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Anti-Lipolytic activity of *Wrightia tinctoria* seeds: *In vivo* & *In vitro* study

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Abstract
The current study was intended to screen the anti-lipolytic activity of *Wrightia tinctoria* seeds. Dried seeds of *Wrightia tinctoria* were powdered and extracted with methanol followed by screening of anti-lipolytic activity using lipid emulsion loaded Wistar rat model *in vivo*. Further fractionation of methanolic extract was done to prepare n-hexane, chloroform, ethyl acetate, n-butanol and remainnig aqueous fraction. All fractions were studied for *in vitro* pancreatic lipase inhibitory activity. The results revealed that in the rats treated with crude methanolic extract of *Wrightia tinctoria*, plasma triglycerides levels 3 hours after oral administration of lipid emulsion were significantly reduced compared to the untreated group. Among all tested fractions of methanolic extract of *Wrightia tinctoria* seeds, n-hexane fraction showed highest lipase inhibitory action i.e. 73.26±1.84 %. In conclusion to above study it has been seen that *Wrightia tinctoria seeds* were found to have noticable anti lipolytic action and can be effective remedy to prevent fat absoption for weight loss treatment.

Keywords: methanolic extracts, anti-lipolytic, lipid emulsion, pancreatic lipase

1. Introduction
Obesity is one of the non-communicable lifestyle disorders characterized by excess adipose tissue mass with body mass index (BMI) > 25Kg/m^2. It can be considered as a cosmetic problem associated with various other lifestyle disorders like dyslipidemia, diabetes, hypertension cardiovascular diseases, musculoskeletal disorders, cancer etc [1]. Absorption of dietary triglycerides in small intestine involves their hydrolysis into free fatty acids by pancreatic lipase enzyme. Targeting the inhibition of pancreatic lipase enzyme involved in lipid metabolism is the best option for weight loss treatment [2].
Wrightia tinctoria (Apocynaceae), a small deciduous tree is also known as Sweet indrajao, Ivory tree, Easter tree etc. in India, it is recognized by its different local names as Indrajava, Svetkutaja (Sanskrit), Kalakuada (Marathi) and Mitha indrajau (Gujarati) [3]. Different parts of plant like seeds, bark, leaves & roots are enriched with valuable medicinal properties and have a long-standing experience of its use by different parts in India [4]. The plant has antidysenteric, stomachic, astringent carminative, aphrodisiac, diuretic properties and it is used in the treatment of skin diseases, abdominal pain & bilious affections [5]. The seeds are useful as anthelmintic, antidiarrhoeal, antidysenteric, astringent, febrifuge, seminal weakness and as an aphrodisiac [6]. Preliminary phytochemical studies of different parts of *Wrightia tinctoria* indicated presence of lipid, saponin, tannin, alkaloid, phenol, steroid, flavonoid, and some other chemical constituents [7, 8]. In Ayurvediac literature seeds of *Wrightia tinctoria* are mentioned for fat reduction property, but till date there is no scientific evidence is available proving this claim. Therefore, present study was designed to investigate antilipolytic activity of *wrightia tinctoria* seeds using reported *in vivo* & *in vitro* models.

2. Materials & Methods
2.1 Collection & Identification of Plant Material
The dried seeds of *Wrightia tinctoria* were procured from local market of Vadodara, Gujarat, India. Seeds were identified by comparision with description given in standard texts. It was further authentified by botanist Dr. P K Patel, Head of Department, Sheth P.T Arts and Science College, Godhra, Gujarat bearing voucher specimen number PPDC/COG/2015/002 as *Wrightia tinctoria* seeds belong to the family Apocynaceae. Seeds were powdered in grinder, passed through 60# sieve & stored in airtight container at room temperature for further use.

2.2 Extraction & Fractionation
Powdered seeds of *Wrightia tinctoria* (1 kg) were extracted with methanol (2.5 L) by maceration method at room temperature for 7 days, followed by filtration using filter paper. Dried marc was again extracted with methanol (2.5 L) by same method for 7 days. Two percolates were mixed, concentrated and evaporated to dryness to give methanolic extracts of *Wrightia tinctoria* (MEWT) (yield: 18.9 % w/w). The dried extract was suspended in water (250 ml) and subsequently extracted with n-hexane, chloroform, ethyl acetate & *n*- butanol (250 ml each) by liquid- liquid partition in separating funnel. All solvent fractions were evaporated to dryness under reduced pressure and designated as HFWT (n-hexane), CFWT (chloroform), EAFWT (ethyl acetate), BFWT (n-butanol) & remaining (aqueous) AQFWT.

2.3 Animals
Healthy Female Wistar rats were kept in clean polypropylene cages (3 rats /cage) and maintained under controlled room temperature (22 ± 2°C) and humidity (55 ± 5%) with 12 hours light & 12 hours dark cycle. The animals were given free access to food and water. After adaptation

to these conditions for 1-week, healthy animals were used in the present study. Study was performed according to the guidelines prescribed by the Committee for Purpose of Control & Supervision of Experiments on Animals (CPCSEA). The protocol for study was approved by the Animal Ethics Committee of Institute bearing protocol number OGECT/PPDC/IAEC/2018/15/2.

2.4 *In Vivo* Anti Lipolytic Activity [9, 10]

Lipid emulsion when administerd orally results in an increaseed load of triglycerides in blood. The lipid elimination can be measure for lipolytic action. Female Wistar rats weighing 180-200 gm were used for study. Two groups (n=5) of rats were treated daily with two doses of MEWT (200 mg & 400 mg /kg) & one group (n=5) of rats with standard drug orlistat (30 mg/kg) over *a* period of 5 days. On the fifth day, two hours after the last administration of the test compounds, lipid emulsion (composed of 80 mg cholic acid, 2 mg cholesteryl oleate, 6 ml saline, and 6 ml of ground nut oil) was given orally to the all groups animals. Food was withheld during the test. Blood samples were collected from the ophthalmic venous plexus 0, 1, 2, 3, 4 and 5 hours after the oral administration and centrifuged at 2,000 x *g* for 15 min. Triglyceride levels were measured using standard kits.

Treatment protocol

Group 1: Positive Control (0.5% CMC for 5 days & 5ml/kg lipid emulsion orally)

Group 2: MEWT 200 (MEWT 200 mg/kg suspended in 0.5% CMC for 5 days & 5ml/kg

Lipid emulsion orally)

Group 3: MEWT 400 (MEWT 400 mg/kg suspended in 0.5% CMC for 5 days & 5ml/kg Lipid emulsion orally)

Group 4: Standard (Orlistat 30 mg/kg suspended in 0.5% CMC for 5 days & 5ml/kg Lipid emulsion orally)

2.5 *In Vitro* Lipase Inhibitory Activity [11]

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 0.1 M phosphate buffer (pH 8) was prepared and emulsified. Porcine pancreatic lipase (0.5gm) 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 that different concentrations of fractions of MEWT (50, 100, 150, 200 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 T_1. The test tubes were incubated at 37°C for 30 min and at the end of the incubation; the absorbance at 450 nm was recorded and designated as T_2. The variation in absorbance = [A450 (T_1) - A450 (T_2)] was calculated for both control and the treatment. The % inhibition was calculated using the formula:

% inhibition = ([ΔA450 Control - ΔA450 Extract] / ΔA450 Control) × 100

2.6 Data Analysis

All the experimental results were expressed as mean ± standard error mean (SEM). The IC_{50}values of all test samples were calculated from concentration-inhibition curves. Statistical significance of differences between means was calculated by analysis of variance (ANOVA) followed by dunnett's multiple comparisons test. $P < 0.05$ was considered statistically significant.

3. Results & Discussion

3.1 *In Vivo* Anti Lipolytic Activity

In this study fat emulsion was used as a tracer substance for plasma triglyceride levels. To evaluate the antilipolytic effects of methanolic extract of *Wrightia inctorial* seeds *in vivo*, we analyzed plasma triacylglycerides levels after oral administration of lipid emulsions with or without treatment with methanolic extracts of *Wrightia tinctoria* seeds to rats. Table1. Shows the serial changes in plasma triacylglyceride concentration when lipid emulsion with (MEWT 200/400 mg/kg & orlistat 30 mg/kg) or without test samples was administered orally to rats. At 3 and 4 h after administration of lipid emulsion, the plasma triacylglyceride concentrations were significantly ($P<0.05$) lower in group administered orlistat & 400 mg/kg of MEWT than those in the positive control group. There was no significant reduction in plasma triacylglyceride levels at the dose of 200 mg/kg body weight (Fig 1).

Table 1: Effect of MEWT on plasma triglyceride levels at different time intervals

Group	Triglyceride levels (mg/dl)					
	0 hr	1 hr	2hr	3 hr	4 hr	5 hr
Positive control	93.76±11.37	117.33±11.95	156.05±8.97	191.2±5.86	206.7±4.22	170.67±4.86
MEWT 200	90.35±8.87	109.97±13.21	128.66±10.26	144.62±12.35	126.36±12.74	112.62±11.41
MEWT 400	82.86±4.06	98.42±3.69	134.42±4.43	115.96±4.18*	101.99±3.81*	90.87±6.37*
STANDARD	88.11±7.56	101.9±9.87	118.8±9.13	104.12±2.77*	97.41±3.13*	91.16±2.44*

Values are expressed as mean ± SEM (n = 5)

* $p < 0.05$ statistically significant when compared to Positive control group

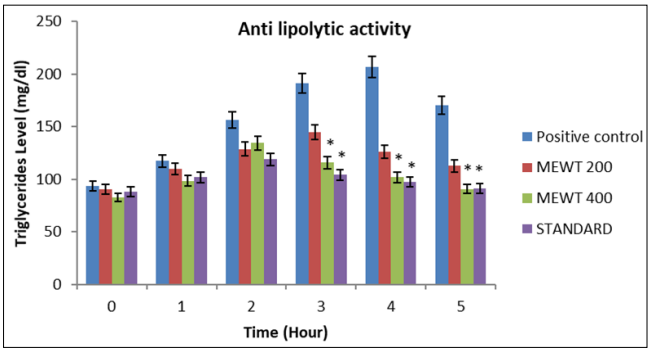

Fig 1: Effect of MEWT on plasma triglyceride levels at different time intervals

3.2 *In Vitro* Lipase Inhibitory Activity

In present study *in vitro* lipase inhibitory effect of different fractions of methanolic extracts of *Wrightia tinctoria* was evaluated using release rate of oleic acid from triolein. Results of % lipase inhibitory activity of different concentrations of all fractions & standard drug orlistat are shown in table 2. The present findings revealed statistically significant ($P<0.05$) lipase inhibitory action of HFWT & standard drug orlistat as compared to other fractions. (Fig.1) IC_{50}value of HFWT was found to be 112.26±2.68 µg/ml which was comparable with standard drug orlistat having IC_{50} Value 42.36±1.43 µg/ml (Fig.2).

Table 2: Inhibitory effects of different concentrations of different fractions of MEWT on pancreatic lipase

Test sample	Concentration (µg/ml)	Inhibition (%) *
HFWT	50	32.75±0.68
	100	49.39±0.97
	150	58.31±0.49
	200	73.26±1.84
CFWT	50	25.56±0.22
	100	37.41±0.56
	150	51.72±1.32
	200	64.11±2.08
EAFWT	50	21.48±0.28
	100	29.11±0.63
	150	34.77±0.59
	200	42.73±2.82
BFWT	50	12.47±1.29
	100	15.26±0.61
	150	22..13±1.06
	200	27.12± 1.26
AQFWT	50	9.32±0.21
	100	11.42±0.76
	150	12.79±0.18
	200	16.74±1.34
ORLISTAT	50	64.22±1.45
	100	79.87±2.25
	150	88.23±1.63
	200	92.54±3.36

* Values are expressed as mean ± SEM (n = 3)

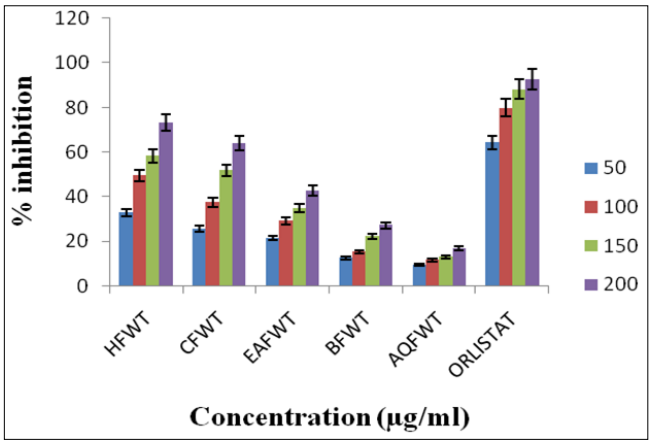

Fig 2: Inhibitory effects of different concentrations of different fractions of MEWT on pancreatic lipase

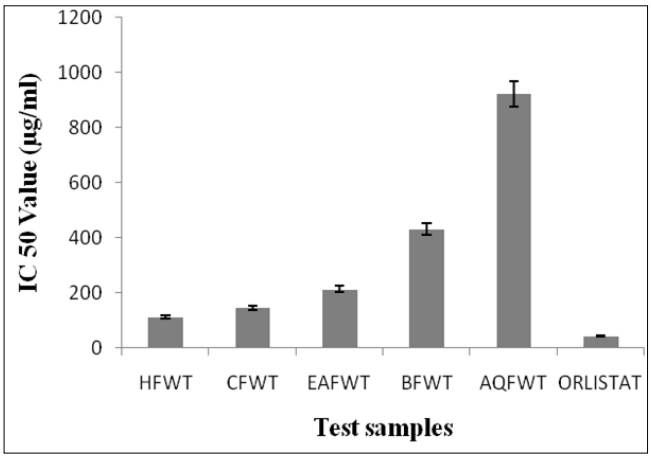

Fig 3: IC50 values of different fractions of MEWT on pancreatic lipase activity

4. Conclusion

These results show that *Wrightia tinctoria* seeds methanolic extracts have inhibitory effect on dietary fat absorption & pancreatic lipase and may be useful in the wight loss treatment & other metabolic disorders. Till date, *Wrightia tinctoria* seeds methanolic extracts have not been reported on lipase and dietary fat absorption activities. Thus, it is worthwhile to further explore these extracts for their potential pharmacological effects in weight loss therapy and attempt should be made to characterize bioactive compounds to be used as safer therapeutic agents in future.

5. Acknowledgement

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6. References

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