"Concomitant Management of Type-II Diabetes and its Associated Hyperlipidemia: Role of ethyl 2-[5-(acetyloxy)pently] benzoate"

Presented By

Mr. BHUSHAN P. PIMPLE

P. E. Society's Modern College of Pharmacy, Nigdi, Pune

at the

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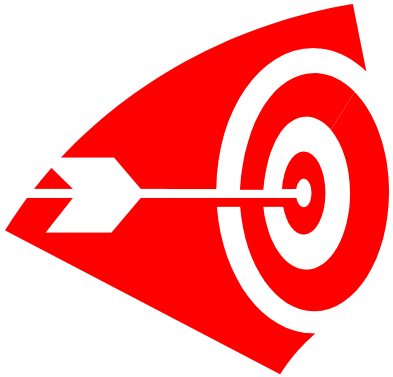
Contents:

1. Introduction

- Diabetes [1, 2]
  - 3470 lacs diabetic
  - 2012: 15 lacs death worldwide
  - 2014: 2 lacs death India

- Associated Complications
  - Renal [3]
  - CVS complications [3]
  - Retinal complications [4]
  - Gastrointestinal complications [5, 6]

2. Objectives



a. **Isolation of ethyl 2-[5-(acetyloxy)pently] benzoate from *Origanum majorana* Linn**

b. **Investigation of ethyl 2-[5-(acetyloxy)pently] benzoate for its antidiabetic AND anti-hyerlipidemic potential**

c. **Comparison of bio-efficacy of ethyl 2-[5-(acetyloxy)pently] benzoate against Pioglitazone & Mevastatin**

3. Material and Methods

3.1. Plant material

Origanum majorana Linn [7, 8, 9]

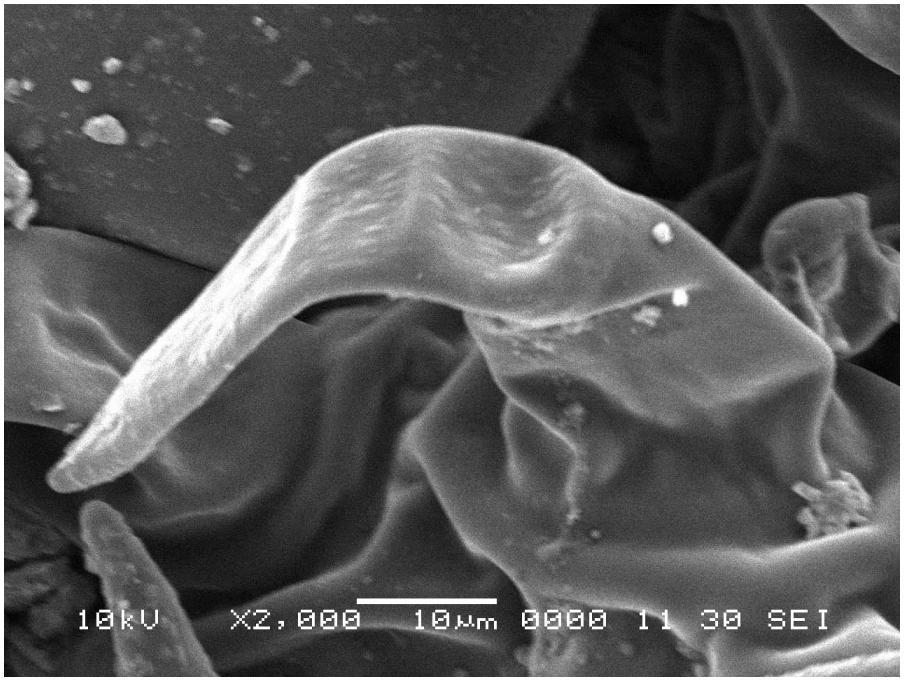
- Synonym: *Origanum hortensis* Moench,
  Majorana majorana (L.) H. Karsh
- Common name: Sweet majorana
- Family: Lamiaceae
- Part Used: Leaves
- Extracts: Methanol extract
- Fraction: Ethyl acetate

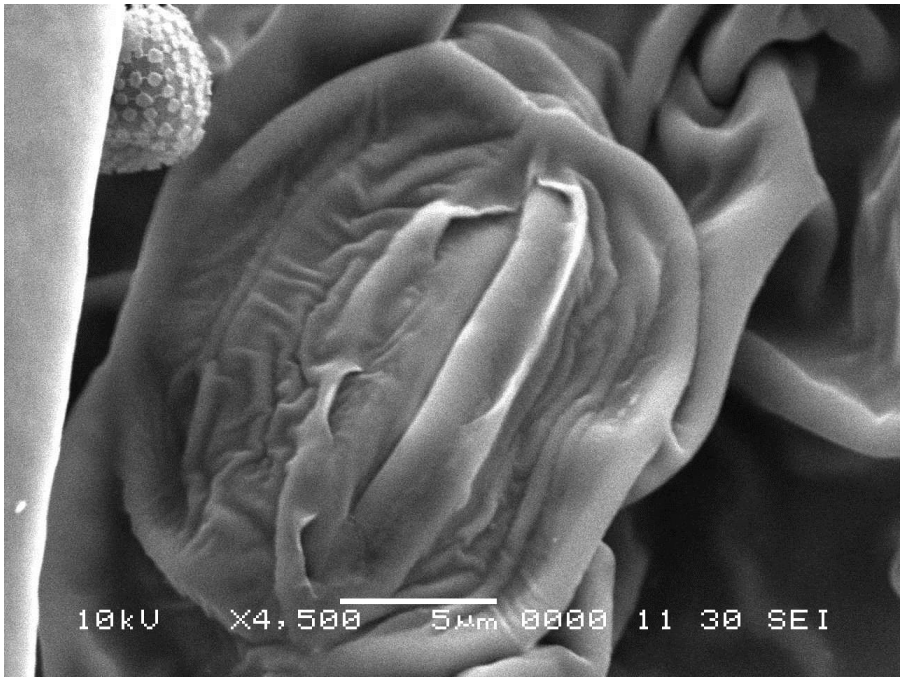
***Origanum majorana* leaves**

3. Material and Methods

Scanning Electron Microscope
Make: FEI
Model: Nova NanoSEM 450

4.1 Results (pharmacognosy)



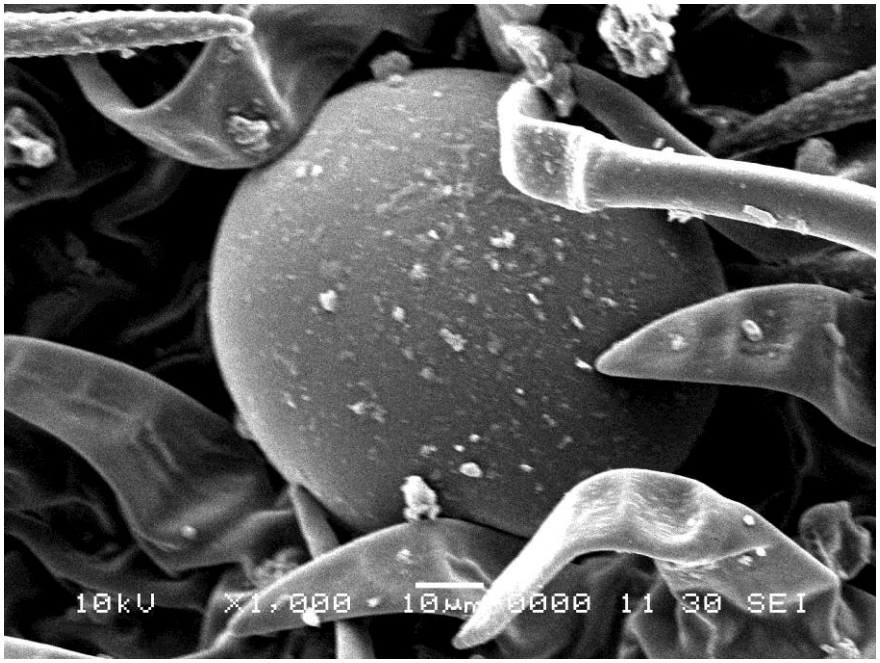


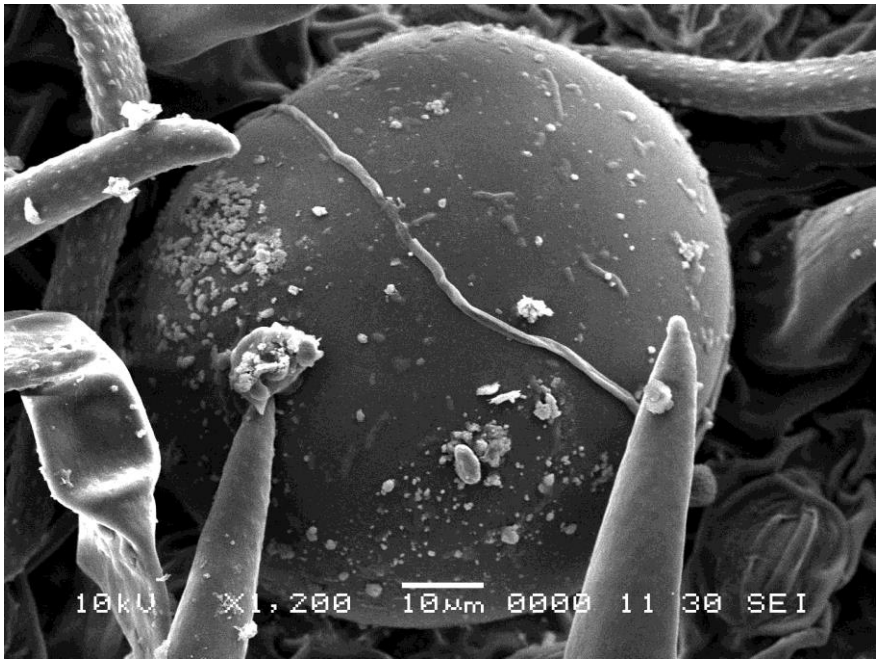
Trichomes
Type: multicellular
Length: 23.46 µm

Diacytic stomata
Diameter:18.66 µm
Area: 273.39 sq µm
Stoma: 12.83 µm in length
Guard cells: 6.556 µm X 18.04 µm

SEM of *Origanum majorana* leaf

4.1 Results (pharmacognosy)





Salt Glands
Shape: spherical
Diameter: 74.96 µm
Crystals size: 2.93 µm

SEM of *Origanum majorana* leaf

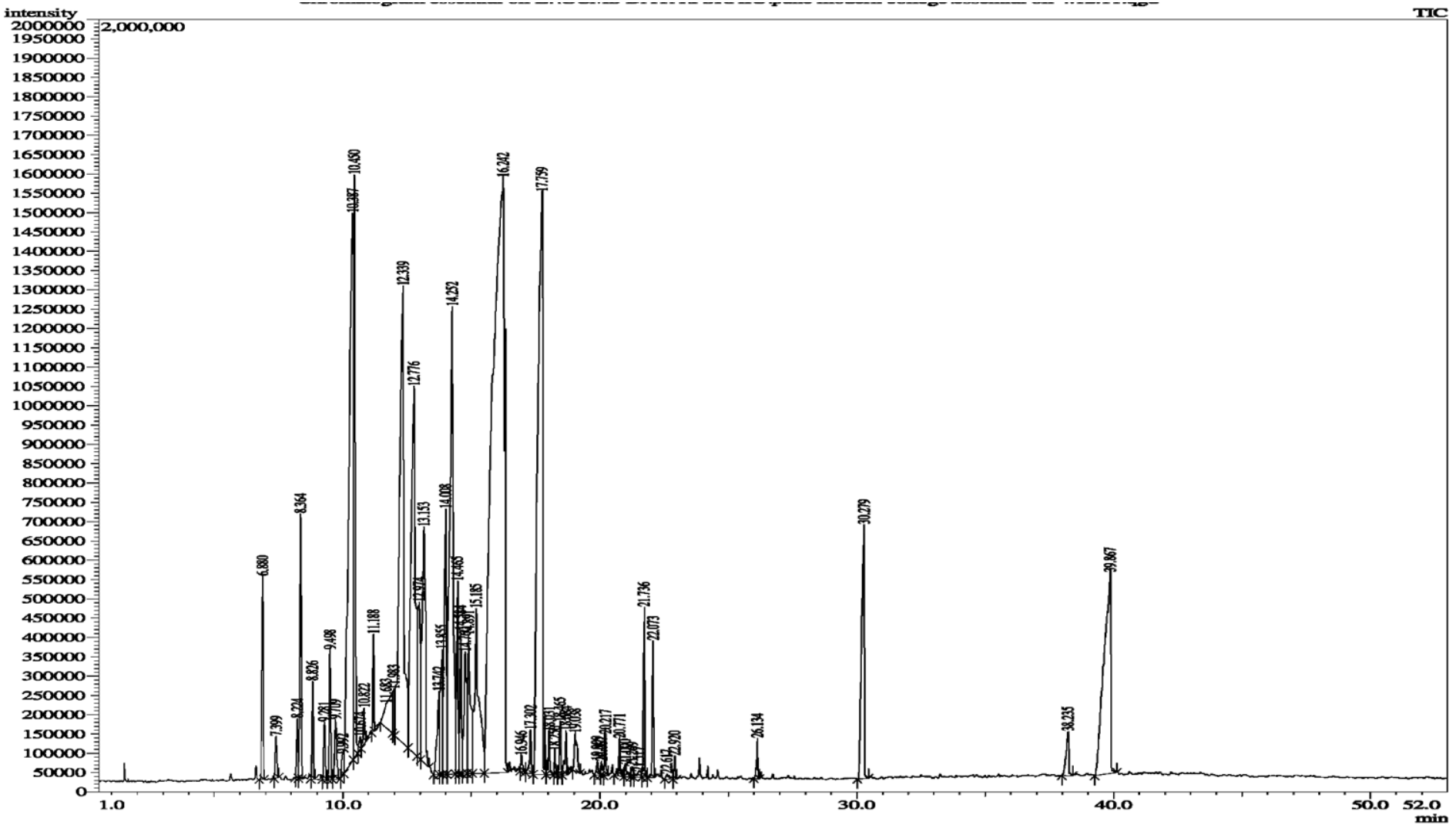
4.1 Results (pharmacognosy)

Physiochemical parameters of *Origanum majorana* Linn. oil

Parameters of Oil	Observations
Color	Pale yellow
Odor	Aromatic, fresh herbaceous
Appearance	Mobile liquid
Oil Yield (g $100g^{-1}$)	01.72 ± 0.09
Refractive Index @ 20 ^{0}C	1.477 ± 0.02
Density @ 20 ^{0}C	0.893 ± 0.03
Boiling point @ 760 mm Hg	265.3 ± 0.20 ^{0}C
Solubility in ethyl acetate	soluble 1 in 2 parts
Solubility in ethanol (75 % v/v)	soluble 1 in 2 parts
Solubility in water	insoluble

Values are expressed as mean ± standard deviation.
The oil sample was analyzed in triplicate.

4.2 Results (phytochemistry)



GC chromatogram of *Origanum majorana* oil

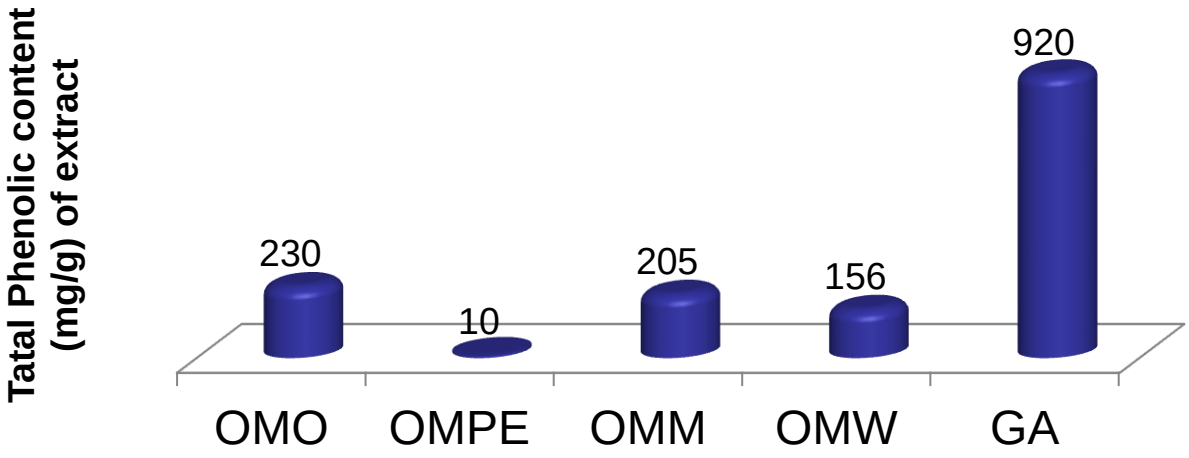
4.2 Results (phytochemistry)

Major compounds of *Origanum majorana* oil

Area %	Height	Height %	Name
1.30	685314	3.47	(+)-3-Carene
8.55	1417611	7.17	D-Limonene
3.19	1510701	7.64	Eucalyptol
7.11	1183782	5.99	(+)-4-Carene
5.68	950698	4.81	(-) β-Linalool
1.57	403795	2.04	2-Butanol, 3,3'-oxybis-
2.66	610093	3.09	Dihydromyrcenol
2.25	687375	3.48	3-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-
1.14	498969	2.52	Cyclohexanone, 5-methyl-2-(1-methylethyl)-, cis-
1.22	315874	1.60	Acetic acid, phenylmethyl ester
1.45	358818	1.82	Borneol
2.49	427057	2.16	Terpinen-4-ol
25.55	1542948	7.81	α-Terpineol
10.02	1511900	7.65	(+)-Linalyl acetate
2.52	646452	3.27	Benzyl Benzoate
4.95	510328	2.58	2-(p-Nitroanilino)tropone

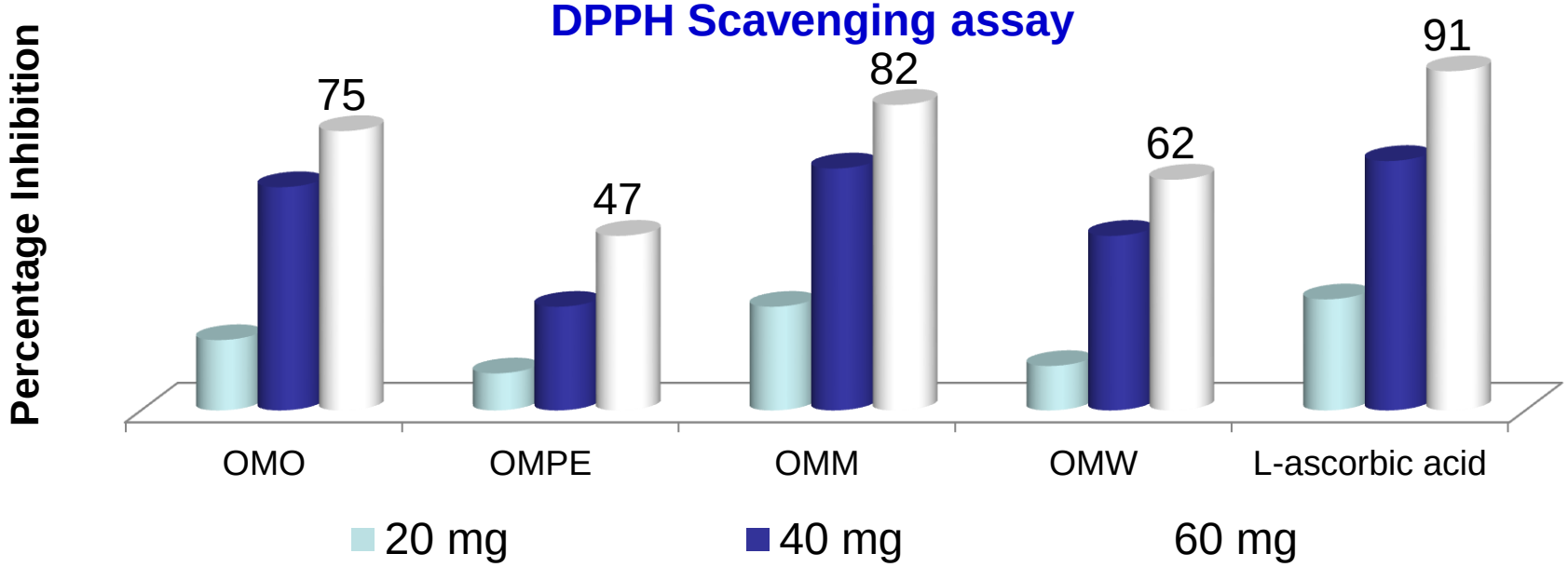
4.2 Results (phytochemistry)

Total Phenolic Content

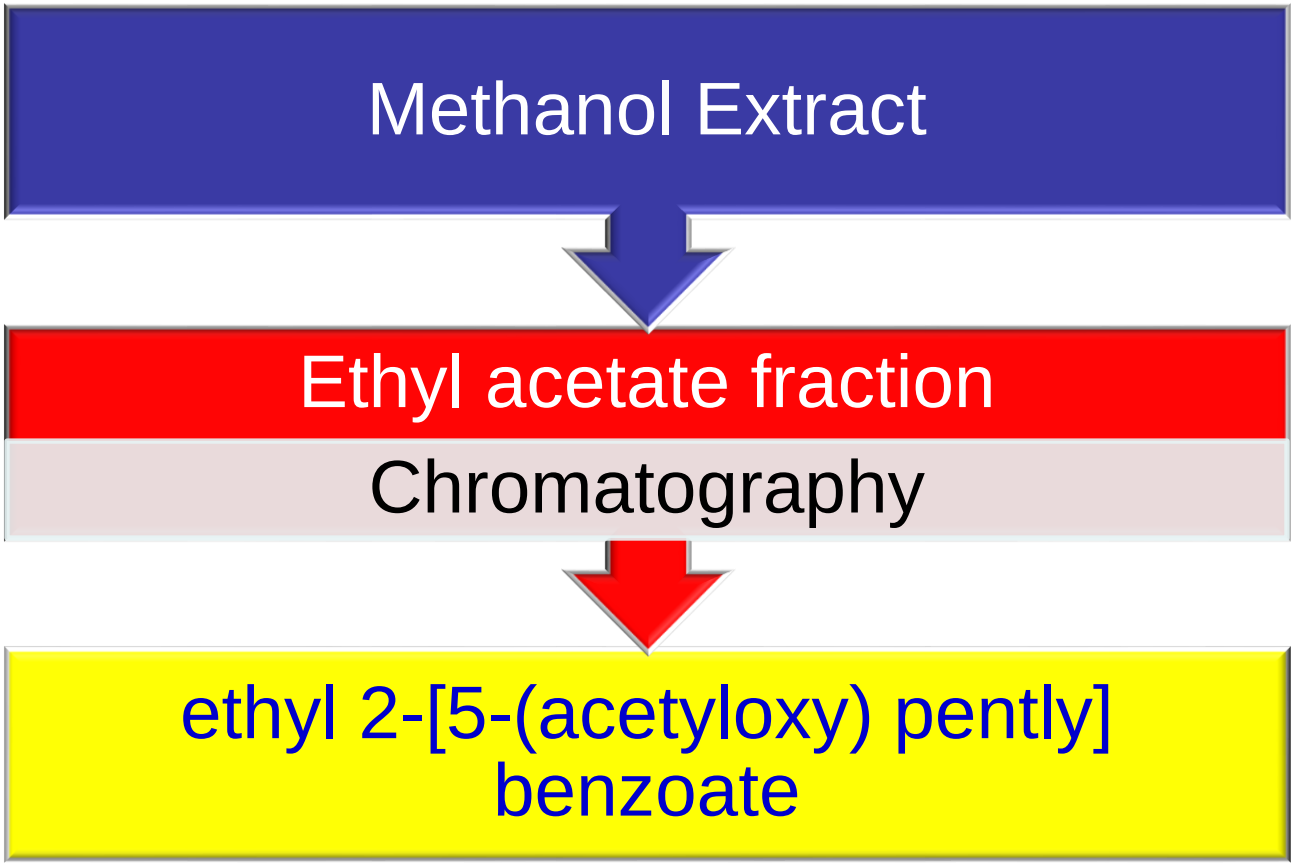


OMO = *O. majorana* oil
OMPE = *O. majorana* pet ether extract
OMM = *O. majorana* MeOH extract
OMW = *O. majorana* Aq. extract

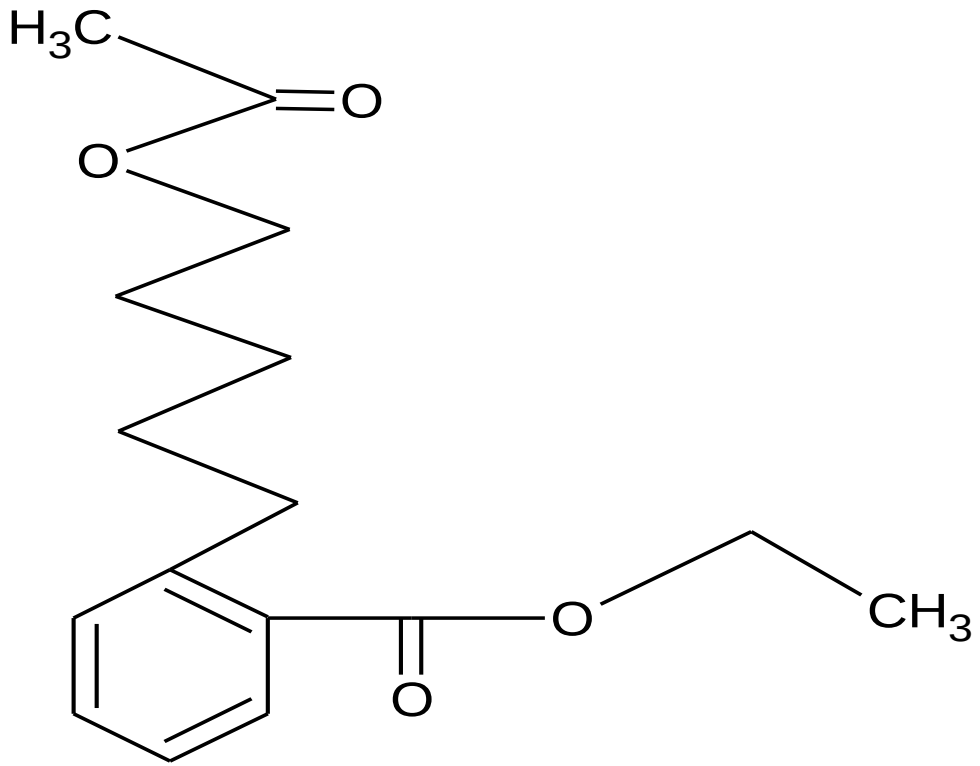
DPPH Scavenging assay



Isolation of ethyl 2-[5-(acetyloxy) pently] benzoate



4.5 Results (characterization of isolated compound)

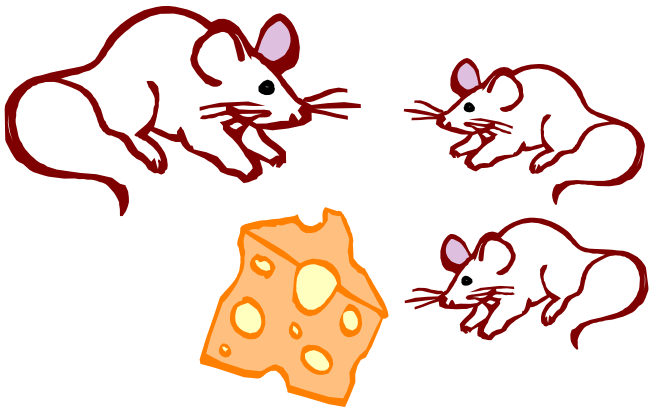


ethyl 2-[5-(acetyloxy)pentyl]benzoate

3. Material and Methods

3.4. Acute toxicity study [22]

3.5. Selection of animals [23]



- Wistar strain male albino rats, weighing 200 ± 20 g
- FBG: 85 ± 10 mg/dl

3.6 Experimental design [24] (MCP/IAEC/02/2009)

- 28 day study
- STZ 65 mg/kg *i.p*. + Nicotinamide 120 mg/kg *i.p.*
- Glucose estimation: 48 hrs FBG > 225 mg/dl

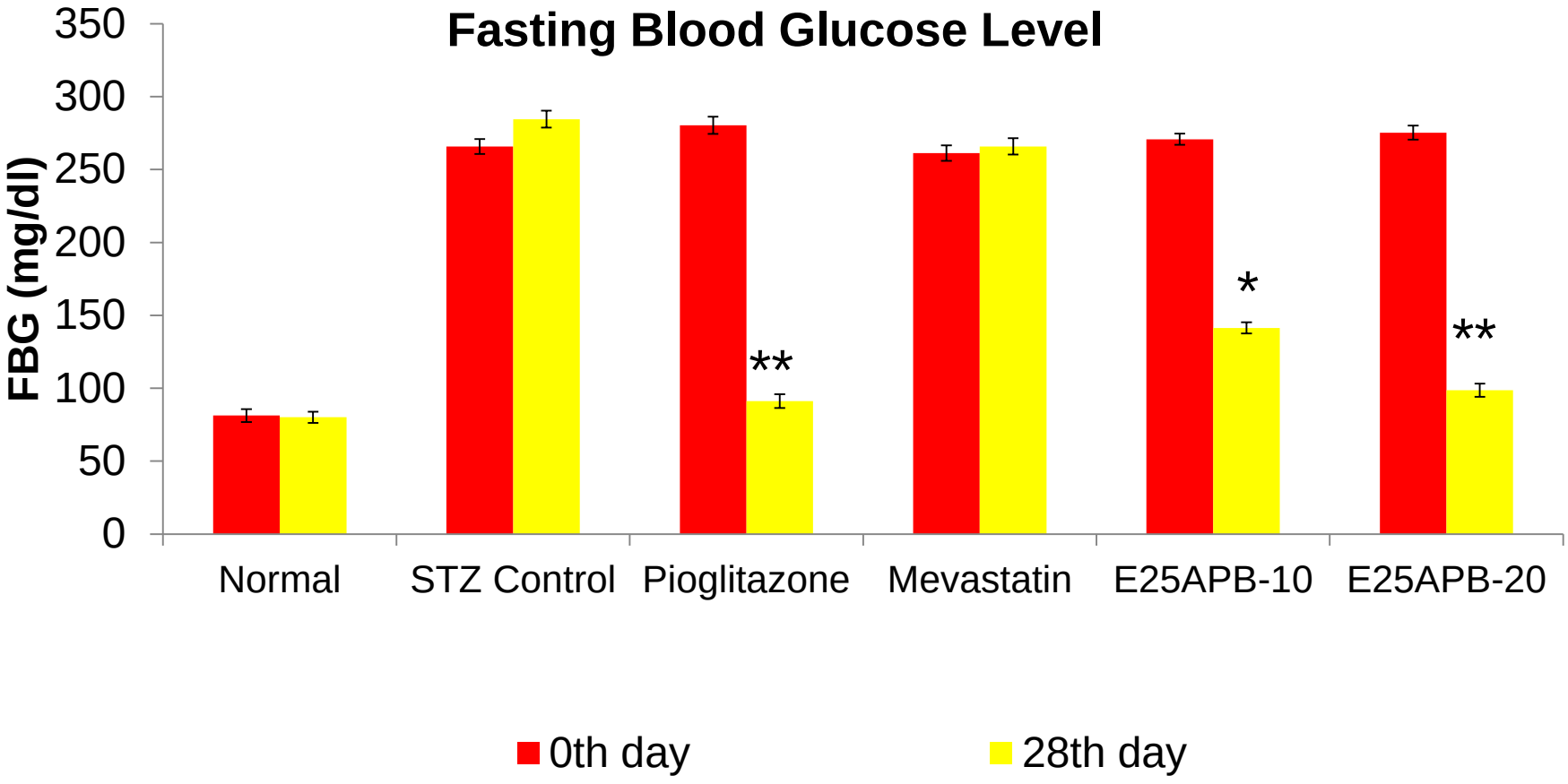
3. Material and Methods

Treatment Protocol

1 control + 5 diabetic = 6 groups

Groups	Treatment
Normal	-
STZ control	-
Pioglitazone	Pioglitazone (20 mg/kg *p.o.*)
Mevastatin	Mevastatin (100 mg/kg *p.o.*)
E25APB – 10	ethyl 2-[5-(acetyloxy) pently] benzoate (10 mg/kg *p.o.*)
E25APB – 20	ethyl 2-[5-(acetyloxy) pently] benzoate (20 mg/kg *p.o.*)

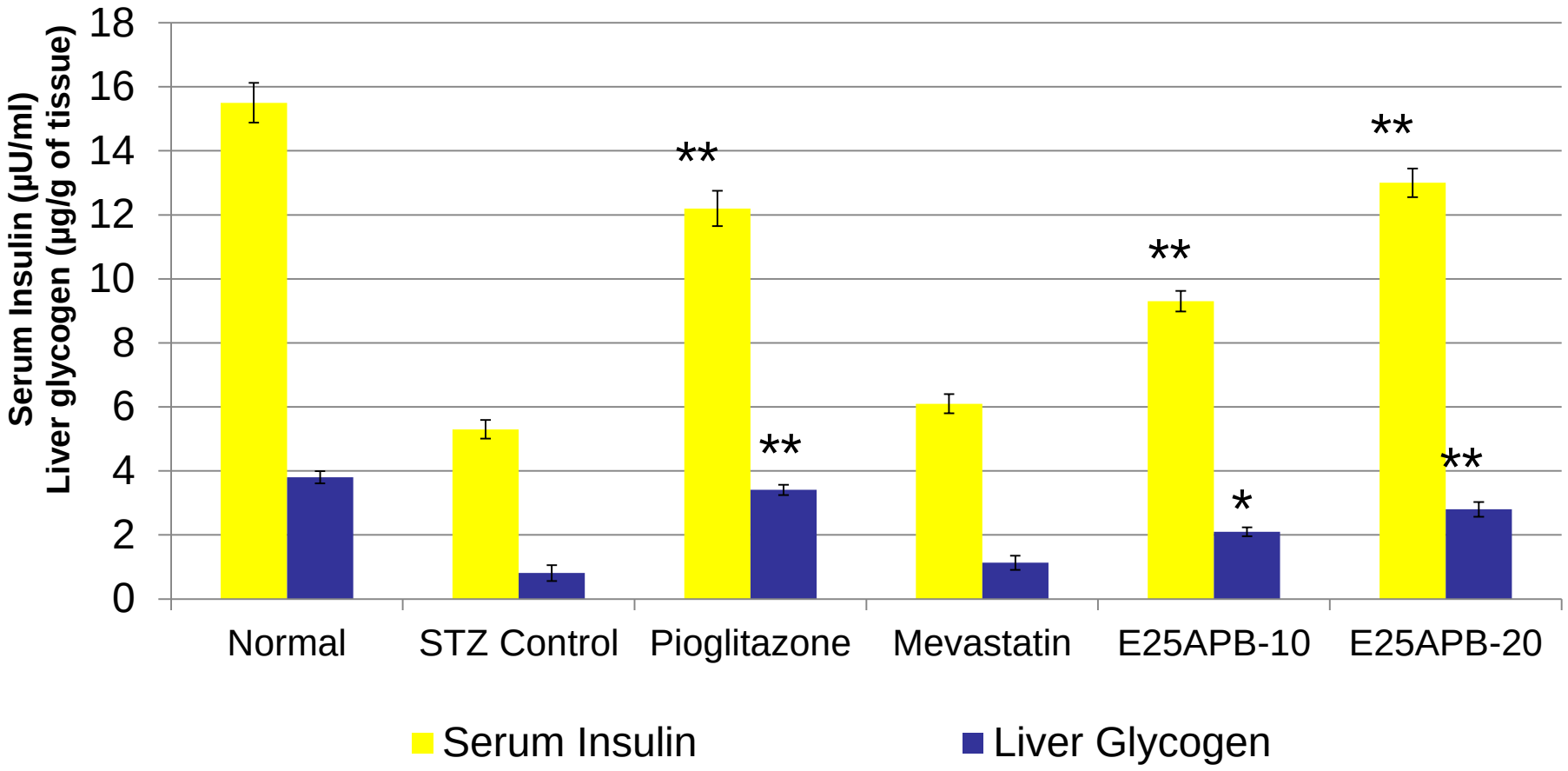
4.3 Results (antidiabetic study)



* = p < 0.05, ** = p < 0.01, n = 6 ANOVA –Dunnett test

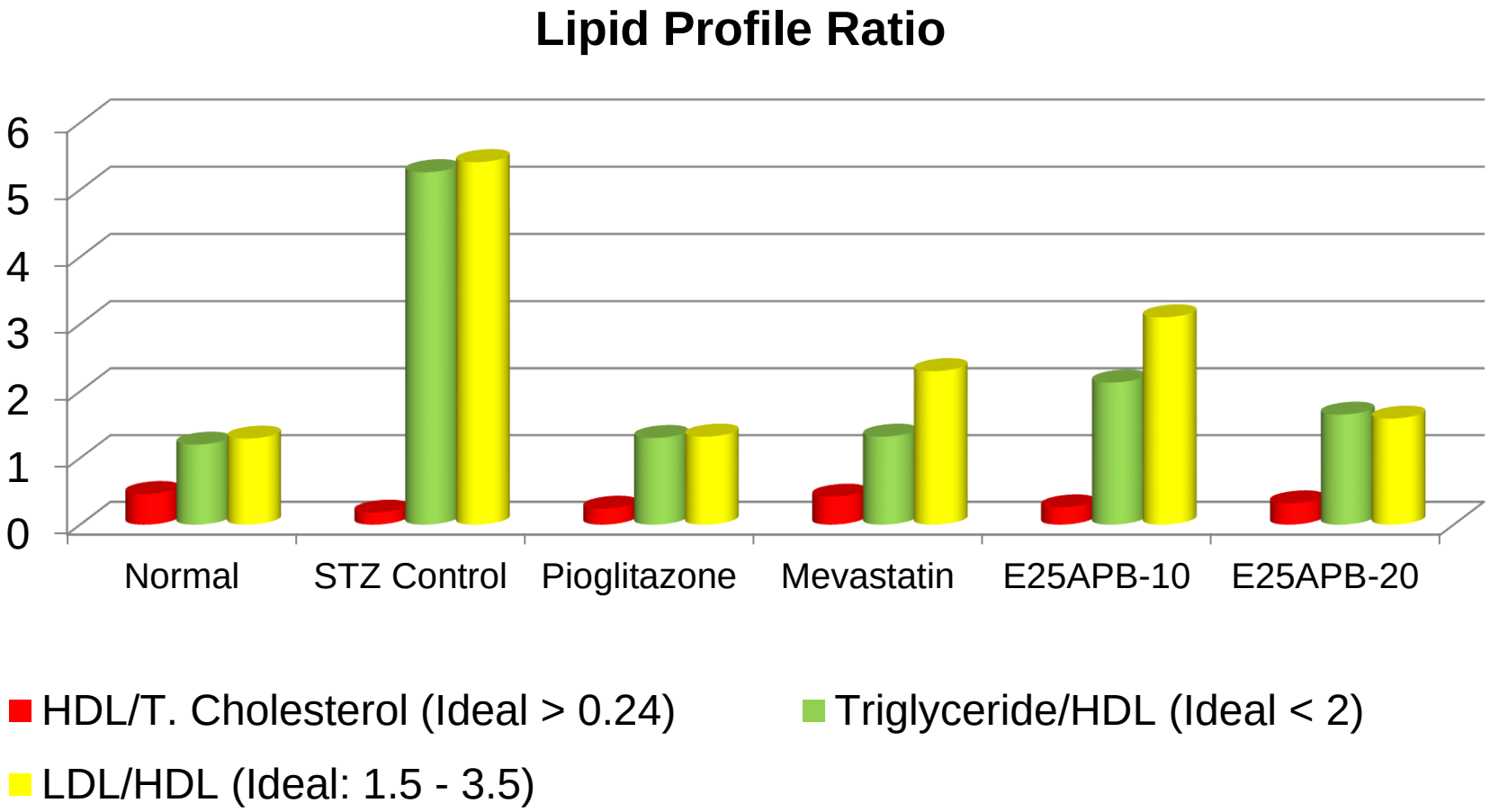
4.4 Results (antidiabetic study)

Effect on Serum insulin and Liver glycogen

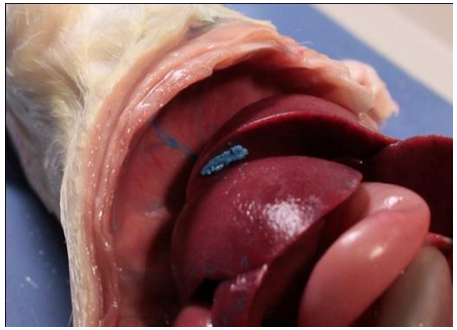


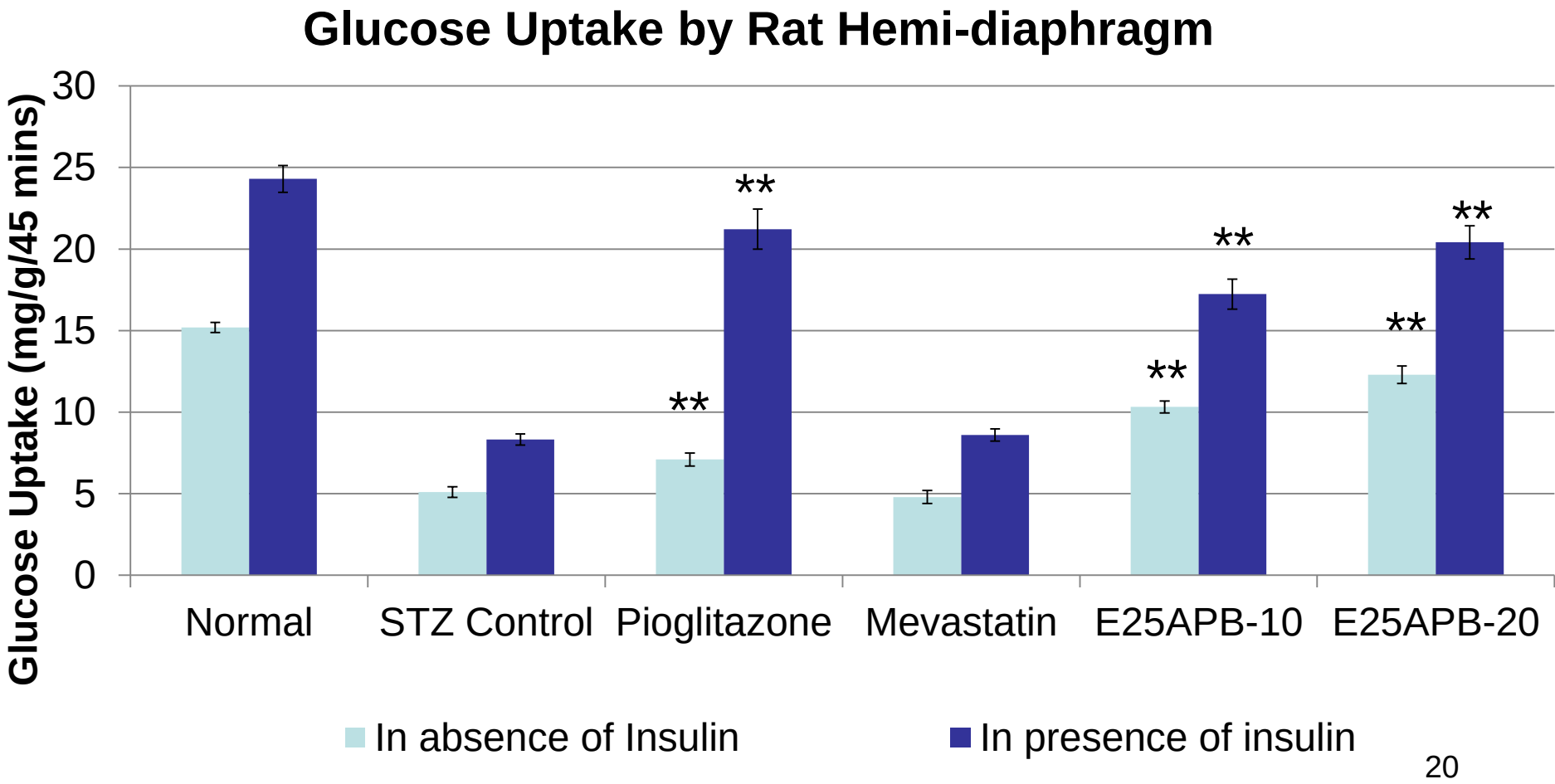
* = p < 0.05, ** = p < 0.01, n = 6 ANOVA –Dunnett test

4.3 Results (anti-hyperlipidemic study in diabetic rats)



4.6 Results




Glucose Uptake by Rat Hemi-diaphragm
Glucose Uptake (mg/g/45 mins)
30
25
20
15
10
5
0
**
**
**
**
**
Normal
STZ Control
Pioglitazone
Mevastatin
E25APB-10
E25APB-20
In absence of Insulin
In presence of insulin

4.7 Results (docking of ethyl 2-[5-(acetyloxy)pently] benzoate)

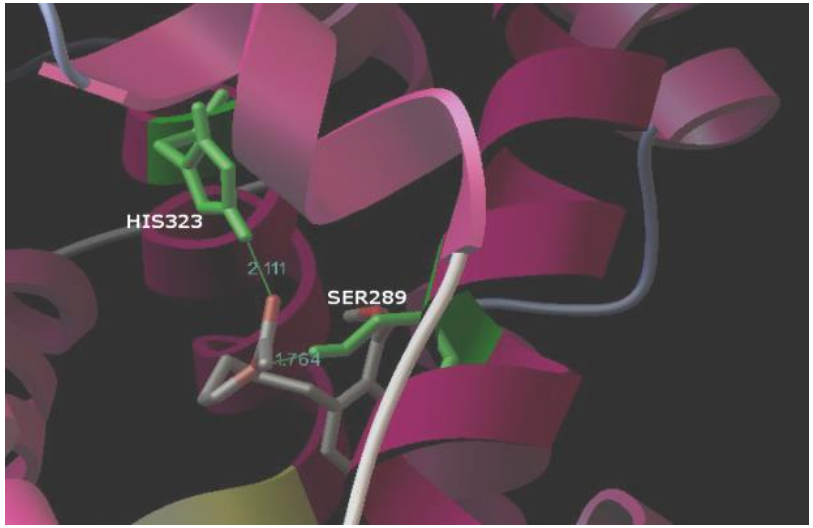
Software Assisted Molecular Docking Studies

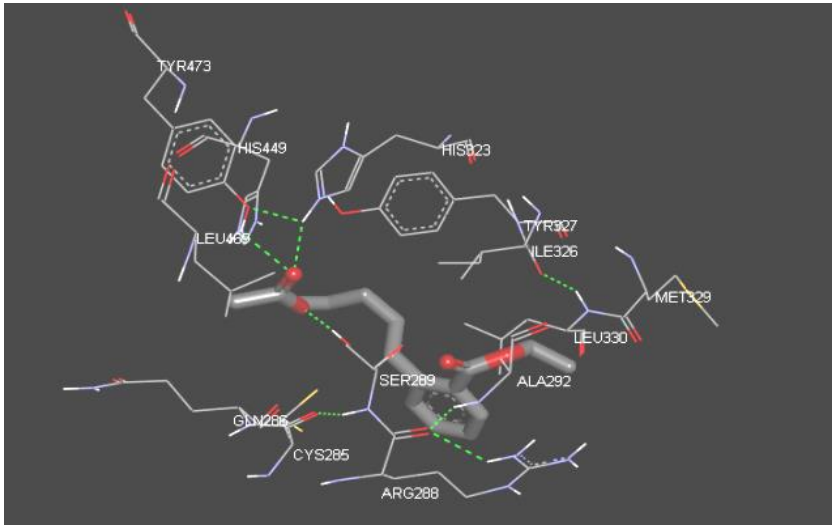
Name of lead molecule	Binding Energy	H-bonds	Participating residues	VdW + Hbond + desolv Energy
Ligand1-2XKW (38^{th} conformation)	-6.95 Kcal/mol	2	SER:289 HIS:323	-9.8
Pioglitazone (12^{th} conformation)	-5.0 Kcal/mol	1	SER:289	-7.69

4.7 Results (docking of ethyl 2-[5-(acetyloxy)pently] benzoate)

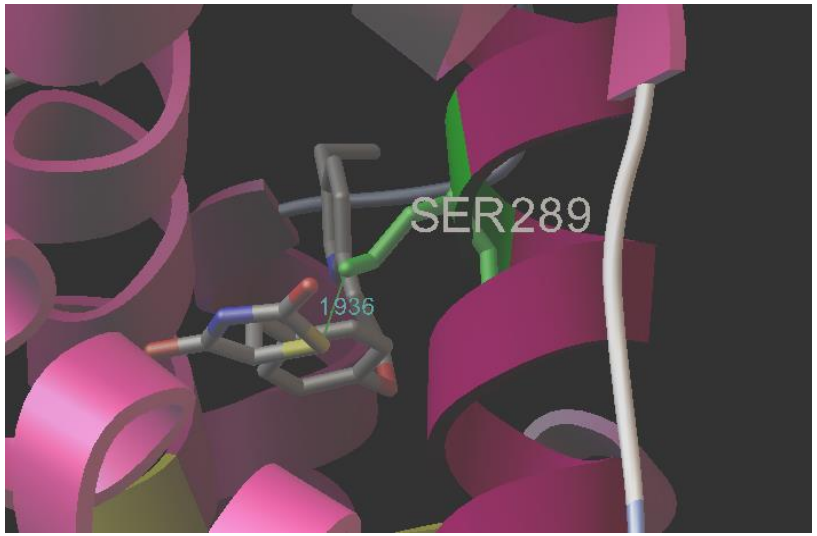
Software Assisted Molecular Docking Studies

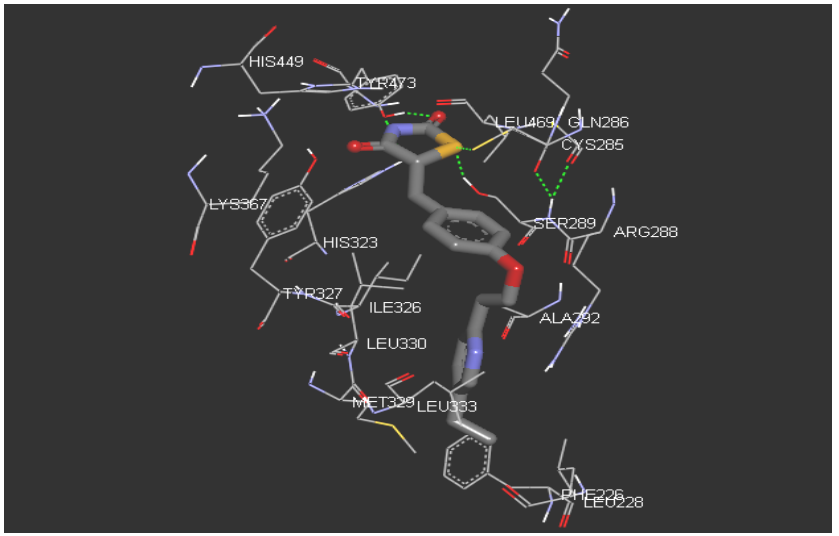
Ligand + PPAR γ





Pioglitazone + PPAR γ





4.7 Results (docking of ethyl 2-[5-(acetyloxy)pently] benzoate)

Comparison of Molecular Properties

Properties	ethyl 2-[5-(acetyloxy)pently] benzoate	Pioglitazone
Molecular Formula	$C_{16}H_{22}O_4$	$C_{34}H_{40}N_2O_8S$
Molar Refractivity	77.14 ± 0.3 cm^3	98.2 ± 0.3 cm^3
Molar Volume	262.6 ± 3.0 cm^3	282.8 ± 3.0 cm^3
Parachor	652.1 ± 4.0 cm^3	1331.68 cm^3
Refractive index	1.499 ± 0.02	1.611
Surface Tension	38.0 ± 3.0 dyne/cm	54.9 ± 3.0 dyne/cm
Density	1.059 ± 0.06 g/cm^3	1.3 ± 0.1 g/cm^3
Polarizability	30.58 ± 0.5 10^{-24} cm^3	38.9 ± 0.5 10^{-24} cm^3
Lipinski's "Rule of 5"/Ghose Rule parameters		
Mol. Weight (< 500)	278.343 Da	636.756 Da
H bond Donors (< 5)	0	1
H bond acceptors (< 10)	4	4
Log P (< 5)	3.15	2.94
Veber rule parameters		
Polar surface area (< 140)	52.61 A^2	68.295 A^2
Rotatable bond count (< 12)	10	7

5. Conclusions

- Salt glands of *O. majorana*
- The OMO & OMM of *O. majorana*: are rich source of phenolics and antioxidants
- The strong antidiabetic property of E25APB
    - a) protecting the β-cells
    - b) stimulating insulin release
    - c) Potentiating glycogenesis
- OM in the treatment of hyperlipidemia, CVS complications
- Glucose uptake & docking studies: PPAR-γ modulation effect

  ethyl 2-[5-(acetyloxy)pently] benzoate > pioglitazone
- Increased dietary intake of OM : concomitant management of diabetes and obesity

6. Research Publications

Asian Pacific Journal of Tropical Disease (2012)S897−S903

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage:www.elsevier.com/locate/apjtd

Document heading doi:

Microscopic evaluation and physicochemical analysis of *Origanum majorana* Linn leaves

BP Pimple[1*], AN Patel[2], PV Kadam[2], MJ Patil[2]

[1] *P. E. Society's Modern College of Pharmacy, Nigdi, Pune, Maharashtra, India. 411 044 and Jawaharlal Nehru Technological University, (JNTU), Hyderabad, Andhra Pradesh, India 500072*

[2] *Marathwada Mitra Mandal's College of Pharmacy, Thergaon, Pune, Maharashtra, India 411 033*

6. Research Publications

Orient Pharm Exp Med (2012) 12:41–50
DOI 10.1007/s13596-011-0047-x

Springer

RESEARCH ARTICLE

Comparative antihyperglycaemic and antihyperlipidemic effect of *Origanum majorana* extracts in NIDDM rats

B. P. Pimple · P. V. Kadam · M. J. Patil

6. Research Publications

312

Asian Pacific Journal of Tropical Disease (2012)312-318

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage:www.elsevier.com/locate/apjtd

Document heading

Ulcer healing properties of different extracts of *Origanum majorana* in streptozotocin–nicotinamide induced diabetic rats

BP Pimple[1,2*], PV Kadam[3], M J Patil[3]

[1]*P. E. Society's Modern College of Pharmacy, Nigdi, Pune-411044, Maharashtra, India*

[2]*Jawaharlal Nehru Technological University, Hyderabad, Andhra Pradesh, India*

[3]*Marathwada Mitra Mandal's College of Pharmacy, Thergaon, Pune-411033, Maharashtra, India*

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UGC

University Grants Commission

Thank you