Research Article

Comparative study between the five different species belongs to *Inula* genus as inhibitors of pancreatic lipase

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Received: 26 April 2021

Revised: 9 June 2021

Accepted: 24 June 2021

Abstract

Background: Pancreatic Lipase enzyme is the key enzyme responsible for the absorption of dietary fat and is associated with obesity. FDA approved drug Orlistat is a potent inhibitor of pancreatic lipase enzyme, but banned due to some serious side effects. **Objective:** This study aim to investigate relationship between pancreatic lipase inhibition and total phenolic content of plant extracts. **Material and methods:** The in vitro anti lipase activity of six plant species i.e *Inula britannica*, *Inula racemosa*, *Inula helenium*, *Inula magnifica* and *Inula viscosa* belongs to *Inula* genus and family Asteraceae were evaluated by using spectrophotometric method. **Results and conclusion:** All the tested plant extracts belongs to *Inula* genus showed mixed type of inhibition towards porcine pancreatic lipase enzyme. *Inula racemosa* showed the highest potency towards the inhibition of porcine pancreatic lipase whereas *Inula britannica* exhibit the least inhibitory properties against porcine pancreatic lipase. The study also confirmed the relation between phenolic content and pancreatic lipase inhibition. From the results it was also clear that all the plant extracts exhibit phenolic content that is related to the inhibitory activity of pancreatic lipase. The total phenolic content varies remarkably in different plant species of *Inula* genus. The fluctuation in total phenolic content may arise because of the different type of phenols and different percentage of phenolic content present in different plant extracts of selected *Inula* genus species.

Keywords: Anti pancreatic lipase, total phenolic content, *Inula* genus, anti-obesity, In-vitro assay.

Introduction

Obesity is a common health problem and is associated with various other cardiovascular health disorders and is considered as a global burden. The conventional therapy of obesity mainly involves the use of synthetic substances, surgical procedures that includes various harmful side effects and thus disturbs the quality of individual's life (Karri et al., 2019; Conroy et al., 2011; Jaradat et al., 2017). Over a decade of intense research on traditional, complementary/alternative, and herbal medicines

has led to the knowledge that plant extracts show remarkable use in the treatment of obesity (Jaradat et al., 2017). The market of these kinds of medicines are growing rapidly and expanding continuously. On the contrary the current practice enumerates the role of nutraceuticals or phytopharmaceuticals in the management of obesity and its comorbidities instead of conventional medicines (Nijhawan et al., 2020; Kim et al., 2003; Zhou et al., 2003). In various developed or developing countries herbal remedies, nutraceuticals or phytopharmaceuticals are predominantly used for the treatment of obesity due to its less side effects (Zhou et al., 2003; Heck et al., 2000).

Orlistat is a prototype drug for weight loss and is a potent gastrointestinal lipase inhibitor that competes with dietary fat site on lipase molecule and blocks the absorption of dietary fat (Jaradat et al., 2017; Heck et al., 2000). Orlistat

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DOI: <https://doi.org/10.31024/apj.2021.6.3.1>

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also reduces the absorption of free fatty acids and monoglycerides (obtained from lipolysis) and inhibit the hydrolysis of triglycerides (Zhi et al., 1999; Hadvary et al., 1998). Therefore if a plant shows a potential inhibitory activity against pancreatic lipase it indicates its anti-obesity activity. This strategy should consider as one of the most important screening strategies in the discovery of anti-obesity natural plant extracts (Jaradat et al., 2017; Zhou et al., 2003; Hadvary et al., 1998).

In this study the various plants of *Inula* genus i.e *Inula britannica*, *Inula racemosa*, *Inula helenium*, *Inula magnifica* and *Inula viscosa* were selected for screening the in vitro anti lipase activity of these plant extracts. Genus *Inula* are perennial herbs belongs to Asteraceae family. This genus includes more than 100 species and most of them are traditionally indicated in ayurveda for their potential pharmacological actions. Herbs from this genus either use alone or with combination of other compatible herbs for the treatment of various metabolic disorders (Abid et al., 2003; Shakya et al., 2019).

In this study five different plant extracts of species belongs to *Inula* genus were tested for its phenolic content and anti-lipase activity by using spectrophotometric assay (Birari et al., 2007; Zheng et al., 2010). Many studies found that there is a connection between the total phenolic content of plant extract and anti-pancreatic lipase activity. The phenol content of the plant is related to the inhibitory activity of pancreatic lipase determined by Molecular docking studies (Alejandra et al., 2017). Our current study mainly emphasize on the relation between phenolic content of plant extract and lipase inhibitory activity by various selected plant species of *Inula* genus. The % inhibition activity of pancreatic lipase of all plant extracts were compared; in addition to that the results were also compared with Orlistat in order to assess their potential use as an alternative to this chemical agent (Abid et al., 2003; Shakya et al., 2019; Bustanji et al., 2010).

Material and methods

Plant extracts were either collected as gift samples or purchased from the available vendors. *Inula racemosa* plant extract was obtained from Amsar Private Limited, *Inula magnifica* plant extract was obtained from RHS Pharma Extracts, *Inula helenium* liquid plant extract was obtained from Hawaii Pharma, Gift samples of *Inula britannica* and *Inula viscosa* was obtained from Special Chem India and Good Scent Company respectively. Certificate of Analysis (COA) of all plant extracts were collected at the time of receiving. After receiving the samples were stored in air tight containers.

Plant extract used for experiment

Five different species were selected on the basis of availability of

Table 1. List of *Inula* genus species (Plant Extracts) selected for experiment.

S.No	Name of the Plant	Source (From COA)	Vendor
1	<i>Inula britannica</i>	Flowers	Special Chem India
2	<i>Inula helenium</i>	Whole Plant	Hawaii Pharma
3	<i>Inula magnifica</i>	Whole Plants	RHS Pharma Extracts
4	<i>Inula racemosa</i>	Roots	Amsar Private Limited
5	<i>Inula viscosa</i>	Roots	Good Scent Company

their plant extracts, and were evaluated for total phenolic content and in-vitro pancreatic lipase activity. The list of the plant and source from which the extract is obtained is mentioned below in Table 1.

Determination of Total Phenolic Content (TPC)

Preparation of Standard Gallic Acid for Calibration Curve

Total phenolic contents (TPC) of all five different plant extracts of selected species belongs to *Inula* genus listed in Table 1 was determined with the help of Folin–Ciocalteu colorimetric method with some alterations as described by Singleton (Singleton et al., 1999). Standard stock solution of gallic acid (1mg/ml) was prepared by dissolving 20 mg of gallic acid in 20 ml of methanol. Stock solution of 10% Folin–Ciocalteu reagent (FCR) was prepared by dissolving 10 g of Folin–Ciocalteu reagent (FCR) in 100 ml of purified water and stock solution of 7% Na₂CO₃ was prepared by dissolving 7 g of purified water in 100 ml respectively. Aliquots of gallic acid solution at different concentrations of i.e. 50, 100, 300 and 500 µg/mL were prepared from the standard stock solution. In order to make a final volume of 10 ml, 5 mL of 10% Folin–Ciocalteu reagent (FCR) and 4 mL of 7% Na₂CO₃ were added to each aliquots. A blue colored mixture was obtained; the mixture was well shaken for 2-3 minutes and was incubated in water bath at 40°C for 30 minutes. The absorbance of each dilution was measured at 760 nm against blank by UV-visible spectrophotometer (Singleton et al., 1999; Barkatullah et al., 2012; Ong et al., 2016). All the experiments were carried out in triplicates. The calibration curve was plotted by obtaining the average value at different concentrations.

Preparation of Samples for Total Phenolic Content

Various dilutions at different concentrations (i.e. 50, 100, 300 and 500 µg/mL) of all the plant extracts were prepared. The procedure as described in step 2.2.1 for the preparation of standard gallic acid was followed, and absorbance for each dilution of different concentration of different plant extracts was recorded by UV-visible spectrophotometer. All the dilutions were prepared in triplicates. The

calibration curve was plotted by obtaining the average value at different concentrations to determine the level of phenolics content in the different plant extracts. Total phenolic content of the different plant extracts was expressed as mg gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g). The total phenolic contents in all the samples were calculated by the using the formula:

$$C = c(V/M)$$

where C = total phenolic content mg GAE/g dry extract, c = concentration of gallic acid obtained from calibration curve in mg/mL, V = volume of extract in mL, and m = mass of extract in gram.

Pancreatic Lipase Inhibition Assay

Preparation of stock solution of different plant extracts

5mg/ml stock solution of different six different plant extracts of selected species of *Inula* genus listed in Table 1 was prepared by dissolving 100 mg of each plant extract in 20 ml volume of ethanol. The stock solution of each plant extract was kept in amber colored borosilicate sample bottles containing unique identification labels. From the stock solution 200 μ l of plant extract was used for lipase inhibition assay (Lewis et al., 2012).

Preparation of Porcine pancreatic lipase stock solution

The Porcine pancreatic lipase enzyme solution was prepared immediately before use. Stock solution of Porcine pancreatic lipase was prepared by suspending 1 g of Porcine pancreatic lipase in 100 ml of 20 mM tris-HCl buffer PH 8 to provide a final concentration of 10 mg/ml. The suspension was then stirred for 15 minutes using magnetic stirred, after that the suspension was centrifuged at 1500 RPM for 10 minutes. The clear supernatant was recovered and was kept in well stoppered amber colored borosilicate sample bottle (Jaradat et al., 2017; Alejandra et al., 2017).

Lipase Inhibition Reaction

The inhibitory activity of plant extracts was measured by using the modified Lipase inhibition reaction previously reported by (Bustanji et al., 2010). The lipase activity was determined measuring the hydrolysis of p-nitrophenol butyrate (p NPB) to p-nitrophenol at 410 nm using UV visible spectrophotometer. 200 μ l of each plant extract were taken in different test tubes after that 100 μ l of Porcine Pancreatic Lipase solution was added to it. The mixture of both was incubated for 5 min at 37°C in biological incubator, and then 10 μ L of the pNPB substrate (100 mM in acetonitrile) was added to it. The volume was make up to 1ml using 20 mM tris-HCl buffer. The pNPB release was estimated as the increment increase in absorbance against blank. Orlistat was used as positive control whereas ethanol was used as

negative control (Alejandra et al., 2017; Zheng et al., 2010). All the experiments were carried out in triplicates. Lipase inhibition activity was expressed as the percentage decrease in the enzyme activity when porcine pancreatic lipase was incubated with the test compounds. % Lipase Inhibition was calculated by using the below mentioned formula.

$$\text{Lipase Inhibition \%} = \frac{(\Delta A_{\text{Control}} - \Delta A_{\text{Sample}})}{\Delta A_{\text{Control}}} \times 100$$

Where: $\Delta A_{\text{Control}} = A_{\text{Control}} - A_{\text{Blank}}$

$\Delta A_{\text{Sample}} = A_{\text{Test}} - A_{\text{Blank}}$

Minimum Inhibitory Concentration IC50

IC50 is the concentration of different plant extracts at which 50% lipase inhibition was observed at different concentrations of plant extract ranging from 50 to 500 μ g/mL. From the data obtained from the experiment the IC50 was calculated from regression analysis and the curve of IC50 was plotted against percentage inhibition using Microsoft excel (Zheng et al., 2010; Firdous et al., 2018).

Results and discussion

Total Phenolic Contents (TPC)

TPC of different plant extracts listed in table 2 were determined by using (F-C) i.e. Folin-Ciocalteu method using gallic acid as standard. Calibration curve was obtained by using different concentration of Gallic acid. TPC of different plant extracts was calculated by using regression analysis. The regression equation obtained from calibration curve of gallic acid was $y = 0.0062x + 0.0392$ (where $R^2 = 0.9991$). All the TPC values were expressed as mg gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g).

From the study it was analyzed that among all the five selected species of *Inula* genus, *Inula racemosa* was observed to exhibit the highest TPC value i.e. 217.41 ± 5.28 GAE/g and the lowest was observed in *Inula britannica* i.e. 159.46 ± 3.65 GAE/g. Similarly the value was 165.61 ± 3.78 GAE/g for *Inula magnifica*, 192.12 ± 5.54 for *Inula helenium* and 179.50 ± 2.50 for *Inula*

Table 2. Total Phenolic content in different plant extracts of *Inula* Genus

S.No	Samples	50 μ g/mL	100 μ g/mL	300 μ g/mL	500 μ g/mL	Mean TPC Value GAE/g	Coefficient of Variation
1	<i>Inula britannica</i>	155.25	157.34	160.29	164.97	159.46 ± 3.65	0.022
2	<i>Inula helenium</i>	186.34	188.26	193.22	200.67	192.12 ± 5.54	0.029
3	<i>Inula magnifica</i>	161.44	163.98	166.78	170.25	165.61 ± 3.78	0.022
4	<i>Inula racemosa</i>	211.22	215.26	217.46	225.68	217.41 ± 5.28	0.024
5	<i>Inula viscosa</i>	175.78	178.92	180.75	182.56	179.50 ± 2.50	0.014

viscosa respectively (Table2).

Lipase Inhibition Assay

Five plant extracts belong to *Inula* genus listed in Table 2.1 were investigated for their anti-lipase activity at different concentrations i.e. 50, 100, 300, 500 µg/ml for Porcine Pancreatic Lipase inhibition activity. The lipase inhibitory activity for different plant extracts was investigated and the findings are displayed in Table 3 and Figure 1 and 2.

The inhibitory effect of reference drug (Orlistat) was also evaluated for comparison. Different aliquots of reference drug and plant extracts were prepared in escalating doses shown in Table 3. From the results it was observed that the inhibitory

Table 3. Mean absorbance and IC₅₀ values of plant extract at different concentrations.

Concentration	Orlistat	<i>Inula britannica</i>	<i>Inula helenium</i>	<i>Inula magnifica</i>	<i>Inula racemosa</i>	<i>Inula viscosa</i>
0	0.00	00.00	00.00	00.00	00.00	00.00
50	65.43±0.93	15.22±1.13	35.46±1.10	19.56±1.22	42.67±0.87	22.36±0.78
100	93.65±0.76	27.11±1.21	56.87±1.02	32.45±1.31	64.22±1.33	39.76±0.98
300	150.98±2.35	39.67±0.98	74.66±1.17	59.87±0.86	89.67±1.24	52.25±1.56
500	200.67±1.55	51.62±0.97	80.76±2.34	72.49±0.76	100.12±0.84	65.77±1.43
IC ₅₀ (µg/mL)	40.35	485.00	80.78	258.77	68.27	226.98

Figure1. Inhibition of porcine pancreatic lipase activity by Orlistat, I. Britannica, I. helenium, I. magnifica, I. racemosa, and I. viscosa. Value are the mean ± SD, n=3. Where: SD= Standard Deviation.

Figure 2. Inhibition of porcine pancreatic lipase activity by I. Britannica, I. helenium, I. magnifica, I. racemosa, and I. viscosa. Value are the mean ± SD, n=3. Where: SD= Standard Deviation

activity of porcine pancreatic lipase increased by increasing the concentration of reference drug (Orlistat) and plant extracts. The IC₅₀ value for the reference drug (Orlistat) and plant extract was calculated and a graph is plotted against % inhibition and concentration shown in Figure 1 and 2. The results also showed that the inhibitory effect of reference drug and plant extracts against pancreatic lipase are dose-dependent.

All the five species showed significant inhibition of in vitro Porcine pancreatic lipase enzyme as demonstrated using Lipase inhibition assay. Throughout the investigation it was observed that *Inula racemosa* showed most potent activity and *Inula britannica* showed least potent activity against pancreatic lipase among all the plant extracts. *Inula racemosa* exhibit lowest IC₅₀ value i.e. 68.27 µg/ml whereas *Inula britannica* exhibit IC₅₀ value of 485.00 µg/ml respectively.

In addition to that IC₅₀ of all plant extracts listed in Table 1 were compared with the reference standard (Orlistat). The results for the same are shown in Table 2 and Figure 1.

The effect of five species of *Inula* genus was monitored and was compared along with that the results was also compared to that of reference drug orlistat (potent inhibitor of pancreatic lipase). The results showed that the following order of % Lipase Inhibition i.e. *Inula britannica* < *Inula magnifica* < *Inula viscosa* < *Inula helenium* < *Inula racemosa*. *Inula racemosa* is a ayurvedic plant that is well documented for the treatment of various metabolic disorders whereas *Inula britannica* is less known in the treatment of metabolic disorders.

Direct correlation of total phenolic content and pancreatic lipase inhibition was observed during the experiment. As the dose of the plant extract increases the lipase inhibition activity also increased. All the plants extracts were compared side by side for its lipase inhibition activity along with that the % inhibition of lipase also compared with the reference drug orlistat and it was observed that the IC₅₀ of *Inula racemosa* and *Inula helenium* nearly equal to IC₅₀ of Orlistat. Thus the study suggests that *Inula racemosa* and *Inula helenium* can be further used to explore the in-vivo anti-pancreatic activity. *Inula viscosa* and *Inula magnifica* also exhibit some anti-lipase properties and can also further explored for better findings.

In fact many previous studies also supports the relationship between total phenolic content and pancreatic lipase inhibitory activity, whereas few studies failed to establish a relation between TPC and Lipase inhibition. But our present study supports the fact that there is a relationship

between TPC and lipase activity that will act as a useful tool for the discovery of new anti-obesity herbs that exhibit the potential to remarkably inhibit the activity of pancreatic lipase (Bhutani et al., 2007; Conforti et al., 2017).

Conclusion

In summary all the tested plant extracts belongs to *Inula* genus showed mixed type of inhibition towards porcine pancreatic lipase enzyme. Among all five plants extracts *Inula racemosa* tested to have strongest inhibition activity towards pancreatic lipase with IC₅₀ value 68.27 µg/mL and *Inula britannica* exhibited to have the lowest inhibition activity with IC₅₀ value 485.00 µg/mL. The study also confirmed the relation between phenolic content and pancreatic lipase inhibition. From the results it is also clear that all the plant extracts exhibit phenolic content that is related to the inhibitory activity of pancreatic lipase. The total phenolic content varies remarkably in different plant species of *Inula* genus. The fluctuation in total phenolic content may arise because of the different type of phenols and different percentage of phenolic content present in different plant extracts of selected *Inula* genus species. In-vitro testing of plant extract against pancreatic lipase proved that the phenolic content is related to inhibit enzyme activity. But dose dependent inhibition was observed throughout the experiment. This means increasing the dose can leads to have better effects but it should not exceed from the safety doses of the plant extract.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Acknowledgement

The authors received support from Rayat institute of pharmacy for encouragement in carrying out her college work.

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