Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 13 [1] December 2023 : 429-434

Online ISSN 2277-1808
Journal's URL: http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Evaluation of *In Vitro* Anti-Obesity activity of Ethanolic Seed Extract of *Cassia tora L.*

M. Hima Saila [*1], Y.Sasi kala[*2],M. Hema Madhuri[*3], S. Navya Sree[*4], R. Geethika[*5] ,D.Ranganayakulu[*6]
[1] Associate professor, Department of Pharmacology, Sri Padmavathi School of Pharmacy, Tiruchanoor, Tirupati, Andhra Pradesh, India, 517503.
[2,3,4,5] B. Pharmacy, Department of Pharmacology, Sri Padmavathi School of Pharmacy, Tiruchanoor, Tirupati, Andhra Pradesh, India 517503.
[*1] **Corresponding Author's** E-mail: himasaila.spsp@gmail.com

ABSTRACT
The present study evaluates the in vitro anti-obesity activity of ethanolic seed extract of Cassia tora L. The ethanolic extract (EE) of Cassia tora L. was obtained sequentially then it was used to estimate porcine pancreatic lipase enzyme [PPA] and pancreatic lipase [PL] inhibitory activities because Inhibition of these enzymes could be beneficial in weight reduction. the inhibitory activity of the ethanolic seed extract of Cassia tora L. on pancreatic lipase and alpha-amylase was found to be dose-dependent. For PPA inhibition, IC_{50} (µg/mL) of the extract was found to be115.83, whereas acarbose shows 44.971, while for PPL inhibition, IC_{50} (µg/mL) of the extracts was 110.51, and that of Orlistat 59.59. The Attempts to determine the therapeutic effects and identification of bio-active principles from herbal prescriptions have become the prime focus in the validation of their folkloric usage and drug discovery programs. Therefore, the present study aimed to determine the anti-obesity effects of ethanolic extract of Cassia tora L. seeds.
KEY WORDS: Obesity, porcine pancreatic amylase enzyme [PPA], pancreatic lipase enzyme [PL].

Received 15.07.2023 Revised 21.11.2023 Accepted 15.12.2023

INTRODUCTION

One of the non-communicable lifestyle illnesses known as obesity is defined by excess adipose tissue mass with body mass index (BMI) greater than 25 kg/m^2 [1]. It is possible to think of it as a problem connected to a number of other lifestyle conditions, such as cancer, diabetes, dyslipidemia, hypertension, cardiovascular diseases, and musculoskeletal ailments. A change in lifestyle combined with regular exercise is less beneficial for long-term weight loss for treating obesity.

The best method for assessing a drug's ability to prevent obesity is to target the inhibition of one or more enzymes involved in lipid and carbohydrate metabolism. In the small intestine, the pancreatic lipase enzyme hydrolyzes dietary lipids into free fatty acids for absorption [2]. On the other hand, absorption of carbohydrates in small intestine involves their hydrolysis into simple sugars by amylase enzyme. Inhibition of these enzymes could be beneficial in weight reduction treatments.

These plant extracts mainly contain polyphenols, alkaloids, and terpenoids, which could play a significant role in anti-obesity through various signalling pathways and gut microbiota [25].

The inhibition of PL, the most important enzyme in the digestion of dietary triglycerides, is one of the possible approaches to retard the uptake of fat, and consequently, reduce weight and obesity [23]. Polyphenols, including flavonoids and phenolic acids, from natural origin are regarded as a major class of the PL inhibitors [24]. However, flavonoids, especially the catechin-types, are presumed to possess more PL inhibitory and anti-obesity effect. The presence of galloyl moiety in the structural backbone of the polyphenolics plays an important role in their ability to inhibit PL.

The *Cassia tora* Linn. tree has a straight, wooden trunk that is abundantly branched, although hairy stem terminal areas are uncommon. It has around 10-cm-long pinnate leaves. About 30 to 50 rhombohedral, brown, *Cassia tora* seeds make up one plant. The shrub produces fruits in the winter and blossoms throughout the wet season. *Cassia tora* is well known medicinal plant commonly found in India and other tropical countries It is mainly found in the states of Uttar Pradesh and Madhya Pradesh, in India. In Himachal Pradesh it mostly grows as a weed in waste disposal sites, roadsides, field border etc [3]. It occurs frequently in hot, wet and tropical climates [4,5]. The leaves and seeds can be used to treat bronchitis,

cough, dyspepsia, leprosy, ringworm, colic, constipation, and dyspepsia. Pods are used to cure eye conditions as well as diarrhoea. Root is well recognised for its bitter, tonic, stomachic, and antidote properties [6,7].

Research reports available on *Cassia tora* showed presence of alkaloids, saponins, flavonoids, phenolics, tannins, carbohydrates, carboxylic acids, resins, quinine. A number of in vivo and/in vitro studies have explained a wide spectrum of pharmacological properties of crude extracts of *Cassia tora* seeds, anti-oxidant activity [8], antiproliferative [12], antigenotoxic [13], hepatoprotective [10], anti-diabetic [9], hypolipidemic [15], anti nocieptive [14], anti -mutagenic [16,17], anti-fertility activity [11], anti-helminthic [18].

Therefore, the present study was designed to investigate *in vitro* anti-obesity action of different fractions of ethanolic extracts of *Cassia tora* by using lipase inhibitory and amylase inhibitory assays by reported methods.

MATERIAL AND METHODS

Collection of plant material

The seeds of Chakramarda were collected from local market in andra pradesh and it was authenticated by Dr.K. Madhava chetty. cleaned, dried in the shade. After drying, seeds were ground into coarse powder using blender and powder was transferred into airtight containers with proper labelling for future use.

Authentication of plant material

The authentication of seed of *Cassia tora* was done by Dr.K. Madhava chetty [plant taxonomist], he is an assistant officer in department of botany in sri venketeswara university (Tirupati), and giving voucher number 0379 to the *Cassia tora* plant.

Preliminary phytochemical screening of ethanolic extract *Cassia tora* l. Seeds

Solid residue of the alcoholic extract of *Cassia tora* was then subjected to phytochemical screening to test for presence of metabolites such as alkaloids flavonoids, phenol, tannins, saponins, sugar, glycosides, steroids, Carbohydrates, glycosides, carboxylic acid, Resin and anthraquinone, which were qualitatively analyzed [26].

Procedure for alcoholic extract of seeds of *cassia tora*

A thimble composed of thick filter paper and 50 grams of coarsely ground Chakramarda seeds (*Cassia tora* Linn) were inserted into the Soxhlet extractor's main chamber. A Soxhlet extractor was set up on a distillation flask with 500 ml of ethanol as the extraction solvent. Afterwards, a condenser was added to the Soxhlet. The solvent was reflux-heated. A distillation arm was used to transport the solvent vapour, which then poured into the chamber holding the sample thimble. Any solvent vapour was guaranteed to cool and trickle back down into the chamber containing the solid substance thanks to the condenser. Warm solvent gently filled the chamber holding the sample. A syphon side arm automatically empties the Soxhlet chamber when it is almost full, allowing the solvent to flow back down to the distillation flask. Four more repetition of this cycle were permitted. The ethanol was removed after extraction, and it was then dried over a water bath. 5.78 grams of ethanolic seed extract of *Cassia tora L*. was obtained. It had a strong nature. After that, the extracts were stored in sterile vials in a refrigerator until use [22].

Determination of alpha-amylase inhibition assay

Porcine pancreatic alpha-amylase inhibitory activity of the ethanolic seed extract of *Cassia tora* L. was determined by using assay method. The plant solutions were prepared in DMSO and range from 25 to 200 microgram per ml. The enzyme solution was made by dissolving alpha-amylase (1 mg) in phosphate buffer (100 mL, 20 mM, pH 6.9). A mixture of an extract solution(0.5mL) and the enzyme solution (0.5 mL), then this mixture was incubated at 25 °C for 30 minutes. Following that, add1 mL of a starch solution (0.5% w/v in distilled water) to the above mixture, again the mixture was incubated for 3 min at 25 °C, after that add 1 mL of DNS (3,5-dinitrosalicylic acid) to the mixture,then it was heated in a water bath at 85 °C for 15 minutes. 9 mL of distilled water was added to the mixture then immediately measure the absorbance at 540 nm. The percentage inhibition was estimated according to the given below Equation[21].

$$\% \text{ inhibitory activity} = Ac - As / As \times 100$$

Ac=Absorbance of control at 540 nm

As=Absorbance of sample at 540 nm

Blank= DNS solution was added before the addition of starch solution.

Acarbose was used as a positive control.

Pancreatc lipase inhibition assay

Lipase inhibitory activity of ethanolic seed extracts of *Cassia tora* L. was determined by using a method. The rate of release of oleic acid from triolein was determined for measuring lipase inhibitory action. A suspension containing 1% (v/v) of triolein, and 1% (v/v) tween 40 in 0.1M phosphate buffer (pH 8) was prepared and emulsified. Porcine pancreatic lipase (0.5 gm) was dissolved in 15 mL 0.1 M phosphate buffer

(pH 8). 800 µL of the triolein emulsion was added to 200 µL of porcine pancreatic lipase and to those different concentrations of fractions of ethanolic seed extract of *Cassia tora* L. (25,50, 100, 150,200 and 250 µg/mL) were added. Orlistat, a potent pancreatic lipase inhibitor was taken as reference standard drug. Immediately after mixing the contents the absorbance was measured at 450 nm and designated as T1. The test tubes were incubated at 37°C for 30 minutes, and at the end of the incubation, the absorbance at 450 nm was recorded and designated as T2 [21].
The variation in absorbance = [A450 (T1) - A450 (T2)] was calculated for both control and the treatment. the % inhibition was calculated using the formula [19,20].

% inhibitory activity=Ac-As/As×100

Ac=Absorbance of control at 450 nm
As=Absorbance of sample at 450 nm
Orlistat was used as a positive control.

RESULTS

Preliminary phytochemical screening of ethanolic extract *Cassia tora l*. Seeds:

Table -1 [26]: **Preliminary phytochemical screening of ethanolic extract *Cassia tora l*. Seeds:**

S.no	Phytochemicals	Ethanol extract of *Cassia tora L s*eeds
1	Alkaloids	+ve
2	Flavonoids	+ve
3	Phenols	+ve
4	glycosides	-ve
5	carbohydrates	+ve
6	Phyto steroids	+ve
7	Tannins	+ve
8	saponins	+ve
9	Resins	+ve
10	quinine	+ve
11	Carboxylic acids	+ve
12	Coumarins	+ve

Pancreatic amylase inhibitory activity

The percentage inhibition of α-amylase activity by the plant extract was estimated with Acarbose as the positive control. Ethanolic seed extract of *Cassia tora L*. exhibited 21.92% inhibition of alpha-amylase activity at 25µg/mL concentration and 75.96 % inhibition at 200µg/mL concentration. The IC_{50} value was found to be 115.83µg/mL. As the positive control, Acarbose exhibited 41.15 % inhibition of α-amylase activity at 25µg/mL and 86.73% inhibition at 200µg/mL concentration. IC_{50} value of Acarbose was found to be 44.971µg/mL and was shown in Table 2 and fig 1.

Table 2: % inhibition of alpha-amylase enzyme activity at various concentration.

Sample	Conc (µg/ml)	% enzyme inhibition	IC 50 (µg/ml)
Acarbose	25	41.15	
	50	54.23	
	100	66.15	
	150	75.19	
	200	86.73	44.971
Cassia tora L. seeds	25	21.92	115.83
	50	37.3	
	100	42.88	
	150	56.73	
	200	75.96	

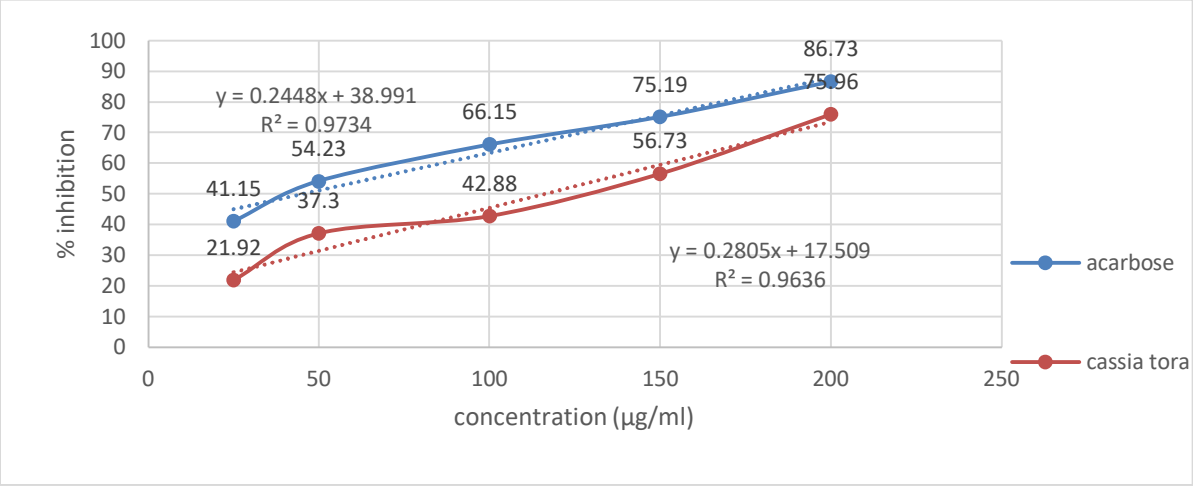

Fig 1. Percentage inhibition of α-amylase against different concentrations of Acarbose and *Cassia tora **L.*** the IC_{50} value of the Acarbose and the extract were 44.971µg/mL and 115.83 µg/mL.

Pancreatic lipase inhibitory activity:

The percentage inhibition of pancreatic lipase activity of the plant extract was estimated with orlistat as the standard drug. ***Cassia tora L.*** exhibited 38.08% inhibition of pancreatic lipase activity at 25µg/mL concentration and 70.85 % inhibition at 200µg/mL concentration. The IC_{50} value of *Cassia tora **L.*** was found to be 110.51µg/mL. orlistat, the positive control, exhibited 41.37 % inhibition of α-amylase activity at 25µg/mL and 84.7% inhibition at 200µg/mL concentration. IC_{50} value of Acarbose was found to be 59.59µg/mL. and was shown in table 3 and fig 2.

Table 3: % inhibition of pancreatic lipase enzyme activity at various concentrations.

Sample	Conc (µg/ml)	% enzyme inhibition	IC 50 (µg/ml)
Cassia tora L. seeds	25	38.08	110.51
	50	42.07	
	100	45.57	
	150	54.23	
	200	66.15	
	250	70.85	
orlistat	25	41.37	59.59
	50	51.37	
	100	55.29	
	150	69.4	
	200	73.92	
	250	84.7	

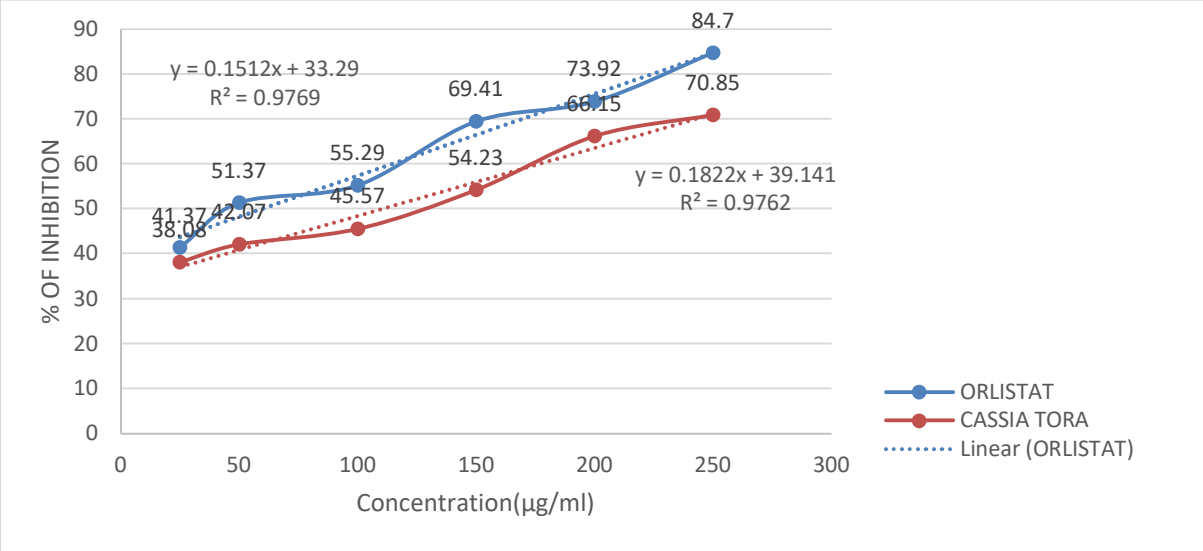

Fig 2. Percentage Inhibition of pancreatic lipase against different concentrations of orlistat and ***Cassia tora L.*** The IC_{50} value of the ***orlistat*** and the ***Cassia tora L*** were 59.59 µg/mL and 110.51 µg/mL, respectively.

DISCUSSION

Obesity is a chronic metabolic disorder characterized by increased adipose tissue mass due to positive energy balance. The main reason for obesity is energy absorption higher than consumption, especially related to the excessive absorption of fat and carbohydrates. Therefore, lipase and α- amylase inhibitory activities were selected as functional indicators to reduce energy absorption (*in vitro*).

Percentage inhibition of alpha-amylase enzyme was estimated by using the standard method [20]. The study was carried out at different concentrations (25, 50, 100,150,200µg/mL). Ethanolic seed extracts of *Cassia tora* L. seeds showed 75.96% of inhibition at 200 µg/ml where as the standard drug(acarbose) showed 84.7 % of amylase inhibition at 200µg/mL. IC50 (µg/mL) value of ethanolic seed extract of *Cassia tora* L. was found to be115.83 while that of acarbose 44.97.

Estimation of percentage inhibition of lipase enzyme by using standard method [21]. The study was carried out at different concentrations (25,50,100,150,200,250µg/mL), ethanolic seed extracts of *Cassia tora* L. was shown 75.96% of inhibition at 250 µg/ml, whereas the standard drug (orlistat) showed 84.7% of inhibition at 250 µg/ml. IC_{50} (µg/mL) value of ethanolic seed extract *Cassia tora* L. was found to be 110.51 while that of orlistat was 59.59.

The inhibitory activity of the ethanolic seed extract of *Cassia tora* L on pancreatic lipase and alpha-amylase was found to be dose-dependent. The anti-obesity activity of ethanolic seed extract *Cassia tora* L. was attributed to the presence of polyphenols, alkaloids, and terpenoids.

CONCLUSION

The present study on *Cassia tora L.* seeds indicated that ethanolic extract had signified potential in inhibiting lipid and carbohydrate metabolizing enzymes and the inhibitory activity of the ethanolic seed extract of *Cassia tora L.* on pancreatic lipase and alpha-amylase was found to be dose-dependent. The anti-obesity activity of ethanolic seed extract *Cassia tora* L. was attributed due to the presence of polyphenols, alkaloids, and terpenoids, further pharmacological studies were needed to find out the exact phytocompounds responsible for the anti-obesity activity

REFERENCES

1. Hu FB, Li TY, Colditz GA, Willett WC, Manson JE. (2003). Television watching and other sedentary behaviors in relation to risk of obesity and type 2 diabetes mellitus in women. J of American Med Assoc. 289:1785-179
2. Verger R. (1997). Interfacial activation of lipases: facts and antifacts. Trends in Biosciences.; 15:32-37.
3. Jain SK, (1968). Medicinal Plants, 37.
4. Acharya TK, Chatterjee IB, (1975). Isolation of chrysophanic acid-9- anthrone, the major antifungal principle of *Cassia tora*, Lloydia, 38, 218–220.
5. Hatano T, Uebayashi H, Ito H, Shiota S, Tsuchiya T, Yoshida T, (1991). Phenolic constituents of *Cassia tora* seeds and antibacterial effect of some naphtholenes and anthraquinones on methicillin resistant staphylococcus aureus, Chemical and Pharmaceutical Bulletin. 47, 121–127

6. Kapoor LD, (2001). CRC Handbook of Ayurvedic Medicinal Plants, CRC Press, 108-109. 35.
7. K, Rao SS, (1984). Jaundice: Tribal Medicine. Ancient Sci Life, 3, 209-212
8. Chakrabarty K, Chawla H, (1983). Terpenoids and phenolics from *Cassia tora* stem bark, Indian J Chem, 22, 1165-1166.
9. Nam J, Choi H, (2008). Effect of butanol fraction from *Cassia tora* L. seeds on glycemic control and insulin secretion in diabetic rats. Nutr Res Pract, 2, 240-246.
10. Ignacimuthu S, Dhanasekaran M, Agastian P, (2009). Potential hepatoprotective activity of ononitol monohydrate isolated from *Cassia tora* L. on carbon tetra chloride induced hepatotoxicity in wistar rats, Phytomedicine, 16, 891–895.
11. Cho IJ, Lee C, Ha TY, (2007). Hypolipidemic effect of soluble fiber isolated from seeds of *Cassia tora* Linn, in rats fed a high cholesterol diet, J Agric Food chem, 55, 1592-1596.
12. Rejiya CS, Cibin TR, Abraham A, (2009). Leaves of *Cassia tora* as a novel cancer therapeutic-An invitro study, Toxicol Vitro, 23, 1034-1038. 57.
13. Wu CH, Yen GC, (2004). Antigenotoxic properties of Cassia tea (*Cassia tora* L.): Mechanism of action and the influence of roasting process, Life Sci, 76, 85-101.
14. Chidume FC, Kwanashie HO, Adekeye JO, Wambebe C, Gamaniel KS, (2002). Antinociceptive and smooth muscle contracting activities of the Methanolic extract of *Cassia tora* leaf, J Ethnopharmacology, 81, 205-209.
15. Grow-Chin Y, Horn-wen C, Pin-der D, (1998). Extraction and Identification of an antioxidative component from *Cassia tora* Linn, J Agri Food Chem, 1p998, 46, 820-824.
16. Roopshree TS, Raman D, Shobha Rani RH, Narendra C, (2008). Antibacterial activity of antipsoriatic herbs: Cassia tora, Momordica charantia and Calendula officinalis, Int J Appl Res Nat Prod, 1, 20-28.
17. Choi JS, Lee H, Park KY, (1998). In vitro antimutagenic effect of alateranin and rubrofusarin gentiobioside from roasted *Cassia tora*, Natural Product Sciences, 4, 100 -104.
18. Acharya TK, Chatterjee IB, (1975). Isolation of chrysophanic acid –9- anthrone, The major antifungal Principle of Cassia tora, Lloydia, 38, 213.
19. Mark S. Smeltzer, Mark E. Hart and John J. Iandolo. Quantitative Spectrophotometric Assay for Staphylococcal Lipase. Appl Environ Microbiol, 1992; 58(9): 2815-2819.
20. Etoundi C.B., Kuate D., Ngondi J.L. and Oben J. Anti-amylase, anti-lipase and antioxidant effects of aqueous extracts of some Cameroonian spice. J Nat Prod, 2010; 3: 165-17
21. Saeed R., Ahmed D. Bioactive compounds from *Carissa opaca* roots and xanthine oxidase and alpha-amylase inhibitory activities of their methanolic extract and its fractions in different solvents. Pharmacognosy. Res. 2015; 7:295–301.
22. Anoma, G.S. *et al.* (2017) "Alcoholic extraction and Phyto-chemical evaluation of CHAKRAMARDA seeds (*Cassia tora* Linn.)," *International Journal of Research in Ayurveda & Pharmacy*, 8(3), pp. 157–161.
23. Rahim AT, Takahashi Y, Yamaki K. Mode of pancreatic lipase inhibition activity ***in vitro*** by some flavonoids and non-flavonoid polyphenols. Food Res Int. 2015; 75:289–94.
24. Lunagariya NA, Patel NK, Jagtap SC, Bhutani KK. Inhibitors of pancreatic lipase: State of the art and clinical perspectives. EXCLI J. 2014; 13:897
25. Nakai M, Fukui Y, Asami S, Toyoda-Ono Y, Iwashita T, Shibata H, et al. Inhibitory effects of oolong tea polyphenols on pancreatic lipase ***in vitro***. J Agric Food Chem. 2005; 53:4593–8.
26. Han-Ning Wang, Jin-Zhu Xiang, Zhi Qi & Min Du (2022) Plant extracts in prevention of obesity, Critical Reviews in Food Science and Nutrition, 62:8, 2221-2234.

.

CITATION OF THIS ARTICLE

M. Hima Saila, Y.Sasi kala,M. Hema Madhuri, S. Navya Sree, R. Geethika ,D.Ranganayakulu. Evaluation of *In Vitro* Anti-Obesity activity of Ethanolic Seed Extract of *Cassia tora L*. Bull. Env.Pharmacol. Life Sci., Vol 13 [1] December 2023: 429-434