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Formulation & Evaluation of Levamisole Tablet

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Abstract: *The aim of current study was to develop suitable gastroententive tablet of levamisole HCL for prolonging the retention of levamisole in stomach, floating tablets were prepared by using HPMC K4M and carbopol 934p in combination and their effect on floating, swelling and release of levamisole HCL was studied. optimization of drug release were carried out by taking different concentration of HPLC K4M and carbopol 934p. It was found that HPMC and carbopol with concentration 32 % and 22% in tablet give satisfactory release and batch was selected for 32 factorial design*

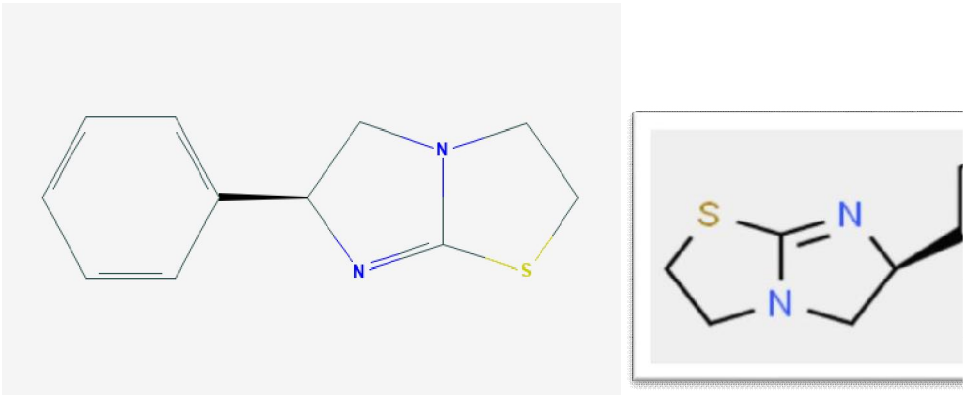
Keywords: gastroententive

I. INTRODUCTION

LITERATURE REVIEW

• Name of the Drug – LEVAMISOLE
• IUPAC Nomenclature - 6-phenyl-2,3,5,6-tetrahydroimidazo[2,1-b] [1,3] thiazole
• Molecular Formula - C11H12N2S
• Identity – CAS No. 16595-8-5; Merck Index 9055; Janessan Res. Code R12564.
• Synonyms – L – Tetramisole, (-)-Tetramisole, Ergamisol®, Wormicid, Vermisol 150, Ketrax
• Drug Class – Organo-heterocyclic compound – Imidazothiazoles
• Drug Category – Anthelmintic, Anti-infective, Anti-parasitic, Anti-nematodal, Immunomodulator, Anti-rheumatic, Insecticide – Dewormer, Adjuvant therapy for Cancer.
• Commercial Preparation – Levamisole is the laevorotatory S- (-) isomer of Tetramisole.

Structures –



(a) (b)

Fig.1. (a) and (b) Chemical structure of Levamisole

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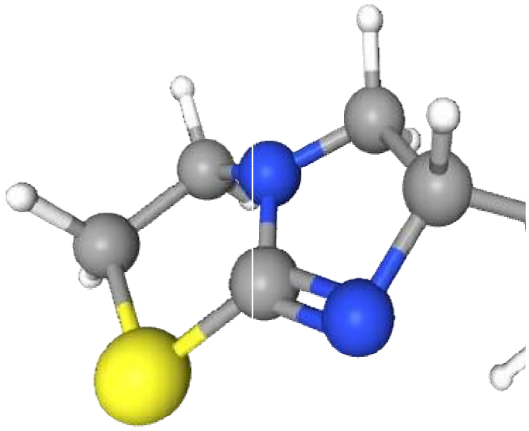

Fig.2. Three-Dimensional Chemical Structure of Levamisole

DISCOVERY, HISTORY, AND BACKGROUND OF LEVAMISOLE

Levamisole is an anti-helminthic drug that was commonly used for the treatment of parasitic, viral, and bacterial infections. It was manufactured by Janssen and first used in 1969 as an agent to treat worm infestations. Levamisole was approved by the FDA in 1990 as an adjuvant treatment for colon cancer. Before this, levamisole was used as an antirheumatic therapy in the 1970s and 1980s for patients with rheumatoid arthritis. Because of its immunomodulatory effects, this drug has been studied in the treatment of various immune- mediated diseases, with some studies showing positive results. This drug has also been used in combination with other drugs for the treatment of various cancers. Levamisole was withdrawn from the American market in 2000 due to its ability to cause serious adverse effects, including agranulocytosis.

In the early sixties during routine screening activities, imidazothiazole derivatives were discovered to have anthelmintic properties. Further research programmes, trying to optimize potency and safety, produced in 1964 the racemic compound tetramisole (R8299), which soon found a wide application in the treatment of helminth infections. It was rapidly discovered that the anthelmintic activity of the racemate resided in the Levo-isomer levamisole (R12564). At present, levamisole continues to be a major broad-spectrum anthelmintic for the treatment of lungworm and gastrointestinal nematodes in man as well as in animals and is used as a single 5- 40 mg/ kg dose, dependent on the parasite species involved (Miller, 1980). From the very start of the clinical use of levamisole, people noticed that this drug did more than just kill worms and had some beneficial effect on host defense mechanisms (Janssen, 1976). The work of Renoux & Renoux (1971) formally confirmed the latter property and triggered multi-disciplinary research efforts to explore this revolutionary characteristic. Briefly, stated, levamisole was found to possess anti-anergic properties as it restored depressed immune functions in both animals and men. But the initial enthusiasm and expectations gradually also gave rise to a profound skepticism fed by the drug's sometimes unpredictable behavior and the poor correlation between immunological changes and the effect on the disease. Eventually, the occurrence of some side effects (Symoens, Veys, Mielants & Pinals, 1978) further contributed to a gradually declining interest in levamisole as an immunotherapeutic drug in the eighties. In 1989/1990, however, interest in levamisole was re-activated by three major multicentre studies which identified levamisole as an efficacious adjunct in the therapy of melanoma (Quirt et al., 1991) and of colon carcinoma (Laurie et al., 1989; Moertel et al., 1990).

Levamisole is highly effective against a wide variety of gastrointestinal nematodes, filariids, and lungworms parasitizing cattle, sheep, goats, 189 pigs, cats, dogs, and poultry. In cattle, levamisole shows high activity against the mature and immature nematodes of the gastrointestinal tract, and the lungworms, Dictyocaulus viviparus at a dose of 7.5 mg/kg. When administered subcutaneously at a dose of 5-10 mg/kg, levamisole exhibited 98-99% activity against

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D. viviparus in calves. The drug is also active against the adult worms of Parafilaria bovicola and Stephanofilaria okinowaensis in cattle at a dose of 7.5 and 15 mg/kg, respectively, given for two days.

Levamisole possesses better activity than its racemic form tetramisole. At a single oral dose of 2.5-5 mg/kg, the drug has been found to give 100% cures against ascariasis in humans. The recommended dose of levamisole for treating A. lumbricoides is 40-80 mg for children and 150 mg for adults. It also exhibits high activity against the hookworms, A. duodenale, and N. americanus. A typical treatment using 2.5 mg/kg of levamisole may bring out about 90% cures against ascariasis and 80% cures against hookworm infections. However, its activity against enterobiasis, trichuriasis, and strongyloidiasis is not very encouraging.

PHARMACEUTICAL MARKET REVIEW OF LEVAMISOLE

Levamisole was discovered in 1966. It is on the World Health Organization's List of Essential Medicines. It is not commercially available in the United States. It was manufactured by Janssen and first used in 1969 as an agent to treat worm infestations. Levamisole was approved by the FDA in 1990 as an adjuvant treatment for colon cancer. Before this, levamisole was used as an antirheumatic therapy in the 1970s and 1980s for patients with rheumatoid arthritis. Levamisole was withdrawn from the American market in 2000 due to its ability to cause serious adverse effects, including agranulocytosis. Similarly, this withdrawal of drug affected the global market. It reduced the demand for the drug in the market and only veterinary use was recommended. Levamisole was then used as a cutting agent or bulking agent in various illicit drugs such as Cocaine and Heroin. This aspect increased its demand and also illegal ways of supplying and manufacturing.

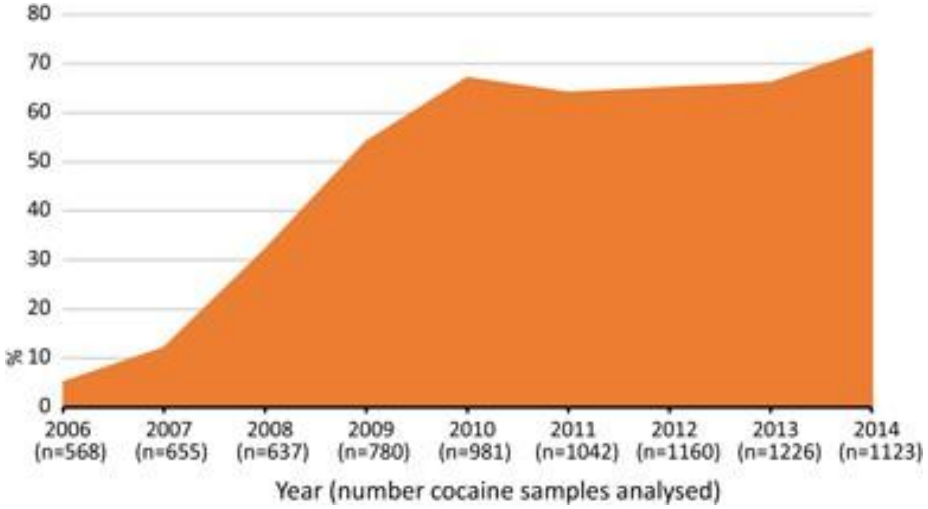

Fig.3. Percentage of Cocaine-Tainted with Levamisole since 2006 to 2014

In the global pharmaceutical market, China shares a large percentage in the production of Levamisole HCl drug. The major players in the Levamisole HCl market include:

- Guilin Nanyao
- Haisheng Pharmaceutical
- Wuhan Dongkangyuan Technology
- Yangzhou Huaxing Chemical
- Shenzhen Simeiquan Biological Technology

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It is available in various pharmaceutical formulations such as tablets, syrups, powder preparation, capsules, cream, gel, ointment, and ointment. Levamisole was marketed under the brand name Ergamisol®. After the expiry of the patent, it is available in various trade names:

Dicaris	Ethnor	Paediatric Tablet	50mg
Vermisol	Klandelwal Laboratories Ltd.	Syrup	50mg/5ml
Dewormis	Biddle Sawyer	Tablet	150 mg
Vermisol	Khandelwal Laboratories Ltd.	Tablet	150 mg
Dicaris	Ethnor	Tablet	150 mg
Vapal	owan Bioceuticals	Tablet	150 mg
Dewormis	Biddle Sawyer	Tablet	50 mg
Levomol	Cipla Limited	Tablet	50 mg
Vermisol	Khandelwal Laboratories Ltd.	Tablet	50 mg

Table. No. 1. Trade names of various Levamisole HCl products

II. DRUG PROFILE

2.1. Physicochemical properties of Levamisole: -

• Physical state: - Solid
• Appearance: - White crystalline powder (Stable)
• Molecular weight: - 204.29 g/mol
• Melting Point: - 227 - 229ºC
• Odour and Taste: - Bitter and Characteristic Odour
• Partition coefficient (Log P): - 1.84 (in Water and Octanol)
• Optical Rotation: - [α] 30 = -124º ± 2º (c=0.9 in water)
• Refractive Index: - 1.713
• UV-Visible Maxima: - 214nm ±2nm in 0.1N HCl
• Solubility: - Freely soluble in water, soluble in ethanol (96%), slightly soluble in methylene chloride.
• Marketed as: - Salt form – Levamisole HCl
• Source: - Synthetic
• Chemistry of Drug: - Levamisole belongs to the class of organic compounds known as imidazothiazoles. These are organic polycyclic compounds containing an imidazole ring fused to a thiazole ring. Imidazole is a 5-membered ring consisting of three carbon atoms, and two nitrogen centres at the 1- and 3-positions. Thiazole is a 6- membered ring that contains both sulfur and nitrogen.

2.2. Mechanism of Action of Levamisole: -

The mechanism of action of levamisole as an antiparasitic agent appears to be tied to its agonistic activity towards the L-subtype nicotinic acetylcholine receptors in nematode muscles. This agonistic action reduces the capacity of the males to control their reproductive muscles and limits their ability to copulate. It has long been used as an anti-helminthic where its primary action is through the opening of acetylcholine receptor channels. It is widely used as an immunomodulatory drug through its action on dendritic cells and by enhancing the release of cytokines like interleukins 12 and 10. This imidazothiazole derivative has a thiol group and an imidazole ring.

The imidazole ring with its cholinergic properties helps in interleukin 2 [IL-2] induced lymphocyte proliferation which is responsible for its immunomodulatory effect. The antioxidant action of levamisole is by the enhancing effect on the major cellular redox systems like glutathione, enzymes like superoxide dismutase, catalase, and also possible effects on glutathione-related enzymes. On the other hand, the anti-inflammatory action is by the inhibition of TNF-alpha (tumor necrosis factor) and interleukin-6. Anti-neoplastic action of levamisole is by two ways that are, through its apoptotic action causing cell cycle inhibition and increased endothelial cell adhesion and the second action is through its anti-angiogenic property.

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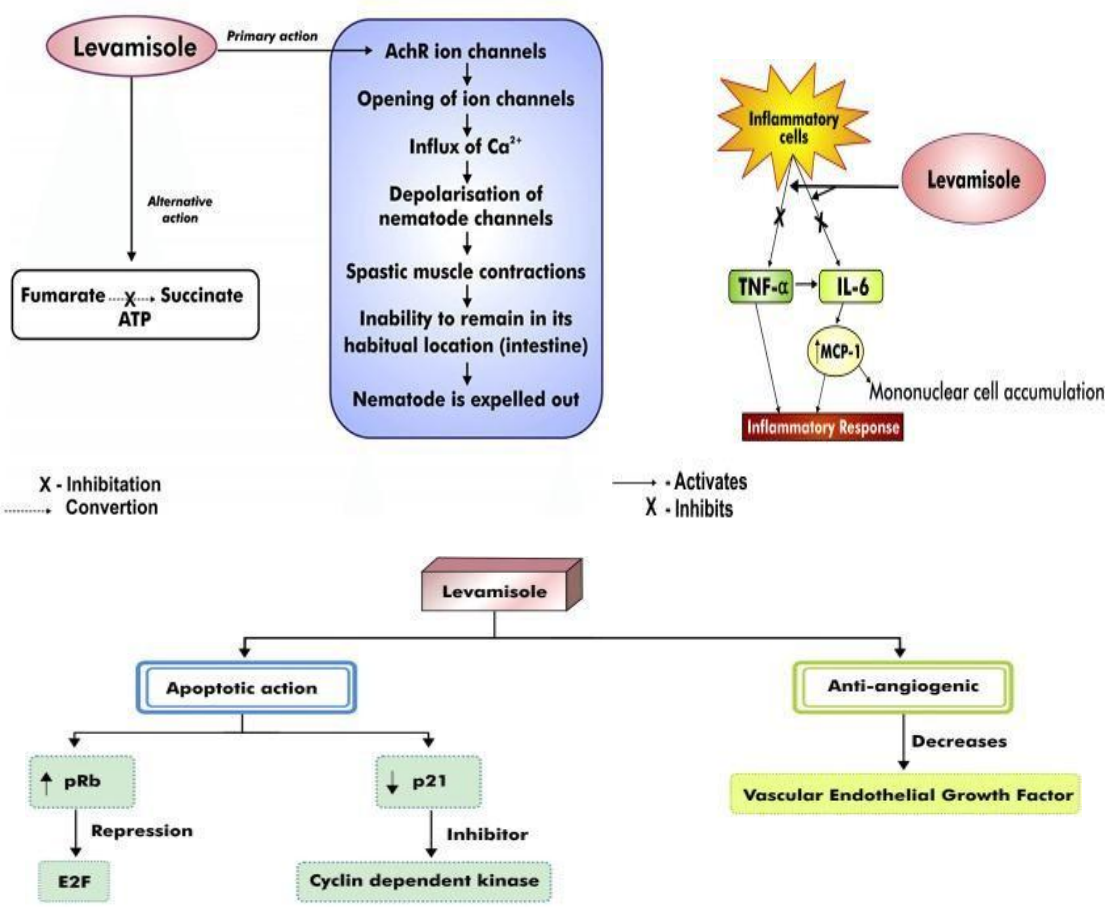

Fig.4. Modes of action of Levamisole

2.3. Pharmacological aspects of Levamisole: -

1) Drug Pharmacokinetics:

• Absorption: - Levamisole is readily absorbed independent of the route of administration. It is rapidly absorbed from the gastrointestinal tract and peak plasma concentration is achieved within 1.5 to 2 hours.

• Distribution: - Levamisole is well distributed in body fluids such as blood, plasma, and urine. It shows protein binding in plasma around 20-25%. It has a plasma half-life (t1/2) of 4 to 5 hours.

• Metabolism: - Levamisole is rapidly metabolized to a large number of metabolites. The metabolism occurs primarily hepatic (extensive) with both active and inactive metabolites in the Liver. The metabolite half-life is 16 hours. The major pathways seen in all species included scission of the thiazolidine ring followed by aliphatic oxidation to a carbonyl and hydrolysis to a thiohydantonic acid to form metabolite R92535. R92535 metabolite is a major (26 – 47%) metabolite and found in urine readily. Hydrolysis of the thiazolidine ring followed by S-methylation and sulfoxidation (R43037). The other major pathway in dogs, pigs, sheep, and cattle was dehydrogenation in the imidazolidine ring followed by sulfoxidation (R66003). With human hepatocytes, other major metabolites were a result of dehydrogenation in the imidazolidine ring (R45714) and aromatic hydroxylation (R9313).

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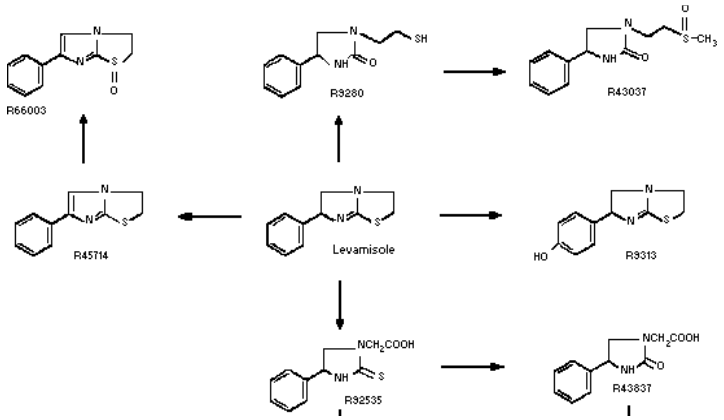

Fig.5. Major metabolites of Levamisole

Excretion: - Levamisole's excretion is primarily through the kidneys, with about 70% being excreted over 3 days and small amounts in the faeces. Only about 5% is excreted as unchanged levamisole. The plasma elimination half-life is fairly quick at 4.4–5.6 hours (biphasic).

2) Adverse Reactions and Side-effects:

 Common side-effects: Nausea, vomiting, diarrhoea, abdominal pain, dizziness, and headache. Fever, influenza-like syndrome, arthralgia, muscle pain, rash, taste disturbances, and cutaneous vasculitis.

 Potentially Fatal: Agranulocytosis, leucopoenia, thrombocytopenia.

 Rare side-effects: Confusion, Paranoia, Tardive dyskinesia, Muscle Tremors, blurred vision, Liver damage, Seizures.

 One of the more serious side effects of levamisole is agranulocytosis or the depletion of the white blood cells. In particular, neutrophils appear to be affected the most.

3) Contraindications:

 Levamisole is contraindicated in pregnancy. It is a drug of Category C: Either studies in animals have revealed adverse effects on the foetus (teratogenic or embryocidal or other) and there are no controlled studies in women or studies in women and animals are not available. Drugs should be given only if the potential benefit justifies the potential risk to the foetus.

 Levamisole is contraindicated in lactating women because as it may pass into breast milk.

 Levamisole is contraindicated in patients with a history of pre-existing blood disorders, severe kidney problems, and swelling (inflammation) of joints.

 Levamisole is contraindicated in patients with a known hypersensitivity to the drug or its components.

4) Drug Interactions:

Levamisole can cause serious problems when taken with few medicines at the same time or have recently taken any of the following –

• Phenytoin
• Tegafur
• Doxifluridine
• Warfarin
• Capecitabine
• BCG vaccine
• Live polio vaccine
• Typhoid vaccine
• Influenza virus vaccine

No specific interactions with food have been reported yet.

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5) Overdosing:

The effects of an overdose may be lethal and need immediate medical attention. Symptoms include nausea, vomiting, increased salivation, frequent urination and defecation, colicky pain in the abdomen, dizziness, headache, muscle tremors, anxiety, irritability, clonic convulsions, depression, rapid respiration, difficulty in breathing, collapse, bleeding in the intestines, inflammation of bowels, severe liver damage, and congestion of blood in the spleen.

6) Precautions and Warning:

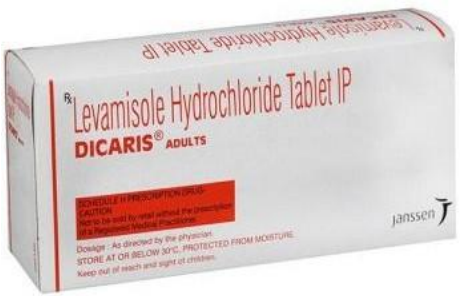
1. Levamisole should not be consumed with alcohol as it may increase the risk of some side effects. It has been reported to produce ―ANTABUSE‖ like side effects.
2. You may feel dizzy or tired while taking this medicine. Do not drive unless you are feeling well.
3. Special precautions need to be taken in rheumatoid arthritis, Sjogren's syndrome, epilepsy, and hepatic impairment where dosage adjustment may be necessary.

2.4. Various uses of Levamisole: -

1. Levamisole was originally used as an anthelminthic to treat parasitic worm infections in both humans and animals. It was the drug of choice for the treatment of Ascariasis in humans. After being pulled from the market after the year 2000, it was only recommended for veterinary use by FDA.
2. Most current commercial preparations of Levamisole are intended for veterinary use as a dewormer in cattle and livestock.
3. Levamisole has also recently gained prominence among aquarists as an effective treatment for Camallanus roundworm infestations in freshwater tropical fish.
4. Levamisole has also been used experimentally and historically to treat various autoimmune disorders in humans due to its Immuno-modulatory action. It is used as an immunomodulator in the treatment of drug-resistant T.B.
5. The combination of the antimetabolite 5-fluorouracil plus the immunomodulator levamisole is used as adjuvant therapy following surgical tumor removal in patients with Dukes' stage C colon cancer, this combination is now recommended as standard therapy in these patients.

2.5. Various Marketed Products in India: -

1. Bio-vam by Biochemix
2. Dewormis by GSK pharma
3. Dicaris by J&J (Ethnor)
4. Jetomisol by J&J (Ethnor)
5. L-vin by Intra labs
6. Levomol by Cipla
7. Vermisol by Khandelwal labs (Xenon)
8. Eradix by Sresan (Combination therapy)
9. Exit by Brawn (Combination therapy)



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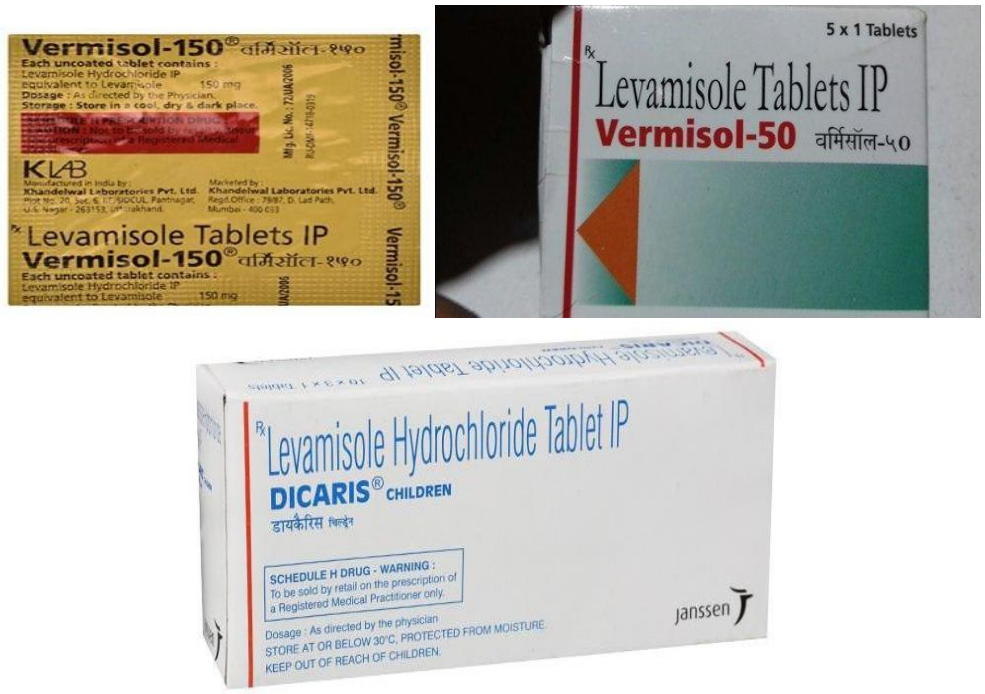

Fig.6. Various marketed products in India

III. METHODS OF SYNTHESIS

3.1. Isolation from Tetramisole Racemate

Initially, Tetramisole is synthesized from various reactants and further, the racemate is treated for isolation of Levamisole. The resolution of tetramisole into levamisole, L-(-) [α]D 25 = -
54.7 (H20), and D-(+) [α]D 25 = + 83.0 (H20) enantiomers has been achieved using d-10- camphorsulphonic acid.

a) From α-Bromoacetophenone: -

From α-bromoacetophenone, which is reacted with 2-imino-1,3-thiazolidine to make the N- alkylated product 1-phenacyl-2-imino-1,3-thiazolidine (38.1.23). Acylating the product with acetic anhydride makes the 2-acetylimino derivative (38.1.24). Reduction of the ketone carbonyl group in this compound with sodium borohydride leads to the formation of the key product of the synthesis—1-(2-phenyl-2-hydroxyethyl)-2-acetylimino-1,3-thiazolidine (38.1.25). Replacing the hydroxyl group in this compound with chlorine using thionyl chloride and subsequent treatment of the product with acetic anhydride results in a heterocyclization reaction to a racemic mixture of (-)- 2,3,5,6-tetrahydro-6-phenylimidazo[2,1-b]thiazoles (38.1.26), which is also called tetramisole. Treating this with D-10-camphorsulfonic acid isolates the desired L-isomer— Levamisole.

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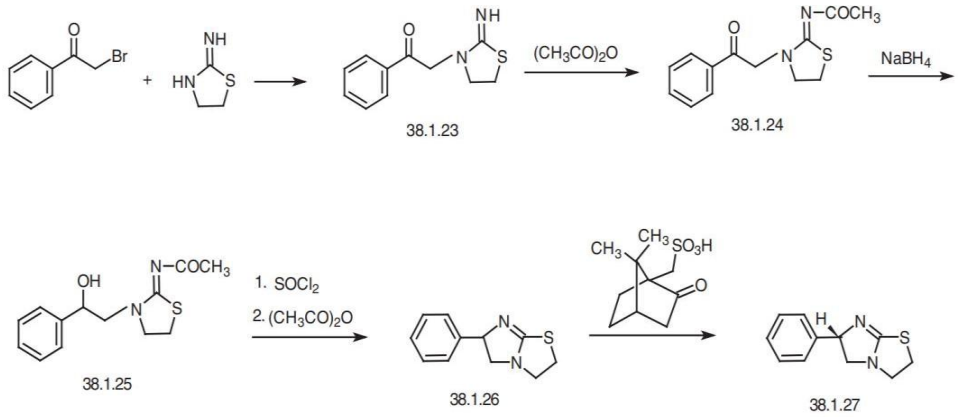
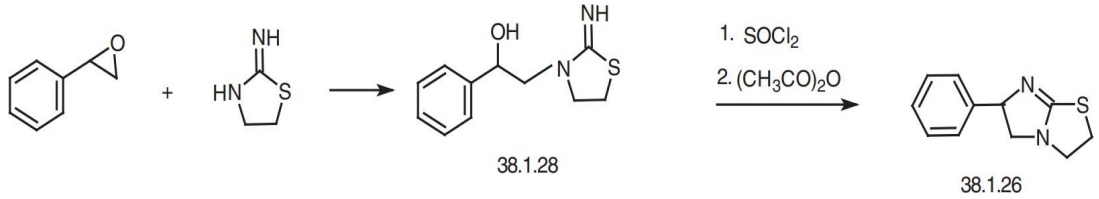


Scheme.1. Synthesis of Levamisole from α-Bromoacetophenone

b) From Styrene oxide: -

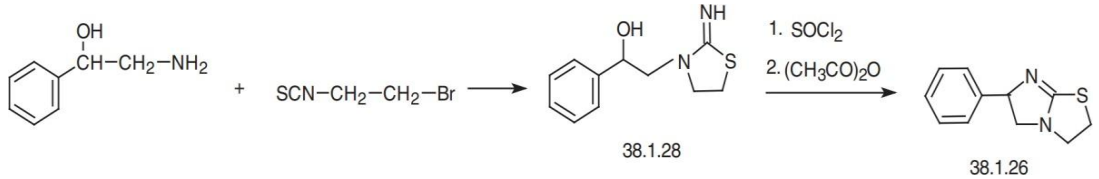
A reaction of styrene oxide and 2-imino-1,3-thiazolidine and subsequent treatment of the resulting product 1-(2-phenyl-2- hydroxyethyl)-2-imino-1,3-thiazolidine (38.1.28) with thionyl chloride and then with acetic anhydride, which leads to the formation of tetramisole. Treating this with D-10-camphorsulfonic acid isolates the desired L-isomer—Levamisole.



Scheme.2. Synthesis of Levamisole from Styrene oxide.

c) From 2-amino-1-phenyl ethanol: -

Reacting 2-amino-1-phenyl ethanol with 2-Bromo ethyl isothiocyanate. This leads to the direct formation of the key 1-(2-phenyl-2- hydroxyethyl)-2-imino-1,3-thiazolidine (38.1.28), which is transformed to tetramisole by a subsequent reaction with thionyl chloride, and then with acetic anhydride.



Scheme.3. Synthesis of Levamisole from aryl amino alcohols.

3.2. Chiral synthesis of Levamisole: -

1. Raghu et al. have prepared optically active imidazolidinones (39), which were cyclized under different conditions to form levamisole though with a rather poor enantiomeric excess (ee = 21-33%). Optically active imidazolidinone is prepared by reacting phenacyl bromide and 4-amino-1-methoxy ethane via forming intermediate N-(1-methoxy ethane) Acetophenone (38). Then it is N-acylated with acyl chloride and further reduced in presence of catalysts. In the last, it is treated with P2S5 in HCl resulting in cyclization and formation of S(-)- Tetramisole which is Levamisole.

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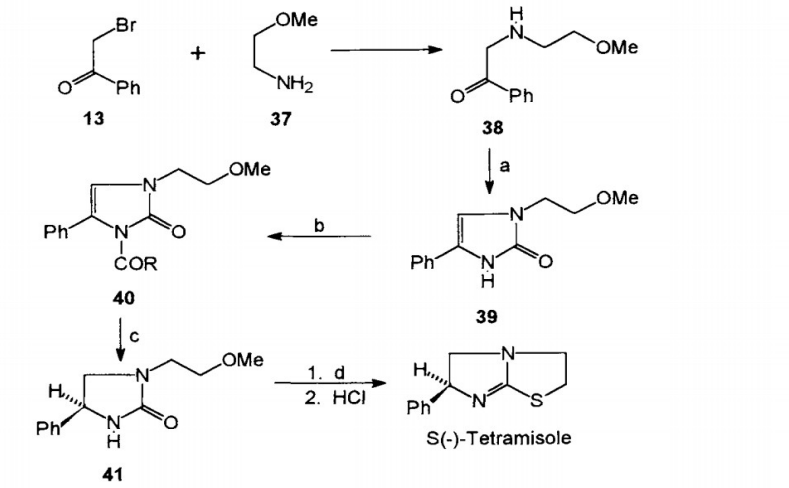

(a) KOCN; (b) RCOCl; (c) hydrogenation in presence of cyclooctadiene-$RhCl_3$ dimer and (+)-2,3-O-isopropylidene-2,3-dihydroxy-1,4-bis(diphenylphosphino)-butane or (+)-DIOP-Rh (cyclooctadienyl)chloride as catalysts; (d), (i) P_2S_5, (ii) HCl.

Scheme.4. Chiral synthesis of Levamisole by Raghu et. Al.

2. Rama Rao et al. have reported a chiral synthesis of S(-)-tetramisole starting from the optically active alcohol (44), which was prepared with 97% ee by reducing phenacyl chloride
(42) with oxazaborolidines (43) in the presence of BH3. It is reacted with methanol in presence of a base (2M NaOH) to give styrene oxide (45). It is treated with NaN3 in DMF to form 2-azido-1-phenyl ethanol (46). Treating it with Diethyl Azidocarboxylate (DEAD) in presence of Triphenylphosphine (TPP) and N2H4 yields 1-amino-1-phenyl ethan-2-amine
(47). On further reduction with palladium on carbon and CS2 in KOH forming a cyclic intermediate compound 2-mercapto-5-phenyl imidazole (48) which is finally treated with dibromoethane in presence of KOH which leads to S(-)-tetramisole which is Levamisole.

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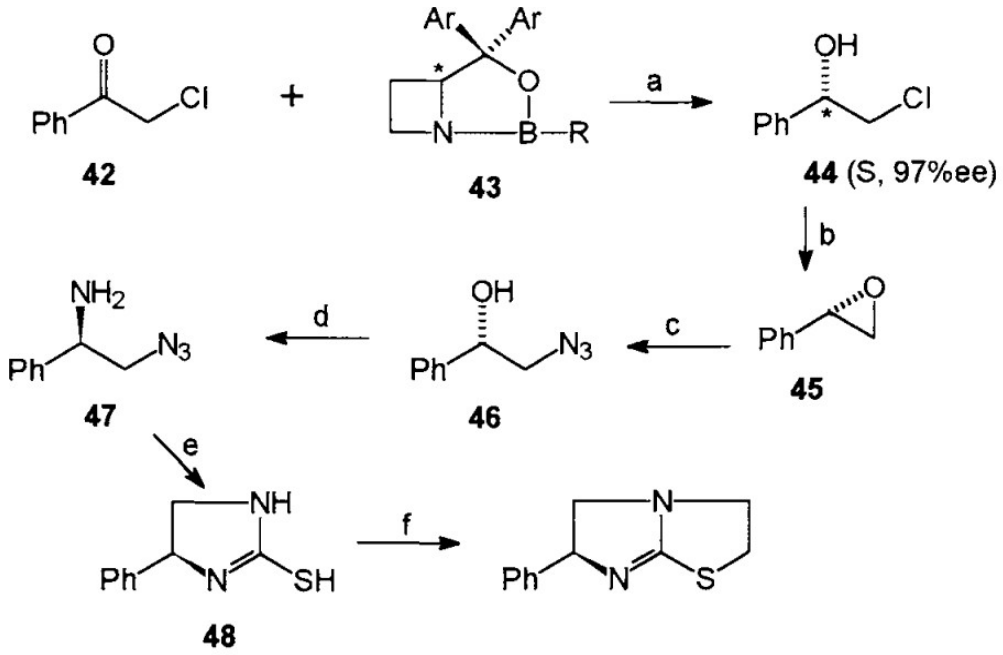

(a), BH_3; (b), 2M NaOH-MeOH; (c), NaN_3, DMF; (d), (i) DEAD, TPP, (ii) N_2H_4; (e), (i) Pd-C, H_2, (ii) CS_2,KOH; (f), as in ref. 3 and 5.

Scheme.6. Chiral synthesis of Levamisole by Rama Rao et al.

3.3. Asymmetric Strecker reaction: -

The synthesis of levamisole depicted features an asymmetric Strecker reaction of N- benzhydryl aldimine A with trimethylsilyl cyanide catalyzed by oxazoline (R, R)-B (5 mol%) as the key step. The chiral α-aminonitrile intermediate C was generated in 90% yield and 90% ee. A study of the scope of the asymmetric Strecker reaction (18 examples) revealed that both alkyl and aryl N-benzhydryl aldimines participate in the reaction to give the corresponding α-aminonitriles in good yield and generally >80% ee with some exceptions.

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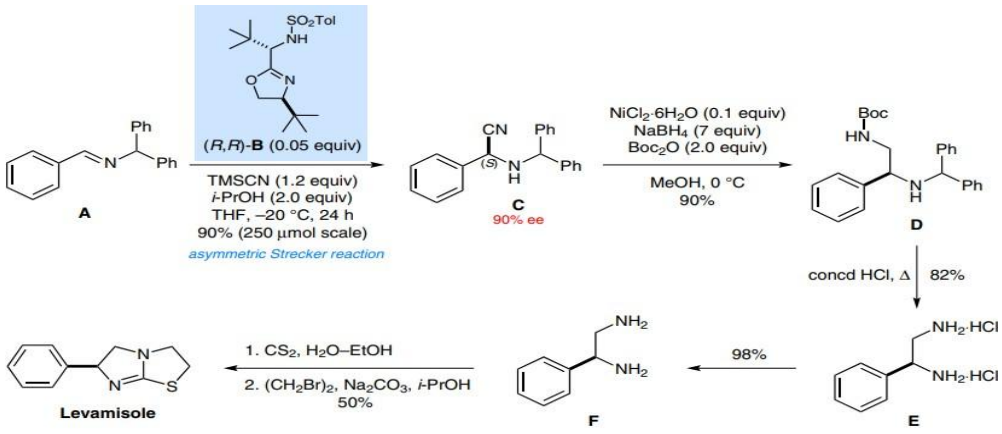

Scope of the substrates in the asymmetric Strecker reaction catalyzed by (*S*,*S*)-B:

X	Yield (%)	ee (%)
Me	90	94
OMe	93	96
F	78	91
Cl	90	82
Br	93	80
NO_2	90	82

R	Yield (%)	ee (%)
Bn	90	91
CH_2Bn	90	87
t-Bu	91	93
i-Bu	94	90
Hex	90	71
CH=CHPh	93	90

85% (60% ee)

94% (82% ee)

Scheme.7. Asymmetric Strecker reaction for synthesis of Levamisole.

CONCLUSION

 It is concluded that the ODT tablets have shown that higher disintegration time and improved dissolution and bioavailability of the drug.

 Here the flow properties of both drug and poly- mers is good.

 Here the FT-IR studies is to determine the no

 chemical interaction between Levamisol and pol- ymers used in the study are compatible to each other.

 Here the tablets preparation was good and with- out any chipping capping and sticking.

 All the formulations shows good results of physi- co-chemical evaluations.

 levamisole tablets were successfully formulated by optimizing the concentration of super disintegrants such as sodium starch glycolate,

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