Methodology
Chapter 3

Methodology

3.1 Study Site

The study was conducted in medical wards of K.L.E.S’s Dr. Prabhakar Kore Hospital and Medical Research Center, with 2200 bed capacity. The hospital is a multi-specialty tertiary healthcare teaching hospital providing both inpatient and out patient services to people in and around Belgaum district.

3.2 Study Design

This is a prospective, pharmacist based study involving all the in-patients admitted to medicine wards fulfilling the inclusion criteria. Ethical clearance (No - KLEU/07-08/D.8612 ) was obtained from the Institutional Ethics Committee for Human (Annexure-1) and a separate ethical clearance No 1/3/2007,23/11/2007 was obtained from institutional animal ethics committee (IAEC reg. No. 627/02/a/ CPCSEA) constituted as per the norms of CPCSEA to conduct animal experiments(Annexure-2).
3.3 Inclusion Criteria

1. Patients of either sex admitted to the wards of medicine units.

2. Patients on multiple drug therapy; with minimum of three drugs.

3. All patients above the age of 18 years

4. Patients willing to participate [written informed consent]

3.4 Exclusion Criteria

1. Patients on additional alternative medicines.

2. Patients with ADRs seeking admission

3.5 Study Material

All the necessary and relevant data were collected during clinical rounds in consultation with the treating physician.
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Table 3.1: Study Material

<table>
<thead>
<tr>
<th>Source</th>
<th>Data Collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients history record</td>
<td>Relevant history with diagnosis, laboratory reports of investigations ordered and past history regarding ADRs experienced for any drug.</td>
</tr>
<tr>
<td>Treatment Chart</td>
<td>List of all the medications prescribed, along with dose, frequency, route and duration of treatment</td>
</tr>
<tr>
<td>Inpatient follow-up records</td>
<td>Disease course, development of new signs and symptoms and change in laboratory repeat investigations if any</td>
</tr>
</tbody>
</table>

### 3.6 Study Procedure

#### 3.6.1 Data Collection

#### 3.6.1.1 ADR

On admission and after administering informed consent in vernacular language (Annexure-3,4,5,6) the patients demographic data, current medications, laboratory investigations, past medical and medication history was collected from the patients profile form. The demographic data collected includes the patients age, sex, weight, occupation etc. The current medication data includes all the drugs, their dosage, route of administration with frequency, date of drugs started and stopped.

The past medical and medication history data collected includes the patients previous allergies, and the drugs received previously. The laboratory data collected includes the relevant laboratory investigations ordered to confirm the ADR.

ADRs detected during the ward rounds were notified by using the notification form developed for the study. This form includes all information regarding the
patients admission status and other details regarding reaction, date of onset of reaction and the list of suspected drugs. An ADR reporting and documentation form was developed (Annexure-8) to gather maximum information about the developed ADR. The suspected ADRs were analyzed to establish causal relationship with suspected drug using various standard scale to categorize them as certain/definite, probable, possible, unassessable/unclassable, unlikely, conditional/unclassified, conditional etc.

3.6.1.1.1 Causality assessment

All the reported suspected ADRs were investigated thoroughly using previously published literature and causality relationship between the reaction and the suspected drug was established and assessed. Assessment was done using two scales viz.

- Naranjo’s probability scale (Annexure-11)
- WHO probability scale (Annexure-9)

The reactions were categorized as certain, probable, possible, unlikely, unassessable and conditional.

3.6.1.1.2 Severity Assessment

All reported suspected ADRs were investigated thoroughly and assessed by level of severity using scale viz.

- Hartwig et al. severity scale (Annexure-13)


3.6.1.1.3 Preventability Assessment

All reported suspected ADRs were investigated thoroughly and assessed by using preventability scale viz.

- Modified Schumock and Thornton scale (Annexure-12)

The reactions were categorized as definitely preventable, probably preventable and not preventable.

3.6.1.2 Drug-Drug Interaction

During clinical rounds in consultation with the treating physician potential drug-drug interactions suspected were noted from the patient daily progress record.

The patients demographic data, current medications, laboratory investigation, past medical and medication history, was collected from the patients progress record, treatment chart, laboratory reports and patient history record.

As for notifying an ADR patients demographic data was collected. The prescribed medications with their dosage, route of administration, date of drug started and stopped, and onset of the adverse event related to the suspected pair of drugs were recorded (Annexure-8).

Similarly, the past medical and medicine history, the patients previous allergies, co-morbidities and the drugs received previously along with the laboratory data if any in support of suspected drug-drug interactions were also noted. After thorough review of literature in support of the suspected adverse drug - drug interactions, the animal experiments were planned to confirm or rule out the same.
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Each of the potential drug-drug interaction was categorized according to their level of significance. The level of significance relates to the magnitude of the effect, to the likelihood of occurrence, and subsequently to the necessity of monitoring the patients or altering therapy to avoid potential adverse consequences (Annexure-10).

The primary factors that define level of clinical significance include significance rating, the time of onset of the interactions, and the documentation that an interaction occurs clinically. The following discussion defines the guidelines used to designate these factors.
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Categorized as per Criteria for significance rating and assigned the score as shown below. A significance rating of one to five was assigned to each interactions and the definition for these number ratings is as given below.

<table>
<thead>
<tr>
<th>Significance Rating (A)</th>
<th>Criteria</th>
<th>Management Rating (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Not listed</td>
<td>0</td>
</tr>
</tbody>
</table>
| 1                      | **Severity - Major:** The effects are potentially life-threatening or capable of causing permanent damage.  
                          **Documentation:** interaction is suspected, probable or established | 1                     |
| 2                      | **Severity - Moderate:** The effects may cause deterioration in a patients clinical status. Additional treatment, hospitalization, or an extended hospital stay may be necessary.  
                          **Documentation:** interaction is suspected, probable or established | 2                     |
| 3                      | **Severity - Minimal:** The effects are usually mild; consequences may be bothersome or unnoticeable but should not significantly affect the therapeutic outcome. Additional treatment is usually not required.  
                          **Documentation:** interaction is suspected, probable or established | 3                     |
| 4                      | **Severity - Major or Moderate**  
                          **Documentation:** Interaction is possible | 4                     |
| 5                      | **Severity:** Minor  
                          **Documentation:** possible OR  
                          **Severity:** Major, Moderate or Minor  
                          **Documentation:** interaction is unlikely | 5                     |
3.6.1.2.1 Criteria for severity

The potential severity of the interaction is important in assessing the risk vs. benefit of the treatment and that of therapeutic alternatives. Many times harmful effects of drug drug interactions can be avoided with appropriate dose adjustments or modification of the administration schedule.

3.6.1.2.2 Minor

ADRs that are having mild effects on patients health and their consequences may be bothersome or unnoticeable but not significantly affect the therapeutic outcome.

3.6.1.2.3 Moderate

The adverse effect that causes deterioration in the patients clinical status and needs additional treatment or hospitalization or extension of hospital stay.

3.6.1.2.4 Major

The effects are potentially life threatening or capable of causing permanent damage.

3.6.1.2.5 Evaluation of suspected Adverse Drug - Drug Interactions in experimental animals

During ward rounds patients receiving Glipizide + Omeprazole and similarly with Glipizide + Ranitidine experienced the adverse effects, which were suspected
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to be due to drug drug interaction. As literature survey indicated scanty information, hence the animal experiments were planned to confirm or rule out the same.

3.7 Materials and Methods

3.7.1 Animals

Healthy adult rats wistar strain weighing 150-250gm were selected for the study and were procured from licensed breeder, Bangalore.

3.7.2 Chemicals

1. Vehicle 2% micronised gum acacia powder by triturating 2 gm in 100 ml water
2. Glucose estimation kit
3. Motor and Pestle, capillary tubes, needles No. 26, alcohol, micropipette, 1 ml pipettes, eppendroffs tubes, centrifuge, 10 ml centrifuge tubes, incubator, distilled water etc.
4. Desiccator
5. Anaesthetic ether

3.7.3 Estimation of blood glucose by GOD-POD Method

Trinder’s method (1969) using two enzymes Glucose oxidase (GOD) and Peroxidase (POD) along with chromogen L-amino antipyrine and phenol was used to
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estimate the blood glucose. This method is intended for in-vitro quantitative
determination of glucose in serum/plasma or cerebrospinal fluid. There was no
interference due to substances like creatinine, fructose, galactose, reduced glutathione, ascorbic acid and xylose. Haemoglobin or bilirubin upto 10mg% does
not affect the test.

3.7.3.1 Principle

Glucose is estimated by enzymatic oxidation by GOD enzyme to give D-gluconic acid and hydrogen peroxide. Hydrogen peroxide in the presence of POD enzyme oxidises phenol which combines with 4-amino antipyrine to produce a red coloured quinoneimine dye. The intensity of colour is directly proportional to the glucose concentration.

\[ D - \text{glucose} + H_2O = O_2 \xrightarrow{\text{GOD}} D - \text{gluconic acid} + H_2O_2 \]

\[ H_2O_2 + 4 - \text{amino antipyrine} \xrightarrow{\text{POD}} \text{Quinone imine dye} + H_2O_2 \]

3.7.3.2 Kit Contents

1. Glucose reagent 5 Vials

2. Glucose standard (10mg/dl) 1 X 5 ml vial
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3.7.3.3 Reagent contents

1. Glucose oxidase 2000 IU/L
2. Peroxidase 3250 IU/L
3. 4-Aminoantipyrine 0.52 m mol/L
4. 4-Hydroxybenzoic acid 10 m mol/L
5. Phosphate buffer 110 m mol/L

3.7.3.4 Storage and Stability

1. 2-8 °C and protect from light
2. Avoid contamination of the reagents
3. Close the reagent bottle immediately after use


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3.7.3.5 Estimation of Plasma Glucose

Equipment  Semi auto analyser

Programme  The basic assay parameters

<table>
<thead>
<tr>
<th>Mode</th>
<th>End Point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength</td>
<td>505 mm (490-550 nm)</td>
</tr>
<tr>
<td>Temperature</td>
<td>37 °C</td>
</tr>
<tr>
<td>Optical path length</td>
<td>1 cm</td>
</tr>
<tr>
<td>Blanking</td>
<td>Reagent blank</td>
</tr>
<tr>
<td>Incubation</td>
<td>10 min at 37 °C</td>
</tr>
<tr>
<td>Sample volume</td>
<td>10 µl</td>
</tr>
<tr>
<td>Working reagent volume</td>
<td>1 ml</td>
</tr>
<tr>
<td>Concentration of standard</td>
<td>100 mg/dl</td>
</tr>
</tbody>
</table>

Linearity  Up to 500 mg/dl

Stability of colour  1 hour

Units  mg/dl

The blood samples were collected in Eppendroffs tubes containing heparin (200IU/ml of blood). The samples were centrifuged for separation of plasma. In separated and non haemolysed plasma, the glucose concentration is generally stable for 3 hours at room temperature and up to 72 hours at 2 - 8 °C. The reagents were added for colour development as below.

3.7.3.6 Procedure

Pipette 3 ml solutions into respective cuvettes of Blank, standard and test, mix well, incubate at 37 °C for 10 minutes. The amount of glucose was estimated using semi auto analyser.
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Table 3.2: Quantity of reagents to be added for the estimation of blood glucose

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Blank</th>
<th>Standard</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working reagent</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Distilled water</td>
<td>0.01 ml</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Standard</td>
<td>–</td>
<td>0.01 ml</td>
<td>–</td>
</tr>
<tr>
<td>Test</td>
<td>–</td>
<td>–</td>
<td>0.01 ml</td>
</tr>
</tbody>
</table>

Glucose concentration in mg % = \( \frac{A_{of\ (T)}}{A_{of\ (S)}} \times 100 \)

where A - Absorbance; S - Standard; T - Test

3.7.3.7 Hypoglycaemic activity

Percentage reduction in blood glucose level at any given time “t” was calculated with reference to the basal blood glucose level using the following formula

% Blood glucose reduction at time “\( t'\)" = \( \frac{A - B}{A} \times 100 \)

Where A = Initial blood glucose level before drug administration; B = Blood glucose levels time “\( t'\)” after drug administration

3.7.3.8 Housing of the Animals

Rats were housed in groups of six in clean polyacrylic cages. The bedding materials of the cage was changed every day. Animals were kept for one week to acclimatize to laboratory conditions before starting the experiment. They had free access to tap water and were fed with standard rat chow. The animals were maintained under natural day and night cycle throughout the study. The animals were experimentally naye and were fasted overnight prior to the day of experimental procedures. The institutional animal ethics committee approved the experimental protocol and care of animals was taken according to CPCSEA guidelines.
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3.7.3.9 Mode of Drug Administration

All the drugs are insoluble/poorly soluble in water, hence all these drugs were suspended individually in freshly prepared 0.2 - 0.3 ml 2% w/v acacia suspension for proper administration through oral route.

3.7.3.10 Blood Sampling

The rats were anaesthetized by light anesthetic ether. The blood was collected from retro-orbital sinus of the rat eye in eppendorff tubes containing heparin as anticoagulant. After collecting blood, the eye was cleaned with cotton dipped in saline and little pressure was applied to stop bleeding. Collected blood sample was centrifuged at 5000 rpm for 10 minutes and the separated plasma was analyzed immediately. After each blood sampling, the animals were given 0.5 ml saline as a fluid replacement to avoid an possibility of shock.

3.7.3.11 Experimental Procedure

1. Glipizide + Omeprazole

Wistar albino rats of either sex (150 - 250 g), maintained under standard conditions, were randomly distributed into 2 groups with six animals in each. The experiment was conducted in two phases. In the first phase, after overnight fasting all the animals of groups 1 were administered glipizide 40 mg/kg per oral. Blood samples were collected at 0,1,2,4,6,8,12,18,24, hours from retro-orbital sinus of the rat eye after drug treatment. Blood glucose levels were estimated using GOD POD method. In the second phase, these animals received Omeprazole 30 mg/kg orally for a period of
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7 days. During this period the animals had free access to food and water supplied ad libitum. On the 7th day, the rats were fasted for 18 hours, with water supplied ad libitum. These animals fasted overnight received glipizide + Omeprazole on 8th day. Blood samples were collected at the above mentioned prefixed time intervals to estimate the blood glucose levels.

2. Glipizide + Ranitidine

Following the above mentioned procedure another suspected pair glipizide + ranitidine for drug drug interactions was evaluated in animal experiments.

3.7.3.12 Statistical Analysis

The data were statistically analyzed by student “t” test and $P \leq 0.05$ is considered as statistically significant.

3.7.3.13 Awareness of ADR reporting

In pre-intervention study, health care professionals including 58 Doctors, 72 Nurses, 20 Pharmacists were surveyed using a questionnaire to assess their knowledge and attitudes regarding ADR and its reporting, to obtain their suggestions to improve ADR reporting(Annexure-14). After this survey, 2 workshops were conducted on pharmacovigilance activities, totally 110 health care personnel’s representing Medical, Nursing and Pharmacists participated. In addition one teaching program was exclusively conducted to 100 nursing staff. Informative booklets on guidelines for reporting ADR were prepared and distributed to all health care professionals(Annexure-16). To facilitate ADR reporting, ADR drop box and display banner with email ID and phone number were placed at various locations viz. nursing station, near doctor duty room etc. (Annexure-15) After
Methodology

this a post intervention survey was conducted to reassess the knowledge and attitudes of health care professionals regarding ADR reporting (Annexure-14). The results were statistically analyzed using paired “t” test.