Ind. J. Pharm. Edu. Res., 2023; 57(3s):s718-s723.
https://www.ijper.org

Original Article

Evaluation of Anti-obesity Activity of n-hexane and Methanolic Extracts of *Cajanus cajan* Linn. Seeds Using Enzymatic Methods

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ABSTRACT

Objectives: To evaluate the *in vitro* anti-obesity activity of *Cajanus cajan* Linn. seeds. **Materials and Methods:** The phytochemical screening and *in vitro* studies are performed using pancreatic lipase and α-glucosidase activity using methanolic and n-hexane extract of *Cajanus cajan* Linn. seeds using different doses (100, 200, 300, 400 and 500 µg/mL) at the end the percentage inhibition in pancreatic lipase and α-glucosidase was measured. **Results:** The results demonstrated clearly that the alkaloids, flavonoids, phenolics, saponins, terpenoids, carbohydrates, and glycosides occurring in a variety of plants have been well-cited as the sources of anti-obesity agents. *Cajanus cajan* Linn. seeds extracts possess dose-dependent pancreatic lipase and α-glucosidase inhibitory activities which might be due to the existence of alkaloids, steroids, saponin, and/or carbohydrates constituents along with their free radical scavenging potential. Hence, n-hexane, and methanolic seed extracts of *Cajanus cajan* Linn. show good anti-obesity activity. **Conclusion:** Overall preliminary and *in vitro* screening studies reveal the significant anti-obesity potential of *Cajanus cajan*. Thus, these plants act as an alternative herbal approach for treating anti-obesity effects and its complication.

Keywords: Obesity, Enzymes, *Cajanus cajan*, Pancreatic lipase, Inhibition.

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Received: 11-11-2022;
Revised: 30-03-2023;
Accepted: 26-06-2023.

INTRODUCTION

Obesity is a serious medical condition that is increasing globally. It is estimated that nearly half a billion of the world's population are considered to be obese and overweight.[1] Moreover, it can be defined as the unnecessary fat and adipose tissue accumulation in the body resulting in an increased food intake and downgrades the energy expenditure.[2] It was estimated that obesity and overweight increased by 8.1 to 13.4% in girls from past thirty years.[3]

According to the WHO database, obesity can be classified in two ways viz. chronic and severe disease that occurs in both developed and developing countries affecting both adults and the children.[4] Numerous marketed drugs are obtained in the market for treating obesity. It is not a life-threating condition; and can be prevented through various measures like lifestyle changes or by using medicine. A drug should meet the following criteria to be an ideal anti-obesity drug. The criteria include a sustained reduction in body weight and obesity- related complications, secondly, the drugs possess significant benefit-risk ratio.[5,6] Various drugs like Amphetamine, Sibutramine, and Rimonabant had already withdrawn from the market due to the higher risk of psychiatric diseases, and some non-fatal myocardial infarction or strokes. Orlistat is a single drug currently available in the market for treating obesity. Additionally, it is safer in cardiovascular events and diabetes too.[7] Recently some novel approaches are taken into account for treating obesity. One of them is, by inhibiting enzyme pancreatic lipase (which helps in the conversion of dietary triglycerides into free fatty acid) i.e., a major source of excess calorie[8] production. Pancreatic lipase is secreted from pancreatic acinar cells, which play a major role in digestion of dietary triglycerides in the small intestine. The partial hydrolysis of dietary triacylglycerol into free fatty acids and diacylglycerols takes place mainly due to gastric and lingual lipase enzymes. Moreover, due to partial digestion (in stomach), they form larger fat molecules that undergo emulsification with bile salts to form small droplets of fat.[9] In Addition to this, α-glucosidase enzyme inhibits the absorption of carbohydrate by competitively blocking the enzyme activity that convert complex polysaccharides into simple monosaccharides.[10] Leptin is another important peptidal hormone. It is a satiety hormone that gives the negative feedback

DOI: 10.5530/ijper.57.3s.81

to the brain mainly in hypothalamus resulting in controlled energy expenditure and hungerness as depicted in Figure 1.[11]

Based on the literature survey, the plant *Cajanus cajan* Linn. (Fabaceae) commonly known as pigeon pea in english and arhar in hindi was selected for the study as represented in Figure 2. To date, no scientific work is reported experimentally proving the role of this plant part in obesity. Therefore, this current research was designed to perform the preliminary phytochemical screening and anti-obesity study of *Cajanus cajan* Linn. seeds to justify its traditional claim. The criteria for obesity estimation are represented in Table 1.

MATERIALS AND METHODS

Chemicals

Pancreatic lipase, alpha-glucosidase and *p*-nitro phenyl butyrate were purchased from sigma aldrich. Orlistat was taken as a gift sample from Biocon Pvt. Ltd., Bangalore. 2,2-diphenyl-2-picrylhydrazyl (DPPH), Hydrogen peroxide 30% (molychem), Acarbose, Acetic acid, Fehling A, Fehling B (Himedia), Mayer's reagent, Magnesium tunning (SD Fine Chemicals Ltd.) Potassium chloride, Sodium hydroxide, Phosphate buffer, n- Hexane, Glacial acetic acid, Ethanol (Rankem India Ltd.,) and other chemicals which used were of HPLC grade.

Plant Material

Cajanus cajan Linn. Fabaceae (Leguminosae) family is a perennial legume with subfamily Papilionoideaea and it is widely distributed in many developing countries in the semi tropics and subtropics which is an erect shrub about 1.5 to 3 m height.[13] The seeds were collected from the market of Uttarkhasi (Uttarakhand), between the month of October-November. The collected seeds were dried and crumbled. Methanolic and n-hexane extracts were prepared by soxhlet extraction. By using a rotary evaporator, the dried extract was taken and stored in deep freeze for future studies.

Phytochemical screening

Phytochemical screening of *Cajanus cajan* seeds were done using the methanolic and n-hexane extracts to confirm the presence or absence of certain compounds useful for anti-obesity activity.

Enzyme inhibition Procedure

Pancreatic lipase activity[14]

This method was adopted from *sharma et al.* with some modifications. Inhibitory activity of porcine pancreatic lipase was determined by using substrate (p-nitrophenyl butyrate) and potassium phosphate as a buffer with 0.1M concentration (7.2 pH and tween 80 at a concentration of 0.1%). Potassium phosphate buffer (95µL) was taken and it was added in 96 well microplate subsequently by adding (25 µL) porcine pancreatic lipase extracts at the concentration of 1 mg/mL. Then different concentration of 30 µL of extracts (100, 200, 300, 400 and 500 µg/mL), and standard (30 µL) orlistat (100, 200, 300, 400 and 500 µg/mL) was taken and then incubated for 30 min at a temperature of 37°C. The reaction was started by 50 µL p-nitrophenyl butyrate (10 mM) and again incubated for 40 min. After incubation amount of release of (p-nitrophenol) in the reaction was measured (415nm) using iMark Microplate Reader. 100% of enzyme activity was showed by control sample which didn't contain any kind of plant extract. The reading was taken in triplicate manner and up written in ±SEM.

The % inhibition was measured by following formula,

$$\% \text{ inhibition} = Ac - [As - Ao]/ Ac \times 100$$

(Ac represent absorbance of control and as represent absorbance of sample with enzyme).

α-glucosidase inhibitory activity[14]

The solution was prepared by using 100mM of phosphate buffer saline at 6.9 pH. The phosphate saline buffer (95 µL) was added to wells microplate 96 and then 25 µL of α- glucosidase (0.5 U/mL) was added. 30 µL of extract and fractions (100, 200, 300, 400 and 500 µg/ml) or Acarbose (100, 200, 300, 400 and 500 µg/mL) was added to the mixture and incubated for 20 min at 37°C temperature. Then p-nitrophenyl-α-D-glucopyranoside at 50 µL concentration (5mM) was added and incubated for 45 min at a temperature of 37°C. Using iMark Microplate reader the absorbance was measured at 415nm (Enzyme with substrate was positive control). 100% of enzyme activity was showed by control sample which didn't contain any kind of plant extract.

The % inhibition was measured by following formula,

$$\% \text{ inhibition} = Ac - [As - Ao]/ Ac \times 100$$

(Ac represent absorbance of control and as represent absorbance of sample with enzyme).

Table 1: Elucidation of BMI result (kg/m²) for adults by WHO.[12]

Weight	BMI(Kg/m²)
Underweight	18.0-18.5
Heavily Underweight	Not more than 16
Adequate Underweight	16.0-17.0
Mild Underweight	17.0-18.5
Healthy person	18.5-25.0
Overweight	More than 25
Severe obesity	More than 30
Category I	30.0-34.98
Category II	35.0- 39.99
Category III	More than 40

Statistical analysis

All the analysis were carried out in triplicate and the result were expressed in mean±standard error of mean (S.E.M) by using Graph Pad Prism 5.0 software. The comparison between orlistat and extracts (n-hexane and methanolic) at various concentration was analysed.

RESULTS AND DISCUSSION

Phytochemical screening

Preliminary phytochemical screening of *Cajanus cajan* L. leaves n-Hexane and Methanolic extract (HECC and MECC) showed the presence of carbohydrate, saponin, flavonoids, steroids, glycosides, alkaloids as depicted in Table 2.

Pancreatic lipase inhibition assay

The pancreatic lipase activity of HECC and MECC was studied in the concentration range of 100 to 500 µg/mL. At lower concentration i.e., 100 µg/mL the HECC and MECC showed (16.28 and 29.28%) percentage inhibition and at higher concentration i.e., 500 µg/mL extracts gave (32.72 and 56.10) percentage inhibition respectively as shown in Table 3. Both the extracts showed concentration dependent inhibition but MECC has more potential to inhibit pancreatic lipase as compared to HECC as depicted in Figure 3. Orlistat showed 81.74 percentage inhibition at 500 µg/mL. The IC_{50} value of MECC and HECC extract was (424.2812 and 905.5121µg/mL) and orlistat have 146.8623 µg/mL.

α-glucosidase inhibition activity

In this assay, HECC and MECC shows inhibition against α-glucosidase. The n-hexane and methanolic extract of *Cajanus cajan* Linn. was studied in the concentration range of 100 to 500 µg/mL for inhibiting α-glucosidase enzyme (Table 4). HECC at 100, 200, 300, 400 and 500 µg/mL concentrations showed 11.23, 17.57, 22.78, 26.13, 29.96 and MECC showed 25.42, 32.11, 38.53, 43.12 and 52.19 percentage inhibition respectively. By increasing concentration increase in percentage inhibition was observed as depicted in Figure 4. The maximum enzyme inhibition was showed by the standard drug i.e., acarbose from 52.34 to 79.23%. The IC_{50} value are depicted in Table 4.

DISCUSSION

Obesity is a condition in which there is excess of fat accumulation takes place within the body, and it is becoming one of the serious health issues in the world, particularly in the industrialized countries. With this growing rate it acts as an epidemic, that, if not controlled or the serious attention not given, it will produce a severe influence on the patient health.[15] Orlistat, Phentermine and Sibutramine are commercially available synthetic drugs

Table 2: Preliminary phytochemical screening *Cajanus cajan* Linn. seeds extract.

Chemical constituent	Chemical test	n-Hexane	Methanolic
Carbohydrate	Molisch test	-	+
	Fehling test	-	+
	Reducing sugar	-	+
	Benedict test	+	+
Monosaccharaides	Barfoed test		-
Saponin	Foam test	-	+
Flavonoids	Lead acetate test	-	+
	Shinoda test	-	+
Protein	Millon test	+	+
	Biuret test	-	-
Steroids	Salkowaski test	+	-
	Libermann Burchard test	-	-
Glycosides	Keller Kilaani test	+	-
	Legal test	-	-
Tannins	Ferric chloride Test	+	+
	Lead aetate test	-	+
Antraquinone glycosides	Borntrager's test	-	-
Alkaloids	Mayer's test	+	+
	Wagner test	+	-
	Dragendroff's test	-	+

"+" shows the presence of phytoconstituents and "-" shows the absence of phytoconstituents.

Table 3: Effect of various concentration of plant extract of n-hexane and methanolic extract on pancreatic lipase.

Sample	Conc. of Sample (µg/mL)	Percentage Inhibition (%)	IC_{50} value (µg/mL)
HECC	100	16.28±0.0011	905.51
	200	21.32± 0.017	
	300	26.11± 0.0014	
	400	29.44± 0.0012	
	500	32.72± 0.0015	
MECC	100	29.28± 0.0017	424.28
	200	36.23± 0.0020	
	300	42.34± 0.0011	
	400	47.21± 0.0014	
	500	56.10± 0.0011	
Orlistat (Standard)	100	45.38± 0.0013	146.86
	200	57.19± 0.0012	
	300	60.45± 0.0014	
	400	73.05± 0.0022	
	500	81.74± 0.0011	

Table 4: Effect of various concentration of plant extract of n-hexane and methanolic extract on α-glucosidase inhibition activity.

Sample	Conc. of Sample (µg/mL)	Percentage Inhibition (%)	IC_{50} value (µg/mL)
HECC	100	11.23 ± 0.0017	918.95
	200	17.57 ± 0.0020	
	300	22.78 ± 0.0011	
	400	26.13± 0.0014	
	500	29.96± 0.0012	
MECC	100	25.42± 0.0017	481.28
	200	32.11± 0.0021	
	300	38.53± 0.0011	
	400	43.12± 0.0011	
	500	52.19± 0.0017	
Acarbose (Standard)	100	52.34 ± 0.0088	27.79
	200	63.03 ± 0.0017	
	300	69.71 ± 0.0014	
	400	74.49 ± 0.0011	
	500	79.23 ± 0.0020	

for obesity. However, these drugs are reported to cause various side effects such as gastrointestinal symptoms viz. diarrhoea, flatulence, abdominal pain, oily stools etc.[16] Therefore, search for safe and effective medication from the natural sources is the need of hour.

The pancreatic lipase shown a better result in terms of percentage inhibition as compared to α-glucosidase. It has been reported that alkaloids, flavonoids, phenolics, saponins, terpenoids, carbohydrates, and glycosides occurring in variety of plants have been well-cited as source of anti-obesity agents. *Cajanus cajan* Linn. seeds extracts possess dose dependent pancreatic lipase and α-glucosidase inhibitory activities which in term might be due to the presence of alkaloids, steroids, saponin and/or carbohydrates constituents along with their free radical scavenging potential. Hence, n-hexane and methanolic seeds extracts of *Cajanus cajan* Linn. showed good anti-obesity activity.

However, present study is a preliminary work and needs more effort on extracts for phytochemical analysis to determine the active constituents and further elucidation of anti-obesity activity by *in vivo* methods, which may help in discovering newer and

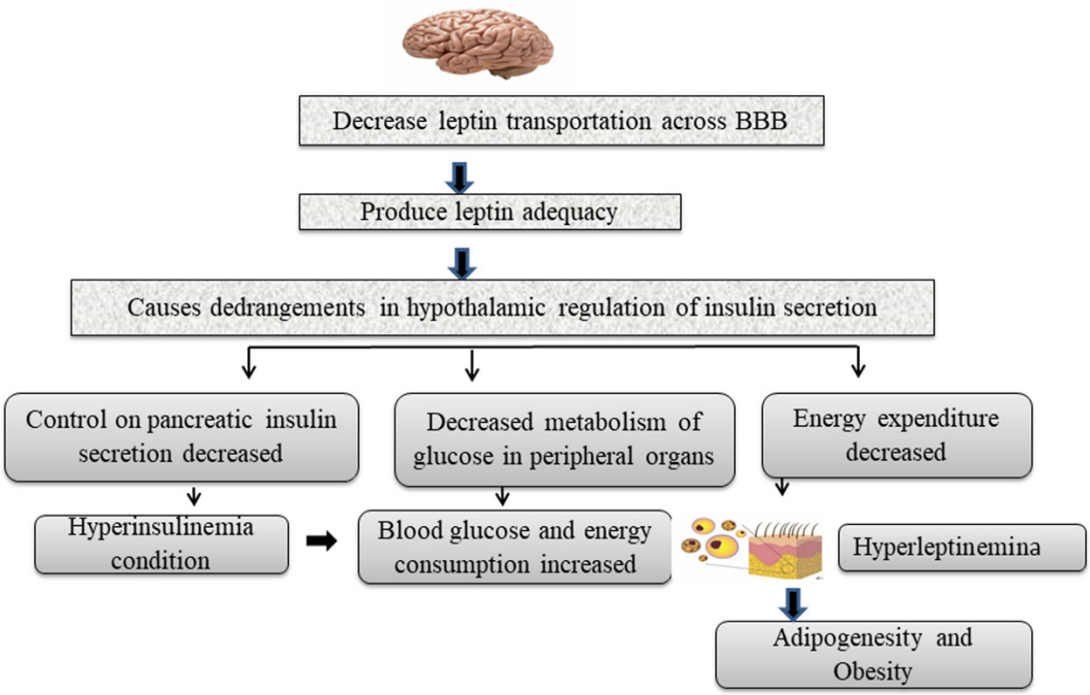

Figure 1: A diagrammatic representation depicting relationship of leptin adequacy on glucose secretion and obesity.

Figure 2: Leaves with pods of *Cajanus cajan* Linn.[13]

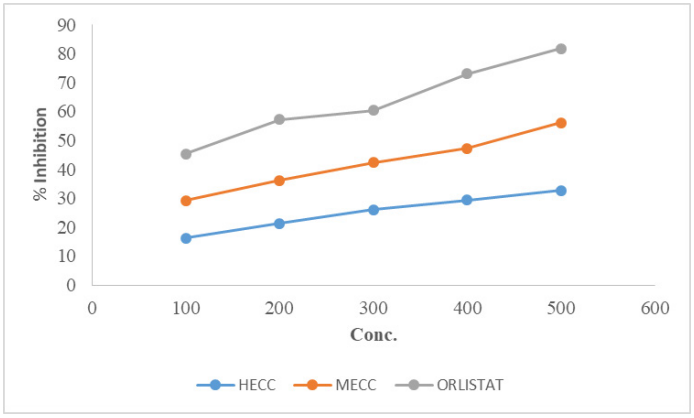

Figure 3: Effect of pancreatic lipase inhibition activity on HECC, MECC and orlistat.

more potent anti-obesity agents. Furthermore, toxicological studies of these extracts can also be carried out to determine the therapeutic use in human beings.

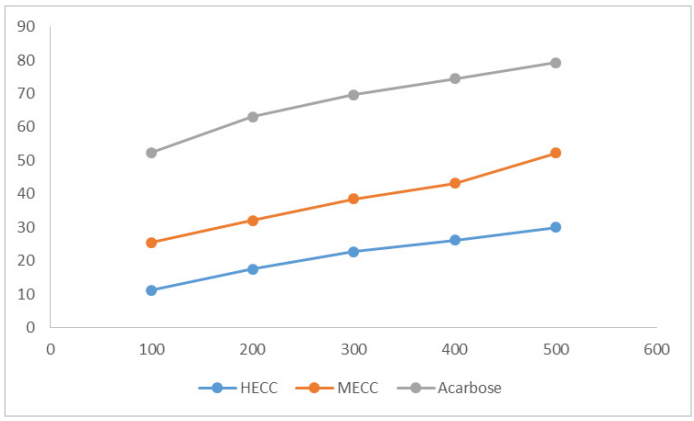

Figure 4: Effect of α-glucosidase inhibition activity on HECC, MECC and acarbose.

CONCLUSION

The potent anti-obesity and the antioxidant activity exerted by the *Cajanus cajan* Linn. seeds seem to be based on the presence of phytoconstituents present in the plant and this could be attributed to the additive effects of the phytoconstituents existing in the n-hexane and the methanolic extract. The study reveals that the methanolic extracts could be added in the list of herbal preparations beneficial in obesity. *Cajanus cajan* Linn. can be examined as a major addition to the therapeutic candidate for the treatment of obesity. Further studies can be undertaken to further demonstrate its mechanism in detail. The current probe has also opened approach for further research chiefly with reference to the

evolution of potent formulation for obesity from *Cajanus cajan* Linn. seeds.

ACKNOWLEDGEMENT

The authors are very thankful to the management of Institute of Pharmaceutical Sciences (Kurukshetra University), Kurukshetra, for providing the infrastructure and support to carry out this Research Work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

BMI: Body Mass Index; **SEM:** Standard Error Mean; **WHO:** World Health Organization; **HECC:** Hexane Extarct *Cajanus cajan*; **MECC:** Methanolic Extract *Cajanus cajan*.

SUMMARY

- Obesity is a serious medical condition which is increasing globally, it is estimated that nearly half a billion of the world population are consider to be obese and overweight or it is also defined as the unnecessary fat and adipose tissue accumulation in the body which results in an increase in food intake and downgrades the energy expenditure
- The aim of the current study is to determine the anti-obesity activity of *Cajaus cajan* seeds using n-hexane and methanolic extracts by using two *in vitro* enzymatic activities (pancreatic lipase and alpha glucosidase).
- The results obtained are expressive of considerable obesity effects which is significantly opposed by *Cajanus cajan* seeds in dose dependent manner.
- In this research work it was concluded that the anti-obesity effects of *Cajanus cajan* Linn. seeds have a defensive action against the enzymatic activity (pancreatic lipase and α-glucosidase). The methanolic extract shows a more potent effect on enzyme by inhibiting its activity and methanolic extracts could be added in the list of herbal preparations beneficial in obesity.

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Cite this article: Sharma S, Chaudhary M, Sharma D, Rani A. Evaluation of Anti-obesity Activity of n-hexane and Methanolic Extracts of *Cajanus cajan* Linn. Seeds Using Enzymatic Methods. Indian J of Pharmaceutical Education and Research. 2023;57(3s):s718-s723.