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Anti-obese activity of *Ziziphus jujuba* Lam leaves extract in dietary obese rats.

M. S. Ganachari^{1*}, Shiv Kumar², K. R. Alagawadi³

1. Associate Professor, Department of Pharmacology and Toxicology, K.L.E.S's College of Pharmacy, JNMC Campus, Belgaum, Karnataka - 590 010. India

2. Lecturer, Department of Pharmacology, K.L.E.S's College of Pharmacy, Nipani, Karnataka - 591 237. India

3. Associate Professor, Department of Pharmaceutical Chemistry, K.L.E.S's College of Pharmacy, JNMC Campus, Belgaum, Karnataka - 590 010. India

Abstract

Objective: To evaluate the effect of *Ziziphus jujuba* leaves extract in cafeteria diet and atherogenic diet induced obesity. **Methods:** Obesity was induced in albino rats by feeding cafeteria diet/atherogenic diet daily for 40 days in addition to normal diet *ad libitum*. Body weight was measured on day 1 and then on alternate days for 40 days. Daily food intake for group of 6 rats was assessed. Serum glucose and lipid levels and internal organs and fat pad weight analysis was carried out on day 41. **Results:** The *Z. jujuba* leaves extract treatment caused significant reduction in body weight, daily food intake, serum glucose and lipid levels, internal organs and fat pad weights in cafeteria and atherogenic diet fed rats when compared with control group of rats. **Conclusion:** The results of the present study conclude that alcoholic extract of *Z. jujuba* leaves showed anti-obese property by decreasing the body weight, food intake, serum glucose and lipid levels and internal organs and fat pad weights in dietary obese rats. The effect produced was comparable with that produced by standard anti-obese drug, Sibutramine.

Key words: Anti-obese, Atherogenic diet, Cafeteria diet, Obesity, Sibutramine, *Ziziphus jujuba*

1. Introduction

Drug treatment of obesity has often seen as controversial, largely because of failure to understand how it should be used. Due to paucity of data, no particular strategy or drug can yet be recommended for routine use. Currently approved drugs are best, when used in conjunction with diet, exercise and behaviour change regimens. They do not cure obesity, when they are discontinued weight regain

occurs. Thus there is a demand for search of new and safer ones [1].

A large section of world's population relies on traditional remedies to treat plethora of diseases. Medicinal herbs are an indispensable part of traditional medicine practised all over the world due to low costs, easy access and ancestral experience [2]. The main reason for this popularity is the belief that most herbal

* Corresponding author
Email : ganachari@hotmail.com

medicines, due to their “natural” origin, are harmless and without side effects [3]. *Ziziphus jujuba* is a small sub- deciduous tree belonging to family Rhamnaceae, which is cultivated and grown wild in many parts of the world [4]. The leaves of *Z. jujuba* are reported to have wide range of activities such as hypoglycaemic [5], hypolipidemic [6], permeability enhancement [7], anti-ulcer [8] and immunomodulatory [9] activity. In Ayurvedic and Unani system of medicine, the leaves of *Z. jujuba* are traditionally used to cure kapha, biliousness, diarrhoea, stomatitis, gum bleeding, syphilitic ulcers, asthma, to reduce obesity and as anti-pyretic and anthelmintic [4]. However the effect of *Z. jujuba* leaves on experimental models of diet induced obesity is not scientifically documented. Hence, we thought it is worthwhile to study the effect of *Z. jujuba* leaves extract on body weight, food intake, serum glucose and lipid levels and internal organs and fat pad weights in albino rats. Sibutramine, a noradrenaline and 5-hydroxytryptamine reuptake inhibitor has been chosen as standard anti-obese drug for comparison [10].

2. Experimental

2.1 Plant material

The leaves of *Z. jujuba* were collected from the damp fields around Belgaum, Karnataka, in May 2004 and were positively identified by Prof. S.B.Sasalatti, Head, Department of Botany, K.L.E.S’s R.L.Science Institute, Belgaum, Karnataka, where a voucher specimen has been deposited.

2.2 Preparation of extract

The leaves were shade dried at room temperature and powdered until able to pass through sieve no. 40. The crude extract of the dried leaves was obtained by percolation using 70 % ethanol at room temperature for 24 h. The dark green solution was concentrated under

reduced pressure at 500 C using Rota vapour apparatus to give a viscous mass, which was then stored at 40°C until used. The crude extract (ZJE) was subjected to preliminary phytochemical screening to characterize the phytoconstituents present.

2.3 Test drug

The alcoholic extract of *Z. jujuba* leaves was insoluble in water. Hence for animal testing, the extract was prepared as an aqueous suspension using 1% Carboxymethyl cellulose. The alcoholic extract failed to produce any lethality or change in behaviour when given up to 2500 mg/kg doses orally in rats. Hence the arbitrary dose selected for experimental studies was 500 mg/kg per day.

2.4 Experimental animals

Adult, female, Wistar strain albino rats weighing 150-180 g obtained from Central Animal House, J.N.Medical College, Belgaum, were used for the study. They were housed in acrylic cages under standard laboratory conditions at 22 -25°C with light (12 h): dark (12 h) cycle. The animals were provided with normal pellet chow and water *ad libitum*. Before conducting the experiment, the animal ethical clearance was obtained from Institutional Animal Ethics Committee (IAEC).

2.5 Cafeteria and Atherogenic Diets

The cafeteria diet [11] (CD) consisted of 3 diets– (condensed milk, 48g + bread, 48g), (chocolate, 18g + biscuits, 36g + dried coconut, 36g) and (cheese, 48g + boiled potatoes, 60g). The 3 diets were presented to group of 6 rats on day 1,2 and 3 respectively and then repeated for 40 days in same succession in addition to normal pellet chow. The Atherogenic diet [12] (AD) consisted of cholesterol-1% (Loba Chemie), cholic acid - 0.5% (Loba Chemie) and lard oil -5% (Sigma). It was provided in addition to normal pellet chow to group of six rats every day for 40 days.

2.6 Grouping of rats for the experiment

Animals were divided into seven groups each consisting of 6 rats as follows.

Group I-Normal control, which received normal pellet chow and water *ad libitum*.

Group II-Cafeteria diet control, which received CD along with normal pellet chow and water *ad libitum*.

Group III-CD + ZJE (500 mg/kg, p.o. per day)

Group IV-CD + Sibutramine (5 mg/kg, p.o. per day)

Group V-Atherogenic diet control, which received AD along with normal pellet chow and water *ad libitum*.

Group VI- AD + ZJE (500 mg/kg, p.o. per day)

Group VII-AD + Sibutramine (5mg/kg, p.o. per day)

2.7 Parameters studied and procedures [13]

2.7.1 Body weight

The body weight (g) was recorded on day 1 and then on alternate days for 40 days in each group.

2.7.2 Food intake

The daily food intake for group of 6 rats was measured daily for 40 days and expressed as mean daily food intake for group of 6 rats.

2.7.3 Serum glucose and lipid levels

On day 40, blood was collected by retro-orbital puncture in anaesthetised rats and was subjected to centrifugation to obtain serum. The serum levels of glucose, total-cholesterol, HDL-cholesterol and triglycerides were estimated using the biochemical kits (Beacon Diagnostics). The serum VLDL-cholesterol and LDL-cholesterol levels were calculated using Friedwalds formula.

VLDL-cholesterol = Triglycerides/5

LDL-cholesterol = Total cholesterol - (HDL-cholesterol + VLDL-cholesterol).

2.7.4 Organ and fat pad weights

The animals were sacrificed by excess of ether anaesthesia and then different organs like kidney, liver, heart, spleen and fat pads like mesenteric left and right perirenal and uterine fat pads were removed and weighed immediately.

2.8 Statistical analysis

The results are expressed as mean \pm SEM. The data were analysed with student's *t*-test and one way analysis of variance (ANOVA) followed by Dunnett's *t*-test as applicable and P values less than 0.05 were considered to be statistically significant.

3. Results

3.1 Effect on body weight

CD and AD fed rats showed significant ($P < 0.001$ and $P < 0.01$) increase in body weight between day 1 and day 40 as compared to normal control group. Administration of ZJE at a dose of 500mg/kg elicited a significant ($P < 0.01$) reduction in body weight difference between day 1 and day 40 in CD and AD fed rats as compared to their respective control group. The average body weight difference between day 1 and day 40 observed in seven group of animals is 20.8 ± 3.51 , 74.8 ± 2.94 , 32.8 ± 2.65 , 30.5 ± 3.64 , 43.16 ± 1.01 , 19.5 ± 1.38 and 19.33 ± 2.07 respectively. (Refer Table 1 Fig 1 and 2)

3.2 Effect on food intake

CD and AD fed rats showed significant increase ($P < 0.01$ and $P < 0.001$) in daily food intake when compared to normal control rats fed with normal diet. Treatment with ZJE caused significantly decreased the daily intake of food in CD ($P < 0.01$) and AD ($P < 0.001$) fed rats as compared with their respective control group. (Refer Table 2)

Table 1. Effect of hydroalcoholic extract of *Ziziphus jujuba* leaves on body weight.

| Group | Treatment | Body weight difference (g) between day 1 and day 40 |
|-------|---------------------------|---|
| I | Control | 20.84 ± 3.51 |
| II | Cafeteria diet control | 74.82 ± 2.94** |
| III | CD + ZJE (500mg/kg) | 32.82 ± 2.65 ^a |
| IV | CD + Sibutramine (5mg/kg) | 30.54 ± 3.64 ^b |
| V | Atherogenic diet control | 43.16 ± 1.01* |
| VI | AD + ZJE (500mg/kg) | 19.50 ± 1.38 ^a |
| VII | AD + Sibutramine (5mg/kg) | 19.33 ± 2.07 ^a |

Values are Mean ± S.E.M. for group of 6 rats per day.

*P<0.01, **P<0.001 as compared to normal control group. (Students' *t*-test)

^aP<0.01, ^bP<0.001 as compared to respective control group. (ANOVA followed by Dunnett's *t*-test)

3.3 Effect on serum biochemical parameters

Feeding of CD and AD caused significant (P<0.001) increase in serum glucose, total-cholesterol, LDL-cholesterol, VLDL-cholesterol and triglyceride levels when compared to normal diet fed rats. Treatment of ZJE resulted in significant reduction in serum levels of glucose, total-cholesterol, LDL-cholesterol and VLDL-cholesterol and significant increase in serum HDL-cholesterol levels in CD and AD fed rats when compared with their respective control group of rats. (Refer Table 3)

Table 2. Effect of hydroalcoholic extract of *Ziziphus jujuba* leaves on daily food intake.

| Group | Treatment | Average food intake (g) per day. |
|-------|---------------------------|----------------------------------|
| I | Control | 137.87 |
| II | Cafeteria diet control | 158.76* |
| III | CD + ZJE (500mg/kg) | 139.12 ^a |
| IV | CD + Sibutramine (5mg/kg) | 140.65 ^a |
| V | Atherogenic diet control | 165.62** |
| VI | AD + ZJE (500mg/kg) | 131.12 ^b |
| VII | AD + Sibutramine (5mg/kg) | 135.0 ^b |

Values are mean food intake for group of 6 rats per day.

*P<0.01, **P<0.001 as compared to normal control group. (Students' *t*-test)

^aP<0.01, ^bP<0.001 as compared to respective control group. (ANOVA followed by Dunnett's *t*-test)

3.4 Effect on organ and fat pad weights

There was significant (p<0.001) increase in weight of internal organs like heart, liver, spleen, kidneys and fat pads like mesenteric fat pad, perirenal fat pad and uterine fat pad in CD and AD fed rats when compared to normal control group. Administration of ZJE extract produced significant reduction in weight of heart, liver, spleen, kidney, mesenteric fat pad, perirenal fat pad and uterine fat pad in CD and AD fed rats when compared with their respective control group of animals. (Refer Table 4)

Table 3: Effect of hydroalcoholic extract of *Ziziphus jujuba* leaves on serum biochemical parameters.

| Group | Treatment | Glucose (mgs %) | Total- Cholesterol (mgs %) | HDL (mgs %) | LDL (mgs %) | VLDL (mgs %) | TGs (mgs %) |
|-------|---------------------------------|----------------------|----------------------------------|----------------------|----------------------|----------------------|----------------------|
| I | Normal Control | 58.16 ± | 74.3 ± | 12.3 ± | 48.03 ± | 16.96 ± | 84.83 ± |
| | | 1.13 | 1.68 | 0.84 | 1.33 | 0.39 | 1.95 |
| II | CD Control | 71.16* ± | 89.6* ± | 14.0 ± | 52.2 ± | 24.8* ± | 124.0* ± |
| | | 1.46 | 2.04 | 0.96 | 1.76 | 0.51 | 2.58 |
| III | CD + ZJE (500mg/kg) | 62.48 ^c ± | 76.6 ^d ± | 17.33 ^b ± | 40.3 ^d ± | 19.03 ^d ± | 95.16 ^d ± |
| | | 2.20 | 1.52 | 1.02 | 2.24 | 0.49 | 2.45 |
| IV | CD + Sibutramine (5mg/kg) | 65.76 ^a ± | 77.33 ^d ± | 19.5 ^d ± | 39.7 ^d ± | 18.23 ^d ± | 91.16 ^d ± |
| | | 1.52 | 1.98 | 0.56 | 1.69 | 0.41 | 2.05 |
| V | AD Control | 92.03* ± | 148.66* ± | 14.33 ± | 110.10* ± | 24.23* ± | 121.66* ± |
| | | 2.17 | 1.74 | 1.20 | 1.45 | 0.34 | 2.57 |
| VI | AD + ZJE (500mg/kg) | 74.0 ^d ± | 86.5 ^d ± | 32.5 ^d ± | 34.16 ^d ± | 19.83 ^d ± | 99.16 ^d ± |
| | | 1.77 | 2.60 | 1.11 | 2.22 | 0.35 | 1.77 |
| VII | AD + Sibutramine (5mg/kg) | 76.55 ^d ± | 66.5 ^d ± | 28.0 ^b ± | 21.46 ^d ± | 17.03 ^d ± | 85.16 ^d ± |
| | | 2.23 | 1.76 | 1.03 | 1.27 | 0.16 | 0.83 |

Values are mean ± S.E.M for group of 6 rats.

*P<0.001 as compared to normal control group. (Students' *t*-test)

^aP<0.05, ^bP<0.02, ^cP<0.01 and ^dP<0.001 as compared to respective control group. (ANOVA followed by Dunnett's *t*-test)

Table 4. Effect of hydroalcoholic extract of *Ziziphus jujuba* leaves on organ and fat pad weights (g).

| Group | Treatment | Liver | Heart | Spleen | Kidney | | Mesenteric fat pad | Perirenal fat pad | Uterine fat pad |
|-------|---------------------------------|---------------------|---------------------|---------------------|---------------------|---------------------|-----------------------|----------------------|---------------------|
| | | | | | Left | Right | | | |
| I | Normal Control | 7.15± | 0.77± | 0.76± | 0.68± | 0.71± | 0.44± | 1.75± | 0.93± |
| | | 0.07 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| II | CD Control | 8.76**± | 0.89**± | 0.95**± | 0.78*± | 0.88*± | 1.87**± | 3.15**± | 2.27**± |
| | | 0.04 | 0.02 | 0.03 | 0.02 | 0.05 | 0.02 | 0.13 | 0.04 |
| III | CD+ZJE (500mg/kg) | 7.93 ^b ± | 0.81 ^c ± | 0.84 ^a ± | 0.77± | 0.81 ^b ± | 1.37 ^c ± | 2.36 ^c ± | 1.72 ^c ± |
| | | 0.20 | 0.02 | 0.01 | 0.01 | 0.02 | 0.02 | 0.11 | 0.06 |
| IV | CD+ Sibutramine (5mg/kg) | 7.25 ^b ± | 0.85± | 0.93± | 0.76± | 0.81 ^b ± | 1.30 ^c ± | 2.20 ^c ± | 1.43 ^c ± |
| | | 0.20 | 0.02 | 0.05 | 0.02 | 0.02 | 0.03 | 0.04 | 0.03 |
| V | AD Control | 9.79**± | 1.35**± | 1.85**± | 1.18**± | 1.20**± | 2.74**± | 2.87**± | 2.03**± |
| | | 0.25 | 0.45 | 0.24 | 0.28 | 0.26 | 0.28 | 0.25 | 0.47 |
| VI | AD+ZJE (500mg/kg) | 7.30 ^c ± | 0.72 ^c ± | 0.84 ^c ± | 0.78 ^c ± | 0.80 ^c ± | 1.58 ^c ± | 2.32 ^b ± | 1.62 ^b ± |
| | | 0.25 | 0.32 | 0.32 | 0.26 | 0.09 | 0.25 | 0.56 | 0.26 |
| VII | AD + Sibutramine (5mg/kg) | 7.61 ^c ± | 0.82 ^c ± | 0.99 ^c ± | 0.72 ^c ± | 0.75 ^c ± | 1.60 ^c ± | 1.41 ^c ± | 1.42 ^b ± |
| | | 0.35 | 0.25 | 0.24 | 0.12 | 0.15 | 0.29 | 0.56 | 0.21 |

Values are mean g + S.E.M for group of 6 rats.

*P<0.01, **P<0.001 as compared to normal control group. (Students' *t*-test)

^aP<0.01, ^bP<0.02 and ^cP<0.001 as compared to respective control group. (ANOVA followed by Dunnett's *t*-test)

4. Discussion

In the present study, the anti-obese activity of *Z. jujuba* leaves extract was studied using dietary animal models of obesity that bear close resemblance to human obesity [14]. The results of our study showed that rats fed with a variety of highly palatable, energy rich, high fat foods elicited significant increase in body weight, food intake, serum levels of glucose, total cholesterol, LDL-cholesterol, VLDL-cholesterol and triglycerides along with correspondent increase in liver, heart, kidney, spleen and mesenteric, perirenal and uterine fat pad weights.

Treatment with ZJE has resulted in reduction in body weight in CD and AD fed rats indicating that ZJE possess weight reducing property. Since obesity is associated with hyperphagia, in the present study CD and AD fed rats consumed more food than normal diet fed rats. ZJE was effective in decreasing daily food intake in both CD and AD fed rats, indicating that it possesses hypophagic property. The extract has shown significant reduction in serum levels of glucose, total-cholesterol, LDL-cholesterol, VLDL-cholesterol and triglycerides along with significant increase in serum HDL-cholesterol levels in CD and AD fed rats. These results are in agreement with previously reported hypoglycaemic and hypolipidemic actions of *Z. jujuba* [5] and [6]. Since obesity is associated with increase in serum levels of glucose (due to

insulin resistance), total-cholesterol, LDL-cholesterol and triglycerides, along with low levels of serum HDL-cholesterol, ZJE may be useful in treating obesity [15]. On the basis of effect of ZJE on serum glucose and lipid levels in dietary obese rats, it can be stated that the extract contains some active phytoconstituent(s), which affects glucose and lipid metabolism. The preliminary phytochemical characterization of the alcoholic extract has shown the presence of saponins, flavonoids, sterols and tannins. The reduction in serum glucose and lipid levels produced by ZJE may be due to the presence of flavonoids and saponins as they have been previously reported to possess hypoglycaemic and hypolipidemic actions respectively [5] and [16]. Treatment with ZJE also caused significant decrease in weight of different internal organs and fat pads in CD and AD fed rats, suggesting that ZJE reduces adipose tissue formation in rats.

Thus, in conclusion the present study demonstrated that ZJE exerted significant anti-obese activity due to its weight reducing, hypophagic, hypoglycaemic and hypolipidemic effects in rats fed on cafeteria and atherogenic diet. Further, there is a need to identify the exact phytoconstituents responsible for the activity and to formulate a poly herbal anti-obese preparation containing ZJE as a main ingredient along with other novel weight reducing and hypolipidemic herbal drugs.

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