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Research Article

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Anti-obesity activity of *Taraxacum officinale* in high fat diet induced obese rats

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ABSTRACT

Obesity has become a worldwide health problem. Orlistat, an inhibitor of pancreatic lipase, is currently approved as an anti-obesity drug. However, gastrointestinal side effects caused by Orlistat may limit its use. The present study investigated the Anti-Obesity effect of Ethanolic extract of Taraxacum officinale (TOE) on rats fed on High Fat Diet (HFD). Experimental obesity was induced by feeding rats with HFD for 10 weeks. HFD treatment caused significant increase in body weight, body mass index, Weight of different fat depots, serum glucose, cholesterol, TG, LDL, VLDL, and decrease in serum HDL in HFD control group which were assessed as an index of obesity. Ethanolic extract of Taraxacum officinale was administered orally at low and High dose (150 and 300mg/kg) as test drug while Orlistat was used as standard drug. On 10th week body weight, lipid parameters, organ weight and fat pad weights were evaluated. ANOVA followed by Turkey's multiple range tests was applied. Over the course of study, it was found that Taraxacum officinale significantly reduced body weight, lipid parameters, organ weight and fat pad weights. Among low and high dose of T.officinale high dose was most effective.

Key words: Orlistat, *Taraxacum Officinale*, Lipid parameters, Obesity, Organ weight, High Fat Diet.

INTRODUCTION

Obesity is a chronic disease with a multifactorial etiology including genetics, environment, metabolism, lifestyle, and behavioral components. Lifestyle factors such as proper nutrition, regular physical activity, and changes in eating behaviors play a major role in combating obesity [1].

It is the accumulation of body fat to the level which might have a negative effect on health. Body Mass Index (BMI) is a widely used diagnostic tool which provides a simple numeric measure of a person's 'fatness'. BMI of 18.5 to 25 may indicate optimal weight a number above 25 may indicate the person is overweight; while a number above 30 suggests the person is obese and over 40, indicates morbidly obese. Indian urban population is experiencing high rates of obesity, as their work often demands less physical exertion. Even rural areas are not immune because of increased mechanization of farming activity leading to reduced physical activity[2].

The prevalence of obesity is increasing worldwide. Numerous diseases are caused or made worse by obesity. These include type 2 diabetes; hypertension; dyslipidemia; ischemic heart disease; stroke; obstructive sleep apnea; asthma; nonalcoholic steatohepatitis; gastroesophageal reflux disease; degenerative joint disease of the back, hips, knees, and feet; infertility and polycystic ovary syndrome; various malignancies; and depression [3].

A compound that selectively limits the intestinal absorption of dietary fat, in excess of that manageable by dietary manipulation alone, could become a useful therapeutic agent for the treatment of obesity. The inhibition of gastrointestinal lipase may be a potential method for decreasing fat absorption. Pancreatic lipase is the key enzyme required for the absorption of dietary triglyceride. Pancreatic lipase may also be indirectly responsible for absorption of cholesterol.

Natural dietary supplements have been considered promising candidates for weight loss and anti-obesity**.** *Taraxacum officinale* is a perennial herbaceous plant of the family *Asteraceae* that has been used for its various medicinal properties [4]. Extracts from this plant have shown hypolipidemic effects and an inhibitory activity of pancreatic lipase, decreasing AUC (area under curve) for the postprandial triglyceride response curve [5].

It is clear that a compound that selectively limits the absorption of ingested fat could be useful in the treatment of obesity and hyperlipidemia, because excessive fat intake is a common etiological factor in the development of both condition [5].

Orlistat is a pharmacological agent promoting weight loss in obese subjects via inhibiting of gastric and pancreatic lipase, an enzyme that is crucial for the digestion of the long chain triglycerides, which at a three daily doses of 120mg reduces fat absorption by 30% and has been proven to be useful in facilitating both weight loss and weight maintenance [6].

However, because Orlistat can result in undesirable side effects, such as fecal incontinence, flatulence, and steatorrhea, its use may be limited. Therefore, it may be worthwhile to search the natural substances that show potent inhibitory activity against pancreatic lipase and have fewer side effects [4].

A comprehensive herbal drug therapeutic regimen offers time tested safe and effective support to conventional therapy in the management of obesity. Combination of adequate dietary management, physical activity and herbal therapy would provide an integrated approach to the management of obesity and hence the plant extract *T. Officinale* has been chosen for the study.

EXPERIMENTAL SECTION

2.1 Plant Extract:

Ethanolic extract of *T.officinale* (Batch No: TOLE/14001) was obtained from Green Chem., Industry, Bangalore as free Sample.

2.2 Chemicals and Reagents:

Triglyceride and Cholesterol Kit (Span Diagnostic, Bangalore, India), Orlistat Capsules 120mg (Torrent Pharmaceuticals LTD, India). All other reagents used in the experiments were of analytical grade and of the high purity.

2.3 Animals:

Male Sprague-Dawley rats were obtained from Central Animal house, Krupanidhi College of Pharmacy, Bangalore, India. The rats were housed under 22±2ºC temperature, 40-60% humidity and 12-12±1 h light-dark cycle. During the course of obesity induction, rats weighing 150-200g were taken for the study and they were broadly divided in to five groups. Group one was normal control (Normal pellet diet was fed) and four groups were fed with HFD (High fat diet) to induce obesity and water *ad libitum*. Experimental protocols were followed as per Institutional Animal Ethical committee guidelines and Animal Ethical committee clearance (CPCSEA No.2014/POCL/02) was obtained for the procurement of animals.

2.4 Experimental design:

The Male Sprague Dawley rats were randomly divided in to five groups (n=6) and fed with normal diet or high fat diet.

1. Normal control (rats will be fed on normal chow diet.)
2. HFD control (rats will be fed on HFD alone.)
3. HFD + Orlistat (200mg/kg Diet) [8]
4. HFD + *T.officinale* leaf extract (150 mg/kg low dose) [9]

5. HFD + *T.officinale* leaf extract (300 mg/kg High dose)

Table No. 1: Description of Diet [7]:

SL.NO.	Composition of High Fat Diet (HFD)	
1.	Fat	
	Lard	230
	Vegetable oil	50
2.	Carbohydrates	
	Dextrin	97
	Corn starch	98
	Sucrose	130
3.	Protein	
	Casein	325
	Vitamin mix	30
	Mineral mix	40
	Total weight (g)	1000
	Energy density (K cals/g)	5.15
4.	% Macronutrients (K cals)	
	Fat	50
	Carbohydrate	25
	Protein	25

Ingredients expressed by weight (g), HFD, high fat diet;

2.5 Measurement of Body weight:
Body weight was measured on every alternative day for 10 weeks.

2.6 Estimation of Plasma Lipid Profiles:
At the end of the experiment, on 10^{th} week blood samples were collected from overnight fasted animals under inhalation of anesthesia by retro-orbital puncture method. Plasma was separated by centrifugation at 2500 rpm for 15 min and was used for further experiments i.e. Total cholesterol, HDL and triglyceride levels were estimated by CHOD-PAP method and GPO-PAP method. LDL levels were calculated by the method of Johnson et al.[10] Blood glucose is estimated by using Glucometer.

2.7 Estimation of Organ weight and Fat pad weight:
On 10^{th} week the animals were sacrificed by cervical dislocation and organs like Liver and kidney weights are taken. Fat pads like Mesenteric and epididymal fat are collected and dried on filter paper and weighed.

2.8 Statistical analysis:
The results will be expressed as mean ± SEM. Comparisons between the treatment groups and control will be performed by analysis of variance (ANOVA) followed by Turkeys' multiple range tests. In all tests the criterion for statistical significance was $p < 0.05$.

RESULTS AND DISCUSSION

3.1 Body weight:
In the present study, the effect of *Taraxacum officinale* was observed on the rats fed with HFD (High fat diet). The study was conducted for about 10 weeks, for first 6 weeks animals were fed only on HFD. After 6^{th} week the treatment was started for next 4 weeks along with HFD. Body weight was measured on every alternative day.

High Fat Diet substantially increased body weights of rats in 6^{th} week, when compared to normal control group. On 10^{th} week, when compared to HFD control, HFD+Orlistat, *T.officinale* low and high dose showed significant activity in decrease in the body weight. *T.officinale* high dose showed similar activity when compared to standard drug Orlistat (Table No. 2).

In the present study, *T.officinale* as well as the standard drug (Orlistat) decreased the body weight when compared to HFD control. This shows that *T.officinale* prevents the absorption of fat from the Intestine, leading to decrease in body weight.

Table No. 2: Effect of *Taraxacum officinale* on Body weight (gm)

	1st Week	6th Week	10th Week
Normal Control	185.00±7.64	251.00±3.26	290.83±1.62
HFD Control	184.17±4.12	310.17±3.50***	380.50±3.74***
HFD+Orlistat	183.17±4.71	303.50±4.19***	329.33±4.36***^{a}
HFD+TOE(low dose)	185.33±3.79	305.17±3.84***	349.17±3.05***^{ay}
HFD+TOE(high dose)	184.50±3.19	304.00±3.28***	310.67±2.75***^{ay}

*All values are Mean ±SEM. ***P <0.001, **P<0.01, *P<0.05 When compared to normal control group, ^{a}P <0.001, ^{b}p<0.01, ^{c}p<0.05 When compared to HFD control, ^{x}P <0.001, ^{y}P <0.01, ^{z}p<0.05 When compared to HFD+Orlistat.*

3.2 Serum Lipid Profiles and Blood Glucose:

Biochemical parameter's such as HDL, Triglycerides and Total cholesterol were measured using biochemical kits. Blood glucose level was measured by using Glucometer. LDL and VLDL were calculated with the formulas respectively.

Rats fed with HFD showed increased levels of serum Triglycerides, LDL, VLDL and total cholesterol and decreased HDL levels. However oral administration of *T.officinale* extract significantly suppressed the rise of Lipid profile and rise in the HDL levels were observed. When compared to HFD+Orlistat, *T.officinale* high dose showed significant difference in reducing the serum Lipid profile (Table No.3).

Treatment groups showed a significant decrease in blood glucose levels when compared to the HFD control group. *T.officinale* high dose showed significant activity in decreasing the blood glucose when compared to HFD+Orlistat (Table No. 3).

Table No. 3: Effect of *Taraxacum officinale* on Lipid parameters

Treatment Groups	TG	Cholesterol	HDL	LDL	VLDL	Blood glucose
Normal Control	99.16±1.70	75±1.67	42.5±1.26	38.17±1.08	16.83±0.70	108.33±3.15
HFD Control	155.5±1.34***	155±2.46***	25.8±0.95***	66.67±1.41***	30.33±1.50***	154.66±3.12***
HFD+Orlistat	144.5±1.31***^{a}	125.33±1.41***^{a}	36±1.98*^{a}	39.83±0.98 ^{a}	24.33±1.33**^{c}	138.66±1.76***^{c}
HFD+TOE(low dose)	152.5±1.34***^{x}	137.83±1.19***^{ay}	29±1.37***^{y}	41.33±1.26 a	26±1.91***	146±4.89***
HFD+TOE(high dose)	147.5±1.06***^{a}	119.17±2.70***^{a}	38.33±0.92 ^{a}	37.17±0.70 a	23.16±1.01*^{b}	133±3.96***^{a}

*All values are Mean ±SEM. ***P <0.001, **P<0.01, *P<0.05 When compared to normal control group, ^{a}P <0.001, ^{b}p<0.01, ^{c}p<0.05 When compared to HFD control, ^{x}P <0.001, ^{y} P <0.01, ^{z}p<0.05 When compared to HFD+Orlistat.*

3.4 Organ weight and Fat pad analysis:

Organ weights included the Liver, Left kidney and Right kidney and fat pad weights such Mesenteric fat, Epididymal fat. Interpretation of the data was done using statistical analysis (ANOVA followed by Tukey's multiple comparison tests). Treatment groups showed a significant activity in decreasing the organ weight and Fat pad weights when compared to HFD control. When compared to standard drug *T.officinale* high dose showed a significant activity (Table No. 4 and 5).

Table No. 4: Effect of *Taraxacum officinale* on Fat pad analysis

Treatment Groups	Epididymal fat (gm)	Mesentric fat (gm)
Normal Control	2.10±0.09	2.48±0.08
HFD Control	7.02±0.11***	6.92±0.07***
HFD+Orlistat	3.28±0.11***^{a}	3.12±0.10***^{a}
HFD+TOE(low dose)	6.13±0.13***^{ax}	5.90±0.13***^{ax}
HFD+TOE(high dose)	3.32±0.08***^{a}	3.47±0.07***^{a}

*All values are Mean ±SEM. ***P <0.001, **P<0.01, *P<0.05 When compared to normal control group, ^{a}P <0.001, ^{b}p<0.01, ^{c}p<0.05 When compared to HFD control, ^{x}P <0.001, ^{y} P <0.01, ^{z}p<0.05 When compared to HFD+Orlistat.*

Table No. 5: Effect of *Taraxacum officinale* on Organ weight

Treatment Groups	Normal Control	HFD Control	HFD+Orlistat	HFD+TOE(low dose)	HFD+TOE (high dose)
Liver wt(gm)	6.15±0.10	10.02±0.09***	7.22±0.10***^{a}	9.02±0.09***^{ax}	7.77±0.10***^{ay}
Left Kidney wt(gm)	1.02±0.03	1.74±0.03***	1.43±0.05***^{a}	1.40±0.04***^{a}	1.18±0.02 ^{ax}
Right Kidney wt(gm)	0.99±0.03	1.71±0.03***	1.41±0.06***^{a}	1.38±0.04***^{a}	1.17±0.02^{ay}

*All values are Mean ±SEM. ***P <0.001, **P<0.01, *P<0.05 When compared to normal control group, ^{a}P <0.001, ^{b}p<0.01, ^{c}p<0.05 When compared to HFD control, ^{x}P <0.001, ^{y}P <0.01, ^{z}p<0.05 When compared to HFD+Orlistat*

It is a well known fact that Obesity does not develop overnight. In short, it is a chronic condition. Also, herbal medications take a longer time to act when compared to synthetic medications. Hence we selected a study period for 10 weeks for both diet and treatment.

HFD has been used to develop experimental obesity characterized with dyslipidemia and insulin resistance in rodents. HFD- fed rats exhibited significant increase in body weight, plasma glucose, insulin, triglycerides and total cholesterol level as compared to normal powdered diet (NPD) fed control rats. High fat diet (HFD) for 10 weeks causes obesity by increasing deposition of fats in the body. The lipogenesis was up regulated by HFD in rats leading to elevation of plasma lipids, which is characterized by elevated TG levels, LDL levels and decreased serum HDL in obese rats. Further, feeding with high fat diet caused hyperglycemia in rats. Therefore the serum lipid levels (total cholesterol, LDL, VLDL, HDL, and triglycerides) and glucose levels were estimated in present study as the marker of hyperlipidemia and hyperglycemia. In present study high fat diet (HFD) induction for 10 weeks led to obesity and dyslipidemia as evidence by gain in body weight, increase in triglyceride levels.

Pancreatic lipase is the key enzyme for dietary fat digestion, and inhibition of the enzyme could be an effective way to alter fat absorption. In-vitro studies have stated that *Taraxacum officinale* has an inhibitory activity on pancreatic lipase enzyme [9]. *T.officinale* leaf has been reported to contain flavonoids such as luteolin and it was demonstrated flavonoids could have pancreatic lipase inhibitory activity [12-13].

CONCLUSION

It can be concluded that *T.officinale* High dose is effective in reducing Body weight, Lipid parameters, immobility time, organ weight mesenteric and epididymal fat when compared to HFD control. Hence *T.officinale* high dose can be potentially used as an Anti-obesity agent.

Further studies to elucidate anti-obesity effects of chronic consumption of *T. officinale* and to identify the active components responsible for inhibitory activity against pancreatic lipase are necessary.

T.officinale low dose was not effective as *T.officinale* high dose in reducing body weight, lipid parameters, blood glucose levels, organ weight and fat pads. Hence low dose is less significant than high dose of *T.officinale.*

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