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Highlights

- Type 2 Diabetes Mellitu
- Nicotine Induced Liver Toxicity
- Case Report on Mesothelioma
- Dicyclomine in Autopsy Material

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A Study on Drug Utilization Review and Potential Drug - Drug Interactions in Chronic Kidney Disease Patients

By Monika K. A., K. S. Charitha, M. Ramana Reddy, K. Vaishnavi, Ramakrishna Prudhivi & Jyothi
Dayananada Sagar University

Abstract- *Background:* Chronic kidney disease (CKD) is a worldwide health crisis. Life expectancy increased significantly among CKD patients due to the extensive use of polypharmacy for prescriptions. This predisposes them to potential Drug–Drug Interactions (DDIs).

Objectives: The aim of the present study is to evaluate the Drug Utilization Review (DUR) and to assess the potential drug-drug interaction in CKD patients.

Methods: This was a prospective observational & analytical study conducted in Sagar Hospitals, Bengaluru. The information collected in the patient profile form, the prescribing pattern was assessed and potential drug–drug interactions were evaluated by using Micromedex, clinirex and drugs.com.

Results: This study reveals that the males were more prone to CKD (63%) than females (37%) and the highest percentage of patients in the age group 61-75 years with the average of 66.40 ± 3.92 years. Among all medications the major class of drugs prescribed were anti-hypertensives & the least were drugs acting on thyroid. A total of 547 potential DDIs were observed of which moderate DDI (64.71%) were highest followed by minor(21.75%) & major(13.34%).

Keywords: *chronic kidney disease (CKD), drug-drug interactions (DDIs), drug utilization review (DUR).*

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Monika K. A. [α], K. S. Charitha [σ], M. Ramana Reddy [ρ], K. Vaishnavi [ω], Ramakrishna Prudhivi [¥] & Jyothi [§]

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Conclusion: The use of polypharmacy for the treatment of multiple co-morbid conditions has been proved to be as one of the most important factors in patients with CKD. Polypharmacy can predispose to drug interactions which results in the failure of the drug therapy and increase in the length of hospital stay. The active participation of clinical pharmacist in clinical activities can help in minimizing the risk and improving the patient care.

Keywords: chronic kidney disease (CKD), drug-drug interactions (DDIs), drug utilization review (DUR).

Author α σ ρ ω: Department of Pharmacy Practice, Dayananda Sagar College of Pharmacy, Bengaluru, India.
e-mails: monikaamarnath@gmail.com, charita.kodumagulla@gmail.com, ramanareddymadathala@gmail.com, vaishnavi.kulkarni71@gmail.com
Author ¥: M. Pharm., (Ph. D), Asst. Professor, Department of Pharmacy Practice, Faculty of Pharmacy, Dayananada Sagar University, Bengaluru, India. e-mail: ramakrishna.prudhivi@gmail.com
Author §: Department of Nephrology, Senior Resident, Sagar Hospitals, Kumarswamy Layout & Tilaknagar, Bengaluru, India.
e-mail: rkjpty6pho945@gmail.com

I. Introduction

Chronic kidney disease (CKD) is defined as a reduction in the Glomerular Filtration Rate (GFR) and/or urinary abnormalities or structural abnormalities of the renal tract.1 One in ten people have chronic kidney disease and about 175,000 new people have chronic kidney disease (stage V CKD) every year in India, requiring dialysis and/or kidney transplantation.2 India has been encountering the major problem of the rising incidence of CKD which may lead to difficulties in health care and economy in future. Indeed, it has been recently estimated that the age-adjusted incidence rate of End Stage Renal Disease (ESRD) in India to be 229 per million population (pmp)3 and >100,000 new patients enter renal replacement programs annually in India.4 The highest prevalence of CKD was observed in Visakhapatnam, Andhra Pradesh (46.8%), Kanpur, Uttar Pradesh (41.7%) and Delhi (41%). The lowest prevalence was observed in Mysore and Bangalore in Karnataka state.5, 6

Patients with CKD have interrelated co-morbidities with shared risk factors, including hypertension, atherosclerosis, glucose intolerance or diabetes, and lipid disorders, that can worsen renal and cardiovascular outcomes.7 The most common cause in the adult population is DM, accounting for approximately 40% of patients on renal replacement therapy. HTN is the second most common cause, accounting for one third of patients on renal replacement therapy.8

Elderly patients often face with polypharmacy when they have multiple disease processes. Declining organ function, as part of the normal aging process, adds to the problem of adverse drug effects in this population. To minimize polypharmacy, prescribers aim to treat multiple disease conditions with a single agent in cases where there is a posibility.9 CKD patients are medically complex to treat and have high risk of adverse reactions. Noncompliance to medication is also a great concern in CKD patients.10 A recent research study suggested that CKD patients have higher prevalence of inappropriate medication prescriptions, mostly antihypertensive and antibiotics.11

Drug use evaluation, sometimes referred to as drug utilization review, is a system of continuous,

systematic, criteria-based drug evaluation that ensures the appropriate use of drugs. It is a method of obtaining information to identify problems related to drug use and if properly developed, it also provides a means of correcting the problem and thereby contributes to rational drug therapy.12 Drug utilization studies in CKD patients help to understand and build evidence for the drug use. CKD patients need to take medicines lifelong, which makes it is very important to study the prescribing trend on a regular basis.

Drug-drug interactions (DDI) can be defined as an appreciably harmful or beneficial process whereby the pharmacological effect of a drug is directly or indirectly influenced and modified by the presence of another drug, which can result in either treatment failure (antagonistic interaction) or drug-induced toxicity (synergistic/additive interaction).13

DDIs are major clinical problem; accounting for 2–6% of all hospital admissions with estimated annual cost to the National Health Service of £500 million in the UK.14 Monitoring the drug-drug interactions may improve the quality of prescribing and dispensing.15 The present study aimed at assessing the drug utilization review and identifying drug-drug interactions.

II. Methodology

a) Study Design and Type

This study was a prospective, observational and analytical study carried out at Nephrology Unit of Sagar Hospitals at Tilaknagar and Kumarswamy layout, Bangalore.

b) Inclusion and Exclusion Criteria

A total of 110 patients were being consented for the study and is carried out for a period of 6 months from September 2017 to February 2018. The patients aged below 18 years, patients with Acute Kidney Injury (AKI), and those who continued to take further treatment in some other hospital were excluded from the study.

c) Study Procedure

The medical case records of all the adult CKD patients were retrieved after a verbal informed consent was obtained from each of them, and the following information was extracted using a pro forma: socio-demographic data, stage of CKD, number and list of medications at the time of last clinic visit for outpatients and at the time of discharge for those who received in-patient care, number and list of co-morbidities.

The estimated glomerular filtration rate (eGFR) was measured using MDRD formula, and CKD staging was done using eGFR as follows: stage 1 (eGFR of ≥90 mL/min with evidence of kidney damage), stage 2 (eGFR of 60–89 mL/min with or without evidence of kidney damage), stage 3 (eGFR of 30–59 mL/min with or without evidence of kidney damage), stage 4 (eGFR of 15–29mL/min with or without evidence of kidney damage), and stage 5 (eGFR <15 mL/min with or without evidence of kidney damage).16

The prescriptions were individually screened to assess the drug utilization and drug-drug interactions in CKD patients. The diagnosis and the drugs prescribed along with dosage schedule, duration were analyzed using Micromedex 2.0 and CIMS. The drug interactions were assessed and checked which were divided into major, moderate and minor using www.drugs.com, Medscape, Clinirex and Micromedex2.0. The interactions checked here were even classified into pharmacokinetic, pharmacodynamics and non-specific type.

d) Data Analysis

In this study the results were analyzed using student's t-test (unpaired) for comparing two groups and one-way ANOVA for finding the statistical differences among more than two groups. Results were expressed in the form of mean ± SD. A p- value < 0.05 was considered statistically significant.

e) Ethical Clearance

Ethical clearance for the study was obtained from the Institutional Human Ethics committee, Dayananda Sagar College of Pharmacy, Bengaluru.

III. Results

A total of 110 patients were reviewed among which 100 patients completed the study, 10 patients continued to take further treatment at different hospital. Our results were based on 100 patients out of which 63% of the patients were male and 37% were female. Most of the patients (54%) were elderly. Major number of patients was diagnosed with end stage renal failure i.e. G5 and the least number are diagnosed with G1. On analysis of type of co morbidities among study population, it was noted that 81% were affected with Hypertension followed by 66% with Diabetes Mellitus, 51% with Anemia, 19% with IHD, 12% with Hypothyroidism, 9% affected with various others diseases such as COPD, Spondylitis, BPH, UTI & LRTI. Maximum number of patients (44%) were appeared with two co-morbidities and 93% of patients had at least one co-morbidity. It was also observed that >15 prescribed drugs were received by 43% of patients and the average number of drugs prescribed per patient was about 13.4± 1.6 drugs. As per the analysis it was reported that the duration of the hospital stay 6-10 days consisted of 43% patients and mean hospital durations was 7.2± 2.2 days. The demographic data with clinical variables was shown in Table1.

The major class of drugs prescribed among patients were antihypertensive drugs constituting 16.48% followed by GIT drugs (14.07%), nutritional supplements (10.88%), chemotherapeutic agents (10.80%), respiratory drugs (8.08%), antidiabetic drugs

(6.29%), analgesics & antipyretics (5.83%), hematinics (4.97%), anti - thrombotics (4.66%), CNS drugs (4.51%), drugs acting on acid-base disorders (4.43%), anti - hyperlipidemics (3.65%), immunosuppresants (1.94%), drugs acting on thyroid are (1.01%) and other drugs (2.33%) as shown in Figure 1.

Among 212 antihypertensive drugs, the most widely prescribed antihypertensive drugs were diuretics (26.41%), followed by CCBs (25.47%), and β blockers (12.26%). Patients having DM as a co-morbidity, received insulin injectables with 64.19%. Anticoagulants occupied 80% of all anti thrombotic drugs. Out of drugs acting on GIT, 46.40% of the prescriptions were prescribed with Proton Pump Inhibitors (PPIs), followed by 24.86% with anti-emetics. The least preferred drugs in this category are H2 antagonists. Anti-depressants were seen in 21 prescriptions. Levothyroxine (13%) is the only drug prescribed for treatment of thyroid. The highest number of antibiotics prescribed as combinations with 30.21% followed by 10.79% with carbapenems,10.07% with macrolide antibiotics,7.91% with cephalosporins,7.19% with Anti-TB,6.47% with Fluoroquinolones,5.03% with Anti-amoebic,3.59% with Anti-fungal and 0.71% with Amino glycosides. Of 64 hematinic drugs 56.24% of the prescriptions were Iron, 34.37% Erythropoietin, 9.37% Vit-B12, and 3.12 % of the prescriptions were prescribed with Combinations. Among nutritional supplements, most of the drugs were prescribed as combinations (60%) and 14.28% prescriptions were with calcium carbonate followed by 12.85% with protein powder. Majority of drugs for acid base disorders were sodium bicarbonate (52.63%) and analgesics & antipyretics were seen in 39 prescriptions. The drug utilization pattern was shown in Table 2.

As shown in Figure 2 there were 547 interactions with an average of 5.4 ±0.9 interactions per each patient in 100 patients, of which major was pharmacokinetic type of interaction accounts for about more than 50% of interactions followed by pharmacodynamic interactions and non-specific. The drug interactions are classified into major, minor & moderate among which moderatedrug interactions accounts for 64.71% followed by minor(21.75%) and major(13.34%).

The interaction between Clarithromycin and budesonide (29.62%) was the most commonly seen major DDI. The list of drugs involved in major DDI was shown in Figure 3.The interaction between levothyroxine and basalog was responsible for 12.06% of moderate DDI and most prevalent interaction seen in minor DDI was between aspirin and pantaprazole (23.72%).

There was no significant difference between male and female in occurrences of drug interactions but there was a statistical significant difference ($p=0.035$**) among different age groups. The maximum numbers of interactions were found in age group of 46-60years. Patients with 2 co-morbidities were experienced predominant number of DDI (43.14%). As there was increase in number of drugs in the prescription chart more number of DDI were noticed. However there was no co-relation between duration of hospital stay and occurrence of DDI (Table 3).

IV. Discussion

In this study, an attempt was made to reveal the prescribing pattern of drugs and potential drug-drug interactions in study population of about 100 patients diagnosed with CKD and who satisfied the inclusion and exclusion criteria for a period of 6 months in Sagar Hospitals, Kumaraswamy layout and Tilaknagar, Bengaluru.

Among 100 patients who were involved in the study, total number of male patients were 63 (63%) and females were 37 (37%), showing that the males were predominant for the development of CKD which was compared and found similar to the study conducted by Rachana PR et al.17 In our study CKD was seen most commonly in the age group above 60 years but it was in contrast to the study conducted by Tamilselvan.et.al, in which age group between 50-60 years age –group were most affected.18 It was observed that, most of the patients admitted to the hospital belonged to G5 stage (48%) and least belonged to G1 stage. Here the staging was done based on GFR rate i.e., by calculating glomerular filtration rate and compared to the study carried out by Anand N et.al, which matched with the study results i.e. here the patients diagnosed with CKD mostly belonged to G5 stage (87.8%).19 Co morbidity is one of the common conditions seen with CKD which leads to the increased rate of morbidity and mortality. In the present study, Hypertension(81%) was the most seen co morbid condition, followed by DM (66%), Anemia(51%), IHD(19%), Hypothyroidism(12%) was inconsistent to the study conducted by Fraser SD et.al. showing that the major common co morbid condition was HTN (88%) followed by anemia (23%).20 44% of study population had 2 co-morbid conditions and only 1% of the population with 5 co-morbid conditions was found homogeneous to Pranavi Dasari et.al study.21

In patients who were diagnosed with CKD, there were several co morbid conditions present. So, wide class of drugs are prescribed for their treatment, of which the most prescribed were anti-hypertensive drugs (16.48%), followed by GIT drugs (14.07%), nutritional supplements (10.08%), respiratory drugs(8.08%) and the least prescribed class was drugs acting on thyroid which was not agreed to the study in which drugs acting on CVS(31%) was major followed by nutritional supplements (15%), hematanics (11%).19 Among the Anti-HTN drugs prescribed in this study the majority of the drugs were diuretics (26.41%), followed by CCB (25.4%), β-blockers (12.26%) and the least prescribed was ACE-I which was compared to the study organized

by Amit Ranjan et.al, showed that the most prescribed drugs in anti-HTN class were Diuretics (78%), followed by α-blockers (40%), CCB (38%) and the least was combination therapy of α-blockers with CCB.22 Among 66 patients diagnosed with DM, there were 81 numbers of prescriptions for anti-diabetic drugs. In this study insulin preparations were most preferred to treat DM than oral hypoglycemic agents. These results were correlated with the study done by Devi DP, George.23 Among all the class of drugs prescribed, analgesic and antipyretic drugs were 5.83%. The most common reason for using this was bone-joint pain, headache, and pain. This was found dissimilarity with Zibgniew et.al study, showing 35% use of NSAID class of drugs.24 Antibiotics were prescribed to minimize the infectious conditions. Combination therapy was most preferred to monotherapy. Other than combination therapy, carbapenems, macrolides, cephalosporins were prescribed respectively and were not found in correlation with the study conducted by Sowmya santra et.al, which demonstrated high prescription of Cefoperazone, metronidazole respectively.25 In spite of high prevalence of anemia (51%), the hematanics class of drugs which include Iron supplements (53.12%), erythropoietin (34.37%), vitamin B-12(9.37%) and oral elemental iron (3.12%) were accounted as the major medications for treating anemia in CKD patients subsequently reducing the requirement of blood transfusion. However, in other studies, conducted by Joshi AD et.al showed erythropoietin was the major prescribed drugs. By this comparison it was concluded that the use erythropoietin would be safer in CKD patients, as it reduces the risk of blood transfusion.26

A total of 547 DDI observed in the study were classified based on their severity assessment and type of interactions. Pharmacokinetic drug interactions (55.39%) were more predominantly seen when compared to pharmacodynamic drug interactions (19.37%). Based on severity DDI were categorized into major, moderate and minor interactions. In which moderate DDI were more commonly seen followed by minor and major which was seen in agreement with the study done by Mr. Sibi C Chacko et.al, which showed that moderate interactions contributed to highest than minor and major.27 Our study reveals that majority of the drug interactions were due to interaction between levothyroxine and basalog which is in divergence with the other studies.27, 28

The factors significantly correlated with the occurrence of DDI were elderly, number of co-morbidities and number of drugs prescribed.

V. Conclusion

Drug utilization evaluation and identification of the potential drug-drug interactions play a key role in providing better patient care. The use of Polypharmacy for the treatment of multiple co-morbid conditions has been proved to be as one of the most important factors in patients with CKD. Polypharmacy can predispose to drug interactions which result in the failure of the drug therapy and increase in the length of hospital stay. The active participation of clinical pharmacist in clinical activities can help in minimizing the risk and improving the patient care.

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Abbreviations

CCBs: Calcium Channel Blockers.
CKD: Chronic Kidney Disease.
DDIs: Drug-Drug Interactions.
DUR: Drug Utilization Review.
IHD: Ischemic Heart Disease.
PPIs: Proton Pump Inhibitors.

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Competing Interests

The authors declare that they have no competing interests.

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Table 1: Demographic Data with Clinical Variables

Variable	No. of Patients	Percentage (%)
Gender		
Male	63	63
Female	37	37
Age		
16-30	4	4
31-45	12	12
46-60	30	30
61-75	44	44
76-90	10	10
CKD Classification (Based on GFR)		
G1	2	2
G2	4	4
G3a	2	2
G3b	6	6
G4	22	22
G5	48	48
No. of Comorbidities		
0	7	7
1	21	21
2	44	44
3	19	19
4	8	8
5	1	1
No. of Drugs Prescribed		
≤ 5	2	2
6-10	22	22
11-15	36	36
>15	43	43
Duration of Hospital Stay		
≤5	35	35
6-10	43	43
11-15	16	16
>15	6	6

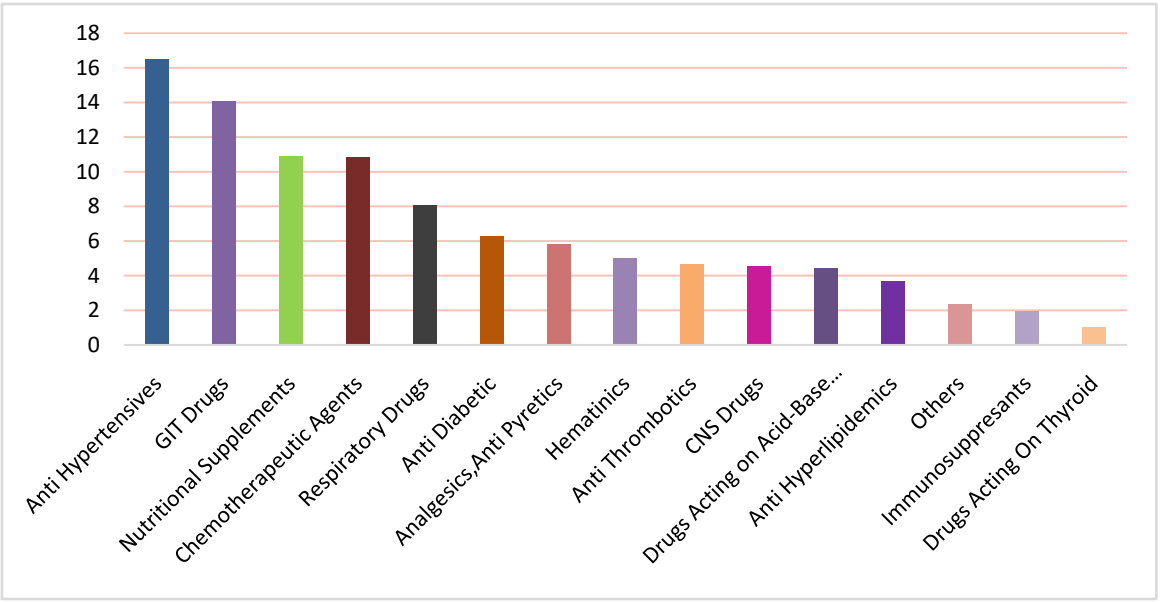

Fig. 1: Classification of Drugs Prescribed

Table 2: Pattern of Various Classes of Drugs Prescribed

Class of Drug	No. of Prescriptions	Percentage (%)
Pattern of Anti-Hypertensive Drugs		
Diuretics	56	26.41
Ccbs	54	25.47
B Blockers	26	12.26
A Blockers	20	9.43
A Agonists	13	6.13
Anti-Anginal	13	6.13
A+B Blockers	9	4.24
Combinations	8	3.77
Centrally Acting Sympatholytics	7	3.30
Anti-Arrhythmic	3	1.41
Arbs	2	0.94
ACE-I	1	0.47
Pattern of Anti-Diabetic Drugs		
Insulin (Injectables)	52	64.19
Gliptins	13	16.04
Sulphonylureas	8	9.87
Combination	5	6.17
A Glucosidase Inhibitor	2	2.46
Biguanides	1	1.23
Pattern of Antibiotics		
Combinations	42	30.21
Others	25	17.98
Carbapenems	15	10.79
Macrolide Antibiotics	14	10.07
Cephalosporins	11	7.91
Anti-TB	10	7.19
Fluoroquinolones	9	6.47
Anti-Amoebics	7	5.03
Antifungals	5	3.59
Aminoglycosides	1	0.71
Pattern of Hematinic Class of Drugs		
Iron Preparations	36	56.24
Erythropoietin	22	34.37
Vit-B12	6	9.37
Pattern of Drugs for Acid-Base Disorders		
Sodium Bicarbonate	30	52.63
Phosphate Binders	22	38.59
Combinations	4	7.01
Calcium Acetate	1	1.75
Pattern of Analgesics & Antipyretic Drugs Usage		
Analgesic & Antipyretic	39	52
Drugs For Gout	18	24
Drugs For Arthritis	10	13.33
Combinations	7	9.33
Antispasmodics	1	1.33
Pattern of Nutritional Supplements Usage		
Combinations	84	60
Ca- Carbonate	20	14.28
Protein Powder	18	12.85
Saline	6	4.28
Vit-E	4	2.85
Others	6	4.28
Vit-D	1	0.71
Folic Acid	1	0.71

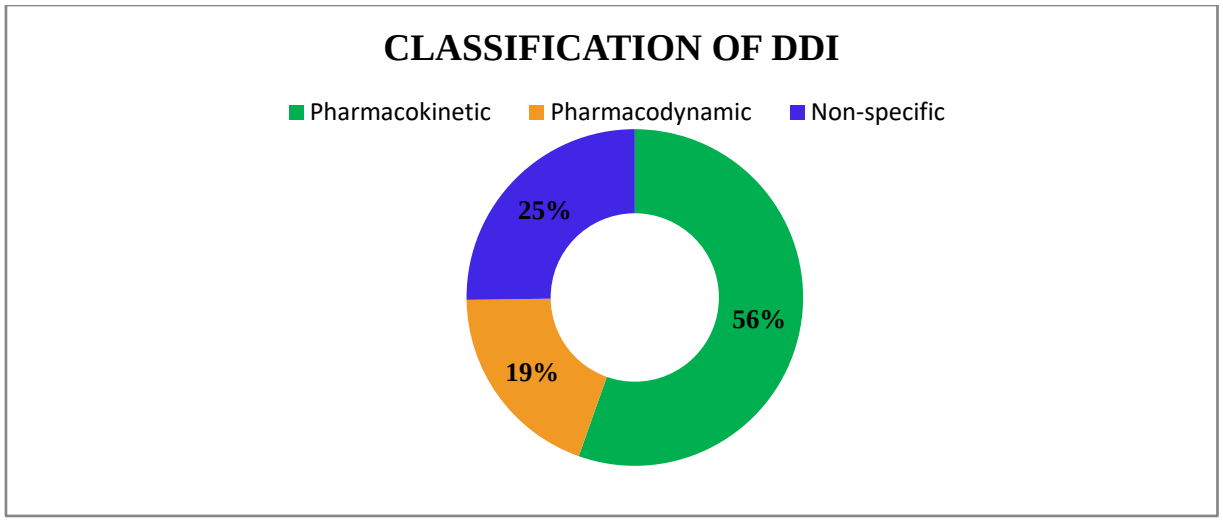

Fig. 2: Classification of DDI

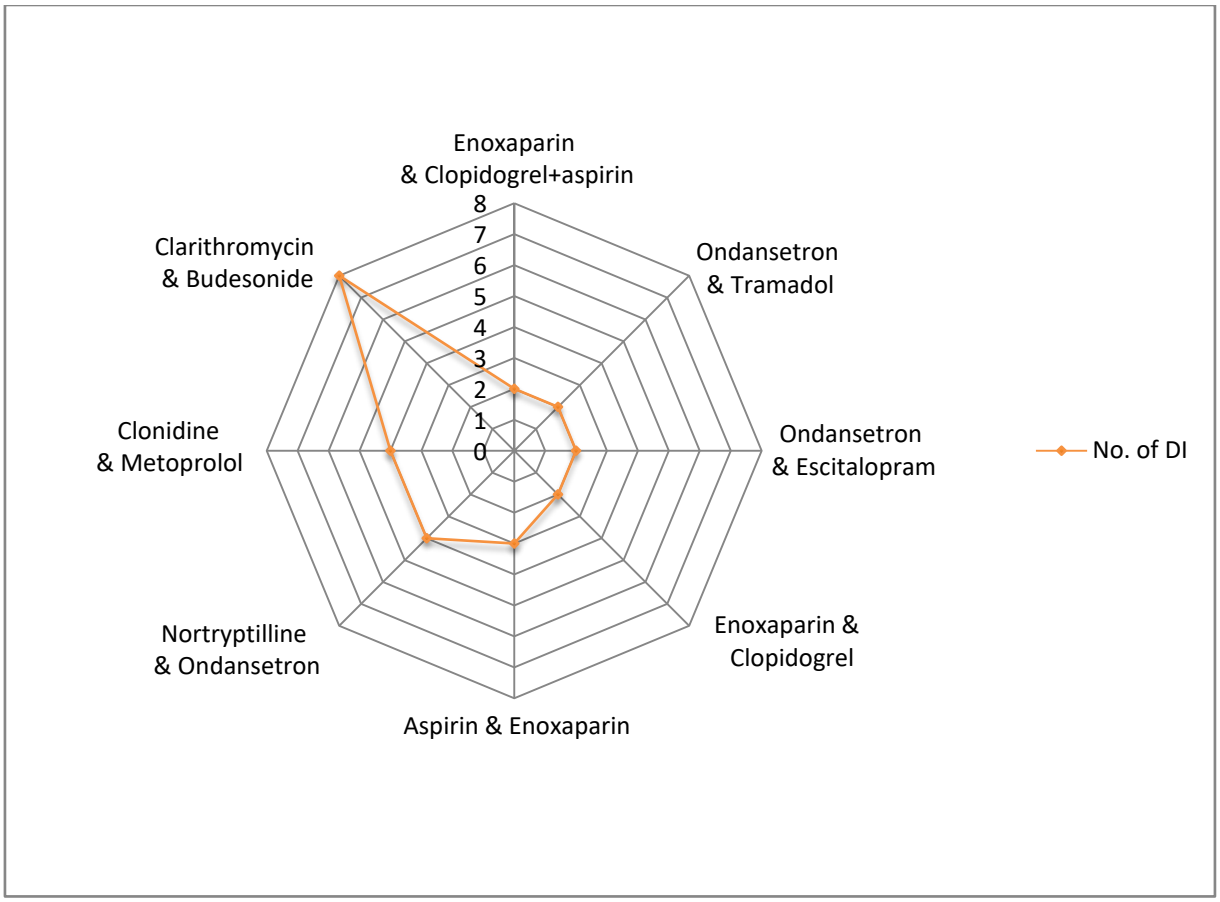

Fig. 3: Description of Major Drug-Drug Interactions

Table 3: Integration of Results

Variable	No. of Patients	Frequency of DDI	*P* - value
Gender			
Male	63	337 (61.6)	0.69
Female	37	210 (38.3)	
Age			
16-30	4	11 (2.41)	
31-45	12	67 (14.69)	
46-60	30	112 (24.56)	0.035**
61-75	40	192 (42.1)	
76-90	10	74 (16.22)	
No. of Comorbidities			
0	7	22 (4.2)	
1	21	118 (21.57)	
2	44	236 (43.14)	0.0019**
3	19	123 (22.48)	
4	8	43 (7.86)	
5	1	5 (0.91)	
No. of Drugs Prescribed			
≤5	3	3 (0.54)	
6-10	22	83 (15.17)	<0.0001***
11-15	37	198 (36.19)	
≥16	36	263 (48.08)	
Duration of Hospital Stay			
≤5	35	202 (36.9)	
6-10	43	214 (39.12)	0.8053
11-15	16	89 (16.27)	
>15	6	42 (7.67)	

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Detection and Identification of Dicyclomine in Autopsy Material

By Dr Vinod Dhingra
Regional Forensic Science Laboratory

Abstract- In this study, autopsy tissues were extracted and cleaned then subjected to TLC using suitable solvent system. A gas chromatographic-mass spectrometric (GC-MS) method is described for the determination of Dicyclomine residue in autopsy tissue. This method allows detection of residual drug in biological tissues by using single-ion monitoring; confirmation by a full scan electron impact (EI) mass spectrum is possible.

The aim of the paper is to use the proposed technique in cases of drug trafficking, illicit drug seizures and as a test for identity in pharmaceutical and forensic toxicological analysis. The focus of present study has been on methods for detection & confirmation of Dicyclomine in autopsy tissues using GC - MS.

Keywords: *dicyclomine, dicycloverine, diocyl, wyovin, TLC, GC-MS.*

GJMR-B Classification: *NLMC Code: QV 705*

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Keywords: dicyclomine, dicycloverine, diocyl, wyovin, TLC, GC-MS.

I. Introduction

Dicyclomine drug is available in the tablet dosage form in the market. It is, also known as Dicycloverine, is chemically 2-(diethyl amino) ethyl-bi (cyclohexane)-1- carboxylate.[1,2] Dicyclomine is used to treat intestinal hyper motility, the symptoms of Irritable Bowel Syndrome (IBS) (also known as spastic colon). It relieves muscle spasms and cramping in the gastrointestinal tract by blocking the activity of acetylcholine on cholinergic (or muscarinic) receptors on the surface of muscle cells. It is a smooth muscle relaxant and it has 72 % of the antimuscarinic power of atropine.[3]

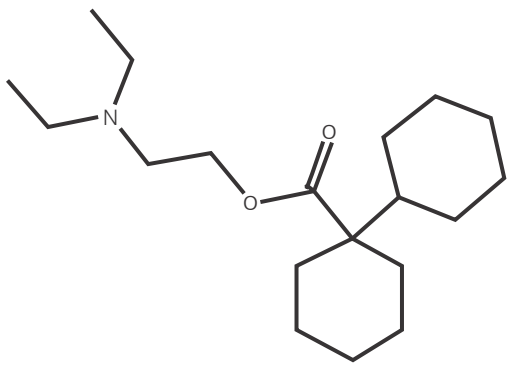

Fig. 1: Chemical structure of Dicyclomine

Literature survey revealed, no article related to TLC and GC MS determination of Dicyclomine in autopsy tissues has been reported. The objective of the present work was to develop an accurate, specific and reproducible method for the determination of Dicyclomine in autopsy tissue s by TLC and GC MS.

An 18-year-old lady found dead in the hostel room of college in Gwalior. The Investigative officer collected tablets from the hostel room and autopsy tissue from post mortem house. All the seized articles were forwarded to the regional forensic science laboratory Gwalior for chemical examination.

The aim of the paper is to use the proposed technique in cases of drug reaction, illicit drug seizures and as a test for identity in pharmaceutical and forensic toxicological analysis. The focus of present study has been on methods for detection & identification of Dicyclomine in autopsy tissues using TLC and GC- MS.

II. Experimental

Standard reagents, Toluene, Acetone, Methanol, Ammonia, Potassium thiocynate, Cobalt chloride and Sodium acetate used were AR grade. Dicyclomine pure, doubly distilled water was used throughout the study.

a) Preparation of Standard Stock Solutions

Standard stock solution of concentration 1000 µg/mL for Dicyclomine were prepared using methanol. From the standard stock solution, the mixed standard solutions were prepared using methanol to contain 100 µg/mL of Dicyclomine. The stock solution was stored at 2-8 °C protected from light.

b) Preparation of Chromogenic Reagent

Potassium thiocyanate (6.06 g), Cobalt chloride (5 g) and Sodium acetate (3.4 g) were dissolved in sufficient water, 2.5 mL of 1 N HCl was added and volume was made up to 25 mL with water. From this solution 20 mL was further diluted to 50 mL with methanol, filtered and stored at room temperature.[4]

Samples: Dicyclomine tablets and autopsy tissue.

c) Extraction of Dicyclomine from Autopsy Tissue and Cleanup of Extracts

In a portion of about 100 g of autopsy tissues (stomach, intestine, lung, liver, spleen and kidney) containing the Dicyclomine drug, 10g Ammonium sulphate was added and minced. Then biological sample was made alkaline with the help of ammonia and extracted with methanol. The filtrate was evaporated. The extracts were subjected to clean up by

Author: Senior Scientific Officer, Regional Forensic Science Laboratory, Gwalior (M.P.) India. e-mail: vdhingraso@hotmail.com

passing through the mixture of silica gel G and activated charcoal filled column having glass wool at the bottom. Finally the collected filtrate was evaporated over hot water bath and used for identification of Dicyclomine.

d) Thin Layer Chromatographic Analysis[5]

Aliquots of standard Dicyclomine and extract obtained were spotted on to the plate, which was developed with toluene: acetone: methanol: ammonia in the ratio of (7: 1.5: 1: 0.1) (v/v/v/v); it gave spot of Dicyclomine at Rf value 0.76 ± 0.02, in a pre saturated TLC chamber, to a height of 10 cm. The plate was removed from the chamber dried in air and sprayed with chromogenic reagent, which forms a blue-colored spots against light pink background.

The Rf value of Dicyclomine can be compared with the obtained spots of extract.

e) TLC Method Optimization and Chromatographic Conditions

The TLC procedure was optimized for estimation of Dicyclomine. The standard stock solution 100 µg/mL of Dicyclomine) were taken and 10 µL samples were spotted on to TLC plates and run in different solvent systems. Initially, toluene, acetone and methanol were tried in different ratios but perfect spots were not obtained. Hence, ammonia was tried along with above mobile phase. Finally for effective separation of Dicyclomine, the mobile phase containing a mixture of toluene: acetone: methanol: ammonia (7: 1.5: 1: 0.1v/v/v/v) was found to be optimum. The above mobile phase improved the spot shape and gave suitable Rf value for Dicyclomine. In order to reduce the neck less effect TLC chamber was saturated for 30 min. The plates were developed for a distance of 80 mm and then dried in hot air, which takes approximately 20 min for complete development of the TLC plate. As Dicyclomine is non UV absorbing compound, it could not be scanned under UV detector. After the TLC plate was developed in mobile phase, derivatizing agent was poured on the plate and dried. Blue spots against light pink background were developed within 20 min as later background starts getting darker.

f) Gas Chromatograph Mass Spectrometer

GC-MS studies were performed on Agilent technologies 5973 inert model mass selective detector using Column HPSMS 0.25 mm id 30 m length, 0.25µ film thickness, 30mx250µmx0.25µm nominal with aux temp 280°, intel temp 250° MS quadrupole 150 ion source 230, column flow 1.0 ml/min He as carrier gas, split mode 20:1 programming 100°C 2 minute hold 20°C/min ramp up to 280°, 500 volt total 16 minute run.

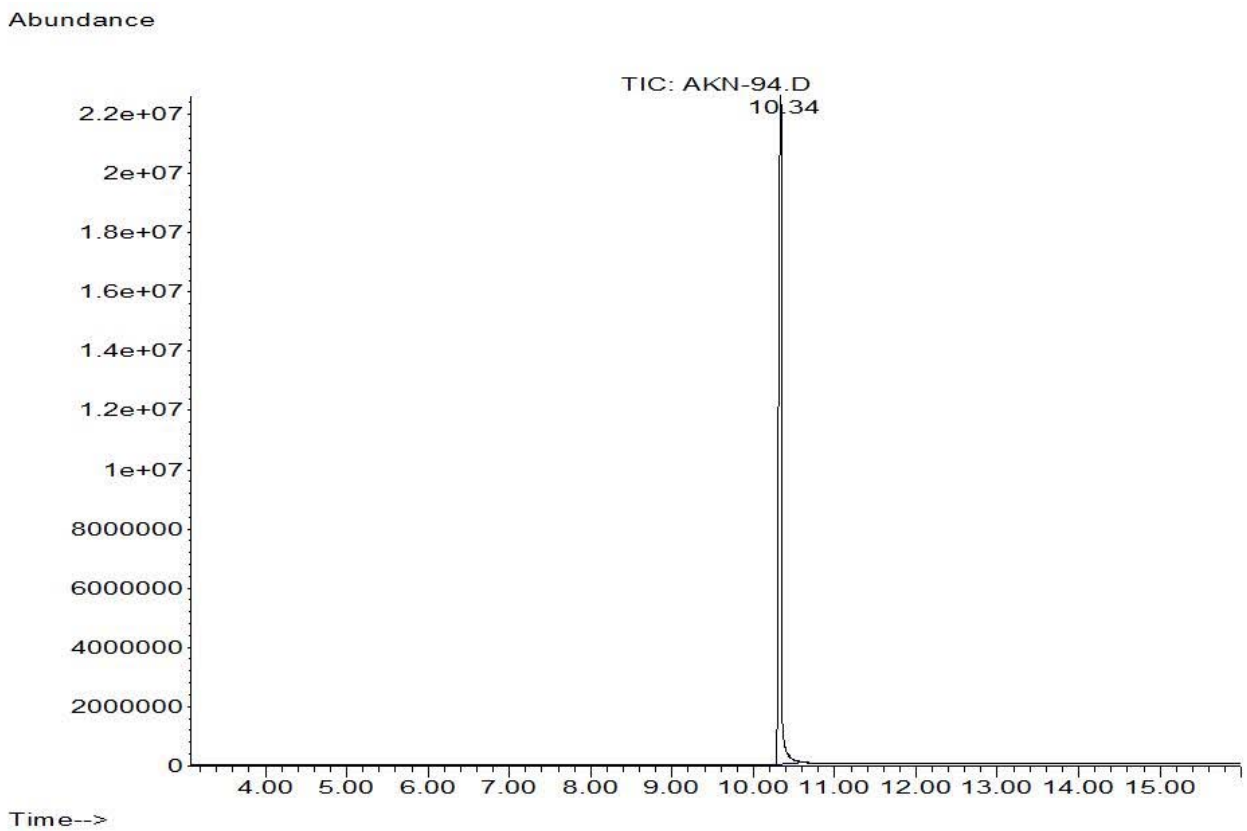

Fig. 1: Total Ion Chromatogram of Extract from Exhibits

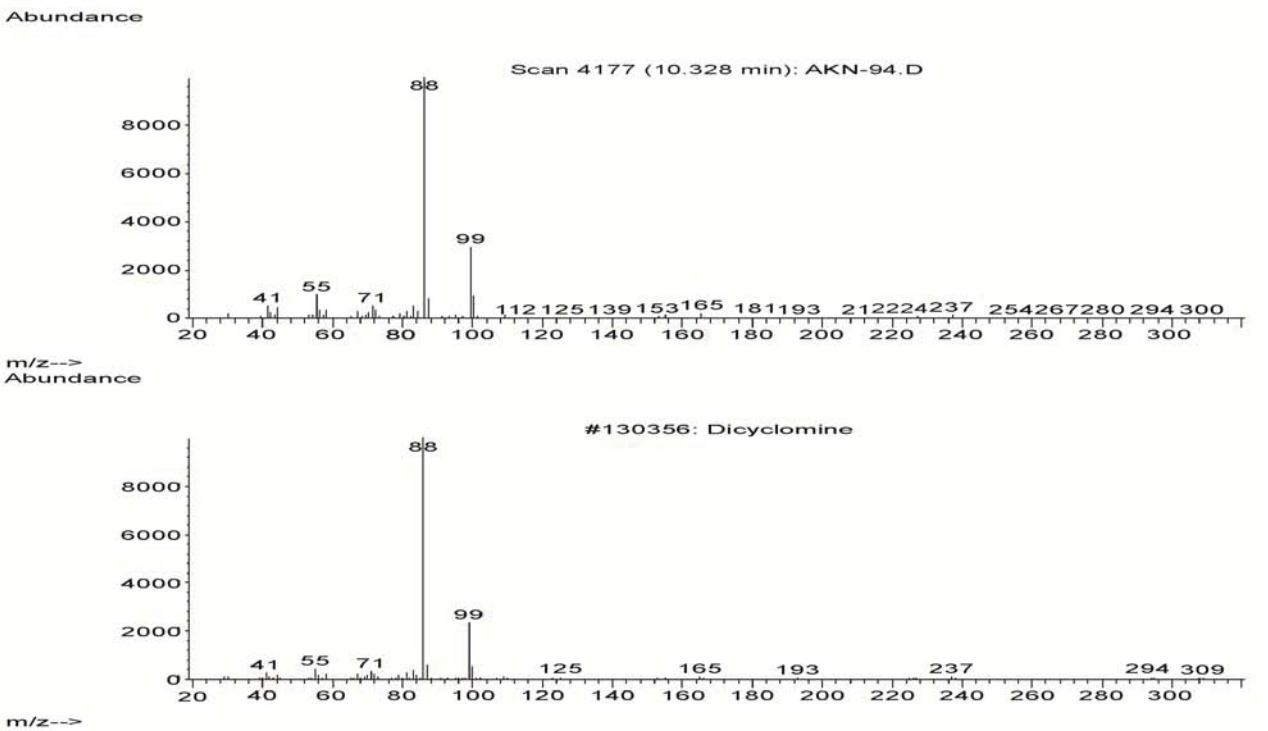

Fig. 2: Mass Spectrum of Extract and their Library Match

III. Results and Discussion

Dicyclomine can be successfully detected on TLC plates with good sensitivity.

The extract was injected into the GC-MS apparatus, and a total ion chromatogram and a full scan mass spectrum were obtained. Figure-1 shows the TIC and Figure-2 shows the mass spectrum of Dicyclomine with their library match, in this work; we attempted to detect Dicyclomine using selected ion monitoring.

The detection limit was determined by analyzing samples at various concentrations with this method, and it was found that 0.01 μg/ml of residual Dicyclomine in a sample can be detected using SIM. Figure 1 shows the total ion chromatogram at equal sensitivity of extracts from blank samples. The peak of Dicyclomine can be clearly identified, thus the detection limit was determined to be at least 0.01 μg/ml in sample. However, many background ions appeared on the full scan mass spectrum of this peak.

IV. Conclusion

Today, TLC is rapidly becoming a routine analytical technique due to its advantages of low operating costs, high sample throughput, and the need for minimum sample preparation. The major advantage of TLC is that several samples can be run simultaneously using a small quantity of mobile phase-unlike HPLC; thus reducing the analysis time and cost per analysis. The developed TLC technique is precise, specific, and accurate. Statistical analysis proves that the method is suitable for the analysis of Dicyclomine as a bulk drug and in pharmaceutical formulation without any interference from the excipients. It may be extended to study the degradation kinetics of Dicyclomine and also for its estimation in plasma and other biological fluids.

The method is simple, fast and reliable with no interference from common drugs. The method developed and the analysis of Dicyclomine in tissues could prove that the Dicyclomine were taken by deceased and the subsequent reaction had caused the death.

Acknowledgement

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Case Report on Mesothelioma

By Saranya K., Sreejith K. & Dr. Ajayakumar
College of Pharmaceutical Sciences

Abstract- A Mesothelioma is an aggressive form of cancer that affects the protective tissues covering the lungs and abdomen. The major clinical manifestations include shortness of breath, cough, tiredness and weight loss. Exposure to asbestos is the common risk factor for mesothelioma. Diagnosis was done by chest x–ray, MRI, and lung function tests. A Biopsy was needed to confirm diagnosis of mesothelioma. Chemotherapy is the only treatment that helps in survival. Combination of cisplatin and pemetrexed is proved to improve quality of life. Treatment regimens involving immunotherapy have yielded variable results.

Keywords: *mesothelioma, cisplatin, immunotherapy.*

GJMR-B Classification: *NLMC Code: QV 55*

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Case Report on Mesothelioma

Saranya K. [α], Sreejith K. [σ] & Dr. Ajayakumar [ρ]

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Keywords: mesothelioma, cisplatin, immunotherapy.

I. Introduction

A mesothelioma is an aggressive form of cancer that progress in the lining of lungs, abdomen or heart. It most commonly starts in the layer of tissues that cover each lung. It may be of two types- pleural mesothelioma which affects the tissues that surround the lungs. It includes chest pain under rib cage, painful coughing, shortness of breath, weight loss etc. Peritoneal mesothelioma – which occurs in tissues of abdomen. The signs and symptoms include abdominal pain, abdominal swelling, lumps of tissue in the abdomen etc. The diagnosis is done by chest X-ray, CT scan, thoracoscopy and biopsy. Possible treatment includes chemotherapy, radiotherapy, and surgery. Radiotherapy involves high energy radiation to kill cancerous area if mesothelioma is diagnosing at very early stage. Deposition of asbestos fibers in the parenchyma of the lung may result in the penetration of the visceral pleura from the fiber. This is then carried to the pleural surface, thus leading to the development of malignant mesothelial plaques.

II. Case Presentation

A 79 year old male patient was admitted to the oncology ward and diagnosed to have mesothelioma. The patient was a smoker and stopped five years back. The patient had complaints of breathlessness and cough for last 1 ½ months. The patient developed hemoptysis for two weeks with progressive dyspnoea on exertion. CT scan reports show moderate right sided pleural effusion and partial collapse of the right lungs. Pleural thickening of 1.2 c.m in the right upper zone with nodular lesions is seen in diaphragmatic pleura. There is no lymph node enlargement. No definite abrasions are present at the lungs. Fine needle aspiration cytology shows right upper lungs benign bronchial epithelial cells and few atypical cells showing drying artifact suspicious of mesothelioma. Blood results show elevated adenosine deaminase level. Immunohistochemistry shows Thyroid transcription factor (TTF) is strongly positive. Pleural fluid cytology showed no malignant cells were present. USG abdomen shows that there is no ascites and no prostatomegaly. Biopsy reports show the presence of metastatic adenocarcinoma and mesothelioma. Treatment was done by chemotherapy using injection Pemetrexed (600mg,every three weeks) and Carboplatin 450mg/100ml, every 28 days) for 3 cycles.

III. Discussion

Mesothelioma is one of the chronic malignancies coming under non small cell lung cancer which is most commonly seen in upper respiratory sites. It commonly affects the lungs and chest wall. Deposition of asbestos fibers in the parenchyma of the lungs may result in the penetration of the visceral pleura from where the fiber can be carried to the pleural surface, thus leading to the development of mesothelioma.

The diagnosis was done by CT scan, biopsy and immunohistochemistry. CT scan reports of the patient show moderate right- sided pleural effusion and partial collapse of the right lungs. Pleural thickening of 1.2 c.m in the right upper zone with nodular lesions is seen in diaphragmatic pleura. There is no lymph node enlargement. No definite lesions are present at the lungs. Fine needle aspiration cytology shows right upper lungs benign bronchial epithelial cells and few atypical cells showing drying artifact suspicious of mesothelioma. Blood results show elevated adenosine deaminaselevel. Immunohistochemistry shows Thyroid transcription factor (TTF) is strongly positive. Pleural fluid cytology showed no malignant cells were present.

Chemotherapy is the mainstay for the treatment of mesothelioma. Here the patient is treated using injection Pemetrexed (600mg, every three weeks) and Carboplatin 450mg/100ml, every 28 days) for three cycles.

IV. Conclusion

A mesothelioma is an aggressive form of cancer that affects the protective tissues which cover the lungs and abdomen. It most commonly starts in the layer of tissues that cover each lung. The signs and symptoms include abdominal pain, abdominal swelling,

Author α σ: Department of Pharmacy Practice, College of Pharmaceutical Sciences, Government Medical College, Calicut, Kerala, India. e-mail: jithuspharma@gmail.com
Author ρ: Department of Oncology, Govt. Medical College, Kozhikode.

lumps of tissue in the abdomen, etc. The diagnosis was done by chest X- ray, CT scan, thoracoscopy and biopsy. Long term survival and cures are exceedingly rare. Chemotherapy is the main treatment that has been proven to improve survival in randomized and controlled trials. Chemotherapy with cisplatin or carboplatin in combination with pemetrexed is the treatment regimen for mesothelioma.

Acknowledgement

We are obliged to the oncology department, Govt. Medical College, Calicut, for their cooperation during the period of case study.

Abbreviations:
CT: Computed Tomography, USG: Ultrasound sonography, TTF: Thyroid transcription factor.

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Nicotine Induced Liver Toxicity in Wistar Albino Rats: Protective effects of Aqueous Extract of Moringa Olifera (Lam)

By Oseni Olatunde Abass, Akindolire Olajumoke & Musbau Aderonke
Ekiti State University

Abstract- Aqueous extract of Moringaolifera (Lam) was evaluated for protective and antioxidant activities in rats. The plant extract showed a remarkable chemo-protective activity on nicotine induced liver toxicity as judged by serum maker enzyme and some antioxidant levels in the liver of male albino rats weighing between 180 and 200g. The animals were grouped into five of six rats each which were intraperitoneally induced with nicotine at 1mg/kg body weight except the control group. Some biochemical parameters (Aspartate aminotransferase (AST), Alanineaminotransferase (ALT), alkaline phosphatase (ALP), creatinine kinase malondialdehyde (MDA)); some antioxidant indices and lipid profiles were monitored. Induction of nicotine produced a significant increase in the level of malondialdehyde and decrease in the levels of superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) as compared to the control animals. Treatment with aqueous extract of Moringaoleifera leaf was found to produce a significant decrease in TBARS and increase in GSH, SOD and CAT in the plasma and liver homogenate of the nicotine induced rats. The levels of total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol in plasma and hepatic tissues of the experimental animals were also monitored. Significant protection was seen in the extract and standard drug (Lisinopril) treated nicotine induced animals. Administration of aqueous extract of the plant brings about a significant restoration towards the control values.

Keywords: *moringaolifera, nicotine, hepatotoxicity, antioxidant property, lipid profile.*

GJMR-B Classification: *NLMC Code: QW 70*

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Nicotine Induced Liver Toxicity in Wistar Albino Rats: Protective Effects of Aqueous Extract of Moringa Olifera (Lam)

Oseni Olatunde Abass [α], Akindolire Olajumoke [σ] & Musbau Aderonke [ρ]

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Keywords: moringaolifera, nicotine, hepatotoxicity, antioxidant property, lipid profile.

I. Introduction

The liver is the key organ regulating homeostasis in the body. It involved with almost all the biochemical pathway related to growth, fight against disease, nutrient supply, energy provision and reproduction. The liver is expected not to only perform physiological functions but also to protect against hazards of harmful drugs and chemicals. In spite of tremendous scientific advancement in the field of chemoology in recent years, liver problems are on rise. Cancer is a major disorder that account high death rate.

Author α: Department of Medical Biochemistry, Faculty of Basic Medical Science, College of Medicine, Ekiti State University, Ado-Ekiti. e-mail: olatunde.oseni@eksu.edu.ng
Author σ ρ: Department of Science Laboratory Technology, Faculty of Science, Ekiti State University, Ado-Ekiti.

Presently a few chemoprotective drugs and that too from natural sources, are available for the treatment of liver disorder. Liver toxicity is an inflammation of your liver in reaction to certain substances to which you're exposed. Liver toxicity can be caused by alcohol, chemicals, drugs or nutritional supplements. In some cases, liver toxicity develops within hours or days of exposure to a toxin.

Nicotine causes vasoconstriction and increased blood pressure[1]. A drop in blood-flow velocity in humans, endothelial damage in rats, and inhibition of platelet aggregation in rabbit's blood, have all been shown experimentally[1]. Nicotine has been reported to up regulate the expression of various proteins such as basic fibroblast growth factor, tumor necrosis factor -α and plasminogen activator inhibitor-1[2]. In addition, nicotine induces mononuclear leukocyte adhesion and expression of adhesion molecules such as vascular cell adhesion molecule-1 and intracellular adhesion molecule in endothelial cells[3]. Most clinical and experimental investigations of the pathophysiology of cigarette smoking have studied the effects of smoke as a whole, while a few studies focused on specific components of cigarette smoke, e.g. nicotine[4]. Nicotine exposure via cigarette smoking has been implicated in pathogenesis of cardiovascular disorders like atherosclerosis and hypertension[1].

Among myriad of plants, Moringaoleifera is one of the best known and most distributed species of Moringaceae family. Moringa is an important tropical crop that is used as human food, medicine and in oil production[5]. Leaves of this plant are traditionally known for or reported to have various biological activities, including hypocholesterolemic agent[6], regulation of thyroid hormone status[7], antidiabetic agent[8], gastric ulcers[9], antitumor agent[10], antihyperglycemic[5] and hypotensive agent[11]. The leaves as well as the flowers, roots, gums, fruits and seeds are extensively used for treating inflammation[12], cardiovascular action, liver disease[13] and hematological, hepatic and renal function[14]. It is generally known in the developing world as a vegetable, a medicinal plant and a source of vegetable oil[15]. Epidemiological studies suggest that specific pharmacologically active agents present in the diet might reduce the relative risk of cancer development[16]. A remarkable surge of interest in

chemoprevention research has led to the identification of many phytochemicals of dietary origin as effective potential chemo preventive agents[10]. Many complications occur as a result of nicotine exposure and its consequence in major and important organs in the body like liver; hence this study was designed to investigate the effect of aqueous extract of Moringaoleiferaleafon nicotine induced hypertension in the liver using wistar albino rats.

II. Materials and Methods

a) Collection and Extraction

The fresh leaves of Moringaolifera was obtained fromIworoko-Ekiti community, Ekiti State and was authenticated at Department of Plant Science, Ekiti State University, Ado-Ekiti.

The leaves were air dried and pulverized. 20% aqueous extract was prepared using distilled water.

b) Animals

Male albino rats of (180-240 g) were used throughout the experiments. Four rats per group(The animals were procured from the Animal House of College of Medicine, Ekiti State University, Ado-Ekiti). The rats were acclimatized for a period of 10 days under standard environmental conditions such as temperature (26 - 30oC), relative humidity (45-55%) and 12 hours dark/light cycle. All the animals were fed with rodent pellet diet and water was allowed ad-libitum under strict hygienic conditions.

c) Experimental Design

The rats were divided into five groups, each consisting of six rats:

Group 1: Normal albino rats treated with normal saline.
Group 2: Nicotine induced rats(1mg/kg body weight).
Group 3: Nicotine induced rats treated with Standard drug (Lisinopril).
Group 4: Treated with 0.5ml of 20% aqueous extract of Moringaoleifera.
Group 5: Nicotine induced rats treated with 0.5ml of 20% aqueous extract of Moringaoleifera.

The nicotine induced groups rats were induced with nicotine intraperitoneally at every other day.

Interval with nicotine (1mg/kg body weight) in normal saline for 21 days to induce hypertension.

d) Preparation of Organs Homogenate

At the completion of the experiment, the rats were quickly dissected; the liver was removed.

10% of the organ homogenate was then prepared in 0.25M sucrose solution using the Teflon homogenizer. The homogenate was centrifuged at 10,000rpm for 10 minutes at 40C to obtain a clear supernatant which was stored at 80C and used for measurement of biochemical contents.

Plasma sample was prepared from the whole blood collected from the heart into EDTA bottles and spinned at 3000 rpm.

e) Biochemical Analyses

Biochemical parameters like aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), creatine kinase (CK), total protein, malondialdehyde (MDA),superoxide dismutase (SOD), catalase (CAT), glutathione (GSH); some lipid profiles like triglyceride, HDL-Cholesterol, LDL-Cholesterol and Total Cholesterol were analysed according to the standard methods.

f) Statistical Analysis

The values were expressed as mean± SD. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Duncan multiple comparison tests. p value <0.05 were considered as significant.

III. Results and Discussion

Table 1: Effect of Aqueous Extract of Moringaoleifera on Some Biomarker Enzymes (U/L) in Nicotine-Induced Diabetic Rats.

	AST		CK		ALT		ALP	
	Plasma	Liver	Plasma	Liver	Plasma	Liver	Plasma	Liver
1	6.785 ± 2.71b	7.24 ± 0.68ab	4.464 ± 1.98b	3.264 ± 4.46b	4.943 ± 0.78b	8.145 ± 1.29a	21.342 ± 4.22a	17.455 ± 2.45c
2	8.382 ± 1.36c	6.66 ± 0.72a	7.338 ± 0.06a	1.264 ± 3.33b	12.11 ± 1.01b	6.404 ± 1.01c	36.347 ± 3.58d	8.352 ± 1.34a
3	6.272 ± 0.67b	8.297 ± 0.49b	3.654 ± 0.52a	3.887 ± 3.65b	5.082 ± 0.8bc	9.676 ± 1.53a	25.345 ± 3.94c	15.541 ± 1.39c
4	4.795 ± 0.19a	12.19 ± 0.59c	2.944 ± 0.54a	3.121 ± 0.22a	2.714 ± 0.43a	14.133 ± 2.23b	16.32 ± 1.39a	26.231 ± 3.55d
5	3.277 ± 1.03a	9.214 ± 7.91b	3.972 ± 0.56b	4.225 ± 3.97c	5.151 ± 0.8bc	8.494 ± 1.34a	25.342 ± 2.49c	16.632 ± 2.34b

Each value is a mean ± SD., n = 3. Values not sharing a common superscript (a-c) differ significantly with each other (P < 0.05) in all the groups

Table 1.0 showed the results of aqueous moringaolifera extracts on some biomarkers (AST, CK, ALT&ALP).In the assessment of the liver damage by Nicotine, determination of AST, CK, ALT and ALP is largely used. This study assessed the effect of nicotine-induced toxicity on the activity of ALP, AST, ALT on the plasma and liver. Generally, necrosis or membrane damage releases the enzyme into circulation and hence it can be the plasma[17]. ALP and CK occur in most tissues of the body as an isoenzyme such as the liver, kidney, bone, placenta and intestine etc. ALP is diagnostic of bone or liver disease or a tumor in these organs;it is found in liver cells and is associated with osteoblastic activity in the bone[18]. As shown in Table 1, from the results obtained in this study, it was observed that there was a significant increase in the activity of ALP in the plasma of animals in group induced with nicotine (group 2) compared to the control and reduction in the enzyme liver level. Healthy and active persons show higher values of serum CK activity. Moreover, CK values are lower in women than men and are usually lower in the morning than in the evening[19]. Significant increase were also observed for ALT and AST in the plasma of nicotine induced group with concomitant reductions in the liver, significant reduction in the concentrations of ALT and AST enzymes were recorded in this study. (This may be attributed to loss of membrane components due to a possible reaction between the drug (nicotine) and the liver tissues. Therefore, enzymes from diseased organs may become manifested in the plasma resulting in increased activity since they must have leaked from the diseased organ. A treatment with the administration of aqueous extract of the plant and the standard drug bring about a significant reduction in the enzymes plasma level, significant restoration towards the control values and significant rise in the biomarker enzyme activities in the liver cells were observed. Nicotine-intoxication caused a significant increase in plasma CK of rats when compared with normal. Furthermore, liver creatinine kinase levels decreased significantly in untreated nicotine induced rats. However, administration of the plant extract significantly reversed the adverse effects of nicotine on both the plasma and liver of the animals. In standard drug treated hepatotoxic rats, CK level in the plasma significantly decreased when compared to normal. Extract alone was able to reverse the nicotine-induced increase in plasma CK levels to value that were statistically similar to normal.

The extract of the plant and lisinoprilsignificantly reversed these changes toward the control ones and minimized the adverse effects of nicotine (Table 1). These findings are similar to the report of 20 that nicotine causes disruptions to membrane of organs thereby compromising the membrane integrity.

Table 2: Effect of Aqueous Extract of Moringaoleiferaon some Antioxidant Enzymes against Nicotine-Induced Hepatoxicity in Rats

	MDA X 10-7		SOD		CAT		GSH	
GP	Plasma	Liver	Plasma	Liver	Plasma	Liver	Plasma	Liver
1	3.008 ± 1.8b	6.692 ± 3.57c	9.120 ± 1.09c	7.680 ± 83.84c	0.003 ± 0.00c	0.002 ± 0.00c	14.561 ± 2.2bc	9.34 ± 1.76b
2	7.897 ± 1.52c	2.662 ± 1.13b	5.665 ± 0.54b	4.267 ± 5.54b	0.001 ± 0.00a	0.001 ± 0.00b	4.28 ± 1.03a	3.12 ± 0.76a
3	3.627 ± 0.55b	3.256 ± 1.41b	14.312 ± 0.19a	2.133 ± 9.81a	0.001 ± 0.00a	0.0004 ± 0.00a	12.855 ± 2.32b	7.49 ± 2.6ab
4	2.505 ± 0.64a	1.708 ± 0.46a	12.509 ± 0.29a	5.760 ± 5.33b	0.002 ± 0.00b	0.002 ± 0.00c	15.322 ± 3.43c	14.35 ± 4.8c
5	2.559 ± 0.41a	1.281 ± 0.89a	14.193 ± 1.19c	2.290 ± 1.29a	0.002 ± 0.00b	0.001 ± 0.00b	12.545 ± 2.45b	9.43 ± 2.09b

Each value is a mean ± SD., n = 3. Values not sharing a common superscript (a-c) differ significantly with each other (P < 0.05) in all the groups

From table 2.0, induction of nicotine with the animals in group 2 there is a significant increase in the levels of lipid perioxidation; as a result of enhance lipid perioxidation leading to tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive free radicals and decrease in the levels of antioxidant (GSH) and antioxidant enzyme (SOD and CAT) when compared to the control animals. Decrease in enzyme activity of superoxide dismutase (SOD) is a sensitive index in hepatocellular damage and is the most sensitive enzyme index in liver injury. These antioxidant enzymes are significantly decreased in the organ (liver) and plasma due to the inadequacy of the antioxidant defenses in combating ROS mediated damage and when they are treated with aqueous leave extract of the plant, the activities of these enzymes was increased and may help to control the free radicals when compared to the hypertensive rats and the effect produced by aqueous leave extract of the plant was comparable with that of standard drug Lisinopril. Treatment with aqueous extract of Moringaoleifera leaf and lisinopril was found to produce a significant

decrease in TBARS and increase in GSH, catalase (CAT) in the liver homogenate of the hypertensive rats. The effect of aqueous extract of Moringaoleifera leaf on group not induced with nicotine showed significant increase in the levels of superoxidedismutase (SOD), catalase (CAT) and GSH and reduction in lipid perioxidation. MDA level is the most important factor indicating increased peroxidative level. Enzymatic antioxidants are important antioxidant for scavenging free radicals. From the figure, these reports suggest that doxorubicin produces renal, cardiac and hepatic injury. The major role of catalase (CAT) is to scavenge H2O2 that has been generated by free radicals or by superoxide dismutase (SOD) in its removal of superoxide anions, and convert it to water[21]. And significant reduction in concentration of superoxide dismutase (SOD), GSH and catalase (CAT) were recorded in the group treated with nicotine.

Table 3: Effect of Aqueous Extract of Moringaoleifera on Some Lipid Profile (mg/dl) in Nicotine-Induced Hepatotoxicity Rats

	Triglycride		HDL-Cholesterol		Total Cholesterol		LDL-Cholesterol	
GP	Plasma	Liver	Plasma	Liver	Plasma	Liver	Plasma	Liver
1	291.59 ± 20.4a	285.86 ± 26.11a	32.98 ± 5.04b	19.70 ± 4.58a	15.804 ± 1.69a	14.68 ± 6.01a	26.37 ± 3.41b	14.49 ± 2.44a
2	440.28 ± 20.4d	335.33 ± 25.55c	24.99 ± 5.83a	18.72 ± 5.83a	29.067 ± 3.04c	30.41 ± 8.44c	67.37 ± 6.43d	21.34 ± 2.58c
3	395.95 ± 35.0b	319.63 ± 39.12b	49.50 ± 11.5c	30.44 ± 0.44c	18.986 ± 3.3ab	22.02 ± 5.32b	34.74 ± 3.55c	17.44 ± 3.38b
4	414.66 ± 11.2c	348.92 ± 36.94c	22.48 ± 2.46a	20.66 ± 4.82a	22.044 ± 0.78b	34.61 ± 16.0d	18.43 ± 2.53a	11.644 ± 1.48a
5	419.33 ± 7.12c	461.79 ± 26.60d	21.14 ± 4.9a	20.83 ± 2.0ab	25.030 ± 3.34c	32.51 ± 7.7cd	17.245 ± 2.44a	37.25 ± 4.56d

Each value is a mean ± S.E.M., n = 3. Values not sharing a common superscript (a-c) differ significantly with each other (P < 0.05) in all the groups

Table 3 shows the levels of total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol in plasma and hepatic tissues of experimental animals. The levels of total cholesterol, and LDL- cholesterol in plasma and the liver of experimental animals increased significantly in the nicotine treated group. Administration of nicotine caused a significant increase in the levels of triglycerides in the plasma and in the liver, compared with positive control rats. This shows the hyperlipideamic effect of nicotine. However, after the rats were treated with the extract, significant improvement in the levels of triglycerides was observed in the plasma. Hypertriglyceridemic patients are at a risk for cardiovascular disease often develops a lipoprotein profile characterized by elevated triglyceride, dense LDL, and low HDL cholesterol which causes myocardial membrane damage. Significant reduction, similar protection and biochemical restoration of levels of Total cholesterol, LDL-cholesterol and Triglyceride were seen in the plant extract and standard drug (Lisinopril) and nicotine treated animals. The group, given only the extract showed the protective potential and effectiveness of the plant in both plasma and the liver. The study suggests that the intake of the extract decreases the absorption of triglycerides and cholesterol, and these findings are in accordance with the report of22that reported Green tea intake also decreases the absorption of triglycerides and cholesterol.

IV. Conclusion

The present study indicates that a decrease in the antioxidant status is one of the main factors contributing to nicotine toxicity to the Liver. The observed significant increase in the LPO and oxidative stress markers and lipid profile in the liver of nicotine-induced animals, suggests that the tissues are subjected to increased oxidative stress. Reversible oxidative/antioxidant, biomarker enzymes and the lipid profile modifications were observed when treated with the aqueous extract of the plant. Treatment found to remove the continuously generated free radicals, to prevent the endogenous antioxidant enzymes decrease and act to prevent oxidative cell damage induced by nicotine.

Conflict of Interests

The authors declare that no conflict of interest exist.

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Quality Guarantee in Parenteral Nutrition: Implementation of Chemical and Microbiological Quality Controls

By Daisy Miranda, Giannina Faúndez, Daniel Navea & Carolina Salas
Hospital Dr. Luis Calvo Mackenna

Abstract- In the preparation of a parenteral nutrition (NP) is always present the risk of errors in the addition or omission of nutrients (chemical composition) or rupture of strict aseptic technique (microbiological quality). Both the Pharmacopoeia and national legislation suggest basic qualitative controls of areas, so the objective of this study is to implement additional analytical and microbiological quality controls, to verify the preparation process, as well as to ensure chemical stability and absence of contamination. For chemical control it included glucose, sodium, calcium, magnesium, phosphorus, potassium and chlorine in 40 samples in the Cobas®B221 and Vitros®4600 equipment, pH, and microbiological control, through NP culture in blood agar after preparation and after 5 days of storage. At day 0, the concentrations of glucose, sodium and calcium correlate with the theoretical concentration. On day 5 of storage glucose, sodium, calcium, magnesium, phosphorus, potassium and chlorine correlated with the theoretical concentration on day 0. No growth of microorganisms was observed in any sample. It is established as a chemical control of elaboration glucose, sodium and calcium; and as chemical stability controls glucose, sodium, calcium, magnesium, phosphorus, potassium and chlorine, maintaining its sterility.

Keywords: parenteral nutrition; chemical control; microbiological control; quality assurance.

GJMR-B Classification: NLMC Code: QW 4

Strictly as per the compliance and regulations of:

Quality Guarantee in Parenteral Nutrition: Implementation of Chemical and Microbiological Quality Controls

Garantía de calidad en nutrición parenteral: Implementación de control de calidad químico y microbiológico

Daisy Miranda [α], Giannina Faúndez [σ], Daniel Navea [ρ] & Carolina Salas [ω]

Resumen- En la elaboración de una nutrición parenteral (NP) está siempre presente el riesgo de errores en la adición u omisión de nutrientes (composición química) o ruptura de la técnica aséptica estricta (calidad microbiológica). Tanto la Farmacopea y legislación nacional sugiere controles básicos de áreas y cualitativos, por ello el objetivo de este estudio es implementar controles de calidad analíticos y microbiológicos adicionales, para verificar el proceso de preparación, así como asegurar la estabilidad química y ausencia de contaminación. Para control químico incluyó glucosa, sodio, calcio, magnesio, fósforo, potasio y cloro en 40 muestras en los equipos Cobas®B221 y Vitros®4600, pH, y control microbiológico, por cultivo de NP en agar sangre posterior a la preparación y a 5 días de almacenamiento. Al día 0, las concentraciones de glucosa, sodio y calcio se correlacionan con la concentración teórica. El día 5 post almacenamiento glucosa, sodio, calcio, magnesio, fósforo, potasio y cloro se correlacionan con la concentración teórica del día 0. En ninguna muestra se observó crecimiento de microorganismos. Se establece como control químico de preparación glucosa, sodio y calcio y como controles de estabilidad química glucosa, sodio, calcio, magnesio, fósforo, potasio y cloro, manteniendo su esterilidad 5 días.

Palabras Claves: nutrición parenteral; control químico; control microbiológico; garantía de calidad.

Summary- In the preparation of a parenteral nutrition (NP) is always present the risk of errors in the addition or omission of nutrients (chemical composition) or rupture of strict aseptic technique (microbiological quality). Both the Pharmacopoeia and national legislation suggest basic qualitative controls of areas, so the objective of this study is to implement additional analytical and microbiological quality controls, to verify the preparation process, as well as to ensure chemical stability and absence of contamination. For chemical control it included glucose, sodium, calcium, magnesium, phosphorus, potassium and chlorine in 40 samples in the Cobas®B221 and Vitros®4600 equipment, pH, and microbiological control, through NP culture in blood agar after preparation and after 5 days of storage. At day 0, the concentrations of glucose, sodium and calcium correlate with the theoretical concentration. On day 5 of storage glucose, sodium, calcium, magnesium, phosphorus, potassium and chlorine correlated with the theoretical concentration on day 0. No growth of microorganisms was observed in any sample. It is established as a chemical control of elaboration glucose, sodium and calcium; and as chemical stability controls glucose, sodium, calcium, magnesium, phosphorus, potassium and chlorine, maintaining its sterility.

Keywords: parenteral nutrition; chemical control; microbiological control; quality assurance.

Author α: Servicio de Farmacia Área Nutrición Parenteral y Nutrición Clínica Hospital Dr. Luis Calvo Mackenna, Santiago Chile.
Author α σ: Facultad de Medicina, Escuela de Química y Farmacia, Universidad Andres Bello, Santiago Chile.
Author ρ ω: Unidad de Laboratorios Clínicos, Hospital Dr. Luis Calvo Mackenna. Providencia, Santiago, Chile.
e-mail: csalas@calvomackenna.cl

I. Introducción

Las nutriciones parenterales (NPs) son mezclas estériles administradas por vía venosa central o periférica, indicada a prematuros, niños y adultos desnutridos o con riesgo de desnutrición secundaria a una patología[1], son consideradas como preparaciones magistrales y con un período de vigencia definido[2]

Hay dos factores importantes que influyen en la estabilidad de la NP, por un lado la compleja composición química ya que pueden contener más de 50 nutrientes (entre ellos agua, glucosa, aminoácidos, lípidos, vitaminas, electrolitos, minerales y elementos trazas)[3], todos contenidos en un envase único[4] y por otro lado al ser preparados estériles se debe asegurar la estabilidad microbiológica, existe controversia en este punto, ya que algunos autores describen como mezclas proclives a contaminarse y otros opinan lo contrario, debido a que se tratan de mezclas de alta osmolaridad y bajo pH[5-7].

El proceso de elaboración de estos productos en nuestro hospital es operador dependiente, realizándose de manera semi automatizada, con adiciones sucesivas de nutrientes, por lo que la existencia de errores por exceso o defecto está presente, además al ser preparados químicamente complejos existen factores internos que pueden afectar la estabilidad de la mezclas ya sea de forma favorable o desfavorable tales como cambio de pH[3], estabilidad de los lípidos y generación delipoperóxidos[3,8-10], precipitados calcio-fosfato[11,12] glucosa[12], aminoácidos[9,12] y factoresexternos como acción de la

luz y oxígeno[13], permeabilidad de contenedores[14] y condiciones de almacenamiento[15]. Otro factor de externo que también se debe considerar es la contribución o aporte no declarados de electrolitos como sodio y magnesio[16,17] de los excipientes de los productos farmacéuticos utilizados en su elaboración.

La vigencia de cada NP también es un tema controversial, es así como la Sociedad Española de Nutrición Parenteral y Enteral (SENPE) y la Sociedad Española de Farmacia Hospitalaria (SEFH) otorgan una vigencia de hasta 4 días a la NP12 y la Sociedad Americana de Nutrición Enteral y Parenteral, ASPEN hasta 9 días.[18] En nuestro hospital considerando estos antecedentes se le otorga una caducidad de 5 días contados desde el día de elaboración.

Actualmente en nuestro país, los controles de calidad realizados a las NP y sugeridos por la legislación vigente son tres:

Gravimétrico: Utilizado para detectar errores de adición o defecto de grandes volúmenes (Diferencia de peso esperado versus peso real de la mezcla igual ± 3-5%).[12]

Visual: Utilizado para detectar cambios de coloración, separación de fases, formación de precipitados, presencia de partículas o filtración del envase.[12]

Control Microbiológico: Para detectar una posible contaminación microbiológica, se realiza al momento de la preparación.[19]

Considerando la importancia e implicancia que tiene para el paciente poder contar con un producto con altos estándares de calidad y seguridad, asimismo tomando en consideración las falencias que pueden existir en relación a los controles de preparación y almacenamiento de la NP, nos propusimos implementar y estandarizar un control de calidad químico cuantitativo y microbiológico de la NP, realizado al momento de la elaboración (control de proceso) y el último día de establecida la caducidad de la NP (control de estabilidad química), adicional a los sugeridos por la actual legislación vigente, los cuales no dan cuenta de comportamiento químico de la mezcla durante los días de vigencia, esterilidad de la mezcla, comportamiento de los nutrientes o su degradación en el tiempo.

II. Materiales y Métodos

Estudio exploratorio, cuantitativo, longitudinal y no experimental. Se analizaron 40 muestras de NP, en día 0 (día de la elaboración) y a los 5 días, para evaluar proceso de elaboración y estabilidad respectivamente.

Cálculo de error gravimétrico:

Se calculó el promedio del error gravimétrico de cada una de las NP incluidas en el estudio a través de la siguiente fórmula:

$$\%\text{Errorgravimétrico} = \left(\frac{\text{Pesomedido} - \text{Pesoesperado}}{\text{Pesoesperado}}\right) \text{x } 100$$

Dónde: Peso medido= Peso real de la NP luego de su preparación; Peso esperado= Peso teórico de la NP, el cual está de acuerdo a la composición de la NP.

a) Control de calidad químico

Los analitos o nutrientes incluidos para realizar el control de calidad químico fueron magnesio (Mg), calcio (Ca), fósforo (P), glucosa (Glu), sodio (Na), potasio (K) y cloro (Cl) medidos en equipo Vitros® 4600 de Johnson& Johnson y Cobas® B221 de Roche.

Para cada muestra se calculó la concentración teórica (calculado en base a la indicación médica) de cada analito, posteriormente se midieron experimentalmente tanto en el día de la preparación (día 0) y día 5 post preparación. Previo a la medición se calculó la concentración de los analitos en promedio en las muestras de NP, para realizar las diluciones correspondientes en relación a la sensibilidad de los equipos.

Una vez elaborada la NP, se extrajeron 4 mL de mezcla, divididas en 2 jeringas con 2 mL cada una, donde la primera se analizó al momento y la segunda se almacenó siguiendo las condiciones utilizadas para almacenar las NP, es decir refrigerada entre 4 y 8ºC. La muestra fue centrifugada 10 minutos a 10.600 revoluciones por minuto (rpm) en centrífuga Abbott®, se separó la fase acuosa en la cual se realizaron las mediciones en los equipos.

b) Control de calidad microbiológico

Se tomaron 3 alícuotas de 50 mL de NP, las cuales se utilizaron para cultivar post preparación (día 0), cultivo 5 días post preparación y la tercera se almacena como contramuestra (se cultiva solo en caso de positividad de la muestra).

La metodología utilizada para detección de contaminación microbiológica de NP fue la utilizada por Montejo y cols(19), es un método de filtración de la muestra en una membrana de 0,45 um y cultivo por 7 días en estufa en una placa agar sangre hasta obtención del resultado.

c) Análisis de los datos

Se cuantificó la concentración de 7 nutrientes (Mg, Ca, P, Glu, Na, K, Cl) en 40 muestras diferentes durante el día de elaboración (día 0) y 5 días después (día 5). A los datos obtenidos, se aplicó el Test de Grubbs para eliminar valores aberrantes (outliers) los cuales fueron eliminados. Con el método de regresión de Passing y Bablok se comparó la concentración esperada según la indicación médica, con la concentración medida por el equipo correspondiente

Por otro lado se realizó una prueba de comparación de medias relacionadas, en la que se determinó si hay influencia de un analito sobre otro, lo cual se evidencia cuando el valor de p es menor a 0.05

III. Resultados

a) Error gravimétrico

Para las 40 NP incluidas en el estudio se obtuvo un promedio de error gravimétrico de 2,3 %.

b) Control de Calidad Analítico

La Tabla 1 muestra las medias de concentración esperada y concentración medida para cada analito durante día 0 y día 5, según regresión Passing-Bablok.

De acuerdo a las regresiones de Passing-Bablok, existe concordancia, el intervalo de confianza (IC) de la pendiente debe contener el valor 1 y el IC del intercepto el valor 0. De acuerdo a lo mostrado en la Tabla 1, para las concentraciones medidas y esperadas para días 0 y 5, se observa lo siguiente:

- K, Cl, P, Mg: No hay concordancia para mediciones en día 0 y 5.
- Ca, Glu: Si hay concordancia para mediciones de día 0, pero no en día 5.
- Na: Si hay concordancia para mediciones de día 0 y 5.

Al observar los valores p en las tablas N°2 y N°3, se puede ver que la única asociación que se repite en ambos días, es la de calcio con fosfato, información relevante cuando se analiza la calidad de estas mezclas nutricionales, ya que la formación de precipitados Ca-P es uno de los puntos críticos y potencial factor de inestabilidad dentro de la NP.

c) Control de Calidad Microbiológico

Las 40 muestras analizadas en donde fue realizado control microbiológico no se observó presencia de crecimiento microbiano en ninguna de ellas a tiempo cero , ni a los 5 días de almacenadas.

IV. Discusión

Controles de calidad cuantitativos, como los propuestos en el presente trabajo, realizados al producto terminado son una herramienta complementaria a controles establecidos, tales como medición de pH, control visual o control gravimétrico. No debemos olvidar que una NP se elabora por adición de diferentes volúmenes, y que los controles de calidad clásicos como los descritos anteriormente no detectan por ejemplo errores en la adición de nutrientes que se agregan en pequeños volúmenes, micronutrientes de estrecho margen terapéutico que pueden ser potencialmente dañinos para el paciente, si se adicionan en exceso o definitivamente no se adicionan como el potasio[20] como lo describen otros autores pero no señalan la metodología ni encontramos estudios que en la literatura que describan medición de electrolitos en la NP , autores describen medición de algunas vitaminas como tiamina por cromatografia de gases[21] o visualizar degradaciones que pueden ocurrir dentro de la mezcla durante su almacenamiento antes de ser administrada, para estos casos la implementación de un control de calidad químico de fabricación como el propuesto en el presente trabajo es de gran utilidad. Para el caso de los macronutrientes, en caso de que ocurran errores en la adición (exceso, déficit o ausencia), podrían ser detectados posterior a la elaboración mediante el control gravimétrico (pesada)[12].

Como preparado magistral se debe considerar que el volumen final de cada NP tiene un error intrínseco por exceso o defecto de nutrientes asociado al proceso de elaboración manual o semi-automatizado y que además, durante su elaboración se utilizan instrumentos no volumétricos como jeringas de diferentes volúmenes. El promedio del error gravimétrico de las muestras analizadas en este estudio fue de 2,3 % p/p, el que está dentro de los rangos aceptables, el cual se define según farmacopea entre un 3 y 5 %p/p por lo cual no se puede considerar como un factor de interferencia en la medición de los diferentes analitos incluidos en el estudio.

De acuerdo a nuestros resultados para los analitos Ca, Na y glucosa el valor obtenido para día cero en comparación a la media esperada es concordante, por lo que reúnen las características para ser considerados como marcadores de elaboración, coincidiendo con lo reportado por otros autores, los que recomiendan medir estos nutrientes (analitos) que se encuentran en estrecho margen terapéutico en la NP3 con fines de control de calidad.

Considerando que el objetivo de este trabajo es implementar controles de calidad complementarios a los descritos en la literatura, utilizando las plataformas analíticas disponibles en la mayoría de los centros asistenciales como son los equipos de laboratorio, se debe realizar a través de la verificación de las concentraciones de cada uno de los analitos seleccionados, ya que en todos los casos nos estamos comparando con una concentración inicial. De acuerdo a los resultados obtenidos para Ca, Na y glucosa, se demuestra que es posible establecer este tipo de controles de calidad complementarios. Es importante mencionar que no es factible medir todos los analitos de la NP, ya sea por un tema de disponibilidad de las técnicas, así como por la metodología analítica recomendada para realizar dicha medición. Previamente es necesario hacer un análisis del tipo de analizo y concentración esperada, además de considerar los CV esperados para cada uno de ellos. En ese sentido, cabe señalar que no fue estimado el efecto matriz previo a las mediciones de estos analitos a fin de comprobar empíricamente la influencia de las preparaciones y sus componentes en la determinación de cada analito, cosa que podría plantearse a futuro con un estudio de mayor envergadura. Sin embargo, los resultados obtenidos entregan datos concretos y objetivos que permiten implementar un sistema de

control químico de las NP, puesto que fue posible con las herramientas disponibles, determinar la concentración de analitos marcadores de control de proceso de elaboración, lo cual constituye un aporte que permite, entre otras cosas, asegurar la calidad de la elaboración de la NP, transformándose en una práctica innovadora asegurando de esta manera la calidad de estos preparados magistrales estériles que se deben administrar al paciente.

Además de las pruebas analíticas, se realizó un control de calidad microbiológico, para evaluar y documentar la esterilidad de los procesos implicados en la elaboración de las NP y la ausencia de crecimiento bacteriano o de hongos en estas muestras. En ninguna de ellas hubo crecimiento microbiológico al cultivar las muestras en placas de agar sangre basado en la técnica de Montejo[19], el cual constituye un control de calidad validado para detección de microorganismos en NP.

Todos los controles microbiológicos resultaron negativos, tanto los realizados a tiempo cero y 5 días post almacenamiento, no obstante, es necesario mencionar que no existe ningún tipo de control microbiológico que podamos conocer su resultado previo a la administración del paciente, ya que el cultivo tarda 7 días, por lo tanto necesariamente los resultados deberán evaluarse en forma conjunta con el comportamiento clínico del paciente.

V. Conclusión

Se logró demostrar empíricamente que se pueden realizar mediciones cuantitativas de algunos analitos que componen una NP utilizando plataformas analíticas de uso rutinario en el laboratorio del HLCM, lo que representa una ventaja significativa al actual sistema de control de calidad de elaboración utilizado en esta institución.

Fue posible definir sodio, calcio y glucosa como marcadores de elaboración de las NP y los analitos medidos en las mezclas se mantienen estables en el tiempo sin una mayor degradación, ya que son sales estables y se pudieron cuantificar. Se determinó que luego de 5 días de almacenadas las NP, refrigeradas entre 2 y 5 °C y sin exposición directa a la luz solar se mantienen estables desde el punto de vista físico químico.

Sin duda, creemos que este estudio representa un avance en pro de poder mejorar la calidad y seguridad de las NP administradas a nuestros niños.

No fue posible determinar la fecha de caducidad de las NP analizadas desde el punto de vista químico, ya que no se cuantificaron otros nutrientes más lábiles tales como lípidos y vitaminas, sin embargo desde el punto de vista microbiológico la caducidad está dada por la esterilidad del preparado a los 5 días, sumado a que los contenedores e infusores (bolsas multicapa que disminuye el paso de oxígeno y colores anaranjados) utilizados en la elaboración de la mezcla favorecen una menor degradación de nutrientes tales como lípidos, vitaminas, aminoácidos debido a los materiales.

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Tabla 1: Medias de concentraciones esperadas y medidas para cada analito durante día 0 y día 5, según regresión Passing-Bablok

					Día 0				Día 5	
Analito	Media esperada	CV	[media] día 0	CV	Pendiente	Intercepto	[media] día 5	CV	Pendiente	Intercepto
Calcio	22,7 (mg/dL)	0,30	20,9 (mg/dL)	0,35	1,05 (0,89-1,32)	-2,27 (-9,28 a 0,93)	23,09 (mg/dL)	0,39	1,18 (0,98 a 1,47)	-7,21 (-14,471 a 2,633)
Cloro	52,48 (mmol/L)	0,53	62,73 (mmol/L)	0,6	1,31 (1,14-1,51)	-6,089 (-12,99 a 1,204)	52,48 (mmol/L)	0,56	1,22 (1,05 a 1,43)	-0,725 (-8,168 a 2,807)
Glucosa	11408,37 (mg/dL)	0,21	10311,12 (mg/dL)	0,22	0,92 (0,79-1,09)	-391,7 (-2115,5 a 1077,7)	10856,06 (mg/dL)	0,26	1,00 (0,84 a 1,29)	-1404,2 (-4501,0 a 337,3)
Fosforo	8,96 (mg/dL)	0,39	17,10 (mg/dL)	0,68	3,82 (2,64-6,17	-16,388 (-39,006 a 7,511)	13,98 (mg/dL)	0,65	3,50 (1,85 a 7,31)	-16,843 (-53,982 a 5,570)
Magnesio	4,06 (mg/dL)	0,39	4,15 (mg/dL)	0,55	1,40 (1,06-1,77)	-1,198 (-2,560 a 0,189)	4,84 (mg/dL)	0,56	2,05 (1,37 a 3,78)	-2,970 (-10,069 a 0,698)
Sodio	49,03 (mmol/L)	0,81	49,41 (mmol/L)	0,67	1,09 (0,89-1,32)	2,06 (-4,75 a 9,43)	49,07 (mmol/L)	0,69	1,06 (0,96 a 1,23)	0,514 (-4,413 a 6,930)
Potasio	25,00 (mmol/L)	0,43	24,17 (mmol/L)	0,59	1,21 (1,03-1,42)	-5,160 (-9,384 a 1,577)	23,34 (mmol/L)	0,54	1,23 (1,06 a 1,38)	-5,515 (-9,289 a 2,192)

Tabla 2: Valores de significación entre las variables al día 0

Día 0	Sodio	Potasio	Cloro	Magnesio	Calcio	Glucosa	Fósforo
Sodio	-	0,013	0,246	0,626	0,217	0,673	0,034
Potasio	0,013	-	0,603	0,594	0,735	0,550	0,035
Cloro	0,246	0,603	-	0,865	0,614	0,930	0,943
Magnesio	0,626	0,594	0,865	-	0,759	0,013	0,881
Calcio	0,217	0,735	0,614	0,759	-	0,711	0,027
Glucosa	0,673	0,550	0,930	0,013	0,711	-	0,522
Fósforo	0,034	0,035	0,943	0,881	0,027	0,522	-

Tabla 3: Valores de significación entre las variables al día 5

Día 5	Sodio	Potasio	Cloro	Magnesio	Calcio	Glucosa	Fósforo
Sodio	-	0,103	0,005	0,040	0,332	0,089	0,785
Potasio	0,103	-	0,329	1	0,213	0,414	0,645
Cloro	0,005	0,329	-	0,772	0,527	0,588	0,894
Magnesio	0,040	1	0,772	-	0,388	0,649	0,005
Calcio	0,332	0,213	0,527	0,388	-	0,083	0,000
Glucosa	0,089	0,414	0,588	0,649	0,083	-	0,987
Fósforo	0,785	0,645	0,894	0,005	0,000	0,987	-

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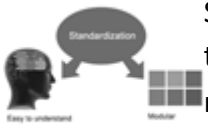

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The board members can also join us as Individual Fellow with 40% discount on total fees applicable to Individual Fellow. They will be entitled to avail all the benefits as declared. Please visit Individual Fellow-sub menu of GlobalJournals.org to have more relevant details.

We shall provide you intimation regarding launching of e-version of journal of your stream time to time. This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.

After nomination of your institution as "Institutional Fellow" and constantly functioning successfully for one year, we can consider giving recognition to your institute to function as Regional/Zonal office on our behalf.
The board can also take up the additional allied activities for betterment after our consultation.

The following entitlements are applicable to individual Fellows:

Open Association of Research Society, U.S.A (OARS) By-laws states that an individual Fellow may use the designations as applicable, or the corresponding initials. The Credentials of individual Fellow and Associate designations signify that the individual has gained knowledge of the fundamental concepts. One is magnanimous and proficient in an expertise course covering the professional code of conduct, and follows recognized standards of practice.

Open Association of Research Society (US)/ Global Journals Incorporation (USA), as described in Corporate Statements, are educational, research publishing and professional membership organizations. Achieving our individual Fellow or Associate status is based mainly on meeting stated educational research requirements.

Disbursement of 40% Royalty earned through Global Journals : Researcher = 50%, Peer Reviewer = 37.50%, Institution = 12.50% E.g. Out of 40%, the 20% benefit should be passed on to researcher, 15 % benefit towards remuneration should be given to a reviewer and remaining 5% is to be retained by the institution.

We shall provide print version of 12 issues of any three journals [as per your requirement] out of our 38 journals worth $ 2376 USD.

Other:

The individual Fellow and Associate designations accredited by Open Association of Research Society (US) credentials signify guarantees following achievements:

- The professional accredited with Fellow honor, is entitled to various benefits viz. name, fame, honor, regular flow of income, secured bright future, social status etc.

- In addition to above, if one is single author, then entitled to 40% discount on publishing research paper and can get 10%discount if one is co-author or main author among group of authors.
- The Fellow can organize symposium/seminar/conference on behalf of Global Journals Incorporation (USA) and he/she can also attend the same organized by other institutes on behalf of Global Journals.
- The Fellow can become member of Editorial Board Member after completing 3yrs.
- The Fellow can earn 60% of sales proceeds from the sale of reference/review books/literature/publishing of research paper.
- Fellow can also join as paid peer reviewer and earn 15% remuneration of author charges and can also get an opportunity to join as member of the Editorial Board of Global Journals Incorporation (USA)
- • This individual has learned the basic methods of applying those concepts and techniques to common challenging situations. This individual has further demonstrated an in–depth understanding of the application of suitable techniques to a particular area of research practice.

Note :

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- In future, if the board feels the necessity to change any board member, the same can be done with the consent of the chairperson along with anyone board member without our approval.
- In case, the chairperson needs to be replaced then consent of 2/3rd board members are required and they are also required to jointly pass the resolution copy of which should be sent to us. In such case, it will be compulsory to obtain our approval before replacement.
- In case of "Difference of Opinion [if any]" among the Board members, our decision will be final and binding to everyone.

"

Preferred Author Guidelines

We accept the manuscript submissions in any standard (generic) format.

We typeset manuscripts using advanced typesetting tools like Adobe In Design, CorelDraw, TeXnicCenter, and TeXStudio. We usually recommend authors submit their research using any standard format they are comfortable with, and let Global Journals do the rest.

Alternatively, you can download our basic template from https://globaljournals.org/Template

Authors should submit their complete paper/article, including text illustrations, graphics, conclusions, artwork, and tables. Authors who are not able to submit manuscript using the form above can email the manuscript department at submit@globaljournals.org or get in touch with chiefeditor@globaljournals.org if they wish to send the abstract before submission.

Before and during Submission

Authors must ensure the information provided during the submission of a paper is authentic. Please go through the following checklist before submitting:

1. Authors must go through the complete author guideline and understand and *agree to Global Journals' ethics and code of conduct,* along with author responsibilities.
2. Authors must accept the privacy policy, terms, and conditions of Global Journals.
3. Ensure corresponding author's email address and postal address are accurate and reachable.
4. Manuscript to be submitted must include keywords, an abstract, a paper title, co-author(s') names and details (email address, name, phone number, and institution), figures and illustrations in vector format including appropriate captions, tables, including titles and footnotes, a conclusion, results, acknowledgments and references.
5. Authors should submit paper in a ZIP archive if any supplementary files are required along with the paper.
6. Proper permissions must be acquired for the use of any copyrighted material.
7. Manuscript submitted *must not have been submitted or published elsewhere* and all authors must be aware of the submission.

Declaration of Conflicts of Interest

It is required for authors to declare all financial, institutional, and personal relationships with other individuals and organizations that could influence (bias) their research.

Policy on Plagiarism

Plagiarism is not acceptable in Global Journals submissions at all.

Plagiarized content will not be considered for publication. We reserve the right to inform authors' institutions about plagiarism detected either before or after publication. If plagiarism is identified, we will follow COPE guidelines:

Authors are solely responsible for all the plagiarism that is found. The author must not fabricate, falsify or plagiarize existing research data. The following, if copied, will be considered plagiarism:

- Words (language)
- Ideas
- Findings
- Writings
- Diagrams
- Graphs
- Illustrations
- Lectures

- Printed material
- Graphic representations
- Computer programs
- Electronic material
- Any other original work

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1. Substantial contributions to the conception and acquisition of data, analysis, and interpretation of findings.
2. Drafting the paper and revising it critically regarding important academic content.
3. Final approval of the version of the paper to be published.

Changes in Authorship

The corresponding author should mention the name and complete details of all co-authors during submission and in manuscript. We support addition, rearrangement, manipulation, and deletions in authors list till the early view publication of the journal. We expect that corresponding author will notify all co-authors of submission. We follow COPE guidelines for changes in authorship.

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Appealing Decisions

Unless specified in the notification, the Editorial Board's decision on publication of the paper is final and cannot be appealed before making the major change in the manuscript.

Acknowledgments

Contributors to the research other than authors credited should be mentioned in Acknowledgments. The source of funding for the research can be included. Suppliers of resources may be mentioned along with their addresses.

Declaration of funding sources

Global Journals is in partnership with various universities, laboratories, and other institutions worldwide in the research domain. Authors are requested to disclose their source of funding during every stage of their research, such as making analysis, performing laboratory operations, computing data, and using institutional resources, from writing an article to its submission. This will also help authors to get reimbursements by requesting an open access publication letter from Global Journals and submitting to the respective funding source.

Preparing your Manuscript

Authors can submit papers and articles in an acceptable file format: MS Word (doc, docx), LaTeX (.tex, .zip or .rar including all of your files), Adobe PDF (.pdf), rich text format (.rtf), simple text document (.txt), Open Document Text (.odt), and Apple Pages (.pages). Our professional layout editors will format the entire paper according to our official guidelines. This is one of the highlights of publishing with Global Journals—authors should not be concerned about the formatting of their paper. Global Journals accepts articles and manuscripts in every major language, be it Spanish, Chinese, Japanese, Portuguese, Russian, French, German, Dutch, Italian, Greek, or any other national language, but the title, subtitle, and abstract should be in English. This will facilitate indexing and the pre-peer review process.

The following is the official style and template developed for publication of a research paper. Authors are not required to follow this style during the submission of the paper. It is just for reference purposes.

Manuscript Style Instruction (Optional)

- Microsoft Word Document Setting Instructions.
- Font type of all text should be Swis721 Lt BT.
- Page size: 8.27" x 11'", left margin: 0.65, right margin: 0.65, bottom margin: 0.75.
- Paper title should be in one column of font size 24.
- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word "Abstract" in bold italics.
- Main text: font size 10 with two justified columns.
- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
- The paragraph before spacing of 1 pt and after of 0 pt.
- Line spacing of 1 pt.
- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

Structure and Format of Manuscript

The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

a) A title which should be relevant to the theme of the paper.
b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.
c) Up to 10 keywords that precisely identify the paper's subject, purpose, and focus.
d) An introduction, giving fundamental background objectives.
e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition, sources of information must be given, and numerical methods must be specified by reference.
f) Results which should be presented concisely by well-designed tables and figures.
g) Suitable statistical data should also be given.
h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.
j) There should be brief acknowledgments.
k) There ought to be references in the conventional format. Global Journals recommends APA format.

Authors should carefully consider the preparation of papers to ensure that they communicate effectively. Papers are much more likely to be accepted if they are carefully designed and laid out, contain few or no errors, are summarizing, and follow instructions. They will also be published with much fewer delays than those that require much technical and editorial correction.

The Editorial Board reserves the right to make literary corrections and suggestions to improve brevity.

Format Structure

It is necessary that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.

All manuscripts submitted to Global Journals should include:

Title

The title page must carry an informative title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) where the work was carried out.

Author details

The full postal address of any related author(s) must be specified.

Abstract

The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Keywords

A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy: planning of a list of possible keywords and phrases to try.

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning searching. Think about the most important concepts related to research work. Ask, “What words would a source have to include to be truly valuable in a research paper?” Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

Numerical Methods

Numerical methods used should be transparent and, where appropriate, supported by references.

Abbreviations

Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

Formulas and equations

Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

Tables, Figures, and Figure Legends

Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.

Figures

Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

Preparation of Eletronic Figures for Publication

Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). Please give the data for figures in black and white or submit a Color Work Agreement form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution at final image size ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs): >350 dpi; figures containing both halftone and line images: >650 dpi.

Color charges: Authors are advised to pay the full cost for the reproduction of their color artwork. Hence, please note that if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a Color Work Agreement form before your paper can be published. Also, you can email your editor to remove the color fee after acceptance of the paper.

Tips for writing a good quality Medical Research Paper

1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

4. Use of computer is recommended: As you are doing research in the field of medical research then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

5. Use the internet for help: An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.

6. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right? It is a good habit which helps to not lose your continuity. You should always use bookmarks while searching on the internet also, which will make your search easier.

7. Revise what you wrote: When you write anything, always read it, summarize it, and then finalize it.

8. Make every effort: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

9. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

10. Use proper verb tense: Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. Arrangement of information: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. Go to seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. Adding unnecessary information: Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. Upon conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear: Adhere to recommended page limits.

Mistakes to avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

Approach:

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

Procedures (methods and materials):

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."

Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

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	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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