CHAPTER - III

STUDIES ON *MOMORDICA DIOICA* Roxb

3.1 Introduction

Momordica dioica Roxb belongs to the family Cucurbitaceae. The other members from the same genus are *Momordica charantia* Linn, *Momordica balsamina* Linn, *Momordica cochinchinensis* Spreng, *Momordica tuberosa* Cogn and *Momordica umbellata* Roxb. It is a perennial dioecious climber with tuberous roots found throughout India, ascending to 5000ft in the Himalayas. Leaves are ovate, mucronate, base emarginate and variously lobed. Fruit 2.5 - 6.3 cm long, ellipsoid, shortly beaked, densely echinate with soft spines. Edible portion of the fruit consists of moisture - 84.1%, protein-3.1%, ether extract 0.97%, carbohydrate - 7.7%, fibre 2.97%, and ash 1.1%. It also contains iron 4.6 mg, calcium 33 mg, phosphorus 42 mg, vitamin A 2,700 IU, thiamine 45.2 mg, riboflavin 176.1 μg, and niacin 0.5 mg/100g. The fruit also contains 275.1 mg of ascorbic acid/100g. Phytohaemagglutinin from the cotyledons have also been reported.

Momordica dioica Roxb (photograph 3a) is commonly known by the following vernacular nomenclature.

Assam - Bhatkarela
Bengali - Ban-Karela
Hindi - Kaksa
Kanada - Karlikai
Marathi - Kartoli
Punjabi - Kakaura, Kirara
Sanskrit - Vahisi
Tamil - Tholoopavai, paluppakai
Telugu - Agakara
Fruits are used as vegetables and also used in the treatment of inflammation caused by lizard excretion, mental and digestive disorders. The whole plant is known for its use in the treatment of eye diseases, poisoning, and fever. Fruit powder or infusion of dried fruits produces a powerful errhine effect in nostrils and provokes a copious discharge from the nasal mucous membrane.
3.2 Literature Survey

3.2.1 Phytochemical Review

Ali and his co-workers\textsuperscript{9} reported Momordicaursenol from the seed of \textit{Momordica dioica}.

\begin{itemize}
  \item 6-methyl-tritriacont-5-on-28-ol(I),
  \item 8-methyl-hentriacont-3-ene(II)
\end{itemize}

\textbf{Pleuchiol (III)}

\textit{stigmata-5,11(12)-dien-3\beta-ol}

\textbf{Momordicaursenol (IV)}

\textit{Urs-12,18(19)-dien-3\beta-ol}

Sadyojatha et al\textsuperscript{10} isolated (V) from the root of this plant

\textbf{\textit{\textbf{\beta-sitosterol (V)}}}

\textit{Stigmast-5-en-3-ol}

From the root, Bryonolic acid(VI), Cucurbitacin-F (VII), Gypsogenin(VIII), Hederagenin(IX), Stearic acid(X), \textit{\textit{\textit{\textit{\alpha}}}-Spinosterol(XI) and Ursolic acid(XII) were isolated by Luo\textsuperscript{11,12}.}
<table>
<thead>
<tr>
<th>Compound</th>
<th>Formula/Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bryonolic acid (VI)</td>
<td><strong>Cucurbitacin-F (VII)</strong>&lt;br&gt;2β, 9α, 16α (23E)-25-(acetoxy-oxy)-2,16, 20-trihydroxy-9-methyl-19-norlanosta-5, 23-dien-3-11, 22-trione-1, 2-dihydro-α-elaterin</td>
</tr>
<tr>
<td>Gypsogenin (VIII)</td>
<td>3-hydroxy-23-oxo-12-oleanen-28-oic acid</td>
</tr>
<tr>
<td>Hederagenin (IX)</td>
<td>23-dihydroxy-12-oleanen-28-oic acid</td>
</tr>
<tr>
<td>Stearic acid (X)</td>
<td>CH$_3$(CH$<em>2$)$</em>{16}$ COOH</td>
</tr>
<tr>
<td>α-Spinasterol (XI)</td>
<td>7,22-stigmastadien-3-o1</td>
</tr>
<tr>
<td>Ursolic Acid (XII)</td>
<td>3-hydroxy-12-ursen-28-oic acid</td>
</tr>
</tbody>
</table>

Ghosh et al$^{13}$ have isolated lectin.
3.2.2 Pharmacological Review

Nematicidal activity was studied by Jyomati et al\textsuperscript{14}. Islam et al\textsuperscript{15} have revealed the pollen viability of this plant affected by storage period and temperature. Sinha et al\textsuperscript{16} have reported differential condensation of chromosome complements in relation to DNA content. Fernandopulle et al\textsuperscript{17,18} identified gastroprotective, ulcer healing and hypoglycaemic activities.

Sexual cross-linking between two genetically female plants and sex genetics of this plant was carried out by Hossain et al\textsuperscript{19}. Rajput et al\textsuperscript{20} have reported sex modification by foliar sprays of silvernitrate. Cytological and polynological investigation was done by De et al\textsuperscript{21}. Antiallergic activity and Nutritive value were estimated by Gupta et al\textsuperscript{22} and Fakir et al\textsuperscript{23} respectively. The effect of intergenic grafting on growth and photosynthesis was studied by Mian et al\textsuperscript{24,25}. Ali et al\textsuperscript{26} have reported the techniques for propagation and breeding. Antimalarial activity against erythrocytic stages of \textit{Plasmodium berghei} was identified by Misra et al\textsuperscript{27}. Autosomal chromosomes carrying sex gene was investigated by Jha\textsuperscript{28}. Removal of aromatic amines and phenols from water by peroxidase produced by cell cultures of \textit{Momordica dioica} was conducted by Chatterjee and his co-workers\textsuperscript{29,30}. Sinha et al\textsuperscript{31} have studied the sex linked polypeptides in dioecious \textit{Momordica dioica}. Anticancer active constituent was reported by Li et al.,\textsuperscript{32}.

3.3 Experimental Methods

3.3.1 Plant Materials

\textit{Momordica dioica} fruits were collected and seeds were separated mechanically during November 2001 from Virudhunagar district of TamilNadu, India and identified by Dr.P.Jayaraman, Taxonomist, Retired Professor, Presidency College, Chennai. A Voucher specimen is preserved at the Department of Pharmacognosy, S.R.M. College of Pharmacy, kattankulathur, India for future reference.
3.3.2 Preparation of extract

Seed was removed from the fruit mechanically. The fruit pulp was shade dried, pulverized using a cutter mill. Pulverized fruit pulp was extracted, according to the flow chart given below by increasing the polarity of solvent.

Extractive value was calculated on dry weight basis and it was found to be 0.5% w/w for HE and 2.6% w/w for EASFME and the extracts were stored in a desiccator for phytochemical and pharmacological activities.
3.3.3 Preliminary Phytochemical screening

The prepared extracts (HE and EASFME) were subjected to a routine qualitative chemical analysis to identify sterols, glycosides, saponins, carbohydrates, alkaloids, flavonoids, proteins, tannins, phenols etc., as per the following chemical tests. The results are represented in Table 3.1.

Table 3.1. Preliminary Phytochemical Screening

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Plant constituents and Tests</th>
<th>HE</th>
<th>EASFME</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Test for Carbohydrates</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>a. Molisch's test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>b. Fehling's test</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>c. Benedict's test</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>d. Barfoed's test</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>e. Test for starch</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Test for Gums and Mucilages</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>a. Alcoholic precipitation</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>b. Molisch's test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>Test for proteins and Amino acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>a. Ninhydrin test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>b. Biuret test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>c. Millon's test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>d. Xanthoproteic test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>e. Tannic acid test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>Test for fixed oils and fats</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>a. Spot test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>b. Saponification test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>Test for Alkaloids</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>a. Mayer's test</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>b. Dragendorff's test</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>c. Wagner's test</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>d. Hager's test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sl. No.</td>
<td>Plant constituents and Tests</td>
<td>HE</td>
<td>EASFME</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------</td>
<td>----</td>
<td>--------</td>
</tr>
<tr>
<td>6.</td>
<td>Test for Saponins</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>a. Frothing test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7.</td>
<td>Test for Glycosides</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>a. Legal's test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>b. Baljet's test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>c. Borntrager's test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>d. Keller-Kiliani test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>e. Cyanogenetic glycoside test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8.</td>
<td>Test for Phytosterols</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>a. Libermann's test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>b. Libermann - Burchard test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>c. Salkowski's test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9.</td>
<td>Test for Flavonoids</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>a. Ferric chloride test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>b. Alkaline - reagent test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>c. Zinc - HCl reduction test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>d. Lead acetate solution test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>e. Mineral acid reaction test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>f. Boric acid test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10.</td>
<td>Test for Tannins and Phenolic Compounds</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>a. Reaction with copper sulphate</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>b. Ferric chloride (5%) test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>c. Reaction with lead acetate</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>d. Reaction with potassium dichromate test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>e. Reaction with potassium ferricyanide test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>f. Gelatin test</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

### 3.3.4 HPTLC Profile

With the phenomenal increase in the demand for herbal medicine in the last two decades, a need has been felt for ensuring the quality, safety and efficacy of the herbal drugs. Phytochemical evaluation is one of the tools for quality assessment, which includes preliminary phytochemical screening, chemoprofiling and marker compound analysis using modern analytical techniques.
HPTLC is a versatile separation technique and is official in most of the pharmacopoeias for determining content uniformity, purity profile, assay values and dissolution rates in unlimited number of monographs. Several samples even of divergent nature and composition can be handled simultaneously. It can be considered as a machine that speed up the work and allows to do many things at a time usually not possible with other analytical techniques.

The term high performance thin layer chromatography is used for the techniques in which substances are accurately and precisely assayed using high performance silica gel. The high performance silica gel gives a more efficient and reproducible separation than conventional grades of silica. Consequently, the plates are smaller, typically 10 cm in length and the development time is shorter, it takes only few minutes.

In the last one decade HPTLC emerged as an important tool for qualitative, semi-quantitative and quantitative phytochemical analysis of herbal drugs and formulations. This includes developing TLC fingerprint profiles and estimation of chemical markers and biomarkers.\textsuperscript{35-38}

TLC fingerprint profile of HE and EASFME of \textit{Momordica dioica} was established using HPTLC. Six concentrations (30, 50, 80, 100, 150 and 200 μg) of both the extracts were spotted on a two different pre-coated silica gel 60 F\textsubscript{254} TLC plate (E.Merck) using CAMAG Linomat IV automatic sample spotter and the plate was developed for HE in the solvent system 20 : 80; ethyl acetate: n-hexane and for EASFME in the solvent system 60: 40; ethyl acetate: n-hexane.

The plates were dried at room temperature and scanned using CAMAG TLC Scanner 3 at UV 254 nm and \( R_f \) values, spectra and peak area of the resolved bands were recorded. Relative percentage area of each band was calculated from peak areas (Fig. 3.1 and 3.2). Photographs were taken for both HE and EASFME at 254 nm, 366 nm and visible region (Photograph 3b, 3c, 3d, 3e and 3f).
3.4 Pharmacological screening

3.4.1 Toxicity studies

3.4.1.1 Introduction

The usage of medicinal plant is accepted as the most common form of traditional medicine. Among the entire flora, it is estimated that 35,000 to 70,000 species have been used for medicinal purposes. Some 5000 of these species have been studied in biomedical research. In developing countries, herbal medicines continue to play an important role in primary health care, especially where coverage of health service is limited. In industrialized countries, herbal medicines are more popular. However, the expanded use of herbal medicine has led to concerns relating to assurance of safety, quality and efficacy. One of the main attractions of herbal treatments is their apparent lack of side effects compared with the drug therapies used in allopathic medicines. Most of the ingredients have a high therapeutic index and are unlikely to cause toxicity even if used in considerable excess, but there are a few materials with well recognized toxicity that are still in common usage or that may be given erroneously.

Toxicology is the science that deals with the adverse effects of chemicals in living organisms. Toxicity tests are focussed at discerning the complications arising from the therapeutic efficacy of the drug, as the toxic effect may arise from the solvent and animal care during the period of the toxicity tests is of paramount importance.

On the basis of the paramount importance of toxicological testing, the present study was carried out to evaluate the minimum lethal dose (MLD_{50}) of the HE and EASFME of *Momordica dioica*. 
3.4.1.2 Experimental design

Extracts Used

Hexane extract (HE) and ethyl acetate soluble fraction of methanolic extract (EASFME) of the fruit pulp of *Momordica dioica* were used for the present study. 2% Tween 80 solution was used as control vehicle wherever necessary.

Animal Used

Wistar Albino mice (20 - 25g) of either sex were procured from the Tamil Nadu Veterinary and Animal Science University, Madhavaram, Chennai, and maintained at room temperature of 25 ± 2°C, relative humidity of 75 ± 5% and 12 hrs dark - light cycle. Food and water were given *ad libitum*.

Method of evaluation

The animals were grouped (ten per group) and administered with different doses (ranging from 0.1 - 3.2 g/kg) of HE and EASFME in oral and intraperitoneal route separately. In each case, there was a control group, which received 2% Tween 80 solution as vehicle control. The range of doses were administered to the mice followed the method of Lorke\(^39\). The animals were under observation in open field condition for 72 hours after the administration of HE and EASFME as mentioned earlier and the number of deaths and signs of clinical toxicity were recorded. Finally, the minimum lethal dose (MLD) and 95% confidence limits were calculated by the method of Litchfield and Wilcoxon\(^40\).

3.4.1.3 Results

The results of the observations have been furnished in Table 3.2, 3.3, 3.4 and 3.5.
### Table 3. 2 Determination of minimum lethal dose (MLD) of HE (p.o.)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (g/kg)</th>
<th>No. of Animals</th>
<th>No. of Survival</th>
<th>No. of Death</th>
<th>MLD (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (2% Tween 80 Solution)</td>
<td>10 ml/kg</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>HE</td>
<td>0.1</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.6</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.2</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>&gt; 3.2 g/kg</td>
</tr>
</tbody>
</table>

### Table 3. 3 Determination of minimum lethal dose (MLD) of EASFME (p.o.)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (g/kg)</th>
<th>No. of Animals</th>
<th>No. of Survival</th>
<th>No. of Death</th>
<th>MLD (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (2% Tween 80 Solution)</td>
<td>10 ml/kg</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>EASFME</td>
<td>0.1</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.6</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.2</td>
<td>10</td>
<td>8</td>
<td>2</td>
<td>3.2 g/kg</td>
</tr>
</tbody>
</table>
Table 3.4 Determination of minimum lethal dose (MLD) of HE (i.p.)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (g/kg)</th>
<th>No. of Animals</th>
<th>No. of Survival</th>
<th>No. of Death</th>
<th>MLD (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (2% Tween 80 Solution)</td>
<td>10 ml/kg</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>HE</td>
<td>0.1</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.6</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.2</td>
<td>10</td>
<td>8</td>
<td>2</td>
<td>3.2 g/kg</td>
</tr>
</tbody>
</table>

Table 3.5 Determination of minimum lethal dose (MLD) of EASFME (i.p.)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (g/kg)</th>
<th>No. of Animals</th>
<th>No. of Survival</th>
<th>No. of Death</th>
<th>MLD (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (2% Tween 80 Solution)</td>
<td>10 ml/kg</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>EASFME</td>
<td>0.1</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.6</td>
<td>10</td>
<td>7</td>
<td>3</td>
<td>1.6 g/kg</td>
</tr>
</tbody>
</table>
3.4.1.4 Discussion and Conclusion

No acute mortality was observed even at the dose > 3.2 g/kg of both the HE and EASFME on oral and i.p. administration and all animals were found to be normal during the observation. The results showed that a very high oral and i.p. dose (> 3.2 g/kg) is well tolerated by the mice without producing any acute toxicity symptoms. Literature reveals the presence of flavonoids and ursolic acid may have helped in reducing the toxicity. Some compound like ursolic acid have been reported to possess hepatoprotection involving the inhibition of toxicant activation and the enhancement of the body defense systems which reduces the toxicity further even at a dose of 3.2 g/kg\textsuperscript{41}. This is the basic principle in the use of crude plant extracts in traditional medicine, where the adverse effect of one component will be nullified by the protective effect of the other components, without interfering with their therapeutic properties.

Therefore, the minimum lethal dose is now estimated from the smallest number of animals possible. In this experiment as described earlier the MLD of the HE was found to be 3.2 g/kg and more than 3.2 g/kg for intraperitoneal and oral route respectively. The MLD of EASFME was found to be 3.2 g/kg for oral route and 1.6 g/kg for intraperitoneal route.

3.4.2 Analgesic Activity

3.4.2.1 Introduction

Many, if not most, ailments of the body cause pain. Pain is mainly a protective mechanism for the body. It occurs whenever any tissue is damaged and it causes the individual to react to remove the pain stimulus.

Analgesics are agents that relieve pain by elevating the pain threshold without disturbing consciousness or altering other sensory modalities. All persons in good health have the ability to perceive pain, through the pain receptors in the skin and other tissues of all free nerve endings.
Pain can be elicited by multiple types of stimuli. They are classified as mechanical, thermal and chemical pain stimuli. Some of the chemicals that excite the chemical type of pain include bradykinin, serotonin, histamine, potassium ions, acids, acetylcholine and proteolytic enzymes. In addition, prostaglandins and substance P enhance the sensitivity of pain endings but do not directly excite them.

Drugs, which are used presently for the management of pain and inflammatory conditions, are either narcotics eg. opioids, non-narcotics eg. salicylates and corticosteroids eg. hydrocortisone. All these drugs are well known for its side effect and toxic effects. Moreover synthetic drugs are very expensive to develop, since for the successful introduction of a new product approximately 3000-4000 compounds are to be synthesized, screened and tested whose cost of development ranges from 0.5 to 5 million dollars. On the contrary many medicines of plant origin had been used since long time without any adverse effects. It is therefore essential that efforts should be made to introduce new medicinal plants to develop cheaper drugs. Plants represent still a large untapped source of structurally novel compounds that might serve as lead for the development of novel drugs. The lack of potent analgesic and anti-inflammatory drugs now actually in use prompted the present study.

3.4.2.2 Experimental Methods

Analgesic Activity

Two standard methods viz. Chemical and Thermal methods were employed to determine the analgesic activity.

Acetic acid-induced writhing response in mice

Pain is induced by injection of irritants into the peritoneal cavity of mice. The animals react with a characteristic stretching behavior which is called writhings. Constriction of abdomen, turning of trunk (twist) and
extension of hind legs are taken as reaction to chemically induced pain. An irritating agent such as phenylquinone or acetic acid is injected intraperitoneally to mice and the stretching reaction is evaluated.

The analgesic activity was determined by acetic acid induced writhing method\textsuperscript{42} using Wistar albino mice of either sex selected by random sampling technique. Paracetamol (standard drug) at a dose of 50 mg/kg and the extracts (HE and EASFME) were given in a dose of 50 and 100 mg/kg intraperitoneally 30 min. prior to the administration of the writhing agent (0.6 % v/v aqueous acetic acid, 10 ml/ kg). The number of writhings during the following 15 min. period was counted. The analgesic activity data are presented in Table 3.6.

**Hot plate method in mice**

The paws of mice and rats, are very sensitive to heat at temperature which are not damaging the skin. The response are jumping, withdrawal of the paws and licking of the paws.

The analgesic activity was determined by Eddy’s hot plate method\textsuperscript{43} using Wistar Albino mice of either sex, selected by random sampling technique. Mice were placed individually on a hot plate maintained at 55 ± 1°C and the reaction time to first sign in seconds for forepaw licking or jumping was determined. Pentazocine 10mg/kg was used as a standard. One hour after the administration of vehicle, test drugs and standard treated mice were individually placed on the hot plate of the analgesiometer maintained at 55°C. The reaction time for forepaw licking or jumping was determined. The analgesic activity data are presented in Table 3.7.
3.4.2.3 Results

Acetic acid-induced writhing response in mice

In the acetic acid-induced writhing method, HE produced a significant reduction in the number of writhings in mice. This reduction was dose related and was maximum with 100 mg/kg (Table 3.6).

HE exhibited a highly significant (P < 0.001) analgesic activity at a dose of 100 mg/kg, and significant (P < 0.05) activity at a dose of 50 mg/kg, when compared with the standard drug Paracetamol (50 mg/kg). The percentage of analgesic activity for HE was found to be 50.74 and 65.22 at 50 mg/kg and 100 mg/kg dose respectively. EASFME produced 8.0% and 13.04% analgesic
activity at 50 and 100 mg/kg dose respectively. Paracetamol (50mg/kg) exhibited 79.69% analgesic activity.

**Table 3.6 Analgesic activity (Aceticacid - induced writhing reflex method) of *Momordica dioica* fruit pulp extracts.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg) i.p.</th>
<th>No.of writhings</th>
<th>Percentage inhibition of writhings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Tween 80</td>
<td>23.0 ± 1.32</td>
<td>-</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>50</td>
<td>4.67 ± 1.05</td>
<td>79.69</td>
</tr>
<tr>
<td>HE</td>
<td>50</td>
<td>11.33 ± 1.12</td>
<td>50.74</td>
</tr>
<tr>
<td>HE</td>
<td>100</td>
<td>8.0 ± 1.93</td>
<td>65.22</td>
</tr>
<tr>
<td>EASFME</td>
<td>50</td>
<td>21.16 ± 2.12</td>
<td>8.00</td>
</tr>
<tr>
<td>EASFME</td>
<td>100</td>
<td>20.0 ± 2.06</td>
<td>13.04</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SEM of six observations.

*a*P < 0.001, *b*P < 0.05 compared to control.

**Hot plate method in mice**

In the hot plate method, both the extracts (HE and EASFME) produced a significant analgesic activity at 100 mg/kg, (i.p. dose), as compared to that of the standard drug Pentazocine (10 mg/kg, i.p.) (Table 3.7). HE exhibited 35.46% (50 mg/kg, i.p.) and 50.07% (100 mg/kg, i.p.) analgesic activity, whereas EASFME showed 19.95% and 44.50% analgesic activity at 50 and
100 mg/kg i.p. dose respectively. The standard drug Pentazocine exhibited 52.43% analgesic activity at a dose of 10 mg/kg, i.p.

Table 3.7  Analgesic activity (Hot plate method) of *Momordica dioica* fruit pulp extracts.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg) i.p.</th>
<th>Reaction time (Sec)</th>
<th>Percentage inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Tween 80</td>
<td>3.33 ± 0.21</td>
<td>-</td>
</tr>
<tr>
<td>Pentazocine</td>
<td>10</td>
<td>7.0 ± 0.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>52.43</td>
</tr>
<tr>
<td>HE</td>
<td>50</td>
<td>5.16 ± 0.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.46</td>
</tr>
<tr>
<td>HE</td>
<td>100</td>
<td>6.67 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.07</td>
</tr>
<tr>
<td>EASFME</td>
<td>50</td>
<td>4.16 ± 0.31</td>
<td>19.95</td>
</tr>
<tr>
<td>EASFME</td>
<td>100</td>
<td>6.0 ± 0.36&lt;sup&gt;b&lt;/sup&gt;</td>
<td>44.50</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SEM of six observations.  
<sup>a</sup>P < 0.001,  <sup>b</sup>P < 0.05  compared to control .
3.4.2.4 Discussion

In order to distinguish between the central and peripheral analgesic action of the extracts both the methods were carried out. Acetic acid-induced writhing response in mice was used to examine the peripheral action. This method is not only simple and reliable but also affords rapid evaluation of peripheral type of analgesic action. In this test, the animals react with characteristic stretching behaviour which is called writhing. It was found that HE significantly inhibited the acetic acid-induced writhing response. The abdominal contraction is related to the sensitisation of nociceptive receptors to prostaglandins. It is therefore possible that HE exerts an analgesic effect probably by inhibiting synthesis or action of prostaglandins.

The hot plate method was originally described by Woolfe and Mac Donald.44 This test has been found to be suitable for evaluation of centrally but not of peripherally acting analgesics. The validity of this test has been shown even in the presence of substantial impairment of motor performance.45 The present findings indicate that HE may have central action more than EASFME.

3.4.2.5 Conclusion

Based on the results of this study, it is concluded that, HE has marked analgesic activity which is both centrally and peripherally mediated. EASFME has moderate analgesic activity, which is only centrally mediated and thus support the claimed use of this plant in the ayurvedic system of medicine.

3.4.3 Anti-inflammatory Activity

3.4.3.1 Introduction

Inflammation is the reactive state of hyperemia and exudation from blood vessels with consequent redness, heat, swelling and pain which a tissue manifests in response to physical or chemical injury or bacterial invasion. It is a tissue reaction by the body to injury and involves a complex array of enzyme
activation, mediator release, extravasations of fluid, cell migration, tissue breakdown and repair. Three components of the inflammatory response have been distinguished and these may involve vasoactive substances, chemotactic factors, degradative enzymes, superoxides and the neuropeptides.

Rheumatoid arthritis is the commonest form of chronic inflammatory joint disease. Arthritis is one of the most distressing and disabling syndromes encountered in medical practice, an estimated 1-2% of adult population is affected. In the United States approximately 0.1% of the population experience rheumatoid arthritis in childhood.

Mainly steroids and NSAID are used in the treatment of inflammatory conditions.

These agents produce severe adverse effects such as adrenal suppression, gastric ulceration, perforation etc., which seriously limit the frequent use of these agent in the inflammation therapy. Efforts have been made to reduce the side effects of these drugs. An ideal anti-inflammatory drug is expected to inhibit prostaglandin synthesis mediated by COX-2 while sparing COX-1. Selective COX-2 inhibitors such as Celecoxib and Rofecoxib are marketed recently for the management of inflammation but ulcer complication and high risk of thrombosis are fraternated with these drugs.

Nature endows the world with medicinal plants to take care of health needs. The potentials of plants as sources of drugs have long been recognized. There are representative anti-inflammatory herbs in almost each family in the plant kingdom with lesser side effects. A good number of plants are employed in the treatment of inflammatory disorders by natural healers. Some of these plants include Aloe vera, Consolida regalis, Chasmanthera dependens, Culcasia scandens, Crataeva religiosa, Tanacetum vulgare, Holmskioldia sanguinea, Mitracarpus scaber, Turner ulmifolia, Curcuma longa, Moringa oleifera and Syzygium cumini.
Some active anti-inflammatory principles have been identified, isolated and characterized. They include - lupeol⁶⁸, prennazole⁷⁷, (+)- usnic acid⁷⁸, (+)- pinitol⁷⁹, zanhasaponins A & B⁸⁰, sasanquol⁸¹ and parthenolide⁶⁹. These compounds could provide drugs with comparative advantage over existing agents and may as well serve as leads for further development into more active drugs with lesser adverse effects.
3.4.3.2 Experimental

Acute anti-inflammatory activity

Among the many methods used for screening anti-inflammatory agents one of the most commonly employed technique is based upon the ability of such agents to inhibit the oedema produced in the hind paw of the rat after injection of a phlogistic agent. Many phlogistic agents (irritants) have been used such as brewer's yeast, formaldehyde, dextran, egg albumin, kaolin, sulfated polysaccharides like carrageenan, naphthoyl heparamine. Usually the volume of the injected paw is measured before and after applications of irritant and the paw volume of the treated animal is compared to the control.

Carrageenan-induced paw oedema in rats

The anti-inflammatory activity was determined by carrageenan-induced paw oedema method\textsuperscript{82} in Wistar Albino rats of either sex using plethysmograph. Diclofenac sodium (standard drug) in a dose of 5mg/kg and extracts (HE and EASFME) in a dose of 50 and 100mg/kg were administered intraperitoneally 30 minutes prior to the administration of carrageenan (0.1 ml of 1% w/v) in to the plantar region of the paw. The paw volume was measured plethysmometrically at 1,2,3,4 and 5 h after the injection of carrageenan. The anti-inflammatory activity data are presented in Table 3.8.

3.4.3.3 Results

In carrageenan-induced paw oedema method (acute model), the standard anti-inflammatory drug (Diclofenac sodium 5 mg/kg, i.p.) produced a significant reduction in the volume of paw oedema in rats as compared to the control rats. The extracts (HE and EASFME) showed maximum inhibition of the carrageenan-induced rat paw oedema at the end of 3 h (Table 3.8). Oedema suppressant effect of 100 mg/kg dose of HE and EASFME treated groups were found to be highly significant (P < 0.001) as compared to control. Both the
Table 3.8: Anti-inflammatory activity of *Momordica dioica* fruit pulp extracts on carrageenan-induced paw oedema model

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Dose (mg/kg) i.p.</th>
<th>Paw oedema volume (ml)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1h</td>
<td>2h</td>
<td>3h</td>
<td>4h</td>
<td>5h</td>
</tr>
<tr>
<td>I.</td>
<td>Control</td>
<td>Tween 80</td>
<td>0.47 ± 0.04</td>
<td>0.62 ± 0.04</td>
<td>0.78 ± 0.03</td>
<td>0.74 ± 0.05</td>
<td>0.71 ± 0.06</td>
</tr>
<tr>
<td>II.</td>
<td>Diclofenac</td>
<td>sodium 5</td>
<td>0.22 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.19 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.17 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.26 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.27 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(53.19)</td>
<td>(69.35)</td>
<td>(78.20)</td>
<td>(64.86)</td>
<td>(61.97)</td>
</tr>
<tr>
<td>III.</td>
<td>HE</td>
<td>50</td>
<td>0.40 ± 0.03</td>
<td>0.37 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.31 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.39 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.42 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(14.89)</td>
<td>(40.32)</td>
<td>(60.26)</td>
<td>(47.30)</td>
<td>(40.84)</td>
</tr>
<tr>
<td>IV.</td>
<td>HE</td>
<td>100</td>
<td>0.35 ± 0.04</td>
<td>0.29 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.23 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.32 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.33 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(25.53)</td>
<td>(53.22)</td>
<td>(70.51)</td>
<td>(56.76)</td>
<td>(53.52)</td>
</tr>
<tr>
<td>V.</td>
<td>EASFME</td>
<td>50</td>
<td>0.41 ± 0.03</td>
<td>0.41 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.36 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.46 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.49 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(12.76)</td>
<td>(33.87)</td>
<td>(53.85)</td>
<td>(37.84)</td>
<td>(30.98)</td>
</tr>
<tr>
<td>VI.</td>
<td>EASFME</td>
<td>100</td>
<td>0.39 ± 0.04</td>
<td>0.32 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.28 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.38 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.39 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(17.02)</td>
<td>(48.39)</td>
<td>(64.10)</td>
<td>(48.65)</td>
<td>(45.67)</td>
</tr>
</tbody>
</table>

Each value is the mean ± SEM of six rats.
Figure in parentheses indicate the percentage anti-inflammatory activity

<sup>a</sup>P < 0.001; <sup>b</sup>P < 0.05 compared to control
extracts of *Momordia dioica* fruit pulp produced a dose dependent inhibition on carrageenan-induced rat hind paw oedema. At the end of 3h the inhibition was found to be 78.20% for Diclofenac sodium (5 mg/kg, i.p.), 60.26% and 70.51% for HE (50 and 100 mg/kg, i.p.), 53.85% and 64.10% for EASFME (50 and 100 mg/kg, i.p.) respectively (Fig. 3.3).

3.4.3.4 Discussion

The present study demonstrated that HE extract was effective in animal model of acute inflammation. Among the many methods used for screening of anti-inflammatory drugs, one of the most commonly employed techniques is based upon the ability of such agents to inhibit the oedema produced in the hind paw of the rats after injection of phlogistic agents. The time course of oedema development in carrageenan induced paw oedema model in rats is generally represented by a biphasic curve. The first phase occurs within an hour of injection and is partly due to the trauma of injection and also to the serotonin component. Prostaglandins (PG) play a major role in the development of the second phase of reaction which is measured around 3 hrs. The presence of PGE$_2$ in the inflammatory exudates from the injected foot can be demonstrated at 3 hrs and periods thereafter. Carrageenan-induced paw oedema model in rats is known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of non steroidal anti-inflammatory agents which primarily inhibit the enzyme cyclooxygenase in prostaglandin synthesis.

3.4.3.5 Conclusion

Based on the reports it can be inferred that the inhibitory effect of HE extract on carrageenan-induced inflammation in rats could be due to inhibition of the enzyme cyclooxygenase leading to inhibition of prostaglandin synthesis. In this acute anti-inflammatory model, both HE and EASFME showed inhibition at 3h. Based on the results of this study, it may be concluded that *Momordica dioica* fruit pulp as potential anti-inflammatory activity and thus support the claimed use of this plant in the ayurvedic system of medicine.
3.4.4  Hepatoprotective Activity

3.4.4.1 Introduction

A therapeutic approach to modern drug development can provide many invaluable drugs from traditional medicinal plants. Search for pure phytochemicals as drug is time consuming and expensive. Numerous plants and polyherbal formulations are used for the treatment of liver diseases.

Medicinal plants play a key role in human health care. About 80% of the world populations rely on the use of traditional medicine, which is predominantly based on plant materials. It is estimated that about 7,500 plants are used in the local health traditions in, mostly, rural and tribal villages of India. Out of these, the real medicinal value of over 4000 plants are either little known or hitherto unknown to the mainstream population.

Liver has a pivotal role in regulation of physiological processes. It is involved in several vital functions such as metabolisms, secretion and storage. Furthermore, detoxification of a variety of drugs and xenobiotics occur in liver. The bile secreted by the liver has an important role in digestion. Liver diseases are among the most serious ailment. They may be classified as acute or chronic hepatitis (inflammatory liver diseases), hepatosis (non-inflammatory liver diseases) and cirrhosis (degenerative disorders resulting in fibrosis of the liver).

Liver diseases are mainly caused by toxic chemicals like

- certain antibiotics
- chemotherapeutic agents
- peroxidised oil
- aflatoxin
- carbon tetrachloride
- chlorinated hydrocarbon
- excess consumption of alcohol.
- infections
- autoimmune disorders
Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages in liver\textsuperscript{86-89}.

In India, more than 87 medicinal plants are used in different combinations in the preparation of 33-patented herbal formulations\textsuperscript{90-93}. Most commonly used plants in herbal formulations are \textit{Andrographis paniculata, Boerhaavia diffusa, Eclipta alba, Picrorhiza kurroa, Oldenlandia corymbosa, Asteracantha longifolia, Apium graveolens, Cassia occidentalis, Cichorium intybus, Embelia ribes, Tinospora cordifolia and Trachyspermum ammi}.

Several plants were reported as hepatoprotective includes, \textit{Acacia catechu}\textsuperscript{94}, \textit{Azadirachta indica}\textsuperscript{95}, \textit{Ocimum sanctum}\textsuperscript{96}, \textit{Phyllanthus niruri}\textsuperscript{97}, \textit{Ricinus communis}\textsuperscript{97}, \textit{Moringa oleifera}\textsuperscript{98}, \textit{Withania somnifera}\textsuperscript{99} and \textit{Sida cordifolia}\textsuperscript{100}.

\textbf{3.4.4.2 Materials and Methods}

\textbf{Dose and route of administration}

For inducing acute hepatic damage, Paracetamol suspension was administered for 3 days at a dose of 2g/kg body weight, p.o.\textsuperscript{101} followed by the standard hepatoprotective drug Silymarin (200mg/kg, p.o.) and two extracts for 7 days (400mg/kg, p.o.).

\textbf{Chemicals used}

Serum glutamate oxaloacetate transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT), Serum alkaline phosphatase (ALP), Serum bilirubin, total proteins and albumins were estimated by using standard kits from M/S Ranbaxy Laboratories Ltd., New Delhi, India. All the reagents used were of analytical grade. Silymarin (Silybon, M/S Micro Labs) was used as standard drug.
**Experimental procedure**

The animals were divided into five groups of six rats each. A suspension of Paracetamol was prepared in 2% v/v Tween 80 and administered orally at the dose of 2g/kg body weight. Silymarin and extracts were also administered in a similar way.

Group I comprised of control rats, which received 2% v/v Tween 80 for 10 days

Group II received Paracetamol orally (2 g/kg) once daily for 3 days

Group III received Paracetamol orally for 3 days followed by Silymarin 200mg/kg orally for the next 7 days.

Group IV received Paracetamol orally for 3 days followed by HE 400mg/kg orally for the next 7 days.

Group V received Paracetamol orally for 3 days followed by EASFME 400mg/kg orally for the next 7 days.

At the end of the treatment (on the 11th day) period, rats of each groups were anaesthetized using anaesthetic ether, and then blood samples were collected by direct cardiac puncture, and centrifuged at 2000 rpm at 4°C for 10 min. to separate the serum for different biochemical analysis. The rats were sacrificed by cervical dislocation. The livers were immediately excised to study its histopathology.

**Serum enzyme assay**

SGOT and SGPT were determined by the method of King (1965). Serum ALP was estimated by the Kind and King method (1954). Serum bilirubin was estimated by the Malloy and Evelyn method (1937).
Serum protein estimation

The serum was also used to determine the levels of total protein, albumin, globulin and the albumin-globulin ratio. Total protein was estimated by the method of Lowry et al., (1951) and the albumin was estimated by the method of Doumas et al., (1971).

Detailed procedure for the estimation of serum enzymes and serum proteins

Serum glutamate oxaloacetate transaminase (SGOT)

The method of King\textsuperscript{102} was adopted for the assay of serum glutamate oxaloacetate transaminase.

Reagents

1. Phosphate buffer : 0.1 M, pH 7.4
2. Substrate: 2.66 g of DL-aspartate and 38 mg of $\alpha$– ketoglutarate were dissolved in 20.5 ml of 1N sodium hydroxide with gentle heating. This was made up to 100 ml in water.
3. 2,4-dinitro phenyl hydrazine reagent (DNPH): 1 mM dinitrophenyl-hydrazine in 2N hydrochloric acid.
4. Sodium hydroxide: 0.4N solution.
5. Standard pyruvate: 10mg of sodium pyruvate was dissolved in 100 ml of phosphate buffer 0.1M, pH 7.4.

Procedure

In different tubes, 1.0 ml of the buffered substrate was added to 0.1ml of serum and incubated at 37°C for one hour. Then 1.0 ml of DNPH reagent was added to arrest the reaction. To the blank tubes, 0.1 ml of enzyme was added only after the addition of DNPH reagent. The tubes were kept aside for 15
minutes, and then 10 ml of 0.4N sodium hydroxide was added and read at 520nm in a Systronic UV spectrophotometer.

The enzyme activity was expressed as IU/litre in serum.

**Serum glutamate pyruvate transaminase (SGPT)**

The reagents and method used were the same as those used for the assay of glutamate pyruvate transaminase\textsuperscript{102} except for the substrate solution and the incubation time was reduced to 30 minutes.

**Assay of Alkaline Phosphatase (ALP)**

Alkaline phosphatase was assayed by the method of Kind and King\textsuperscript{103} using disodium phenyl phosphate as substrate.

**Reagents:**

1. Carbonate – bicarbonate buffer: 0.1 M, pH 10.0
2. Disodium phenyl phosphate solution: 0.01 M
3. Magnesium chloride solution: 0.1 M
4. Folin’s phenol reagent:

   Into a 1,500ml round-bottomed flask, 100g of sodium tungstate, 25 g of sodium molybdate, 700 ml of water, 50 ml of 85% ortho-phosphoric acid and 100 ml of concentrated hydrochloric acid were added and refluxed for 10 hours. Then 150g of lithium sulphate, 50 ml of distilled water and a few drops of bromine were added. The mixture was boiled to remove excess bromine. It was then cooled and diluted to one litre with water. The reagent was diluted 1:2 with distilled water just before use.

5. Sodium carbonate solution: 15%.
6. Standard phenol: 100 mg of recrystallized phenol in 100 ml of water was prepared. 100 μg of phenol per ml was then prepared by proper dilution and used as the working standard.

Procedure

The incubation mixture of 3.0 ml contains 1.5ml of buffer, 1.0 ml of substrate and requisite amount of the enzyme source. The reaction mixture was incubated at 37°C for 15 minutes. The reaction was arrested by the addition of 1.0 ml of Folin’s phenol reagent. The control tubes received the enzyme after arresting the reaction. The contents were centrifuged and to the supernatant, 1.0 ml of 15% sodium carbonate solution, 1 ml of substrate and 0.1ml of magnesium chloride were added and the mixture was incubated for 10 minutes at 37°C. The color was read at 640nm against a blank in Systronic UV spectrophotometer. The standard solution of phenol of varying concentrations was also treated similarly.

The enzyme activity was expressed as IU/litre in serum.

Estimation of serum bilirubin

Serum bilirubin was estimated by the method of Malloy and Evelyn\textsuperscript{104}.

Reagents

1. Absolute methanol
2. Hydrochloric acid, 1.5% v/v with water.
3. Diazo-reagent: Prepare freshly before use by adding 0.8 ml of solution B to 10 ml of solution A.

Solution A: Dissolve 0.1 g of sulfanilic acid in 1.5 ml of concentrated hydrochloric acid and make up to 100 ml with water.

Solution B: Dissolve 0.5 g of sodium nitrite in water and make up to 100ml. Prepare freshly at frequent intervals.
4. Standard solution of bilirubin: Prepare a solution containing 10 mg per 100 ml chloroform. It may be necessary to reflux the mixture gently to dissolve the bilirubin.

**Procedure**

Take two test tubes and place 0.2ml of serum and 1.8 ml of distilled water. To the serum add 0.5ml of the diazo – reagent and to the blank 0.5 ml of 1.5% hydrochloric acid. Finally, to each add 2.5 ml of methanol. After 5 minutes the color developed was read at 540nm against the blank containing water in a Systronic UV spectrophotometer. The standards were read against blank containing chloroform. This gives total bilirubin. To estimate the direct bilirubin, the method is repeated by substituting 29ml of water for absolute methanol.

The values are expressed as mg/dl.

**Estimation of Protein**

The protein content was estimated by the method of Lowry et. al\textsuperscript{105}

**Reagents**

1. Alkaline copper reagent
   - Solution A: 2% sodium carbonate in 0.1 N sodium hydroxide.
   - Solution B: 0.5% copper sulphate in water.
   - Solution C: 1% sodium potassium tartrate in water.
   - 50 ml of solution A was mixed with 0.5 ml of solution B and 1.0 ml of solution C

2. Folin’s phenol reagent: This was prepared as described earlier in ALP.

3. Standard bovine serum albumin:
   - 100 mg of crystalline BSA was dissolved in 100 ml of distilled water.
**Procedure**

An aliquot of the suitably diluted sample (0.1 ml to 10 ml by two serial dilutions) was made up to 1.0 ml with water. 4.5 ml of alkaline copper reagent was added to all the tubes including blank. Blank containing 1.0 ml of water and standard containing aliquots of BSA were also treated similarly. The contents were left to stand for 10 minutes at room temperature. Then 0.5 ml of diluted Folin’s – phenol reagent was added. The blue color developed was read at 640 nm after 20 minutes in a Systronic UV spectrophotometer.

The values are expressed as g/dl in serum.

**Albumin Globulin ratio**

The albumin and globulin content of the serum was estimated by the method of Doumas et al.\(^{106}\).

**Reagents**

1. **Anhydrous Sodium Sulphite Solution**: 25g of sodium sulphite was dissolved in 100 ml of distilled water.

2. **Diethyl ether**.

3. **Biuret reagent**: This reagent was prepared by mixing freshly, 5 parts of 25% sodium hydroxide to 1 part of 2% copper sulphate.

**Procedure**

To 0.04 ml of serum, 4 ml of sodium sulphite solution and 4 ml of diethyl ether was added and centrifuged. After centrifugation 2.0 ml of lower layer was removed. Another test tube contained 2.0 ml of water and 0.02 ml of serum. The blank contained 2 ml of sodium sulphite solution 2.0 ml of Biuret reagent was added to all the tubes and left at room temperature for 15 minutes. The color developed was read at 540 nm in a Systronic UV spectrophotometer. The A/G ratio was calculated by using the formula,
Absorbance of albumin  
\[ A/G \text{ ratio} = \frac{\text{Absorbance of total protein} - \text{Absorbance of albumin}}{\text{Absorbance of total protein} - \text{Absorbance of albumin}} \]

**Histopathological examination**

Small fragments of the liver was washed in ice-cold saline, fixed in 10% formalin solution, dehydrated with ethanol (50%), embedded in paraffin and cut into 5 µm thick sections using a microtome. The sections were stained with eosin-haemotoxylin dye for photo microscopic observation of necrosis, steatosis and fatty change of hepatic cells\textsuperscript{107}.

**Statistical analysis**

The experimental results were expressed as the Mean ± SEM (Standard Error Mean). The statistical analysis was performed by analysis of variance (ANOVA) followed by The Dunnett’s test was used to make a statistical comparison between groups. Results with P < 0.05 were considered statistically significant.

**3.4.4.3 Results**

Rats treated with Paracetamol (2 g/kg, p.o.) developed significant hepatic damage as observed from levels of the hepatospecific enzymes as well as severe alterations of different liver parameters (Table 3.9). Activities of SGOT, SGPT and ALP were significantly increased in Paracetamol treated animals (Group 2). Serum bilirubin level was also significantly enhanced upon Paracetamol treatment. The levels of total proteins and albumins were decreased in rats treated with Paracetamol when compared with control rats (Group-1). Histopathological changes confirmed the hepatic damage when compared to the normal animals liver tissue (Fig.3.4a). Paracetamol treatment showed extensive centriolobular necrosis. There was a mild chronic inflammatory cell infiltrate in the portal tracts. (Fig.3.4b).
Fig. 3.4a  Liver section of a normal rat showing normal hepatic cell architecture.

Fig. 3.4b  Liver section of a rat with Paracetamol-induced hepatotoxicity showing severe focal necrosis.

Fig. 3.4c  Liver section of a rat induced with Paracetamol + standard drug Silymarin showing almost normal hepatic cell architecture.

Fig. 3.4d  Liver section of a rat induced with Paracetamol + HE treated group showing almost normal hepatic cell architecture.

Fig. 3.4e  Liver section of a rat induced with Paracetamol + EASFME treated group showing mild focal necrosis.
Oral administration of *Momordica dioica* fruit pulp HE (400 mg/kg, dose) significantly (P < 0.001) decreased the levels of SGOT, (71.23 ± 6.43 IU/L), SGPT (63.01 ± 3.33 IU/L), ALP (164.68 ± 14.65 IU/L) and Bilirubin (2.9 ± 0.25 mg/dl) when compared to group II 133.66 ± 11.03, 104.42 ± 10.09, 453.96 ± 18.98 IU/L and 3.52 ± 0.64 mg/dl respectively. In fact the elevated level of ALP from 453.96 ± 18.98 to 164.68 ± 14.65 IU/L by HE and standard drug Silymarin (215.06 ± 18.44 IU/L). It reveals that HE has got more power to reduce the elevated level of ALP than the standard drug Silymarin. The increased bilirubin value (3.52 ± 0.64 mg/dl) was reduced to (2.9 ± 0.25 mg/dl) by oral administration of HE, which is below the control value (2.95 ± 0.39 mg/dl) (Table 3.9). The total protein and albumin levels were increased (10.99 ± 0.29 g/dl: 7.71 ± 0.14 g/dl) respectively; in HE treated animals when compared to Paracetamol treated rats. The activity exhibited by standard drug Silymarin was much more higher than the HE treated rats in case of total protein and albumin. The increase in total protein and albumin level in HE treated provides for the protective effect of HE on liver.

The EASFME reduced the elevated marker enzyme levels only to certain extent and bilirubin level has reduced to the normal value. EASFME has increased the total protein content and albumin level remarkably. Both HE and EASFME were compared with the standard herbal drug Silymarin with a dose of 100mg/ kg, bodyweight, p.o. Silymarin has provided a better inhibition, of the elevated level of SGOT, SGPT, ALP and serum bilirubin and also increased the total protein content and albumin level. Overall the activity exhibited by HE was comparable with that of the standard drug Silymarin (Fig. 3.6).

The findings described above were supported by the histopathological study where an oral administration of either standard drug Silymarin (Fig.3.4c) or HE improved the histopathological picture of the liver. The histopathological pattern of the livers of the rats treated with the HE showed a normal lobular pattern with a mild degree of fatty change, necrosis and inflammation (Fig.3.4d). Whereas Silymarin treated group shown a normal hepatic cell architecture (Fig. 3.4c).
<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Dose</th>
<th>SGOT (IU/L)</th>
<th>SGPT (IU/L)</th>
<th>ALP (IU/L)</th>
<th>Bilirubin (mg/dl)</th>
<th>Total Protein (g/dl)</th>
<th>Albumin (g/dl)</th>
<th>Globulin (g/dl)</th>
<th>A/G Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>Control (Normal Saline)</td>
<td>0.1 ml/kg</td>
<td>49.81 ± 1.31</td>
<td>40.50 ± 2.13</td>
<td>111.28 ± 11.59</td>
<td>2.95 ± 0.39</td>
<td>10.47 ± 0.10</td>
<td>7.66 ± 0.07</td>
<td>2.81 ± 0.04</td>
<td>2.72 ± 0.09</td>
</tr>
<tr>
<td>II.</td>
<td>Paracetamol treated</td>
<td>2 g/kg</td>
<td>133.66 ± 1.03</td>
<td>102.42 ± 10.09</td>
<td>453.96 ± 18.98</td>
<td>3.52 ± 0.64</td>
<td>10.01 ± 0.44</td>
<td>7.0 ± 0.13</td>
<td>3.01 ± 0.08</td>
<td>2.32 ± 0.14</td>
</tr>
<tr>
<td>III.</td>
<td>Silymarin</td>
<td>200mg/kg</td>
<td>57.65 ± 1.95*</td>
<td>51.40 ± 5.38*</td>
<td>215.06 ± 18.44*</td>
<td>2.68 ± 0.16*</td>
<td>11.35 ± 0.41</td>
<td>7.72 ± 0.09</td>
<td>3.63 ± 0.15</td>
<td>2.13 ± 0.11</td>
</tr>
<tr>
<td>IV.</td>
<td>HE</td>
<td>400mg/kg</td>
<td>71.23 ± 6.43*</td>
<td>63.01 ± 3.33*</td>
<td>164.68 ± 14.65*</td>
<td>2.90 ± 0.25</td>
<td>10.99 ± 0.29</td>
<td>7.71 ± 0.14</td>
<td>3.28 ± 0.12</td>
<td>2.35 ± 0.09</td>
</tr>
<tr>
<td>V.</td>
<td>EASFME</td>
<td>400mg/kg</td>
<td>106.55 ± 4.45*</td>
<td>84.38 ± 8.00</td>
<td>293.62 ± 9.93*</td>
<td>2.90 ± 0.19</td>
<td>10.18 ± 0.77</td>
<td>7.60 ± 0.11</td>
<td>2.58 ± 0.17</td>
<td>2.94 ± 0.14</td>
</tr>
</tbody>
</table>

Values are given as mean ± S.E.M for six rats in each group.

\*P < 0.001; \*P < 0.05 compared to control
Effect of Momordica dioica fruit pulp extracts during paracetamol induced liver damage.
3.4.4.4 Discussion

Damage to the structural integrity of liver is reflected by an increase in the level of serum transaminases because these are cytoplasmic in location and are released into circulation after cellular damage\textsuperscript{108}. In this study a similar rise in the levels of SGOT, SGPT, and ALP in Paracetamol treated rats were observed. The oral administration of HE of fruit pulp of \textit{Momordica dioica} in the present study seems to offer protection to the structural integrity of hepatocellular membrane. This is evident from the significant reduction in serum SGOT, SGPT and ALP levels, and thus offers protection against Paracetamol-induced liver toxicity in rats.

Decreased bilirubin level observed after the administration of HE could be a further evidence for the protection against Paracetamol-induced hepatotoxicity.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig3.5.png}
\caption{Potential mechanisms of liver cell death resulting from the metabolism of Paracetamol}
\end{figure}
Paracetamol, a widely used over-the-counter analgesic and antipyretic, produces hepatic necrosis when ingested in very large doses. It is metabolized in the liver primarily to glucuronide and sulphate conjugates. Paracetamol toxicity is due to the formation of toxic metabolites when a part of it is metabolized by cytochrome P450.\textsuperscript{101} Induction of cytochrome P\textsubscript{450} or depletion of hepatic glutathione is a prerequisite for Paracetamol-induced hepatotoxicity (Fig.3.5). Therefore the anti-hepatotoxic activity of the drug may be due to, inhibition of cytochrome P\textsubscript{450}, promotion of glucuronidation, stimulation of hepatic regeneration, activation of the functions of the reticuloendothelial systems or inhibition of protein biosynthesis.\textsuperscript{109}

### 3.4.4.5 Conclusion

Thus this study confirms the protective action of the HE of fruit pulp of \textit{M. dioica} against experimentally induced liver damage in rats, which was comparable to that of a standard hepatoprotective drug Silymarin. SGOT, SGPT, ALP and Serum bilirubin are the most sensitive tests employed in the diagnosis of hepatic diseases.\textsuperscript{110} The elevated levels of these parameters were reduced by the treatment with \textit{Momordica dioica} fruit pulp extract. This hepatoprotective activity may be due to the presence of active principle Ursolic acid that was reported to possess hepatoprotectant activity.
3.4.5 Glucose lowering effect and Hypolipidemic Activity

3.4.5.1 Introduction

Diabetes mellitus is a silent chronic disorder characterized by elevated blood sugar levels either due to defective insulin secretion or action or both. It is associated with altered metabolism of carbohydrates, fats and proteins. Elevated blood sugars cause changes in the blood vessels thus affecting the eyes, kidneys, nerves, heart, brain and the feet. There are two main categories of this disease. Type 1 diabetes mellitus also called insulin dependent diabetes mellitus (IDDM) and Type 2, the non-insulin dependent diabetes mellitus (NIDDM)\textsuperscript{111}. Though the former form of diabetes accounts for 5 to 10\% of all cases, the incidence is rapidly increasing in specific regions. It is estimated that incidence of Type 1 diabetes will be about 40\% higher in the year 2010 than in 1997\textsuperscript{112} and yet there is no identified agent substantially capable of preventing this type of disease\textsuperscript{113-115}. NIDDM is far more common and results from a combination of defects in insulin secretion and action. This type of disease accounts for 90 to 95\% of all diabetic patients.

Diabetes mellitus is a major and growing health problem in most countries and an important cause of prolonged ill health and early death. It was the sixteenth leading cause of global mortality in 1990, accounting for 5,71,000 deaths\textsuperscript{116}. The growth will be particularly strong in India and China\textsuperscript{117,118}. The growth in number of people with diabetes is expected to be fast in Pakistan, Indonesia, Egypt and Mexico and somewhat slow in Japan\textsuperscript{117}. Recent studies of geographical and ethnical influences have shown that people of Indian origin are highly prone to diabetes\textsuperscript{119}. It is estimated that currently there are over 32 million diabetics in India and it could be 57 million by 2025 according to World Health Organization reports. India is thus called the Diabetic capital of the world. Hence it is very essential for all adult Indians to test their blood sugar levels, especially if anybody in their family has diabetes or if they are over weight.
Despite the great strides that have been made in understanding and management in this disease, serious problems like diabetic retinopathy\textsuperscript{120}, diabetic nephropathy\textsuperscript{121} and lower extremity amputation\textsuperscript{122}, continue to confront patients and physicians. Certain population subgroups have prevalence rates of disease approaching 50\% and this is strongly related to the epidemic of obesity and socio-economic inequalities that plague our society\textsuperscript{123}.

Multiple defects in the pathophysiology of diabetes are mostly imprecisely understood and therefore warrant not isolating a single drug target to the reversal of all or majority of aspects of the disease\textsuperscript{124}, as biological systems are too complex to be fully understood through conventional experimentation and also because they are nonlinear. The therapeutic approach of several traditional medicinal systems is more holistic. The fundamental mechanisms of these medicinal systems are still unexplainable using modern tools. The medicinal preparations in traditional medicines contain a variety of herbal and non-herbal ingredients that are thought to act on a variety of targets by various modes and mechanisms.

A benevolent number of plants are used in the treatment of diabetes by natural healers. Some of these plants include \textit{Allium cepa}\textsuperscript{125,126}, \textit{Coccinia indica}\textsuperscript{127}, \textit{Ficus bengalensis}\textsuperscript{128,129}, \textit{Gymnema sylvestre}\textsuperscript{130,131}, \textit{Momordica charantia}\textsuperscript{132,133}, \textit{Pterocarpus marsupium}\textsuperscript{134}, \textit{Swertia chirayita}\textsuperscript{135}, \textit{Syzygium cumini}\textsuperscript{136}, \textit{Trigonella foenum-graecum}\textsuperscript{137} and \textit{Zizyphus jujuba}\textsuperscript{138}.

\textbf{3.4.5.2 Materials and methods}

\textbf{Experimental animals}

\textbf{Effect of oral glucose tolerance in rats (OGTT)}

Diabetes was induced by an i.p. injection of freshly prepared alloxan\textsuperscript{139} (120mg/kg body weight dose). Rats with blood glucose levels more than 250mg/dl were considered diabetic and were used for the experiment. Five groups of six animals each were used.
After overnight fasting a 0 min. blood sample (0.2 ml) was taken from the rat in the different groups viz., normal, diabetic + glibenclamide (10 mg/kg), diabetic + HE (400 mg/kg), diabetic + EASFME (400 mg/kg) in 2% Tween 80\(^{140}\). The rats of all the groups were administered glucose solution (2 g/ml, per kg) by gavage without delay. Blood was collected at 30, 60, 90 and 120 min. intervals after glucose administration\(^{141}\). Serum glucose level was measured immediately.

**Study of intraperitoneal administration (acute) of extracts in hyperglycaemic rats**

In the experiment a total of 30 rats (24 diabetic surviving rats, six normal rats) were used. The rats were divided into five groups, six rats in each group.

- **Group I** Normal untreated rats
- **Group II** Diabetic rats
- **Group III** Diabetic rats given Glibenclamide 10 mg/kg i.p.
- **Group IV** Diabetic rats given HE 400 mg/kg i.p.
- **Group V** Diabetic rats given EASFME 400 mg/kg i.p.

Blood samples were collected at zero, 1, 3 and 5 h. after injections. Blood glucose levels were measured immediately.

**Study of oral administration (chronic) of extracts in hyperglycaemic rats**

- **Group I** Normal untreated rats
- **Group II** Diabetic rats
- **Group III** Diabetic rats given Glibenclamide 10 mg/kg orally for 15 days
- **Group IV** Diabetic rats given HE 400 mg/kg orally for 15 days
- **Group V** Diabetic rats given EASFME 400 mg/kg orally for 15 days
On 16th day in fasting condition blood samples were collected from the tail vein and centrifuged at 2000 rpm at 4°C for 10 min. to separate serum for the estimation of various bio-chemical parameters. The change in body weight before and after the treatment and also urine sugar of all the rats were determined on 16th day.

**Glucose**

**Estimation of blood glucose**

Blood glucose was estimated by the method of Sasaki et al\textsuperscript{142}, using o-toluidine reagent.

**Reagents**

1. O-Toluidine reagent: 12.5g of thiourea and 12.0g of boric acid were dissolved in 50ml of distilled water by heating over a mild flame. Exactly 75 ml of o-toluidine (redistilled) and 375 ml of acetic acid (AR) were mixed separately. These two solutions were mixed and the total volume was made up to 500 ml with distilled water. The reagent was left overnight in the refrigerator and filtered.

2. Glucose standard: 100mg of pure glucose was dissolved in 100 ml of distilled water containing 0.01% benzoic acid.

**Procedure**

To 0.1 ml of serum, 4.0 ml of o-toluidine reagent is added and kept in a boiling water bath for 15 minutes. The greenish blue color developed was read at 640nm in a Systronic UV spectrophotometer. Blank containing 2.0 ml of water and standards containing 20 to 40 μg of glucose were also treated similarly.

The values are expressed as mg/dl.
**Serum Cholesterol**

**Determination of Cholesterol**

Cholesterol was estimated by the method of Parekh and Jung\(^{143}\).

**Reagents**

1. **Ferric acetate-Uranyl acetate reagent:** 10 ml of water and 3.0 ml of concentrated ammonia were added to 500 mg of crystalline ferric chloride. The precipitate was washed several times with distilled water and was dissolved in glacial acetic acid and made up to one litre with acetic acid. 100 mg of uranyl acetate was added, shaken well and kept overnight. The reagent was stored in an amber colored bottle. This reagent was stable for 6 months.

2. **Sulfuric acid-ferrous sulphate reagent:** To 100 ml of glacial acetic acid, one gram of anhydrous ferrous sulphate was added and shaken well. 100 ml of concentrated sulfuric acid was added and after cooling, the volume was made up to one litre with concentrated sulfuric acid. The reagent was stable for 6 months.

3. **Cholesterol standard:** The stock standard was prepared by dissolving 200 mg of cholesterol in 100 ml of chloroform. A standard curve was obtained using aliquots containing 10 to 20 \(\mu\)g of cholesterol.

**Procedure**

10 ml of ferric acetate-uranyl acetate reagent was added to 0.1 ml of sample, mixed well and allowed to stand for 5 minutes and centrifuged. 3.0 ml of the supernatant was taken for the analysis. Similarly 0.1 ml of standard cholesterol was mixed, and 3.0 ml of aliquots were taken.
Blank tubes contained 3.0 ml of ferric acetate-uranyl acetate reagent. 2.0 ml of ferrous sulphate-sulfuric acid reagent was added to all the tubes and mixed well. The color intensity was measured at 540nm after 20 minutes in a Systronic UV spectrophotometer.

Serum cholesterol was expressed as mg/dl.

**Serum triglycerides**

**Determination of Triglycerides**

Triglyceride of serum was estimated by the method of Foster and Dunn\(^4^4\).

**Reagents**

1. Activated silicic acid
2. Saponification reagent: 5.0 g of potassium hydroxide was dissolved in 60 ml distilled water and 4.0 ml of isopropanol.
3. Sodium meta-periodate reagent: To 77 g of anhydrous ammonium acetate in 700 ml of distilled water, 60 ml of glacial acetic acid and 650 mg of sodium meta-periodate were added. It was dissolved and diluted to 1.0 litre with distilled water.
4. Acetyl acetone reagent: Acetyl acetone 0.75 ml was added to 20 ml of isopropanol and mixed well. To this 80 ml of distilled water was added and mixed well.
5. Stock solution: 400 mg of tripalmitin dissolved in 100 ml of chloroform.
**Procedure**

0.1 ml of serum was made upto 4.0 ml with isopropanol. It was mixed well and 0.4 gm of activated silicic acid was added. It was shaken in a vortex mixer for 15 minutes and centrifuged.

2.0 ml of the supernatant was taken. Standards ranging from 20-100 mg were made up to 2.0 ml with isopropanol. To all the tubes, 0.6 ml of saponifying reagent was added and incubated at 60-70º C for 15 minutes. After cooling 1.0 ml of sodium meta-periodate solution was added and mixed. To this, 0.5 ml of acetyl acetone was added, mixed and incubated at 50º C for 30 minutes. After cooling, the color was read at 405nm in a Systronic UV spectrophotometer.

The value of triglyceride in serum was expressed as mg/dl.

**Creatinine**

**Estimation of serum creatinine**

This was estimated according to the method of Broad and Sirota\(^{145}\) using Jaffé’s reaction.

**Reagents**

1. Saturated picric acid
2. Sodium hydroxide: 0.75 N
3. Sulfuric acid: 2/3 N
4. Sodium tungstate: 10%
5. Stock standard creatinine: 100 mg of creatinine was dissolved and made up to 100 ml in 0.1 N hydrochloric acid. Working standard was prepared by appropriate dilution of the stock solution.
Procedure

A protein free filtrate was prepared by precipitating 1.0 ml of serum with 8.0 ml of water, 0.5 ml 2/3 N sulfuric acid and 0.5 ml of 10% sodium tungstate. After centrifugation, 5.0 ml of the clear filtrate was taken. To this, 1.5 ml saturated picric acid solution and 1.5 ml of 0.75 N sodium hydroxide were added. The color intensity was measured at 460nm after 15 minutes in a Systronic UV spectrophotometer. Standard and blank were also processed similarly.

The creatinine levels were expressed as mg/dl.

Urea

Estimation of serum urea

Urea was determined by the method of Natelson et al\textsuperscript{146}, using diacetylmonoxime.

Reagents

1. Diacetylmonoxime reagent: 2.0 g of diacetylmonoxime was dissolved in 100 ml of 2.0% acetic acid.

2. Sulphuric acid-phosphoric acid mixture: 25 ml of concentrated sulfuric acid, 75 ml of 85% o-phosphoric acid and 70 ml of distilled water were mixed.

3. Sodium tungstate solution: 10%

4. Sulfuric acid: 0.67 N

5. Standard urea solution: 20 mg of urea dissolved in 100 ml of distilled water.
Procedure

To 0.1 ml of serum was added 3.3 ml of water and mixed with 0.3 ml of 10% sodium tungstate and 0.3 ml of 0.67 N sulfuric acid reagent. The suspensions were centrifuged and to 1.0 ml of the supernatant, 1.0 ml of water, 0.4 ml of diacetylmonoxime and 2.6 ml of sulfuric acid-phosphoric acid reagents were added in that order. Aliquots of standard urea were also treated in a similar manner and heated in a boiling water bath for 30 minutes, cooled and the color developed was measured at 480nm in a Systronic UV spectrophotometer.

The values were expressed as mg/dl.

Statistical analysis

The experimental results were expressed as the Mean ± SEM (Standard Error Mean). The statistical analysis was performed by analysis of variance (ANOVA) followed by Dunnett’s test was used to make a statistical comparison between groups. Results with P < 0.05 were considered statistically significant.

3.4.5.3 Results

Oral Glucose Tolerance Test (OGTT)

In a single oral administration of glucose (2 g/ml per kg) at 30 min. the blood glucose levels increased (180.6 ± 6.4 mg/dl) above the normal value and reduced to 155.4 ± 4.6, 110.5 ± 5.1 and 92.7 ± 4.2 mg/dl at 60, 90 and 120 min. of glucose load respectively.

At 60 min. of glucose load, Glibenclamide treated diabetic animals, the blood glucose value was 172.1 ± 6.4 mg/dl and this value reduced to 122.9 ±
5.2 mg/dl at 90 min. and at 120 min. the glucose value was near to the normal value 106.2 ± 5.5 mg/dl (Table 3.10).

HE treated animals exhibited a highly significant decrease (P < 0.001) in blood glucose at 90 min. and was found to be 143.2 ± 3.3 mg/dl and at 120 min. the blood glucose value was 113.3 ± 3.6 mg/dl, which is very near to the value of standard drug Glibenclamide.

In EASFME treated rats the blood glucose values were only 222.3 ± 6.6, 202.4 ± 5.6, 174.5 ± 4.1 and 136.7 ± 4.6 mg/dl at 30, 60, 90 and 120 min. of glucose load respectively. These values were significant (P < 0.05) at 90 min. and highly significant (P < 0.001) at 120 min. as compared to normal group (Table 3.10).

Table 3.10: Glucose tolerance test in diabetic rats treated with Momordica dioica fruit pulp extracts.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Blood glucose levels (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>I.</td>
<td>Normal</td>
<td>81.1 ± 4.1</td>
</tr>
<tr>
<td>II.</td>
<td>Diabetic control</td>
<td>172.3 ± 7.1</td>
</tr>
<tr>
<td>III.</td>
<td>Diabetic + Glibenclamide (10 mg/kg)</td>
<td>96.7 ± 4.3</td>
</tr>
<tr>
<td>IV.</td>
<td>Diabetic + HE (400 mg/kg)</td>
<td>93.5 ± 3.4</td>
</tr>
<tr>
<td>V.</td>
<td>Diabetic + EASFME (400 mg/kg)</td>
<td>94.9 ± 3.7</td>
</tr>
</tbody>
</table>

Values are given as mean ± S.E.M for six rats in each group.
<sup>a</sup>P < 0.001,  <sup>b</sup>P < 0.05,  compared to control
Table 3.10 gives the blood glucose value of normal, diabetic control, Glibenclamide, HE and EASFME treated animals after oral administration of glucose (2 g/ml per kg). In Glibenclamide and HE treated groups the animals showed significantly decreased blood glucose concentration after 60 min. and 120 min. HE treated animals tend to bring the values near normal. HE (400 mg/kg) was equally effective in reducing blood glucose when compared with glibenclamide. Glibenclamide (10 mg/kg) produced a significant decrease in blood glucose level at 60-120 min. after the administration.

Effect of Intraperitoneal administration of extract in hyperglycaemic rats

The mean glucose value of fasted animals at various time intervals after i.p. administration of HE and EASFME of *Momordica dioica* fruit pulp in hyperglycaemic rats are shown in (Table 3.11). The glucose levels were compared to the values obtained for normal groups.

In hyperglycaemic rats injection of the HE in the dose of 400 mg/kg lowered blood glucose level significantly at 3h (P < 0.001) and the percentage inhibition was 59.28, whereas at 5h the percentage inhibition was found to be 63.99, when compared to group III (Glibenclamide treated), the highest activity was found at 3h (70.48%) and at 5h (65.11%). EASFME does not produce any reduction in the blood glucose level, in i.p. route of administration.
Table 3.11 Effect of intraperitoneal administration of extracts of *Momordica dioica* fruit pulp on blood glucose concentration in diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Blood Glucose (mg/dl)</th>
<th>0 h</th>
<th>1 h</th>
<th>3 h</th>
<th>5 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>Normal</td>
<td>129.89 ± 4.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>125.08 ± 3.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>126.56 ± 3.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II.</td>
<td>Diabetic control</td>
<td>525.64 ± 7.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV.</td>
<td>Diabetic + Glibenclamide</td>
<td>483.28 ± 10.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(10 mg/kg)</td>
<td>(8.06)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>346.01 ± 7.2</td>
<td>(33.57)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>153.98 ± 4.3</td>
<td>(70.48)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>182.76 ± 6.5</td>
<td>(65.11)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V.</td>
<td>Diabetic + EASFME</td>
<td>500.19 ± 10.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(400 mg/kg)</td>
<td>(4.84)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>455.67 ± 7.1</td>
<td>(12.52)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>212.39 ± 5.6</td>
<td>(59.28)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>188.64 ± 6.3</td>
<td>(63.99)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diabetic + EASFME</td>
<td>520.06 ± 9.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(400 mg/kg)</td>
<td>(1.06)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>500.91 ± 9.4</td>
<td>(3.83)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>493.26 ± 8.2</td>
<td>(5.44)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>470.0 ± 7.3</td>
<td>(10.09)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are given as mean ± S.E.M for six rats in each group.

Data in the parenthesis indicate percentage inhibition.

\[ ^{a} p < 0.001, \quad ^{b} p < 0.05 \] compared to control group.
Table 3.12  Effect of treatment with *Momordica dioica* fruit pulp extracts for 15 days on changes in body weight and urine Sugar in diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Body Weight (g)</th>
<th>Urine Sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>Normal</td>
<td>176.7 ± 5.3</td>
<td>187.4 ± 5.8</td>
</tr>
<tr>
<td>II.</td>
<td>Diabetic control</td>
<td>160.6 ± 4.6</td>
<td>139.4 ± 3.9</td>
</tr>
<tr>
<td>III.</td>
<td>Diabetic + Glibenclamide (10 mg/kg)</td>
<td>173.9 ± 4.8(^a)</td>
<td>175.3 ± 4.9(^a)</td>
</tr>
<tr>
<td>IV.</td>
<td>Diabetic + HE (400 mg/kg)</td>
<td>180.4 ± 5.9(^a)</td>
<td>178.4 ± 6.2(^b)</td>
</tr>
<tr>
<td>V.</td>
<td>Diabetic + EASFME (400 mg/kg)</td>
<td>163.9 ± 3.4(^b)</td>
<td>173.5 ± 4.7(^a)</td>
</tr>
</tbody>
</table>

Values are given as mean ± S.E.M for six rats in each group.

\(^a\)P < 0.001, \(^b\)P < 0.05 compared to control

Effect of oral administration of extracts in hyperglycaemic rats

Changes in body weight and urine sugar

Table 3.12 demonstrated the changes in body weight and urine sugar of normal and experimental animals.

Blood glucose

A significant reduction in blood glucose value was observed in diabetic control group from the initial value of 500.0 ± 42.82 mg/dl to the level of 219.0 ± 26.13 mg/dl with HE (P < 0.001) and 205.0 ± 18.46 mg/dl with EASFME (P < 0.001). The standard drug Glibenclamide has reduced the level to 198.33 ± 18.91 mg/dl (Table 3.13).
Serum cholesterol and triglycerides

Serum cholesterol and triglycerides levels in all five groups of animals are given in Table 3.13. The cholesterol and triglycerides levels were significantly higher in the diabetic group (194.66 ± 3.72 and 104.22 ± 5.08 mg/dl respectively) compared to those in normal rats (76.33 ± 3.85 and 76.0 ± 7.78 mg/dl respectively). The HE treated diabetic rats has significantly reduced levels of both cholesterol and triglycerides. (58.17 ± 6.37 and 81.17 ± 3.68 mg/dl respectively) when compared to diabetic control (194.66 ± 3.72 and 104.22 ± 5.08 mg/dl) and brought near to the normal group value. EASFME treated diabetic rats has reduced levels of cholesterol and triglycerides (88.17 ± 6.37 and 71.33 ± 3.62 mg/dl respectively) below the normal value (76.0 ± 7.78 mg/dl) of triglycerides.
Table 3.13: Effect of *Momordica dioica* fruit pulp extracts on serum glucose, cholesterol, triglycerides, total protein, urea and creatinine in diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Glucose (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>Total Protein (g/dl)</th>
<th>Urea (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>Normal</td>
<td>82.17 ± 8.84</td>
<td>76.33 ± 3.85</td>
<td>76.0 ± 7.78</td>
<td>7.38 ± 0.11</td>
<td>28.33 ± 0.88</td>
<td>0.45 ± 0.03</td>
</tr>
<tr>
<td>II.</td>
<td>Diabetic control</td>
<td>500.0 ± 42.82</td>
<td>194.66 ± 3.72</td>
<td>104.22 ± 5.08</td>
<td>6.13 ± 0.24</td>
<td>67.0 ± 4.18</td>
<td>2.12 ± 0.08</td>
</tr>
<tr>
<td>III.</td>
<td>Diabetic + Glibenclamide (10 mg/kg)</td>
<td>198.33 ± 18.91 (72.20)</td>
<td>82.16 ± 3.41 (95.07)</td>
<td>43.16 ± 2.13 (216.45)</td>
<td>6.1 ± 0.13 (2.40)</td>
<td>51.17 ± 2.15 (40.94)</td>
<td>1.3 ± 0.08 (49.10)</td>
</tr>
<tr>
<td>IV.</td>
<td>Diabetic + HE (400 mg/kg)</td>
<td>219.0 ± 26.13b (67.25)</td>
<td>58.17 ± 6.37a (115.35)</td>
<td>81.17 ± 3.68 (81.66)</td>
<td>6.6 ± 0.07b (37.60)</td>
<td>62.33 ± 3.35 (12.08)</td>
<td>1.7 ± 0.08 (25.15)</td>
</tr>
<tr>
<td>V.</td>
<td>Diabetic + EASFME (400 mg/kg)</td>
<td>205.0 ± 18.46b (70.60)</td>
<td>88.17 ± 6.37a (89.99)</td>
<td>71.33 ± 3.62b (116.56)</td>
<td>6.6 ± 0.10b (37.60)</td>
<td>34.17 ± 2.87a (84.90)</td>
<td>0.8 ± 0.09b (79.04)</td>
</tr>
</tbody>
</table>

Values are given as mean ± SEM for six rats in each group.

Figures in the parenthesis indicate the percentage protection in individual parameters from their elevated values. The percentage protection is calculated as 100 x (value of diabetic control - value of samples) / (value of diabetic control - value of control).

^aP < 0.001, ^bP < 0.05 compared to diabetic control
Antidiabetic and hypolipidemic activity of fruit pulp extracts of Momordica dioica
Total protein, urea and creatinine

Table 3.13 shows the levels of total protein, urea and creatinine in all five groups of animals. Diabetic control group has decreased protein value (6.13 ± 0.24 g/dl) and increased urea (67.0 ± 4.18 mg/dl) and creatinine value (2.12 ± 0.08 mg/dl) when compared to normal group (7.38 ± 0.11 g/dl, 28.33 ± 0.88 mg/dl and 0.45 ± 0.03 mg/dl respectively) (Fig. 3.7). HE and EASFME treated diabetic rats has increased protein levels (6.6 ± 0.07 and 6.6 ± 0.10 g/dl respectively). HE has little effect on urea and creatinine (62.33 ± 3.35 and 1.7 ± 0.08 respectively) when compared to EASFME treated group (34.17 ± 2.87 and 0.8 ± 0.09 mg/dl respectively). EASFME treatment has brought this urea and creatinine value to the normal value (28.33 ± 0.88 and 0.45 ± 0.03 mg/dl) when compared to the standard Glibenclamide treated group (51.17 ± 2.15 and 1.3 ± 0.08 mg/dl respectively).

3.4.5.4 Discussion

Intraperitoneal administration of HE extract of *Momordica dioica* fruit pulp produced a statistically significant decrease in blood glucose concentration in alloxan-induced hyperglycaemic rats. Intraperitoneal administration of EASFME of *Momordica dioica* fruit pulp showed only a significant reduction in blood glucose level in hyperglycaemic rats which gives an indication that EASFME had lower tendency to decrease blood glucose level in i.p. route. Alloxan selectively destroys insulin secreting β–cells in the islets of Langerhans and the effect is irreversible\(^{147}\).

Diabetic animals receiving the HE of *Momordica dioica* fruit pulp showed rapid normalization of blood glucose level in comparison to the control and this could be due to the possibility that many β-cells are still surviving and cell regeneration cannot be ignored.
Diabetes is possibly the world's fastest growing metabolic disorder and as knowledge of the heterogeneity of this disorder increases, so does the need for more appropriate therapies\textsuperscript{148}. Traditional plant medicines are used throughout the world for a range of diabetic presentations. The study of such medicines might offer a natural key to unlock a diabetologist's pharmacy for the future. Several reports are available on the hypoglycaemic effect of the roots and other parts of \textit{Momordica dioica}\textsuperscript{149}.

In the light of the above reports, an attempt was made to study the effect of fruit pulp of \textit{Momordica dioica}.

In the present study the hypoglycaemic and hypolipidemic activities of the fruit pulp extract of \textit{Momordica dioica} was evaluated in alloxan-induced diabetic rats. The continuous treatment of \textit{Momordica dioica} extract for a period of 15 days caused a significant reduction in blood glucose level in diabetic rats. Studies on other species of this genus \textit{Momordica charantia} and \textit{Momordica foetida}\textsuperscript{150, 151}, have shown that aqueous extract of \textit{Momordica charantia} L. fruits reduce the fasting glucose levels of both hyperglycaemic and normoglycaemic in mice and foetidin, isolated from \textit{Momordica foetida}, has been shown to lower the blood glucose level of normal rats, but not in diabetic animals\textsuperscript{152}.

However in the present study it was observed that, there was a significant weight loss in the diabetic group and the treatment with \textit{Momordica dioica} fruit pulp extracts in the treated diabetic group resulted in an improvement in their body weights. It indicates that, \textit{Momordica dioica} fruit pulp extract protect, the body weight loss due to its antidiabetic activity and further, it was supported by the increase in protein level in extract treated groups.

Since alloxan treatment causes permanent destruction of beta cells, \textit{Momordica dioica} fruit pulp extract may produce the antidiabetic effect in diabetic rats by a mechanism other than the stimulation of insulin secretion from the $\beta$–cells.
In the alloxan-induced diabetic animals, the rise in blood glucose is accomplished by elevation of serum cholesterol and triglycerides levels. The level of cholesterol and triglycerides were brought to normal by the treatment of HE and EASFME in the diabetic rats. Even the decreased protein value during diabetes has increased appreciably in *Momordica dioica* extract treated rats.

### 3.4.5.5 Conclusion

*Momordica dioica* fruit extract has effect in correcting many of the metabolic abnormalities, due to diabetes and may be concluded that *Momordica dioica* fruit has antidiabetic and hypolipidemic effects in experimental diabetes. Further pharmacological and phytochemical investigations are under way to elucidate the mechanism of glucose lowering and hypolipidemic actions and other bio-chemical parameters of *Momordica dioica*.

### 3.5 Antimicrobial activity

#### 3.5.1 Introduction

In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. The world health organization estimates that majority of the people living in developing countries dwell on traditional system of medicine for the treatment of various ailments. It has been estimated that 14 - 28% of higher plant species are used medicinally, but only 15% plants were investigated both phytochemically and pharmacologically.

A approach that has been used for the investigation of anti-microbial principles from plant is based upon high throughput screening of medicinal plant extracts. The WHO at their meeting in 1985 specified some medicinal
plants such as *Aloe vera*, *Allium sativum*, *Azadirachta indica*, *Cassia fistula*, *Fumaria officinalis* and *Withania somnifera* meant for the treatment of skin diseases.

Pharmacophore such as clausenol\textsuperscript{153}, a carbazole alkaloid isolated from the stem bark of *Clausena anisata*, Atylosol\textsuperscript{154} isolated from *Atylosia trinervia*, Carpesiolin\textsuperscript{155}, isolated from *Carpesium abrotanoides*, Cnicin\textsuperscript{156} isolated from *Centaurea calcitrapa*, Louisfieserone\textsuperscript{157} from *Indigofera suffruticosa*, Hyperforin\textsuperscript{158} isolated from *Hypericum perforatum* were found to possess significant antimicrobial activity.

Infectious diseases account for approximately one half of all deaths in tropical countries. In industrialized nations, despite the progress made in the understanding of microbiology and their control, incidents of epidemics due to drug resistant microorganisms and the emergence of hitherto unknown disease causing microbes, pose enormous public health concerns. Historically, plants have provided a good source of anti-infective agents; emetine, quinine, and berberine remain highly effective instruments in the fight against microbial infections. Phytomedicines derived from plants have shown great promise in the treatment of intractable infectious diseases including opportunistic AIDS infections.

World wide infectious disease is the number one cause of death accounting for approximately one half of all deaths in tropical countries. Perhaps it is not surprising to see these statistics in developing nations, but what may be remarkable is that infectious disease mortality rates are actually increasing in developed countries, such as the United States. Death from infectious disease, ranked 5th in 1981, has become the 3rd leading cause of death in 1992, an increase of 58%\textsuperscript{159}. It is estimated that infectious disease is the underlying cause of death in 8% of the deaths occurring in the US\textsuperscript{159}. 
Similarly, higher plants have made important contributions in the areas beyond anti-infectives, such as cancer therapies. Early examples include the antileukaemic alkaloids, vinblastine and vincristine, which were both obtained from the Madagascan Periwinkle (*Catharanthus roseus* syn. *Vinca roseus*)\(^{160}\). Other cancer therapeutic agents include taxol, homoharringtonine and several derivatives of camptothecin. For example, a well known benzylisoquinoline alkaloid, papaverine, has been shown to have a potent inhibitory effect on the replication of several viruses including cytomegalovirus, measles and HIV\(^{161}\).

Much of the exploration and utilization of natural products as antimicrobials arise from microbial sources. It was the discovery of penicillin that led to later discoveries of antibiotics such as *Streptomycin*, *Aureomycin* and *Chloromycetin*. Though most of the clinically used antibiotics are produced by soil micro-organisms or fungi, higher plants have also been a source of antibiotics. Examples of these are the bacteriostatic and antifungicidal properties of Lichens, the antibiotic action of allicine in *Allium sativum* (garlic), or the antimicrobial action of berberines in goldenseal (*Hydrastis canadensis*)\(^{162}\). Plant based antimicrobials represent a vast untapped source for medicines. Continued and further exploration of plant antimicrobials needs to occur. Plant based antimicrobials have enormous therapeutic potential. They are effective in the treatment of infectious diseases and simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. They are effective, yet gentle. Many plants have tropisms to specific organs or systems in the body. Phytomedicines usually have multiple effects on the body. Their actions often act beyond the symptomatic treatment of diseases.

### 3.5.2 Preparation of Samples

In the antimicrobial activity study, both the extracts (HE and EASFME) were dissolved in dimethyl sulfoxide (DMSO). Both the extracts were tested at the concentrations of 100, 200, 300, 400 and 500 µg/disc. The activity profile
was compared with different standard antibiotics for individual microorganism (Table 3.14).

**Microorganisms used in this Study**

Bacterial strains (Table 3.14) were obtained from the stock culture of National Chemical Laboratory, Pune, India and King Institute, Chennai, India.

**Antimicrobial Assay**

The anti-microbial activity was studied by disc-diffusion\textsuperscript{163-165} method employing 24h culture of eleven different types microorganisms.

**Kirby-Bauer antimicrobial susceptibility test**

A standardized filter paper disc agar diffusion procedure, known as the Kirby-Bauer method was used to determine the susceptibility of microorganisms against the extracts. This method allows for the rapid determination of the efficacy of a drug by measuring the diameter of the zone of inhibition that results from diffusion of the agent into the medium surrounding the disc. In this procedure, filter paper (Whatmann No.1) disc of uniform size were impregnated with specified concentrations of different extracts and then placed on the surface of the agar plate that has been seeded with the organism to be tested. The medium of choice is Mueller-Hinton agar, with a pH of 7.2 to 7.4, which was poured into the plates to a uniform depth of 5 mm and cooled for solidification. Prior to use, the plates were dried in an incubator at 37°C for 10 to 20 min. to remove moisture on the agar surface. The plates were then swabbed with a standardized inoculum by means of a cotton swab to ensure confluent growth of the organism. The disc were aseptically placed on the surface of the agar plate at well-spaced intervals. Once placed, each disc is gently touched with a sterile applicator stick to ensure its firm contact with the agar surface.
Following incubation, the plates were examined for the presence of zone of inhibition and correspondingly readings were obtained.

3.5.3 Results

Anti-microbial activity of the extracts (HE and EASFME) of *Momordica dioica* fruit pulp are represented in Table 3.14.

3.5.4 Discussion

Both the HE and EASFME of *Momordica dioica* fruit pulp exhibited moderate and concentration dependent antibacterial activity. HE was found to be sensitive to *Bacillus cereus*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Proteus vulgaris*, *Salmonella typhi*, *Shigella dysenteriae*, *Staphylococcus aureus* and *Staphylococcus epidermidis*, whereas it was found resistant to *Escherichia coli* and *Pseudomonas aeruginosa*. EASFME was found to be sensitive to *Escherichia coli*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella dysenteriae*, *Staphylococcus aureus* and *Staphylococcus epidermidis*, whereas resistant to *Bacillus cereus*, *Bacillus subtilis* and *Klebsiella pneumoniae*.

3.5.5 Conclusion

Both the extracts possessed some antimicrobial activity against all the tested microorganisms. So, it can be concluded that HE was found to be much more active than EASFME. These results may support the uses of this plant fruits in traditional medicines.
Table 3.14 Antimicrobial activity of *Momordica dioica* fruit pulp extracts

<table>
<thead>
<tr>
<th>Microrganisms</th>
<th>HE</th>
<th>IZ at μg/disc</th>
<th>EASFME</th>
<th>IZ at μg/disc</th>
<th>Standards$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>200</td>
<td>300</td>
<td>400</td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td>G+</td>
<td>-</td>
<td>-</td>
<td>8.0</td>
<td>8.5</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>G+</td>
<td>-</td>
<td>6.0</td>
<td>7.0</td>
<td>8.0</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>G+</td>
<td>-</td>
<td>7.0</td>
<td>8.0</td>
<td>9.0</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>G+</td>
<td>-</td>
<td>-</td>
<td>8.0</td>
<td>9.0</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>G-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>G-</td>
<td>-</td>
<td>-</td>
<td>10.0</td>
<td>11.0</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>G-</td>
<td>-</td>
<td>-</td>
<td>9.0</td>
<td>10.0</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>G-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8.0</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>G-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>G-</td>
<td>-</td>
<td>-</td>
<td>11.0</td>
<td>12.0</td>
</tr>
<tr>
<td><em>Shigella dysenteriae</em></td>
<td>G-</td>
<td>10.0</td>
<td>11.0</td>
<td>12.0</td>
<td>13.0</td>
</tr>
</tbody>
</table>

Values (mean of three replicates) are; IZ, inhibition zone (mm); -, no inhibition.

$^a$ Ce, ceftriaxone (30 μg/disc); Ch, chloramphenicol (30 μg/disc); Er, erythromycin (15 μg/disc); Nv, novobiocin (30 μg/disc); Tr, trimethoprim (5 μg/disc); Te, tetracycline (10 μg/disc); Ci, ciprofloxacin (10 μg/disc); Am, ampicillin (10 μg/disc).
3.6 Antifeedant activity

3.6.1 Introduction

Insect antifeedants are compounds with the ability to reduce or inhibit insect feeding without directly killing the insect. Such compounds offer a number of properties that are highly desirable in environmentally friendly crop protection agents. Although the principles of insect control using antifeedants has been shown to work under field conditions, practical application of these compounds has until now been limited to a few examples, mainly consisting of extracts of the neem tree containing the highly potent tetranortriterpene azadirachtin.

The role of plant secondary metabolites in host plant recognition, especially in oligophagous insects, is of great importance. Substances eliciting feeding responses of the insects have been extensively studied and numerous attempts have also been made in the past decades to isolate antifeedants from plants avoided by insect species. As the most studied of these plant secondary metabolites with antifeedant activity, but also in some cases insecticide activity, one should cite polygodial\textsuperscript{166}, waburganal\textsuperscript{167}, azadirachtin\textsuperscript{168}, toosendanine\textsuperscript{169}, clerodanes\textsuperscript{170} and ecdysteroids\textsuperscript{171}.

Neem oil is a broad spectrum botanical insecticide, nematicide and fungicide derived from the seeds of the neem tree (\textit{Azadirachta indica}). Particularly Azadirachtin-A from the neem seed kernal has been shown to be an effective antifeedent against the desert locusts\textsuperscript{172}. Govindachari et al., have isolated several natural congeners of Azadirachtin-A which possess antifeedant activity\textsuperscript{173}.
3.6.2 Method

*S. litura* L (Lepidoptera), a polyphagous pest of cotton, rice, tomato, tobacco, groundnut, castor and legumes were used as a test insect for antifeedant studies.

Field collected larvae were cultured on castor leaves (*R. communis* L) in the laboratory at 25 ± 2°C. Second generation larvae (third instar) from the laboratory cultures were used for antifeedant bioassay.

The dual choice anti-feedant bioassay\(^{174}\) was performed. Field collected *R. communis* leaves were cut into circular discs (180 cm\(^2\)) with the median vein as the marker between two equal halves. Extracts were dissolved in 1 ml of acetone which was spread with the help of a fine pipette on the right half of the circular leaf disc to have the desired concentration (such as 5, 10 and 20 μg/cm\(^2\) leaf area). The left half of the leaf was treated with 1 ml of acetone which served as control. After air drying, each leaf disc was placed in a Petridish. Five freshly moulted third instar larvae of *S. litura* (from the same egg mass having similar weight) were placed in the center of the leaf and left to feed for 24 h. For each concentration and compound, five replicates were maintained. After 24 h the larvae were removed and unfed area in the treated and control halves were measured using a ΔT area measurement meter. Antifeedant activity (%) was calculated using the following formula.

\[
\text{Antifeedant activity (\%)} = \frac{100 - (\text{Area fed in treated})}{\text{Area fed in control}} \times 100
\]

**Insect used in this study**

The test insect, *S. litura* L., was reared in the laboratory on *R. communis* L., (castor leaves) at 25± 2°C and third instar larvae were used for testing the extracts.
3.6.3 Results

The activity was tested at various concentrations (5 μg/cm², 10 μg/cm² and 20 μg/cm²) for both the HE and EASFME. It was found that EASFME exhibited a significant activity when compared to HE (Table 3.15). A concentration dependant activity was found with both the extracts. However, the *Momordica dioica* fruit pulp exhibited highest activity (61%) in EASFME at 20 μg/cm².

Table 3.15: Antifeedant activity (%) of *Momordica dioica* fruit pulp extracts against *Spodoptera litura*

<table>
<thead>
<tr>
<th>Name of the extract</th>
<th>Antifeedant activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 (μg/cm²)</td>
</tr>
<tr>
<td>HE</td>
<td>12.88 ± 0.13</td>
</tr>
<tr>
<td>EASFME</td>
<td>39.06 ± 0.14</td>
</tr>
<tr>
<td>Azadirachtin-A*</td>
<td>79.32 ± 0.29</td>
</tr>
</tbody>
</table>

Values are mean ± S.D; n = 5

* Treatment at 0.5 (μg/cm²)

3.6.4 Conclusion

The above results suggest that the fruit pulp extract of *Momordica dioica* may be efficiently used in the pest management.
3.7 Phytochemical analysis of Hexane extracts of fruit pulp of *Momordica dioica*.

The above pharmacological studies revealed that, among the two extracts of fruit pulp of *Momordica dioica*, it was found that, HE was more active when compared to EASFME. Hence HE was subjected to column chromatography to identify and separate the constituents.

**Materials and Methods**

HE (1 gm) was weighed and dissolved in chloroform (sufficient quantity) and admixture was prepared using silica gel (3 gm). Column was packed with silica gel 30 gm (mesh 70 - 325) using hexane as a solvent. The column was eluted with solvent in the increasing order of polarity 100% hexane to 15% EtoAc in hexane. 10 ml fractions were collected.

Elution of the column using 7.5% EtoAc in hexane yielded compound (III-1), 120 mg (12%) as a semi solid material.

Further enhancing the polarity 10% ethyl acetate in hexane afforded compound (III-2) 150mg (15% ) as a yellow semi solid material.

With 12.5% EtoAc in hexane afforded mixture of compound (III-3a and III-3b) was obtained as a white solid residue.

**Experimental**

**Compound (III-1): Linoleic acid.**

\[
\text{M.F } C_{18}H_{32}O_2 \quad \text{Mass m/z: } 280 (M^+)
\]

\[
^{1}H \text{ NMR (200 MHz, CDCl}_3\text{) } \delta \text{ ppm: } 0.87 \text{ (t, } 3\text{H, CH}_3\text{), } 1.25 \text{ (m, } 8\text{H, (CH}_2)_4\text{), } 1.62 \text{ (m, } 14\text{H, (CH}_2)_7\text{), } 2.34 \text{ (t, } 2\text{H, (CH}_2\text{)), } 5.36 \text{ (q, } 4\text{H, (CH=CH)}_2\text{) and } 9.03 \text{ (q, } 1\text{H, COOH).}
\]
$^{13}$C NMR (200 MHz, CDCl$_3$) $\delta$ ppm: 14.13, 22.59, 22.71, 27.21, 29.07, 29.16, 29.26, 29.38, 29.45, 29.71, 31.54, 31.94, 34.08, 127.91, 128.07, 129.73, 130.03 and 180.18.

MS (m/z): 280, 256, 241, 227, 213, 199, 185, 171, 157, 143, 129, 111, 97, 83, 73, 53 and 41 (Fig.3.8a).

IR $\nu_{max}$: 3448, 2918, 2849, 1707, 1464, 1411, 1295, 940 and 720 cm$^{-1}$ (Fig.3.8b).

**Compound III-2 Palmitic Acid**

MP 61 - 64$^\circ$C  \hspace{1cm} MF: C$_{16}$H$_{32}$O$_2$. \hspace{1cm} Mass m/z : 256 (M$^+$)

$^1$H NMR (200 MHz, CDCl$_3$) $\delta$ ppm: 0.95 (t, 3H, CH$_3$), 1.25 (m, 26H, (CH$_2$)$_{13}$), 2.7 (t, 2H, CH$_2$), and 9.27 (q, 1H, COOH).

$^{13}$C NMR (200 MHz, CDCl$_3$) $\delta$ ppm: 14.04, 22.53, 22.64, 24.64, 25.58, 27.15, 29.03, 29.10, 29.21, 29.32, 29.40, 29.64, 31.48, 31.89, 34.96, 37.14 and 180.04.

MS (m/z) : 256, 213, 199, 185, 171, 157, 143, 129, 109, 97, 83, 73 and 55 (Fig.3.9a).

IR $\nu_{max}$: 3416, 2927, 2845, 1736, 1639, 1618, 1452, 1384, 1158, 1025, 886 and 609 cm$^{-1}$ (Fig.3.9b).
Compound 111-3a and b, (Mixture of oleic acid and ursolic acid)

**Oleic acid**

**MF:** C_{18}H_{34}O_{2} : \hspace{1cm} \text{Mass (m/z) : 282 (M^+)}

**IR \nu_{\text{max}}:** \hspace{1cm} 3420, 2999, 2847, 1700, 1465, 1293, 1154, 955 and 633 cm\(^{-1}\).

**MS (m/z):** \hspace{1cm} 284, 271, 256, 241, 227, 213, 199, 185, 171, 149, 129, 111, 97, 83, 69, 55 and 43 (Fig.3.10a).

**Ursolic acid**

**MF:** C_{30}H_{48}O_{3} : \hspace{1cm} \text{Mass (m/z) : 456 (M^+)}

**MS (m/z):** \hspace{1cm} 456, 412, 397, 369, 340, 324, 316, 300, 285, 271, 255, 246, 229, 213, 191, 175, 159, 135, 119, 107, 81, 57 and 43 (Fig.3.10a).
3.8 References


3. The wealth of India, A dictionary of Indian Raw materials and Industrial products VI, Ed: Publication and Information Directorate, CSIR, New Delhi, India, 408 (1962).


119 Shaw, J.E., de courten, M.P. and Zimmet, P.Z. in Diabetes in the New Millennium (eds Turtle, J.R., Kaneko, T. and Osato, S.), The endocrinology and Diabetes Research Foundation of the University of Sydney, Sydney, 1 (1999).


