

Studies on Effect of Some Medicinal Plants on Pancreatic Lipase Activity using Spectrophotometric Method

NEHA GOWADIA* AND T.N. VASUDEVAN†

Department of Pharmacy
SGSITS, Indore 452 003, India

Forty medicinal plants were studied for their effect on pancreatic lipase activity. The method involves the prior incubation of enzyme with 10% w/v aqueous decoction of plant drug. The enzyme activity was assayed by colorimetry at 710 nm. Percentage activation or inhibition of enzyme by plant extract was calculated by comparing the activity of pancreatic lipase in presence and absence of plant extract. Results of studies showed that *Eugenia caryophyllus*, *Mesua ferea*, *Terminalia bellerica*, *Terminalia chebula*, *Swertia chirata*, *Withania somnifera* have significant inhibitory effect on the activity of enzyme. *Tribulus terrestris* and *Picrasma excelsa* showed activation of enzyme activity.

INTRODUCTION

Pancreatic lipase is an important enzyme of digestion; it promotes the intraduodenal conversion of dietary long chain triglycerides into more polar free fatty acids. This polarity is apparently required for the products to cross brush border membrane of enterocytes, on their way to interior of cells.¹

Medicinal plants in the form of Ayurvedic drugs being safer and nontoxic are used indiscriminately. The presence of inhibitors of digestive enzymes in food and plants has received much attention since the last few decades. This is because the inhibitors, considered as anti-nutritive factors, may lead to stoppage of degradation of food material in the intestinal tract and lead to disturbances in digestion. Although inhibitors of digestive enzymes have been studied, but pancreatic lipase inhibitors from plants have received far less attention. Therefore, the present study has been carried out to find the effects (activatory/inhibitory) of plant constituents on pancreatic lipase, as various lipases play a major role in the regulation of fat metabolism. Thus the control of lipase activity may be important in the etiology of obesity and atherosclerosis.²

The pancreatic lipase activity determination in the present work is based on the reaction³ of free fatty acids liberated from the substrate by the action of enzyme, with cupric acetate, to form a blue coloured complex which gives maximum absorbance at 710 nm.

†Department of Pharmaceutical Sci., UDCT, Matunga, Mumbai-19, India.

EXPERIMENTAL

Instrument: Spectrophotometer Miltonroy Spectronic 1201

Enzyme: Pancreatin I.P. was used as a source of pancreatic lipase.

Pancreatic lipase activity in given sample was determined by Lazo-wasem method⁴ and it was found to contain 200 Wilson units/g of pancreatic lipase activity. One Wilson unit of lipase is the amount of enzyme that will liberate an amount of fatty acid equivalent to 1 mL of 0.05 N alcoholic sodium hydroxide solution from 1 mL of olive oil in 30 min under the conditions of assay.

Substrate: Olive oil (Elousa)

Cupric acetate reagent: A 5% w/v aqueous solution of cupric acetate was made and filtered, then pH was adjusted to 6.0–6.2 using pyridine.

Phosphate buffer I.P. (pH 7); Oleic acid; Benzene; Ethanol.

All reagent used were of analytical-reagent grade.

Plant material: Plant drugs were purchased from the crude drug market, Mumbai. The plants were then authenticated on the basis of morphological and microscopic characters mentioned in standard text books.

S.No.	Name of plant	Part used	Family
1.	<i>Acorus calamus</i>	Rhizome	Araceae
2.	<i>Adhatoda vasaka</i>	Leaf	Acanthaceae
3.	<i>Aegle marmelos</i>	Fruit	Rutaceae
4.	<i>Alpinia galang</i>	Rhizome	Zingiberaceae
5.	<i>Anethum sowa</i>	Fruit	Umbelliferae
6.	<i>Azadirachta indica</i>	Leaf	Meliaceae
7.	<i>Boerhaavia diffusa</i>	Leaf	Nyctaginaceae
8.	<i>Brassica alba</i>	Seed	Cruciferae
9.	<i>Carum carvi</i>	Fruit	Umbelliferae
10.	<i>Cassia augustifolia</i>	Leaf	Leguminosae
11.	<i>Centella asiatica</i>	Leaf	Umbelliferae
12.	<i>Cephaelis ipecacuanha</i>	Root	Rubiaceae
13.	<i>Coriandrum sativum</i>	Fruit	Umbelliferae
14.	<i>Cuminum cyminum</i>	Fruit	Umbelliferae
15.	<i>Curcuma longa</i>	Rhizome	Zingiberaceae
16.	<i>Elettaria cardamomum</i>	Fruit	Zingiberaceae
17.	<i>Embelia ribes</i>	Fruit	Myrsinaceae
18.	<i>Ephedra sinica</i>	Stem	Ephedraceae
19.	<i>Eugenia caryophyllus</i>	Flowering bud	Myrtaceae
20.	<i>Foeniculum vulgare</i>	Fruit	Umbelliferae
21.	<i>Glycyrrhiza glabra</i>	Root	Leguminosae
22.	<i>Holarrhena antidysentrica</i>	Bark	Apocynaceae
23.	<i>Hyoscyamus niger</i>	Seed	Solanaceae

S.No.	Name of plant	Part used	family
24.	<i>Linum usitatissimum</i>	Seed	Linaceae
25.	<i>Mesua ferea</i>	Seed	Guttiferae
26.	<i>Myristica fragrans</i>	Kernel	Myristiceae
27.	<i>Nordostachys jatamansi</i>	Rhizome	Valerianaceae
28.	<i>Picrasma excelsa</i>	Wood	Simarubaceae
29.	<i>Piper nigrum</i>	Seed	Piperaceae
30.	<i>Psoralea coryfolia</i>	Fruit	Leguminosae
31.	<i>Rheum palmatum</i>	Rhizome	Polygonaceae
32.	<i>Strychnos nuxvomica</i>	Seed	Loganiaceae
33.	<i>Swertia chirata</i>	Aerial part	Gentianaceae
34.	<i>Terminalia belerica</i>	Fruit	Combretaceae
35.	<i>Terminalia chebula</i>	Fruit	Combretaceae
36.	<i>Tinospora cordifolia</i>	Stem	Menispermaceae
37.	<i>Tribulus terrestris</i>	Fruit	Zygophyllaceae
38.	<i>Valeriana wallichii</i>	Rhizome	Valerianaceae
39.	<i>Withania somnifera</i>	Root	Solanaceae
40.	<i>Zingiber officinalis</i>	Rhizome	Zingiberaceae

Procedure

Preparation of standard curve of oleic acid: To study the obeyence of Beer's law standard curve of oleic acid was plotted as major hydrolytic product of olive oil is oleic acid.

Different concentration of oleic acid (2–20 μmol) were placed in test tubes. 5 mL of benzene was added to each test tube to dissolve the acid. Then 1 mL of cupric acetate reagent was added. Tubes were vortexed for 1 min and allowed to stand. After separation of two layers, the adsorbance of upper layer was recorded at 710 nm (Fig. 1).

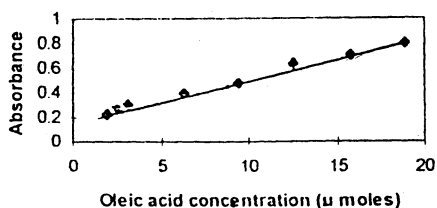


Fig. 1. Standard curve of oleic acid at 710 nm

Screening of medicinal plants for their effect on pancreatic lipase activity: Enzyme solution containing 1 Wilson unit/mL of pancreatic lipase was prepared in phosphate buffer of pH 7. 1 mL of this solution was incubated with 1 mL of plant extract (10% w/v aqueous decoction) for 1 h at 37°C. The mixture was again

incubated for 3 h with 1 mL of substrate at 37°C. At the end of 3 h the reaction was stopped by addition of 1 mL alcohol. Free fatty acids liberated were extracted with 5 mL of benzene. 1 mL of copper acetate reagent was added and vortexed. Allowed the two layers to separate in test tubes. Absorbance (s) of upper layer was recorded at 710 nm.

Blank for it was prepared using same method but instead of enzyme solution 1 mL of phosphate buffer (pH 7) was used. Control and its blank were also prepared in a similar way, but 1 mL distilled water was used instead of plant extract and absorbance (C) of upper layer was recorded at 710 nm.

The percentage activation or inhibition was calculated as follows:

$$\% \text{ Activation /Inhibition} = \frac{C - S}{C} \times 100$$

RESULTS AND DISCUSSION

The results are indicated in the following Table.

S.No.	Name of Plant	Effect	Percentage
1.	<i>Acorus calamus</i>	I	72.40
2.	<i>Adhatoda vasaka</i>	NE	–
3.	<i>Aegle marmelos</i>	NE	–
4.	<i>Alpinia galang</i>	I	37.12
5.	<i>Anethum sowa</i>	NE	–
6.	<i>Azadirachta indica</i>	NE	–
7.	<i>Boerhaavia diffusa</i>	I	53.20
8.	<i>Brassica alba</i>	I	28.14
9.	<i>Carum carvi</i>	NE	–
10.	<i>Cassia augustifolia</i>	I	29.40
11.	<i>Centella asiatica</i>	NE	–
12.	<i>Cephaelis ipecacuanha</i>	I	36.20
13.	<i>Coriandrum sativum</i>	I	38.78
14.	<i>Cuminum cyminum</i>	I	78.70
15.	<i>Curcuma longa</i>	NE	–
16.	<i>Elettaria cardamomum</i>	I	45.60
17.	<i>Embelia ribes</i>	I	61.85
18.	<i>Ephedra sinica</i>	I	70.58
19.	<i>Eugenia caryophyllus</i>	I	83.70
20.	<i>Foeniculum vulgare</i>	I	51.21
21.	<i>Glycyrrhiza glabra</i>	NE	–
22.	<i>Holarrhena antidysentrica</i>	I	66.14
23.	<i>Hyoscyamus niger</i>	I	27.17

S.No.	Name of Plant	Effect	Percentage
24.	<i>Linum usitatissimum</i>	NE	–
25.	<i>Mesua ferea</i>	I	92.40
26.	<i>Myristica fragrans</i>	NE	–
27.	<i>Nordostachys jatamansi</i>	NE	–
28.	<i>Picrasma excelsa</i>	A	38.80
29.	<i>Piper nigrum</i>	I	43.40
30.	<i>Psoralea coryfolia</i>	I	30.28
31.	<i>Rheum palmatum</i>	NE	–
32.	<i>Strychnos nuxvomica</i>	NE	–
33.	<i>Swertia chirata</i>	I	79.19
34.	<i>Terminalia belerica</i>	I	98.60
35.	<i>Terminalia chebula</i>	I	97.06
36.	<i>Tinospora cordifolia</i>	I	41.50
37.	<i>Tribulus terrestris</i>	A	28.62
38.	<i>Valeriana wallichii</i>	NE	–
39.	<i>Withania somnifera</i>	I	87.90
40.	<i>Zingiber officinalis</i>	NE	–

A: Activation of pancreatic lipase activity

I-Inhibition of pancreatic lipase activity

NE: No effect on pancreatic lipase activity

These results reflect the effect of constituents of plants studied on the enzyme. Plants like *Eugenia caryophyllus*, *Mesua ferea*, *Terminalia belerica* and *Terminalia chebula* showed significant inhibition of pancreatic lipase. These plants contain tannins which are known to have enzyme inhibition property due to their ability to bind and coagulate proteins.

In our studies *Curcuma longa* and *Zingiber officinale* extracts have shown no effect on pancreatic lipase while *Zingiber officinale* and curcumin the active constituent of *Curcuma longa* are reported⁵ to have stimulatory effect on lipase. *Piper nigrum* extract in our studies has shown inhibitory effect on pancreatic lipase while piperine the active principle of it stimulates the lipase as reported.⁵ This difference in behaviour can be due to use of isolated compounds in reported studies.

As the saponins are claimed to be the activators of lipase, the saponins containing plant *Tribulus terrestris* has shown stimulatory effect in our results. However, *Centella asiatica* and *Glycyrrhiza glabra* also containing saponin have shown no effect on the enzyme activity in our studies.

Medicinal plants containing volatile oil *Acorus calamus*, *Alpinia galang*, *Azadirachta indica*, *Foeniculum vulgare*, *Coriandrum sativum*, *Cuminum cyminum* and *Elettaria cardamomum* have shown inhibitory effect on enzyme while other volatile oil containing plants *Anethum sowa*, *Myristica fragrans*, *Valeriana wallichii* showed no effect. Mucilage containing drugs like *Aegle*

marmelos and *Linum usitatissimum* showed no effect. *Boerhaavia diffusa*, *Cephaelis ipecacuanha*, *Ephedra sinica*, *Hyoscyamus niger*, *Withania somnifera* and *Swertia chirata* have shown inhibitory effect. This effect may be due to active constituent or combined effect of constituents in a drug.

ACKNOWLEDGEMENT

The authors are thankful to Advanced Biochem Ltd., Thane, for providing gift sample of Pancreatin and to the UGC for providing research grant.

REFERENCES

1. M. Semeriva and P. Desnuelle, in: A. Meister (Ed.), *Advances in Enzymology*, Vol. 48, Interscience Publication, p. 321 (1979).
2. A.M. Rogel, W.L. Stone and F.O. Adebonjo, *Lipids*, **24**, 518 (1980).
3. R.R. Lowry and I.J. Tinsley, *JAACS*, **53**, 470 (1976).
4. E.A. Lazowasem, *J. Pharm. Sci.*, **50**, 999 (1961).
5. K. Patel and K. Srinivasan, *Int. J. Food Sci. and Nutr.*, **47**, 55 (1996).

(Received: 1 March 2000; Accepted: 29 April 2000)

AJC-2010

Analytical Sciences

INTERNATIONAL CONGRSS ON ANALYTICAL SCIENCES 2001

(ICAS2001)

6-10 AUGUST 2001

TOKYO, JAPAN

For more information, contact:

Prof. TSUGUO SAWADA, Chairman
Department of Applied Chemistry
The University of Tokyo
7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8656, Japan
Tel: +81 3 5841 7236 (or 7237)
Fax: +81 3 5841 6037
E-mail: icas2001@laser.t.u-tokyo.ac.jp