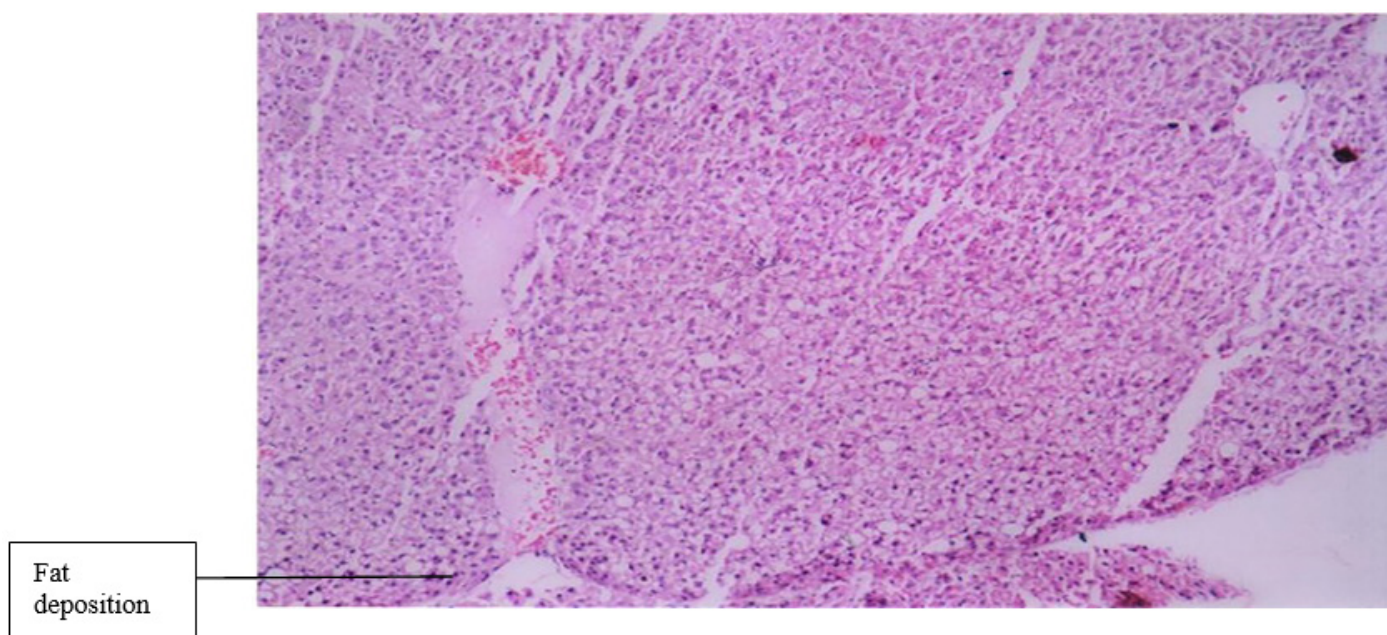
The levels of these biochemical parameters were highly elevated in group of animals who were fed on cafeteria diet when compared with the group of animals who were fed on normal diet. However,



**Table 4:** Effect of aqueous and alcoholic extracts of roots of *Bauhinia variegata* Linn. on organ weights (g) in Cafeteria diet fed rats.

Group	Treatment	Liver	Heart	Kidney	
				Left	Right
I	Normal diet	5.3±0.25	0.68±0.03	0.53±0.03	0.59±0.04
II	Cafeteria diet	7.6±0.11 <sup>++</sup>	1.01±0.12 <sup>++</sup>	0.92±0.04 <sup>++</sup>	0.93±0.03 <sup>++</sup>
III	Cafeteria diet+Aqueous extract	6.23±0.03	0.57±0.03	0.57±0.03	0.61±0.008
IV	Cafeteria diet+Alcoholic extract	6.20±0.08	0.63±0.04	0.60±0.07	0.62±0.004
V	Cafeteria diet+Orlistat	6.3±0.15	0.74±0.02	0.70±0.006	0.69±0.003

Values are MeanSEM for group of 6 rats. \*\* $p < 0.01$  as compared to Cafeteria diet. ++ $p < 0.01$  as compared to Normal diet.



**Figure 2:** T.S. of Liver Tissue from animal belonging to Positive Control Group fed with Cafeteria Diet (H&E, 100X).

the group of animals which received treatment in the form of aqueous, alcoholic extracts of roots of *Bauhinia variegata* Linn. and those which received the std drug Orlistat demonstrated decrease in the level of these parameters as compared to the group which did not receive treatment. Amongst the two extracts, the group which received the aqueous extract as treatment has shown significant decrease in these values of serum biochemical parameters as well slight increase in serum HDL levels (Table 3).

### Effect of Alcoholic extract on Cafeteria Diet induced Obesity

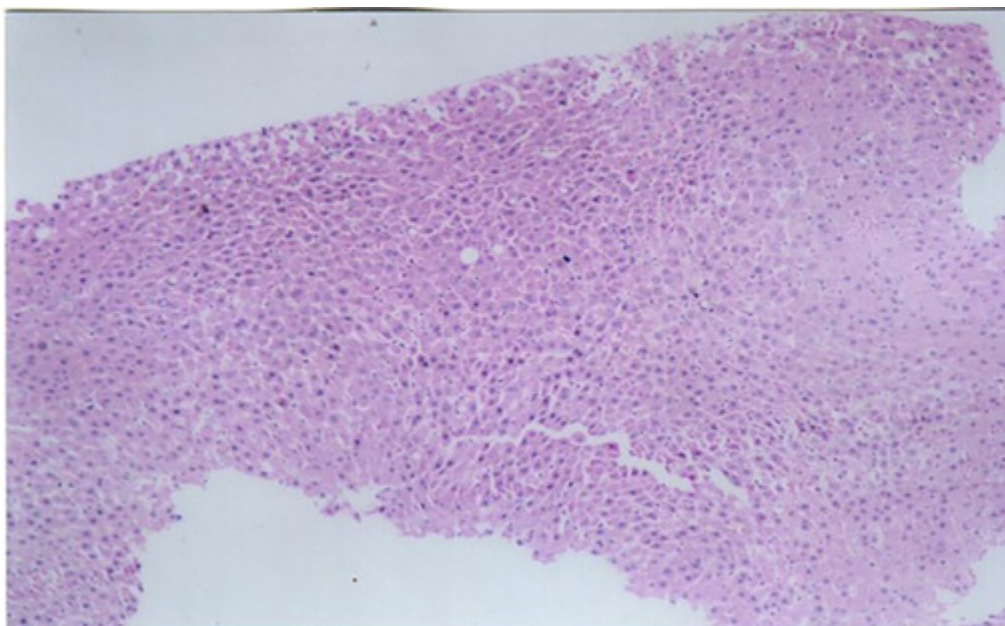
One of the factors associated with obesity is accumulation of adipose tissue which in turn increases the individual organ weight. The weight of internal organs like liver, heart and kidneys in group of animals fed with cafeteria diet is elevated when compared with the group of animals who were fed on the cafeteria diet but received treatment with aqueous and alcoholic extracts of roots of *Bauhinia variegata* and the std drug Orlistat (Table 4).

### Histological Studies

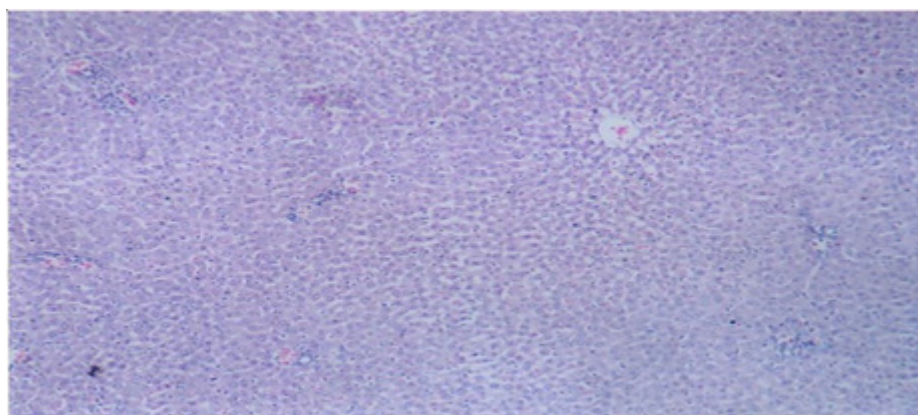
Since the aqueous extract showed significant activity compared to alcoholic extract, group of animals treated with aqueous extract were subjected to histopathological studies of liver for the deposition of fat and the development of NAFLD. The TS of the animals belonging to the group which received cafeteria diet showed excessive deposition of fat and development of NAFLD. When the TS of liver tissue of animals which along with the cafeteria diet received treatment with aqueous plant extract and std drug Orlistat there is a significant decrease in deposition of fat in the liver tissue and development of NAFLD than compared to the positive control group (Figures 1 to 4).

### DISCUSSION

The designing of the cafeteria diet is such that it exerts synergistic effect and causes obesity. This particular model was chosen for the study since it is the closest to the human diet which is



**Figure 3:** T.S. of Liver Tissue from animal belonging to Group Cafeteria diet+Aqueous extract of *Bahunia variegata* Linn. (H&E, 100X).



**Figure 4:** T.S. of Liver Tissue from animal belonging to Group Cafeteria diet+Std drug Orlistat (H&E, 100X).

being followed today majorly comprising of junk food whose main constituents include cheese, chocolates, high sugar like in condensed milk, high starch content like in potatoes. These observations help us to conclude that both the extracts have appetite suppressing activity and that the effect of aqueous extract in controlling weight gain is comparatively more significant than the alcoholic extract. The thermogenesis effect of the aqueous extract was also more significant than the alcoholic extract.<sup>2</sup>

### Serum Biochemical Parameters

One of the characteristic features of obesity is increased levels of serum total cholesterol, LDL-cholesterol and triglycerides accompanied by low levels of serum HDL levels.

Hyperinsulinemia and insulin resistance are features closely and strongly associated with obesity. When the group animals which were fed with cafeteria diet were studied it was observed

that they had a significant increase in serum glucose levels when compared to the normal group of animals. The group of animals which received treatment with aqueous extract, alcoholic extract and std drug Orlistat have shown significant decrease in serum glucose levels. This control on glucose levels was more significant in the groups which received the plant extract as treatment than with the group which received the std drug. Amongst the extracts as well the group which received aqueous extract as treatment has shown significant decrease in glucose values than the group which received the alcoholic extract.<sup>13-16</sup>

### Internal organ weight

One of the factors associated with obesity is accumulation of adipose tissue which in turn causes increase in the weight of internal organs like heart, liver and kidneys. The study showed that the animals which belonged to the group which received only the cafeteria diet have shown a significant increase in the weight



of their internal organs when compared to the animals belonging to the normal group. The animals which received treatment with aqueous and alcoholic extracts have demonstrated significant decrease in the weight of the internal organs as compared to the group of animals fed with cafeteria diet, similarly the group which received the std drug Orlistat also has shown decrease in the weight of internal organs.<sup>7</sup>

### Histological Studies

NAFLD is a continuum to obesity and controlling or preventing weight gain can bring about a holistic prevention of development of NAFLD or its prevention. The aqueous extract of the roots of *Bauhinia variegata* Linn. have prevented the aggravation of NAFLD in animals who were fed cafeteria diet. This clearly is indicative that the aqueous extract of the roots of *Bauhinia variegata* Linn. has the capacity to prevent deposition of adipose tissue and avoid the aggravation of NAFLD, also that this activity of the aqueous extract is significant when compared to the group of animals who received treatment with the alcoholic extract of the roots of *Bauhinia variegata* Linn. and the std drug Orlistat as treatment.

### CONCLUSION

In conclusion we may say that this experimental study which was carried out for 40 days and which involved evaluating and comparing the efficacy of the aqueous and alcoholic extracts of roots of *Bauhinia variegata* Linn. for their anti-obesity activity with each other as well as with the std drug Orlistat has shown that both the extracts have exhibited anti-obesity activity which is at par with that of the std drug Orlistat in controlling weight gain, maintaining homeostasis and body temperature, maintaining serum biochemical parameters, preventing both increase in the weight of internal organs as well as preventing the development of NAFLD. At the same time, it is observed that the aqueous extract of the roots of *Bauhinia variegata* Linn. has shown comparatively better anti-obesity activity than the alcoholic extract roots of *Bauhinia variegata* Linn. It has shown significant effect in controlling the weight gain, preventing decrease in body temperature, controlling the serum biochemical parameters, weight of internal organs and averting the development of NAFLD by inhibiting the deposition of fat in the liver tissue. Since the extracts have shown activity similar to the std drug of Orlistat, it may be concluded that like the std drug Orlistat the extracts of the roots of *Bauhinia variegata* Linn. also are lipase inhibitors, probably preventing digestion of dietary triglycerides, inhibiting absorption of dietary fats and decreasing calorie intake. Overall causing weight loss and acting as an aid in the management of hypertension, type-II Diabetes and hypercholesteremia. Further subjecting the extract to isolation, structural elucidation may help in discovering and formulating newer anti-obesity drug along with reconnoitering the precise mechanism of action.

### ACKNOWLEDGEMENT

The authors are grateful to The Principal, KLE College of Pharmacy, Vidyanagar, Hubli for facilitating research amenities.

### CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

### ABBREVIATIONS

**HDL:** High density lipoproteins; **LDL:** Low density lipoproteins; **GERD:** Gastroesophageal reflux disease; **NAFLD:** Non-alcoholic fatty liver disease; **b.w.:** Body weight; **CD:** Cafeteria diet; **p.o.:** Per oral; **mg/kg:** Milligram per kilogram; **T.S.:** Transverse section **ANOVA:** Analysis of Variance; **NE:** Norepinephrine; **5-HT:** 5-hydroxytryptamine.

### SUMMARY

The present study was done on the roots of *Bauhinia variegata* Linn. which were subjected to hot soxhlet extraction with petroleum ether (40-60°C) followed by extraction with 90% alcohol and maceration with chloroform water to obtain alcoholic and aqueous extracts respectively. The extracts were subjected to preliminary phytochemical investigation followed by screening them for their anti-obesity activity on Wistar Albino rats fed on Cafeteria Diet for 40 days. Simultaneously a group of animals received the Cafeteria diet along with treatment using std. Orlistat drug. On completion of 40days the animals were screened for their body weight, body temperature, serum biochemical parameters, weight of internal organs and the isolated organs were subjected to histological studies. Both the aqueous as well as the alcoholic extracts showed promising anti-obesity activity when compared to the std drug and on being compared with each other the aqueous extract has shown better anti-obesity activity as compared to the alcoholic extract. The aqueous extract has shown promising activity on weight control, has inhibited decrease in body temperature, has shown efficiency in preventing weight gain of internal organs, development of NAFLD and elevation of serum biochemical parameters when compared to the alcoholic extract as well as the std. drug Orlistat. Thus presenting lipase inhibitory activity, appetite suppressant activity and aiding weight loss. Further investigating the extracts for their action on other dietary models, investigating them for their mechanism of action, isolating phytoconstituents responsible for the anti-obesity activity can help us in formulating a newer, potent, and promising anti-obesity drug

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**Cite this article:** Ankalikar AA, Viswanathswamy AHM, Undale VR. Comparative Study of Aqueous and Alcoholic Extracts of Roots of *Bahunia variegata* Linn. on Cafeteria Diet Induced Obesity. *Indian J of Pharmaceutical Education and Research.* 2025;59(1s):s367-s374.