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Highlights

- Evaluation and Ranking of Drug
- A Prospective Observational Study
- Antibiotic use During Pregnancy
- Cross-Linkers in Submicron Particles

Discovering Thoughts, Inventing Future

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Evaluation and Ranking of Drug Release from Different Grades of Guar Gum, Acacia Gum and Polyvinyl Pyrrolidone as Cross-Linkers in Submicron Particles

By Negla Abdulghani Elsayed Yagoub, Dr. Abubakar Osman Mohamed Nur, Fadilah Sfouq Aleanizy & Sarah Ahmed

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Abstract- Due to their unique properties, nanoparticles made of polysaccharides are promising carriers to deliver and protect the physiological properties of hydrophilic drugs. They have been successfully applied as drug delivery systems (83).

Objective: The main goal of this research is to Improve Carbamazepine water solubility and drug release properties by nano sizing, and using guar gum, Acacia Gum and poly-vinylpyrrolidone, each of two viscosity grades, as crosslinking agents. Moreover, the study is extrapolated, utilizing composite index (CI) design and mathematical modelling, in an attempt to locate the most suitable set of the factors that affect nanoparticles produced with optimum specifications.

Keywords: polymer, Guar gum, acacia gum, polyvinyl pyrrolidine, carbamazepine, drug release, composite index.

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Abstract- Due to their unique properties, nanoparticles made of polysaccharides are promising carriers to deliver and protect the physiological properties of hydrophilic drugs. They have been successfully applied as drug delivery systems (83).

Objective: The main goal of this research is to Improve Carbamazepine water solubility and drug release properties by nano sizing, and using guar gum, Acacia Gum and poly-vinylpyrrolidone, each of two viscosity grades, as crosslinking agents. Moreover, the study is extrapolated, utilizing composite index (CI) design and mathematical modelling, in an attempt to locate the most suitable set of the factors that affect nanoparticles produced with optimum specifications.

Methods: The method used nano and submicron particles that were produced in our previous study (Evaluation of different grades of guar gum, acacia gum and polyvinyl pyrrolidone as cross-linkers in producing submicron particles). All runs were subjected to drug release investigations according to which a weighted composite index was generated.

Results: Based on the obtained findings and the associated statistical analysis, particles of run8 were found to be the best ranked as they fulfilled all the constraints.

Conclusion: Acacia gum was found to have the most interesting properties in developing submicron particles with controlled drug release, accordingly the study recommends the need for further investigations.

Keywords: polymer, Guar gum, acacia gum, polyvinyl pyrrolidine, carbamazepine, drug release, composite index.

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I. Introduction

a) Drug Release

A central reason for pursuing nanotechnology is to deliver drugs, hence understanding the manner and extent to which the drug molecules are released is important. The drug loading of the nanoparticles is generally defined as the amount of drug bound per polymer mass (usual moles of drug per mg polymer or mg drug per mg polymer); it could also be given as a percentage relative to the polymer.

Nanoparticles made of polysaccharides, due to their unique properties, are promising carriers to deliver and protect the physiological properties of hydrophilic drugs and have been successfully applied as drug delivery systems (1) As natural biomaterials, polysaccharides are stable, safe, nontoxic, hydrophilic, and biodegradable.

b) Biological benefits of nanoparticles

The property of nanoparticle formulations that make this approach highly beneficial is related to the surface properties imparted on nanometer-sized entities (2). Applying Nano-crystal Technology or one of the alternate nanoparticle formulation approaches to the many formulation and performance issues associated with poorly water-soluble compounds in the pharmaceutical industry provides many benefits.

c) The Solubility Challenge

It is estimated that ~40% of active substances identified through combinatorial screening programs are difficult to formulate as a result of their lack of significant solubility in water (3, 4, and 5). In one sense, this is understandable. If a molecule must penetrate a biological membrane to be absorbed, the molecule generally must possess some hydrophobic or lipophilic characteristics. When these types of situations arise, a nanoparticle formulation approach has proven to be very useful and invaluable in all stages of drug development and has opened opportunities for revitalizing marketed products with suboptimal delivery.

d) Guar gum

Guar gum (GG) is galactomannan derived from Guar *Cyamopsis tetragonolobuskernels* which belong to family *Leguminosae.*

It is biocompatible, biodegradable, non-toxic, low-cost and amenable to chemical modifications, properties that make it an ideal material for developing drug delivery formulations (6). However, native guar gum has also shortcomings such as, uncontrolled rates of hydration, high swelling, thickening effect, instability upon storage, high susceptibility to microbial attack and the difficulty to control viscosity due to relative fast biodegradation (7).

Thermal treatment of guar gum at 70℃ for 10 minutes is an efficient tool to produce guar gum with desired properties for pharmaceutical processing and industries. The treatment has resulted in the production of treated guar gum with improved flowability, swellability, and compressibility. On the other hand, the method of drying seems to have a significant influence on the viscosity of the resultant treated guar powder and verification of such effect might necessitate a more collaborated extended study (8).

e) Acacia Gum

This is the dried exudate of the acacia tree (*Acacia senegal*or related species of *Acacia* Fam. *Leguminosae*. The gum is highly soluble in water. Physically, acacia is considered to be a complex, highly branched, globular molecule, which is closely packed rather than linear, thus accounting for its low viscosity. Rheologically, acacia gum solutions exhibit typical Newtonian behavior at concentrations up to 40%. Above 40%, solutions become pseudoplastic, as is shown by a decrease in viscosity with increasing shearing stress (9).

f) Povidone

PVP is a water-soluble pharmaceutically acceptable polymer. Due to its ability to improve solubility and wettability of poorly soluble drugs, it is frequently used in solid dispersions to enhance solubility and dissolution rate (10, 11), Due to its hydrophilicity and rapid dissolution in an aqueous medium, PVP is very frequently applied as a carrier in immediate release dosage forms. PVP has a long history of use in human drug products and high molecular weight PVPs generally do not get absorbed in the GI tract.

g) Carbamazepine (*CBZ*)

One of the bad soluble active drug substances. Although Carbamazepinehas a high intestinal permeability, its bioavailability is limited by its low water solubility (0.11mgmL−1) (2).

5H-ibenz[b,f]azepine-5-carboxamide A white or almost white crystalline powder. It exhibits polymorphism that is very slightly soluble in water; sparingly soluble in alcohol and in acetone, and freely soluble in dichloromethane.

Carbamazepine is widely distributed throughout the body and is about 70 to 80% bound to plasma proteins. It induces its own metabolism so that the plasma half-life may be considerably reduced after repeated dosage.

The mean plasma half-life of carbamazepine on repeated dosage is about 12 to 24 hours; it appears to be considerably shorter in children than in adults.

Carbamazepine is a dibenzazepine derivative with antiepileptic and psychotropic properties. It is used to control secondarily generalisedtonic-clonic seizures and partial seizures and in some primary generalized seizures.

h) Composite index

A composite index is a grouping of equities, indexes or other factors combined in a standardized way, providing a useful statistical measure of overall market or sector performance over time, and it is also known simply as a "composite." Usually, a composite index has a large number of factors that are averaged together to form a product representative of an overall market or sector (12).

II. Materials and Methods

Materials: The Nano and submicron particles produced in our previous study (Evaluation of different grades of guar gum, acacia gum and polyvinyl pyrrolidone as cross-linkers in producing submicron particles) as in Table1 are used in this study

Table I: Layout of formulation runs according to mixed 3-2 -levels factors and 1- 3-levels factor statistical design

Run	Stirring Rate	Polymer grade	Polymer load	Polymer type
R1	1000	G-non treated	1%	Guar gum
R2	1000	Acacia lower viscosity	1%	Acacia Gum
R3	1000	Povidone K90 higher viscosity	1%	Povidone
R4	1000	G-non treated	10%	Guar gum
R5	1000	Acacia lower viscosity	10%	Acacia Gum
R6	1000	Povidone K90 higher viscosity	10%	Povidone
R7	1000	G- treated	1%	Guar gum
R8	1000	Acacia higher viscosity	1%	Acacia Gum

R9	1000	PovidoneK30 lower viscosity	1%	Povidone
R10	1000	G- treated	10%	Guar gum
R11	1000	Acacia higher viscosity	10%	Acacia Gum
R12	1000	PovidoneK30 lower viscosity	10%	Povidone
R13	500	G-non treated	1%	Guar gum
R14	500	Acacia lower viscosity	1%	Acacia Gum
R15	500	PovidoneK30 lower viscosity	1%	Povidone
R16	500	G-non treated	10%	Guar gum
R17	500	Acacia lower viscosity	10%	Acacia Gum
R18	500	PovidoneK90 higher viscosity	-10%	Povidone
R19	500	G- treated	1%	Guar gum
R20	500	Acacia higher viscosity	1%	Acacia Gum
R21	500	PovidoneK30 lower viscosity	1%	Povidone
R22	500	G- treated	10%	Guar gum
R23	500	Acacia higher viscosity	10%	Acacia Gum
R24	500	PovidoneK30 lower viscosity	10%	Povidone

a) Apparatus

The following instruments were used in the experimental part of this study:

Instrument	Specification and Source
Analytical balance	Reblab ®, Germany
Zetasizer 90 plus	Malvern Panalytical Ltds
U.V. Spectrophotometer	double beam UV-1800, Shimadzu, Japan
Magnetic stirrer	Stuart, England
Scanning electron microscope	Zeiss EVO LS10; Cambridge, United Kingdom

b) Methods

Collected submicron particles from all runs were subjected to the following qualifications.

c) Particle size analysis

By using particle size analyser 90, measurements of polydispersity (PD %) were performed.

A specified amount of dry particles was completely dissolved in ethyl acetate, filtered and transferred to the instrument cell and subjected to the test.

d) Entrapments efficiency of nanoparticles

Dried nanoparticles were dissolved in ethyl acetate (a common solvent for polymers and drug samples). The amount of entrapped carbamazepine that was present in the solution was measured spectrophotometrically at 287 nm (USP, 13).

Drug incorporation efficiency was expressed both as Drug Content (% w/w), also referred to as drug loading in the literature, and Drug Entrapment (%); represented by Eqs. (1) and (2) respectively. The individual values for two replicate determinations and their mean values were reported

$$\text{Drug loading (\% w/w)} = \frac{Mass\ of\ drug\ in\ nanoparticles}{Mass\ of\ nanoparticle}\%100 \quad (1)$$

$$\text{Drug Entrapment (\%)} = \frac{Mass\ of\ drug\ in\ nanoparticles}{Mass\ of\ drug\ used\ in\ formulation}\%100 \quad (2)$$

e) Nanoparticle drug release assessment

All runs were subjected to drug release investigations where the amount of particles equivalent to 1 g of carbamazepine was weighed and transferred to a dissolution test beaker containing 1L of sodium lauryl sulphate. 3ml of each sample was filtered into 100 ml volumetric flask and the absorbance of the samples was determined at 287 nm against water as a blank (14). Making use of the drug calibration curve (as discussed next), the amount of carbamazepine was then estimated. The assay method was derived from the USP carbamazepine tablets dissolution test monograph (USP, 13).

f) Calibration curve

From the reference standard Carbamazepine, 40 mg was accurately weighed and dissolved in 8 ml absolute methanol, 1 ml of this solution was taken and diluted to 10 ml. Serial dilutions were then carried out to obtain solutions of different drug concentrations. The absorbance of each concentration at 287nm was determined spectrophotometrically and a calibration curve was thus generated (USP,13).

g) Composite index design

A weighted composite index was generated for the data to designate a single score utilizing three constraints (15). This was done in order to select the optimized factors setting (polydispersity, Entrapment Efficiency and nanoparticle drug release rate at 60 mints) that could possibly yield the most desired properties for drug granules and tablets. The process of statistical composite index application was aided by the computer Excels program.

III. Results

a) Characterization of produced particles

Table 2 summarizes the polydispersity index (PDI %) and entrapment efficiency (EE %) properties of produced particles within different formulation runs. The Carbamazepine calibration curve and drug release profiles of different formulation runs are depicted in figures 1 and 2, respectively.

Table 2: Polydisperse index (PDI %) and entrapment efficiency (EE %) of yielded particles within different formulation run

Run No.	EE	PDI
R1	52.3%	5.50%
R2	52.3%	0.52%
R3	52.3%	1.76%
R4	13.1%	0.37%
R5	13.1%	0.50%
R6	13.1%	0.43%
R7	52.3%	0.62%
R8	52.3%	0.30%
R9	52.3%	0.67%
R10	13.1%	0.39%
R11	12.5%	4.04%
R12	11.8%	0.40%
R13	39.6%	0.55%
R14	47.0%	0.44%
R15	45.0%	0.69%
R16	12.7%	0.38%
R17	13.1%	1.09%
R18	13.1%	1.04%
R19	52.3%	0.09%
R20	52.3%	0.38%
R21	52.3%	0.56%
R22	13.1%	1.74%
R23	13.1%	0.81%
R24	13.1%	16.64

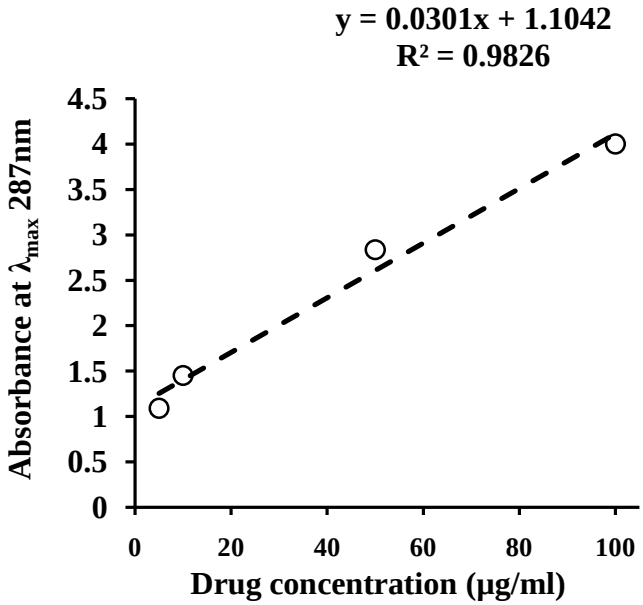

Fig. 1: Calibration plot for determination of Carbamzepine in solutions using UV method. Each data point is the average of 3 determinations, R^2: correlation coefficient

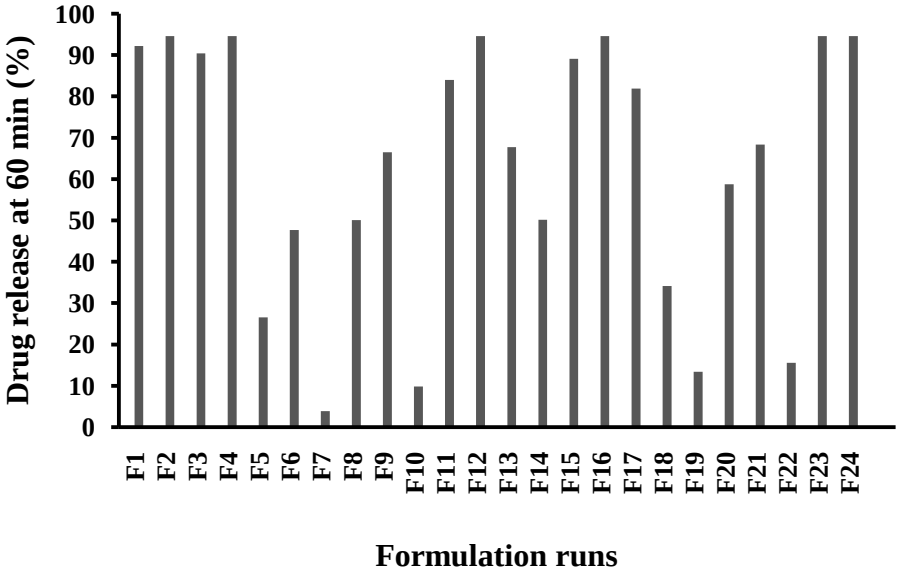

Fig. 2: Cumulative % drug released after 60 Min. of particles within different formulation runs

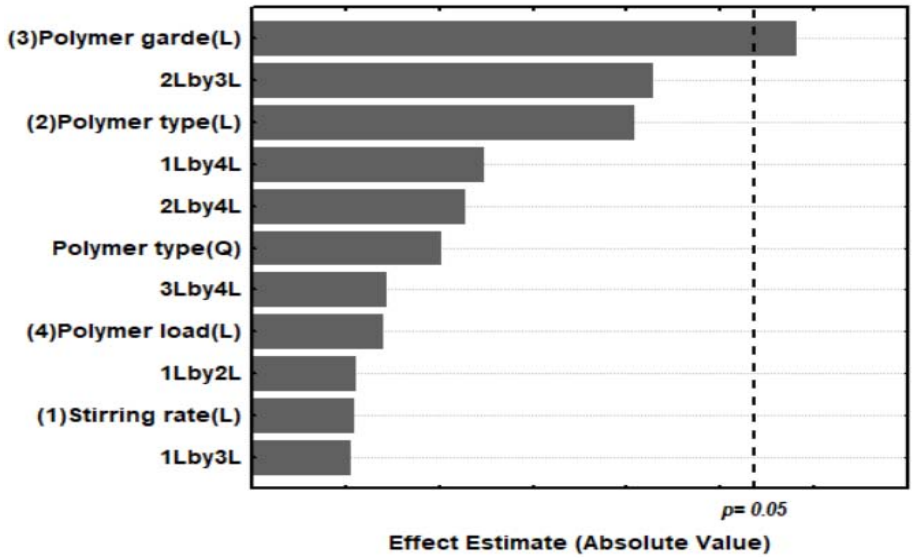

Fig. 3: Estimated effects of the linear (L) and quadratic (Q) and joined influences of the investigated variables on percent drug release at 60 min of different formulations within the experimental design where p= 0.05 denotes cut-off point for significant influences

Composite index scoring and ranking of different formulations of carbamazepine-loaded polymeric particles

Table 3 abridges the composite index scoring and the subsequent ranking of different formulations in the design based on preset of selected 3 constraints of polydispersity index (PDI), entrapment efficiency (EE %) and drug release at 60 min (% $Rel_{60\ min}$).

Table 3: Composite index (CI) and subsequent ranking order of different formulations in the design based on pre-set constraints for particles polydispersity index (PDI %), entrapment efficiency (EE %) and drug release at 60 min (%Rel_{60min})

Run No.	Responses values			Transformed responses			CI	Ranking
	PDI %	EE %	%Rel_{60min}	PDI %	EE %	%Rel_{60min}		
R1	5.50	52.3	70	0	0.14	0	0.14	7
R2	0.52	52.3	73	0	0.14	0.07	0.21	4
R3	1.76	52.3	68	0	0.14	0	0.14	7
R4	0.37	13.1	73	0.16	0	0.07	0.23	3
R5	0.50	13.1	15	0	0	0	0.00	13
R6	0.43	13.1	26	0.04	0	0	0.04	12
R7	0.62	52.3	5	0	0.14	0	0.14	7
R8	0.30	52.3	28	**0.29**	**0.14**	0	**0.43**	**1**
R9	0.67	52.3	45	0	0.14	0	0.14	7
R10	0.39	13.1	6	0.12	0	0	0.12	8
R11	4.04	12.5	62	0	0	0	0.00	13
R12	0.40	11.8	73	0.10	0	0.07	0.17	6
R13	0.55	39.6	46	0	0	0	0.00	13
R14	0.44	47.0	28	0.02	0.08	0	0.10	9
R15	0.69	45.0	67	0	0.06	0	0.06	11
R16	0.38	12.7	73	0.14	0	0.07	0.21	5
R17	1.09	13.1	60	0	0	0	0.00	13
R18	1.04	13.1	12	0	0	0	0.00	13
R19	0.09	52.3	8	0	0.14	0	0.14	7
R20	0.38	52.3	37	0.14	0.14	0	0.28	2
R21	0.56	52.3	46	0	0.14	0	0.14	7
R22	1.74	13.1	9	0	0	0	0.00	13
R23	0.81	13.1	73	0	0	0.07	0.07	10
R24	0.54	13.1	73	0	0	0.07	0.07	10

IV. Discussion

a) Drug release Studies

A central reason for pursuing nanotechnology is to enhance drug delivery, hence understanding the manner and extent to which the drug molecules are released is important. In order to obtain such information most release methods require that the drug and its delivery vehicle be separated (16, 17).

For the drug to be released from the Polymer particles, the Polymer undergoes degradation by hydrolysis or biodegradation through cleavage of its backbone ester linkage into oligomers and finally monomers (18).

b) Calibration curve of standard carbamazepine

The generated calibration curve for standard CBZ in solutions using the validated UV assay method shows high acceptable linear correlation regression between drug concentration and UV absorbance with a highly established correlation coefficient ($R^2 = 0.9826$) in the drug concentration range of 1—100μg/ml (Fig. 1).

c) Effects on drug release characteristics

The effect of different variables on drug release at 60min for different formulations has been studied. Fig 3, showed the linear, quadratic and joined influences of polymer type, polymer grade, polymer load and stirring rate. Among the different variables investigated, the polymer grade has the predominant and significant effect on drug release over the other variables, it has a linear effect with $p > 0.05$ which is the cutoff point. Polymer type (2) has less effect than the polymer grade(3) and when joining their linear effect (2 and3) it appears less than (2) and more than (3). Only the polymer type has a quadratic effect on drug release but it was a non-significant one.

d) Relation between polymer (type, grade) and drug release

Similar to what was found in a study done by Nur et al (19) considering Guar gum, Treated Guar Gum, and Xanthan Gum, as drug fabricating polymers, different drug release profiles were also present in this study. This might be related to their dissimilar hydration and swelling attributes that determine the rate at which the surface viscous barrier (controlling gel) is being formed. These findings along with the effect of particle size and EE% can explain the variation in CBZ release profile from the different gums. Moreover, the statistical work shown in fig 3 reveals the predominated effect of polymer grade (viscosity) as a significant effect over the other factors. Following is a discussion on the effect of different polymer grades on CBZ release.

Considering Native Guar gum, a fast release of 20% to 40% was observed immediately after the addition of loaded particles. This doesn't go along with Nur et al study and it's likely due to a fraction of CBZ present on the surface of the particles being immediately released upon coming in contact with the SLS medium.

However, native guar gum has also shortcomings such as uncontrolled rates of hydration, high swelling, thickening effect, instability upon storage, high susceptibility to microbial attack and the difficulty to control viscosity due to relative fast biodegradation (20). Various strategies were developed in order to overcome these issues, offering the opportunity to tailor the physical and chemical properties of guar gum thus yielding materials that may find a wide range of applications

Regarding Treated Guar gum, the CBZ release was found to be delayed. Less than 30% of the drug entrapped was released within 120 Min., This goes parallel with the results of Nur et al (21), which reported low hydration and swelling capabilities of the treated gum. Accordingly, this is reflected in the enhancement of drug release as a result of the delay in the formation of the gel layer that controls the drug release. Such a result is a good explanation of the poor release profile from the treated guar as for the particle in order to release the entrapped drug, the particle must be swollen to permit the drug release.

In Povidone K_{30} (Lower viscosity) the fastest and uniform release was shown with polymer concentration 1% (R 9 &R21) which has higher EE%. This can be explained by the lower viscosity of the prepared emulsion producing small particles and the high hydrophilicity of povidonek30. All these parameters can increase drug dissolution, which is reported by a study published in ISP Pharmaceuticals (11). The study used low molecular weight PVPs as carriers in solid dispersions due to their higher aqueous solubility, lower viscosity in the diffusion boundary layer, and faster dissolution rate. the study revealed that solid dispersions of indomethacin from co-precipitation and spray drying processes showed faster release from PVP with low molecular weight (PVP K30) than those with high molecular weight (PVP K90) (22).

In our study, the release of CBZ from PVP K30 was very fast in R24. This is can be due to the lower EE% which means that the drug is on the surface of particles not entrapped due to the emulsion's high viscosity as a consequence of increased polymer concentration (10%). This high viscosity renders the drug from diffusing into a polymer molecule and crosslinking with it.

Another study, done by Bharali et al (23), investigated the characteristics of in vitro release of entrapped PVP at low loadings of the compound, which remains in the form of a molecular dispersion inside PVP particles. It was found that when the concentration of dye inside the core of the particle is very high, a part of it is associated or clustered, which has to be dissolved and released more slowly out of the particles. These

phenomena appear clearly in our study in R 21 which has a higher EE% of 52% with a lower release rate.

Regarding Povidone k90 (High viscosity) High molecular weight grade PVP K90 dissolves in a large variety of organic solvents. However, due to its hydrophilicity, its moisture uptake level is high (24) which may result in difficulties in its physical stability leading to drug crystallization in the carrier polymer caused by the plasticizing effect of absorbed water.

The drug release profile of the four runs (3,6,15,18) is strongly linked with EE% as increase EE% increase drug release, R 3 and R15 reached 90% release in 60 minutes as shown in Table 4. The fastest one is in R15 (76% release at 30 mins) can be attributed to the amount of CBZ entrapped (less than 50%) and hence more drugs are on the particle surface leading to burst release (more than 30% in the first 10 mints) (25)

With respect to Lower viscosity, Acacia gum showed the slowest release rate among runs, higher viscosity of acacia, large particle and higher polydispersity as seen in Table2 are the responsible factors. R2 small particle and high EE% these results are not in accordance with relevant published work discussed above. As EE% is a result of how a drug is cross-linked with a polymer, a decreased viscosity will lead to an increase in EE% as less barrier is present, this was seen in R2 (1%polymer concentration produces a solution of lower viscosity) even with large particle sizeR5 with smaller particles (1433.38) than R2 though with lower EE% can be explained the same way.

Considering the higher viscosity of acacia gum runs, a fast release profile was observed which can be relied on for the burst release. More than 20% to 47% of drugs are released in the first 10 minutes with lower EE%, which means the drug is on the particle surface and not entrapped as seen in R 11 and R 23 with less EE%.

In R 8 and R 20 the EE% is high; it has a fast release of 20 % this can be explained by their small particle increasing drug solubility and accordingly enhancing drug release

e) Effect of particle size on drug release

Particle size distribution and morphology are the most important parameters of the characterization of particles. In a study done by (25), it has been found that particle size affects drug release. Smaller particles offer a larger surface area. As a result, most of the drug-loaded onto them will be exposed to the particle surface leading to fast drug release, despite these findings present study found that the smallest particle of R19 (131.72) and R22 (168.25) have the slowest drug release. This may be contributed to the nature of treated guar gum used, thermal treatment of guar gum lead to new gum with odd properties due to degradation of the polymer chain. On the contrary, R1 (native guar) which has a particle size (769.81) showed fast drug release (41.83%) in the first 10 minutes, which support the finding of Robinson (11). Such results can give us a good indication that drug release is mainly affected by polymer characteristics rather than particle size. When we go through the runs we find that R 5 & R 3 have almost the same particle size (1.43 & 1.45) but with different drug releases. R5 (lower viscosity Acacia gum) have 18.83% of drug released in the first 10 minutes while R 3 (povidone lower viscosity) has 45.48% of drug released in the first 10 minutes which support the above finding as seen in Table 4.

Polymer degradation can also be affected by particle size. For instance, the degradation rate of poly (lactic-co-glycolic acid) was found to increase with increasing particle size in vitro (26).

f) Relation between EE% and drug release

The fast drug release in first 10 minutes can be explained by the EE%, as the drug on surface of the particle is released before the entrapped one. This finding appear in R 16 and R 4 (native Guar) with large particle size (3,600.58 &26,450.88) and drug release 34.12% &50.20% respectively

It also ppear in povidone k90 R 12 and R 24 (release 33.66% & EE% 11.49%) (Maximum release 67.02% and EE% 12.88%), respectively (Table 4).

A fast release of 20% to 40% was observed for native guar run just after the addition of loaded particles, likely due to a fraction of CBZ present on the surface of the particles being immediately released in contact with the simulated fluids. The CBZ released in the SLS medium over the total duration of the experiment reached 85 %, indicating that the release of CBZ from the particles can also be controlled by pH.

g) Optimization by composite indexing

Using composite index design as ranking tool prove to be effective in evaluating each factor in an equal way that help in making decision with strong statistical view.

Since the relative contribution of each individual constraint to the true composite score within each step was unknown, the decision was made to assign an arbitrary value of 1/3 to each of the three factors and, accordingly, each test result was transformed to a value between 0 and 0.33. Within each separate step, multi-linear regression equations were applied for the three constraints in order to generate the composite index (CI) for each selected constraint including higher than and lower than ideal values. The run having the highest composite index would be considered as a batch fulfilling the constraints and consequently would be considered as an optimized one.

Table 3 abridged the composite index scoring and the subsequent ranking of the different 24 runs based on the previously mentioned preset 3 constraints of (EE%, PDI and R% at 60 mints) in composite index are summarized in Table 3,

The generated composite index scoring for Runs in this series has ranked R 8 as first run though it has R% 28 at 60 min with increased EE% and the smallest PDI(0.3) lead to increase its efficiency in rank

V. Conclusion

It was found that Acacia gum has the more interesting properties in developing submicron particles like controlling drug release, and hence need to be studied further, while polymer viscosity has large impact on particles behavior.

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What Caused Her Fall? A Clinical Case of Leg Swelling

By N. Stacy Amadife MD & Constance Mere MD

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Abstract- Minimal change disease (MCD) is typically not a disease seen in adults as it comprises only 10-15% of cases (1). Disease can be further characterized as primary/idiopathic or secondary. Typical secondary causes include drugs such as NSAIDs and Lithium and malignancies including Non-Hodgkin Lymphoma. Thus, secondary causes are often the culprit. We present a 47-year-old African-American female patient with a history of Multiple Sclerosis (MS) and HIV who presented with sudden onset worsening lower extremity edema and 6.6 grams (g) urine protein to creatinine ratio with primary MCD.

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What Caused Her Fall? A Clinical Case of Leg Swelling

N. Stacy Amadife MD α & Constance Mere MD σ

Abstract- **Minimal change disease (MCD) is typically not a disease seen in adults as it comprises only 10-15% of cases (1). Disease can be further characterized as primary/idiopathic or secondary. Typical secondary causes include drugs such as NSAIDs and Lithium and malignancies including Non-Hodgkin Lymphoma. Thus, secondary causes are often the culprit. We present a 47-year-old African-American female patient with a history of Multiple Sclerosis (MS) and HIV who presented with sudden onset worsening lower extremity edema and 6.6 grams (g) urine protein to creatinine ratio with primary MCD.**

I. Introduction

Minimal change disease (MCD) is a nephrotic syndrome primarily seen in children and early teens (1). In adults, the major nephrotic disease remain Focal Segmental Glomerulosclerosis (higher prevalence in people of African origin) and Membranous nephropathy (higher prevalence in people of European descent). It is rare to see MCD in adults as it comprises only 10-15% of cases (2). Patients usually present with sudden onset edema, proteinuric kidney injury, and hyperlipidemia. Disease can be further characterized as primary/idiopathic or secondary. Typical secondary causes include drugs such as non steroidal anti-inflammatory drugs (NSAID) and Lithium, infections such as Syphilis, Mycoplasma, allergens, autoimmune disorders like Systemic Lupus Erythematous (SLE), Celiac disease, diabetes, as well as malignancies including Non Hodgkin Lymphoma and bronchogenic carcinoma (1). The pathogenesis hypothesis states that disruption of actin cytoskeleton within the podocyte and basement membrane in conjunction with a disrupted immune system cause an increase in mediating factors leading to filtration of albumin into the urinary system (2).

II. Case Report

We present a case of a 47 -year old African-American woman with biopsy proven MCD.

The patient presented to the Emergency Department (ED) after sustaining a fall at home. She hit her head albeit did not lose consciousness. She reports myalgia, nausea, and acute worsening of paresthesia in her hands and lightheadedness over the past one month. In addition, she notes worsening leg swelling spanning three weeks and involuntary 30 pound weight gain over the past month. She denies any herbal medication use, illicit drug use, or recent illness. The last time she took NSAIDs was for menses four months prior to presentation and totaled no more than six doses.

Her past medical history is significant for Multiple Sclerosis (MS) diagnosed in 2005 and her last flare in 2008. Flares are characterized by fatigue, frequent fall, and dizziness. Her disease is managed with Glatramer injections three times weekly. She also has a history of HIV with undetectable viral load and takes Biktarvy daily. CD4 count at time of admission 976. Finally, patient has leiomyomas and follows with outpatient gynecology.

Her vitals: heart rate 101 beats per minute Blood pressure 150/90mm Hg, 16 Respirations per minute and oxygen saturation of 99% on room air.

Upon admission, lab investigations demonstrated:

C3, serum	95.62 (mg/dl) (79-152)
C4, serum	13.75 (mg/dl) (16-38)
Albumin ,serum	Less than 1.5 (g/dl)
Calcium, serum	7.3 (mg/dl)
Brain natriuretic peptide (BNP)	7.5 (pg/mL) (less than 100)
CPK	9 IU/L (35-230)
D dimer	2.58 (ug/ml) (0-0.48)
White blood cell count	4.36x10 ^ 9 per microliter (3.2-10.6)
Hemoglobin	12.5 (g/dl) (12.1-15.9)
Platelet	120x10 ^ 9 per microliter (177-406)

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Sodium	138 (meq/L)
Potassium	5.3(meq/L)
Chloride	109 (meq/L)
Bicarbonate	26 (meq/L)
BUN	29(mg/dl)
Creatinine	1.3 (mg/dl) (baseline 0.7-0.8)
Glucose	97(mg/dL)

Lipid panel

Cholesterol	341 (mg/dL)	(125-200)
HDL	35.6 (mg/dL)	(>47)
LDL	169.7 (mg/dL)	(less than 130)
Triglyceride	424 (mg/dL)	(less than 150)

Urine studies

Urinalysis:	Amber appearing urine, with greater than 500mg/dL protein with few bacteria, 16-25 WBC (normal 0-4 per high powered field). No nitrites, no leukocyte esterase, and no Redblood cell cast. Specific gravity: 1.032 (normal 1.01-1.03)
Urine protein	>1500 mg/dL
Urine Creatinine	225.66 mg/Dl
Urine BUN	1780 mg/dL
Urine Sodium	20 mg/dL

Imaging

Renal ultrasound	Patent renal veins and normal sized kidneys
Lower extremity Vein Doppler	NEGATIVE for deep vein thrombosis
CT Head and Cervical spine	No acute intracranial process and evidence of multi-level disk disease.

Exam notable for obese woman with generalized edema, normal heart sound intensity, no adventitious breath sounds, and no focal neurological deficits. Patient oriented to person, place, and situation.

Neurology initially consulted due to concern for MS flare and patient completed four day course of daily Solumedrol. Head imaging showed no evidence of acute flare.

Nephrology consulted due to concern for nephrotic syndrome. Urine studies, autoimmune workup including SPEP, UPEP, ANCA, RPR, serum free light chains recommended. Results all negative. ANA positive and reflex to titre pending. Double stranded DNA (dsDNA) quantified as indeterminate. Urine protein: creatinine ratio is 6.64g/day. Interventional Radiology (IR) consulted for kidney biopsy. Patient started on IV Furosemide , IV albumin, and anti hypertensives. Protein and sodium restriction intake enforced. Plan for biopsy of kidney.

Biopsy results on electron microscopy demonstrated effacement of podocytes and absence of tubule-reticular structures. On light microscopy normal appearing glomeruli seen with some evidence of interstitial edema. Immunofluorescence demonstrated no glomerular positivity with IgG, IgA, IgA, C3, C1q, kappa, lambda, or fibrinogen. Faint one plus glomerular positivity seen with IgM, however non specific. No specific tubulointerstitial or vascular positivity with any of the above mentioned immunoreactants.

Patient started on prednisone 80mg every morning. Testing for G6PD negative, and patient started on Dapsone 100mg day for Pneumocystis jiroveci pneumonia (PJP) prophylaxis.

At time of discharge, labs demonstrated

Sodium	138 (meq/L)
Potassium	3.6 (meq/L)
Chloride	99 (meq/L)
Bicarbonate	32 (meq/L)
BUN	17(mg/L)
Creatinine	0.8 (mg/L) (baseline 0.7-0.8)
Glucose	112 (mg/L)

White blood cell count	16.46x10^9 per microliter	(3.2-10.6)
Hemoglobin	10.5 (g/dl)	(12.1-15.9)
Platelet	179x10^9 per microliter	(177-406)
Glucose 6 phosphate dehydrogenase	9 u/g of Hemoglobin	(7-20)

III. Discussion

The incidence of primary MCD in adults is not well defined (1). The hallmark of biopsy results is absence of immunofluorescence staining for varying antigens/immunoreactant (IgG, IgM, IgA, C1, etc.) and effacement of podocytes (1) on electron microscopy. If other features are seen, it cannot be MCD (1). Nonetheless, low intensity staining of C3 and IgM can be normal (8). This was seen in our patient. Typically, this disease has a higher prevalence in children who are often steroid responsive. By two weeks, 50% of kids have responded, whereas the percentages are more sobering in adults. Here, 75% have responded by 13 weeks (8). Furthermore, adults have greater risk for progression to renal failure in adults. In study by Nolasco et. al, ten of nineteen patients progressed to renal failure, with eight of those eventually requiring dialysis (9).

There have been few reports of adults with MCD and even fewer in patients with comorbidities such as HIV and MS, as in our patient. However, given the biopsy results this remains a case of primary MCD. In spite of the patient's history of well controlled HIV, HIV Associated nephropathy (HIVAN) remained on the differential. It is important to recognize that anti retroviraltherapy (ART) does not protect against MCD. In fact, seven of eight patients were diagnosed with MCD while on ART. HIVAN detected in only one case (4). On the other hand, a viral load of greater than 400 was also not a good predictor of HIVAN, as only 37% of such patients diagnosed with HIVAN (6).

While the patient did have abrupt onset edema, hyoalbuminemia, and proteinuria, her serum creatinine was not greater than 2. Above 2 is more typical for HIVAN (5). Variability in labs and presentation echo the importance of biopsy. Biopsy will demonstrate tubular atrophy and dilation as well as flattened epithelial cells in setting of collapsing FSGS (due to podocyte proliferation). Furthermore, a large number of tubular and glomerular cells coated with HIV RNA (4). Important to note that low CD4 count and presence of proteinuria are not predictive of HIVAN. Furthermore, a viral load of greater than 400 was also not a good predictor of HIVAN, as only 37% of such patients diagnosed with HIVAN (6).

Our patient did not have HIVAN in spite of medical history. Similarly, one could postulate MCD secondary to MS drugs. While the patient was treated for presumed flare on admission, there are very little reports in the literature of Glatiramer induced nephrotic syndrome. On the other hand, Interferon gamma B (IFN B) has been linked to MCD after long time use. Kumasake et al. describe case of a woman with MS on IFN B who develops MCD after 21 months on MS treatment (7). Our patient was never treated with IFN B and no evidence seen on renal biopsy.

IV. Conclusion

MCD is a type of nephrotic syndrome, characterized by a urine protein/creatinine of 3500mg and greater. Patients usually present with sudden onset edema, proteinuric kidney injury, and hyperlipidemia. It is believed that disruption of actin cytoskeleton within the podocyte and basement membrane in conjunction with a disrupted immune system cause an increase in mediating factors leading to filtration of albumin into the urinary system and marked proteinuria. Patients need close follow up to ensure steroid responsiveness, as measured by reduction in proteinuria. Due to long duration of steroid therapy, patient's need PJP prophylaxis. This includes Atovaquone or Dapsone. It is prudent to be aware that adults have greater risk for progression to renal failure (than children). In a study by Nolasco et. al, ten of nineteen patients progressed to renal failure, with eight of those eventually requiring dialysis. If adults have truly failed steroid therapy, there will be no improvement after four months. The next step is to discuss the efficacy of second line non-steroidal therapies such as calcineurin inhibitors. This case highlights a case of primary MCD in a woman with HIV and MS, while illustrating that even when patients have other comorbidities or concern for secondary causes of MCD, it is imperative to obtain a renal biopsy to clarify the picture.

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Antibiotic use during Pregnancy: A Retrospective Study of Prescription at the District Health Center of Kangaba, Mali

By Karim Traoré, Seidina AS. Diakité, Mahamadou Ballo, Drissa Konaté, SoryI. Diawawa, Bourama Keita, Abdoulaye Maiga, Modibo Sangaré, Aiguérou A. Guindo, Fatoumata Daou, Moussa Soumana, Ibrahim Sanogo, Fousseyni S. Doucouré, Mahamadou Diakité & Sékou Bah

Abstract- *Background:* Pregnancy is a critical stage in a woman life, and the use of drugs, especially antibiotics calls for concern. The service and choice of antibiotics during pregnancy depends mainly on maternal factors such as health, nutrition, and socio-economic status, as well as the mode of delivery. This study was aimed to assess antibiotic use among pregnant women according to the Food and Drug Administration categorization of drugs based on their risk in pregnancy.

Methods: The study was a retrospective, cross-sectional survey. The sampling consisted of all prescriptions for pregnant women with at least one antibiotic drug and recorded in a registry.

Keywords: *antibiotics, prescription, pregnancy.*

GJMR-B Classification: *DDC Code: 618.2 LCC Code: RG525*

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Methods: The study was a retrospective, cross-sectional survey. The sampling consisted of all prescriptions for pregnant women with at least one antibiotic drug and recorded in a registry. Data included primary demographic data, the nature of the antibiotic medicines, their dosage, the duration of treatment, and the type of prescribed antibiotic combination, were analyzed based on the FDA classification guidelines; Data were analyzed using the statistical software Epi info.

Results: One thousand four hundred and ninety-nine (n=1,499) pregnant women received at least one prescription of antibiotics during pregnancy. The average age was 28 years old, and the most represented age group was 21-25(29.6%); Regarding drug delivery, amoxicillin (36.6%), erythromycin (31.7%), and azithromycin (15.6%) were the most prescribed drugs during the first trimester of pregnancy. Metronidazole (54.9% and 40.1%), erythromycin (29.9% and 20.7%), and azithromycin (9.9% and 29.5%) were the most prescribed molecules during the second and third trimesters of pregnancy, respectively. The frequently prescribed therapeutic class was macrolides, with 65.7%, followed by beta-lactams, with 15.1%. The dosage of the most prescribed drugs was 500mg, with 94.7%.The most used route of administration was oral (96.7%). The duration of treatment in most of the prescriptions was less than one week, with 99.2%. Antibiotics belonging to category B of the FDA classification were the most prescribed with 43.5%, followed by category A at 37.7%, category C at 10.8%, and category D at 8%.

Conclusion: The antibiotics prescribed for pregnant women fell within the FDA risk categories A and B, with rare cases of prescription occurring in categories C and D. The most frequently prescribed antibiotic class was the macrolides.

Keywords: antibiotics, prescription, pregnancy.

I. Background

Maternal mortality and morbidity are high in sub-Saharan Africa due to complications from microbial infections[1]. Managing of complications related to these infections during pregnancy requires the prescription of many drugs, including antibiotics. The best use of antibiotics to treat infectious diseases during the antenatal visits, in addition to iron administration and dietary supplements, could reduce maternal and baby mortality during pregnancy[2]. Reports suggest that antibiotics account for nearly 80% of all prescription medications during pregnancy, and approximately 20–25% of women receive an antibiotic during pregnancy [3-5]. Poor management of antibiotics is one of the leading causes of antibiotic resistance in microbial agents [6]. The use and choice of antibiotics during pregnancy depends on health resources, nutrition status, mode of delivery, and socio-economic factors. A better knowledge of the pharmacokinetics, potential toxicity, and teratogenic risks of these drugs is essential to optimize the efficacy and safety of antibiotic treatment [7]. The pharmacokinetics of antibiotics during pregnancy can be affected by multiple factors, including absorption, distribution, metabolism, and elimination [8]. Some antibiotics can potentially to affect embryo-fetal development at different stages of pregnancy. Teratogenic effects occur mainly during the embryonic period (first trimester of pregnancy) [9]. Prescribing in pregnancy always raises the issue of drug risks to the embryo or fetus, an additional pharmacokinetic compartment related to transplacental drug distribution. The use of medications during pregnancy is a significant concern for patients and prescribers. The incidence of

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thalidomide in the 1960s and the teratogenic effects discovered in 1971 with diethylstilbestrol are some examples of the hazards that prescription drugs may pose to pregnant patients [10, 11]. Pregnancy is associated with changes in the physiological, psychological, and psychosocial aspects of a woman life. Antibiotics are among the more frequently prescribed medicines in pregnant women, and the use of antibiotics is increasing. However, with limited studies available in this population, the safe use of antibiotics in pregnancy remains a concern.

The Food and Drug Administration (FDA) categorization of drugs based on their risk of pregnancy should be considered before prescribing a medication to pregnant women. The health center receives pregnant women for prenatal consultations and various types of care.

No study on antibiotics prescribed in pregnant women and their compliance with the FDA classification on drug safety during pregnancy has been done in this village. This study will contribute to the improvement of antibiotic prescription in pregnant women.

II. Methods

The study was carried out in the district health centers of Kangaba, a malaria-endemic area located 80 km southwest of Bamako. A cross-sectional study was carried out from January to March 2021 to collect data on the use and prescription of antibiotics during the antenatal visits. The sampling consisted of all prescriptions for pregnant women with at least one antibiotic drug and recorded in a registry. The nature of the antibiotic drugs, the dosage, the duration of treatment, and the type of prescribed antibiotic combination were analyzed based on the FDA classification guidelines. A non-compliant prescription was defined as any breach of one or more of the parameters listed above concerning, to the FDA classification guidelines. In the registries, we also collected information about the socio-demographic characteristics (age and sex of the patient). In addition, a report form was administered to all prescriber's Data focusing on their professional qualification and their level of knowledge of the FDA classification.

FDA classification of drug safety in pregnancy[12]

- Category A: No adverse effects in human pregnancies. Safety established using well controlled human studies.
- Category B: Presumed safety in human pregnancies. Limited human studies/no adverse effects in animal studies.
- Category C: Uncertain safety: Limited human studies/adverse effects in animal studies.
- Category D: Adverse effects in pregnancies. Benefits may outweigh associated risks.
- Category X: Adverse effects in pregnancies. Risksoutweigh possible benefit.

Anti-Microbials: D and X FDA drug categories[12]

- Category D: Aminoglycosides: Gentamycin, Streptomycin, Tobramycin, Tetracyclines, Doxycycline, Minocycline, Tetracycline, Voriconazole, Chloramphenicol, Antimycotics (Amphotericin B, 5-flucytosine, Griseofulvin).
- Category X: Quinine, Thalidomide, Ribavirin, Miltefosine, oral contraceptives, statins.

III. Statistical Analysis

Data were collected on a report form, entered into Excel, and analyzed using the statistical software Epi info 6.04.

a) Ethical considerations

Our study protocol was approved by the ethics committee of the Faculty of Medicine and Odontostomatology, and Pharmacy of the University of Sciences, Techniques, and Technologies of Bamako (USTTB). The health and administrative authorities of Kangaba were informed before the beginning of data collection.

The information found in the logs was kept entirely confidential and was not disclosed to anyone outside the study investigators. The personal information concerning each pregnant woman was coded. Only the principal investigator could identify the patients during the data analysis for publication of the results.

IV. Results

Table 1: Antibiotics prescribed during the antenatal visit to the district health center of Kangaba.

Antibiotics	*Age of the pregnancy*			Total n (%)
	First Trimester N (%)	Second trimester N (%)	Third Trimester N (%)	
Amoxicillin	225(36.6)	0(0)	0(0)	225(15)
Erythromycin	195(31.7)	355(54.9)	95(40.1)	645(43)
Azithromycin	96(15.6)	193(29.8)	49(20.7)	338(22.5)
Metronidazole	28(4.6)	64(9.9)	70(29.5)	162(10.8)
Ciprofloxacin	11(1.9)	19(2.9)	16(6.8)	46(3.1)

Doxycycline	0(0)	12(1.9)	7(2.9)	19(1.3)
Cefixime	4(0.7)	0(0)	0(0)	4(0.3)
Gentamycin	53(8.6)	0(0)	0(0)	53(3.5)
Lincomycin	2(0.3)	0(0)	0(0)	2(0.1)
Ceftriaxone Associated	1(0.2) 0(0)	0(0) 4(0.6)	0(0) 0(0)	1(0.1) 4(0.3)
Total	615(100)	647(100)	237(100)	1499(100)

Table 2: The distribution of prescriptions according to the therapeutic class of antibiotics and the age of the pregnancy.

Therapeutic class of antibiotics	Age of the pregnancy			Total n (%)
	First trimester N (%)	Second trimester N (%)	Third trimester N (%)	
Aminosides	53(8.6)	0(0)	0(0)	53(3.5)
Bêta-lactamines	226(36.7)	0(0)	0(0)	226(15.1)
Céphalosporines	4(0.7)	0(0)	0(0)	4(0.3)
Lincosamides	2(0.3)	0(0)	0(0)	2(0.13)
Macrolides	291(47.3)	550(85)	144(60.8)	985(65.7)
Macrolides+bêta-lactamines	0(0)	1(0.2)	0(0)	1(0.06)
Macrolides+ Fusidanines	0(0)	1(0.2)	0(0)	1(0.06)
Macrolides+ Nitroimidazoles	0(0)	1(0.2)	0(0)	1(0.06)
Nitroimidazoles	28(4.6)	63(9.7)	70(29.5)	161(10.7)
Quinolones	11(1.8)	19(2.9)	16(6.8)	46(3)
Tétracyclines	0(0)	12(1.8)	7(2.9)	19(1.39)
Total	615(100)	647(100)	237(100)	1499(100)

Table 3: Dosage frequency per day, dosage form, and duration of treatment of antibiotics prescribed to pregnant women.

Variables	Category	(%)
Dosage of antibiotic in mg	<500mg	77(5.1)
	500mg	1419(94.7)
	1000mg	3(0.2)
	>1000mg	0
Daily frequency of antibiotic use	Once	65(4.3)
	Twice	1216(81.2)
	Thrice	13(0.9)
	Four times	205(13.7)
Forms of antibiotics	Tablet	1450(96.7)
	Injection	49(3.3)
Duration of treatment	<7days	1487(99.2)
	7days	10(0.7)
	>7days	1(0.1)

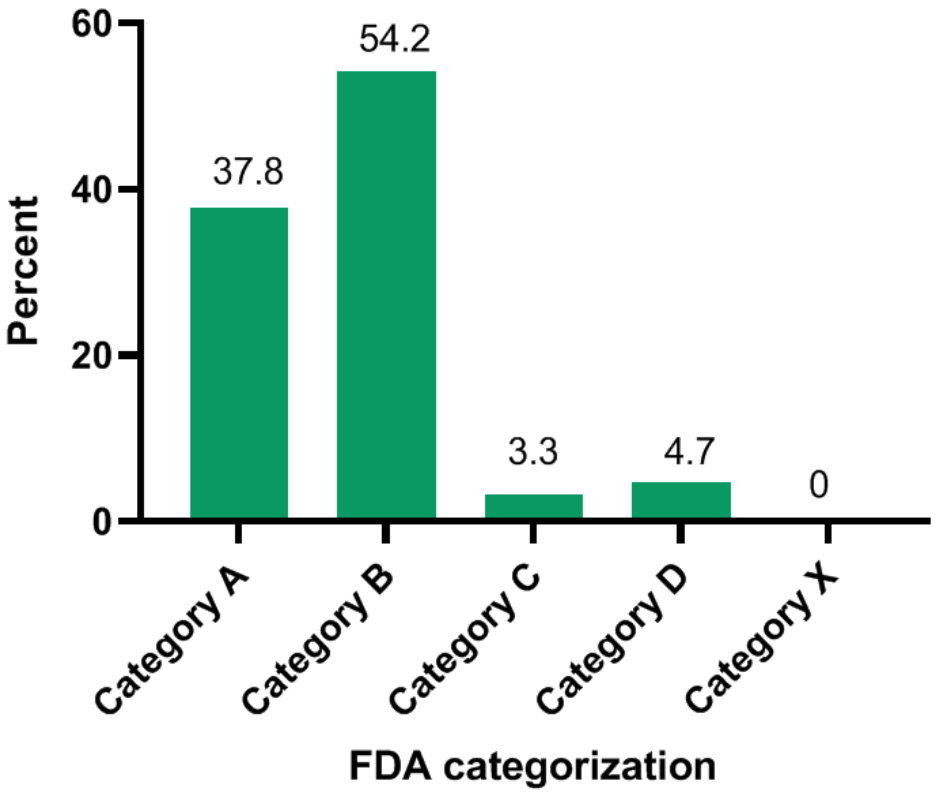

Figure 1: Antibiotics prescribed to pregnant women according to the FDA categorization

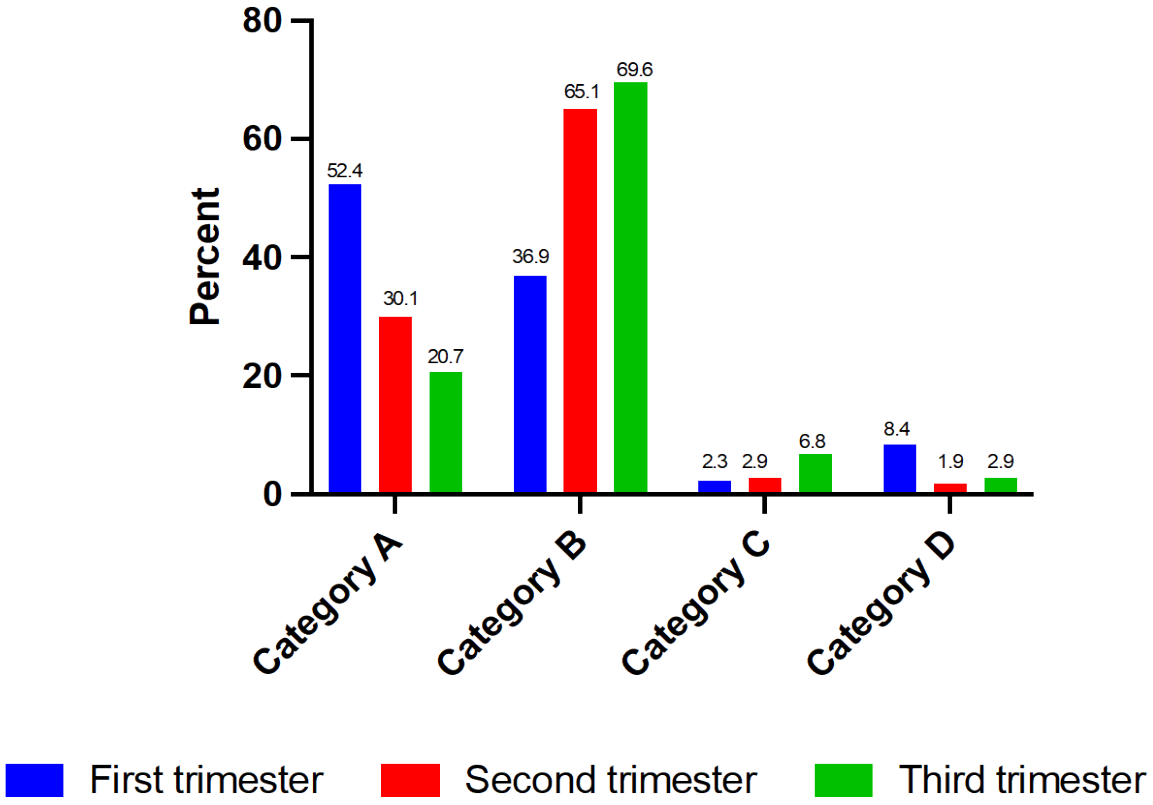

Figure 2: Antibiotic prescribed according to age of the pregnancy and FDA categorization

Table 4: Types of antibiotics prescribed to pregnant women according to FDA classification in the health center

Drug/ FDA recommendation	Age of the pregnancy		
	1st trimester	2nd trimester	3rd trimester
FDA recommended	Amoxicillin, Erythromycin, Azithromycin, Metronidazole, Ceftriaxone, Cefixime	Erythromycin, Azithromycin, Metronidazole, Ciprofloxacin	Erythromycin, Azithromycin, Metronidazole, Ciprofloxacin
Not FDA recommended	Ciprofloxacin, Gentamycin, Lincomycin	Doxycycline	Doxycycline

Pregnant women underwent an antibiogram before the prescription of the antibiotics in 0.5% (8/1,499).

V. Discussion

Most pregnant women are exposed to some type of medication during pregnancy. Drugs prescribed during pregnancy can exercise a teratogenic effect on fetuses, and those prescribed during breastfeeding can also impact on infant health. Antibiotics are among the more frequently prescribed types of medications during pregnancy and lactation [13].

The risk of antibiotic exposure was highest in the first and second trimesters but lowered in the third trimester. Mensah et al. 2017 in Ghana found that the risk of antibiotic exposure was highest in the last trimester. This is reassuring because the acquisition of specific fetal immunity begins in the third trimester, and is highly dependent on the microbiome, which can be altered by antibiotics [14].

Amoxicillin (category A) at 36.6%, erythromycin (category B) at 31.7%, and azithromycin (category A) at 15.6%, were the mainlydrugs prescribed during the first trimester of pregnancy (Table 1). Erythromycin (category B) at 54.9%, azithromycin (category A) at 29.8%, and metronidazole (category B) at 9.9%, were the mainly drugs prescribed during the second trimesters (Table 1). In the third trimesters, erythromycin (category B) at 40.1%, metronidazole (category B) at 29.5%, and azithromycin (category A) at 20.7%, were the mainly drugs prescribed (Table 1). A study carried out in northern Nigeria by Ogboma et al. in 2019 reported that ciprofloxacin (25.3%) and erythromycin (21.7%) were the mainly drugs prescribed during pregnancy[15].

In Kangaba health center, macrolides were the most prescribed antibioticsat 65.7%, followed bybeta-lactamsat15.1%, and nitroimidazoleat 10.7%.Ogboma et al. in 2019 in Nigeria, and Elizabeth C. Ailes et al. in 2018 in the USA reported that fluoroquinolones were the most prescribed class in pregnant women with 46.7% and 32%, respectively [15, 16]. A study carried out in Ghana between 2011 and 215 by Mensah et al. reported that 67% of prescriptions for antibiotics in pregnant women were beta-lactams [14].

Prescribing macrolides during pregnancy is common, as similar results have been reported in the literature[17-20]. The use of macrolides in pregnancy is, however, a growing concern [18]. Significantly, a recent study by Fan et al. followed 104,605 children from birth to 14 years old, and it was concluded that prescribing macrolides in any trimester was associated with an increased risk of genital malformation [18]. Whereas a previous cohort of 1,033 women exposed to macrolides (erythromycin, azithromycin, clarithromycin or roxithromycin) reported that there was no association between this drug and the development of significant abnormalities in the fetus [17].

The dosage in mg of most drugs prescribed was 500mg with 94.7% regardless of the age of pregnancy. This result is similar to that observed by Ogboma et al. in 2019 in Nigeria [15]. The dosage frequency per day of most drugs prescribed was twice with 81.2%. The most common route of administration was oral with, 96.7%. The dosage form of most prescribed drug was tablet (96.7%). The duration of treatment in most of the prescriptions was less than one week (99.2%). This does not appear to be in line with the management of antibiotic resistance, where a minimum of seven days and a maximum of twenty-one days is recommended to avoid resistance that could result from incomplete treatment. The duration of treatment depends mainly on the nature of the disease, the severity, the presentation of the drug (dosage in mg and dosage form), the age of the pregnancy, and the pharmacokinetic of medicarion.

Most drugs fell into category B at 54.2%, and category A at 37.8%. Mensah et al. 2017 in Ghana reported that most of the antibiotics prescribed were of category B at 96.6%, followed by C and D at 2.9% and 0.5%, respectively [14]. Drugs in categories C and D are toxic to the fetus but can be used during pregnancy if the benefits to the mother outweigh the risks to the fetus.

The prescription of, ciprofloxacin (1.85%), gentamycin (8.6%) and, lincomycin (0.3%) in the first trimester of pregnancy does not conform to FDA recommendations. According to the FDA, ciprofloxacin, gentamycin, and lincomycin should be prescribed in the second and third trimesters of pregnancy due to their potential embryotoxicity.

The prescription of, doxycycline (Category D) in second (1.2%) and third (2.9%) trimesters of pregnancy is not recommended by FDA, because doxycyclineis toxic on the fetus.

VI. Conclusion

The antibiotics prescribed for pregnant women fell within the FDA risk categories A and B, with rare cases of prescription occurring in categories C and D. The most frequently prescribed antibiotic in Kangaba was the macrolides.

Singles

FDA: Food and Drug Administration

MRTC: Malaria Research, and Training Center

USTTB: University of Sciences, Techniques, and Technologies of Bamako

Contribution

Karim Traoré, Seidina Diakité, Sékou Bah, and Mahamadou Diakité participated in the conception and design of the manuscript. Karim Traoré, Bourama Keita, Sory I Diawara, and Drissa Konaté performed the statistical analysis, and Karim Traoré Mahamadou Ballo, Modibo Sangaré drafted the manuscript. All authors read, and approved the final version of the manuscript.

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Conflict of interest: None

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PCO2 gap - As an Endpoint of Resuscitation and Predictor of Mortality in Patients with Shock: A Prospective Observational Study

By Dr. Prabhu S, Dr. Vimal Bhardwaj, Dr. V. Viju Wilben & Mr. Vinil Kumar

Abstract- *Introduction:* Endpoint of resuscitation is essential to be determined objectively as we get more substantial evidence supporting the fact that both under resuscitation and over resuscitation is detrimental to overall outcomes. Since carbon dioxide is more diffusible than oxygen it readily gets in to the blood in low perfusion states whereas oxygen doesn't. Hence widening the PCO2 gap. Since this PCO2 gap can be determined easily in the ICU we propose that PCO2 gap can be used as a reliable indicator of endpoint of resuscitation and predictor of mortality in patients with shock.

Aim: To evaluate the association between PCO2 gap and outcome of resuscitation in patients with shock. The Objectives of the project are to study the association between PCO2 difference and in-hospital mortality in patients admitted with shock and to study the correlation between PCO2 difference and lactate clearance.

GJMR-B Classification: *DDC Code: 617.044 LCC Code: RD156*

Strictly as per the compliance and regulations of:

PCO2 gap – As an Endpoint of Resuscitation and Predictor of Mortality in Patients with Shock: A Prospective Observational Study

Dr. Prabhu S [α], Dr. Vimal Bhardwaj [σ], Dr. V. Viju Wilben [ρ] & Mr. Vinil Kumar [ω]

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Aim: To evaluate the association between PCO2 gap and outcome of resuscitation in patients with shock. The Objectives of the project are to study the association between PCO2 difference and in-hospital mortality in patients admitted with shock and to study the correlation between PCO2 difference and lactate clearance.

Materials and methods: 71 adult patients presenting with shock to our ER were enrolled in the study. They were resuscitated according to standard protocols. PCO2 gap was measured at presentation, then every 2 hours until the resolution of shock which were correlated to the lactate clearance, hemodynamics and the IVC index of the patient. The data was then analyzed using the R software and logistic regression was done to analyze various factors associated with mortality. P value less than 0.05 was considered statistically significant.

Results: The correlation between pCO2 gap and the in hospital mortality was statistically significant at 0,2,4,6 and 24 hours. The correlation between pCO2 gap and the end point of resuscitation was statistically significant at 2,4,6 and 24 hours implied by the pearson's correlation. We also found a positive correlation between PCO2 gap and lactate clearance which was statistically significant.

Conclusion: The PCO2 gap can be used a marker of the adequacy of the cardiac output in patients with shock. Using pCO2 gap has potential to avoid administration of unnecessary fluids and inotropes in patients, who have lactate elevated in the absence of tissue hypo perfusion. We suggest using pCO2 gap as a complementary tool to evaluate the adequacy of blood flow to global metabolic demand. A high pCO2 gap on initial presentation was associated with high mortality rates. So it can be used as a predictor of outcomes in patients with shock.

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I. Introduction

Shock is the clinical expression of circulatory failure that results in inadequate cellular oxygen utilization.[1] Shock is a common condition in critical care, affecting about one third of patients in the intensive care unit (ICU), both over resuscitation and under resuscitation can adversely impact the outcomes.[2,3,4] End point of resuscitation has always been a matter of debate, initially continuous SCvo2 monitoring as introduced by Rivers et al had the obvious limitation that normal/high values cannot discriminate whether delivery is adequate or in excess to demand[5,6,7]. High ScvO2 profiles have even been shown to be related to elevated blood lactate concentration and poor survival rates.[8]

Lactate cannot differentiate between different etiologies of shock and it can get elevated in various other conditions.[9] Carbon dioxide (Co2) is highly diffusible and can be a marker of adequacy of venous return, the central venous and arterial CO2 gap, as an easily available clinical monitoring tool. Observational study has shown that Persistence of such a large pCO2 gap after 24 hours of treatment was predictive of higher mortality.[10]

In conclusion, determining the PCo2 gap during resuscitation of critically ill patients is useful in deciding when to stop resuscitation.[11] Central venous-arterial carbon dioxide difference (PCO2 gap) can be a marker of cardiac output adequacy in global metabolic conditions that are less affected by the impairment of oxygen extraction capacity. Assessing the adequacy of oxygen delivery with oxygen requirements is one of the key-goal of hemodynamic resuscitation. Clinical examination, lactate and central or mixed venous oxygen saturation (SvO2 and ScvO2, respectively) all have their limitations. Many of them may be overcome by the use of the carbon dioxide (CO2)-derived variables. The venoarterial difference in CO2 tension ("ΔPCO2" or "PCO2 gap") is not an indicator of anaerobic metabolism since it is influenced by the oxygen consumption. By contrast, it reliably indicates whether blood flow is sufficient to carry CO2 from the peripheral tissue to the lungs in view of its clearance: it, thus, reflects the adequacy of cardiac output with the metabolic condition. We investigate the relation between

the PCO2 gap and serum lactate and its role in resuscitation of patients with septic shock.

II. Review of Literature

Shock is defined as inability to maintain MAP which is refractory to fluid resuscitation. It has a guarded prognosis, there are many upstream and downstream markers for resuscitation, septic shock guidelines endorses Lactate as a prognostic marker; has got its own limitations as it can be elevated in other clinical conditions9 and it cannot differentiate the cause of shock9. With enough evidence coming up about over resuscitation and positive balance being one of the predictor of mortality there is a need for ideal resuscitation marker which can be easily employed bedside with present day equipment used on day to day basis.

CO2 is the end product of aerobic metabolism, PCO2 in the venous blood reflects the global tissue blood flow relative to metabolic demand. CO2 is about 20 times more soluble than O2 so it more reliably diffuses out of ischemic tissues into the venous effluent making it a sensitive marker of hypoperfusion in situations where an O2 diffusion barrier exists (e.g. non-functional and obliterated capillaries), "masking" poor O2 extraction (O2ER) and increased tissue O2 debt, CO2 still diffuses to the venous effluent, "unmasking" the low perfusion state for the clinician when venous-to-arterial CO2 difference is evaluated the gap is a marker of adequacy of venous blood flow to remove CO2 produced rather than a marker of tissue hypoxia or dysoxia[11]

Table 1: PCO2 Gap in Different Shock States

Shock type	Lactate	O2ER	ScvO2	cvaCO2gap
Cardiogenic or hypovolemic	HIGH	HIGH	LOW	HIGH
Anemic or hypoxemic	HIGH	HIGH	LOW	LOW
Distributive	HIGH	LOW	HIGH	HIGH
Cytopathic	HIGH	LOW	HIGH	LOW

As illustrated in table 1, Lactate is high in all types of shock, PCO2 Gap is high in cardiogenic and distributive shock which is amenable to fluid resuscitation and inotropic support and low in hypoxemic and cytopathic shock where fluid resuscitation has no role thus it can be concluded that PCO2 gap is useful in determining when to start and stop fluid resuscitation.[11] Co2 gap is a marker of adequacy of venous blood rather than marker of tissue hypoxia or dysoxia as shown by Vallet et al in an experimental model of isolated limb in which ischemic hypoxia (IH) and hypoxic hypoxia (HH). The authors demonstrated that when DO2 was reduced beyond its critical threshold in IH (dysoxia), this was associated with an increased limb venous-to-arterial PCO2gap.[12]

Conversely, in HH, pCO2 gap did not increase in spite of a marked VO2 and VCO2 reduction.[12] There is a good correlation between Mixed CO2 and Central CO2 difference with Arterial CO2 as demonstrated by Van Beest et al in severe sepsis and septic shock patients, hence Central CO2 can be substituted for mixed CO2 for determining the CO2 gap which acts as surrogate marker for Cardiac Index.[10]

Cushieri J etal conducted study in ICU patients to see the correlation between Central Venous and Arterial CO2 gap and Cardiac index determined by thermodilution technique and showed statistically significant correlation.[13]

Hence CO2 gap can be used as a marker of Cardiac output.

a) Role in Sepsis

In sepsis although Cardiac output may be normal but regional compromise of circulation is well documented phenomenon which may lead to increase in CO2 secondary to micro-circulation compromise. P(cv-a)CO2 could be considered as a better indirect assessment of systemic blood flow than ScvO2 in resuscitated-septic shock patients.[14]

A cutoff value for pCO2 gap of 0.8 kPa (6mmHg) discriminated between high and low lactate clearance and CI.[15,16] In study done by Vallee et al done in septic shock patients compared When the 70% ScvO2 goal value is reached, the presence of a P(cv-a)CO2 larger than 6 mmHg shown to be an useful tool to identify patients who still remain inadequately resuscitated.[14]

We hypothesize that CO2 gap is non inferior to lactate clearance in resuscitation of critically ill patients.

III. Research Question

Would pCO2 gap serve as an ideal bedside marker to predict the outcome of resuscitation in a patient with shock?

IV. Aims and Objectives

Aim of the Project: To study the association between PCO2 gap and outcome of resuscitation in patients with shock.

Objectives of the Project: The Objectives of the project are as follows:

- Primary objectives- To study the association between PCO2 gap and in-hospital mortality in patients admitted with shock.
- Secondary objectives

➢ To study the correlation between PCO2 gap and lactate clearance.

➢ To study the role of PCO2 gap as a marker for endpoint of resuscitation in patients with shock.

V. Methods and Methodology

Study area: Emergency Department and medical intensive care unit, NH Health City, Bangalore

Study population:

- Inclusion Criteria

– All adult patients (more than 18 years of age) in shock requiring vasopressor to maintain MAP of 65mmHg, having a central venous access and arterial line.

- Exclusion Criteria

– Patient Refusal
– Pregnancy
– Advance directive with consensus against active resuscitation
– Disseminated Malignancy

Sample size: 71

Study design: Prospective observational study.

Study intervention: No interventions

Study duration: One Year

VI. Methodology

✧ All shock patients were resuscitated according to the standard protocol with fluid bolus of 30 ml/kg over 1 hour and guided therapy with fluid challenges targeting heart rate, base deficit, urine output and pulmonary congestion as per routine clinical practice.

✧ Lactate clearance was documented every 2nd hourly and VBG from Central line and ABG from Radial Line was analyzed at the same time and CO2 gap was checked every 2nd hourly.

✧ Screening 2D-echocardiography was done at the emergency department and inotropic agent was decided based on heart contractility.

✧ Patient demographic details, diagnosis, SOFA Score, was done in the first 6 hours of resuscitation (two hours apart) and the data was collected. Lactate and Co2 Gap were captured and documented after 24 hours of resuscitation.

✧ Aim of resuscitation was to target MAP of 65 mm Hg and two stable lactate values 2 hours apart. If lactates had not improved then further fluid boluses were decided upon reviewing pulmonary congestion in ultrasound (M mode of lung will be done and if B lines are more than 4 then it is indicative of pulmonary congestion). The corresponding CO2 Gap was noted.

✧ First choice of vasopressor was nor-adrenaline as per the standard infusion dose. If patient requires vasopressor support despite fluid boluses then steroid in the form of injection Hydrocortisone 50mg IV every 6th hourly was administered.

✧ Antimicrobial administration and further management was decided by clinical examination and supportive investigations as per clinician's judgement.

Data collection methods: Proforma

Data collection forms: Attached

VII. Statistical Methods

a) Sample Size Calculation

Sample size was calculated using nMaster software v2.0

In a study done by Beest PV et al, the mortality of patients with sepsis was 24.5% (13 out of 53) and risk of mortality for those with high PCO2 gap ranged from 1.6 to 5.3

Keeping a conservative value in odds ratio as 2.5, with power of 80% and 5% alpha error the minimum required sample size is 71.

b) Statistical Analysis Plan

Data was analyzed using R software. Continuous variable were described using mean and standard deviation. Categorical variables were described using frequency and percentage. Patients were categorized based on PCO2 difference and logistic regression was done to analyze various factors associated with mortality. Correlation between PCO2 difference and lactate was done using appropriate statistical methods. P value less than 0.05 was considered statistically significant.

c) Ethical consideration

Ethical clearance was obtained prior to the study from the ethics committee of the institution. Informed consent was obtained from the patient or guardian before the onset of study. Confidentiality of

patient details are and will be maintained. It was explained to the patient that the study is purely descriptive and merely for data collection. There is no intervention required specifically for the study. Management of these patients were along the standard international guidelines. As the study did not involve any extra procedure, no compensation was offered during and after the study.

VIII. Results

A total of 71 patients were enrolled in the study. 7 patients died from the 48 to 72 hours time period. Their samples were collected and analyzed till the 24th hour of admission. The mean age of the patients was 54 years (SD 16.2; range 18–81 years).

Table 2: Demographic and disease characteristics

Variable					
Age	Median 57	Mean 54	SD 16.2	Minimum 18	Maximum 81
Gender	Male - 24 Female- 47				
SOFA score at enrollment		Mean 9		Minimum 2	Maximum 19
Type of shock	Frequency				
Anemic	1				
Cardiogenic	14				
Distributive	50				
Hypovolemic	3				
Hypoxemic	2				
Neurogenic	1				
Fluid requirement In ml	Median 2000	Mean 2076	SD 998	Minimum 500	Maximum 4500

The primary outcome of the study was the correlation between the PCO2 gap and the in hospital mortality at each of the sampling time points. The correlation between the PCO2 gap and the in hospital mortality was positive at 0, 2, 4, 6 and 24hours. The correlation was statistically significant at 0 and 2 hours. (Table 2)

Table 3: Correlation between the PCO2 gap and the in hospital mortality

Time point	Point biserial correlation (rpb)	Probability (p) value
0 hour	0.309	0.009
2 hours	0.358	0.002
4 hours	0.200	0.108
6 hours	0.096	0.473
24 hours	0.170	0.207

There was a statistically significant negative correlation between end point of resuscitation and pCO2 gap at 2h,4h, 6h and 24 hours as implied by the Pearson's correlation in Table 3.

Table 4: Correlation between the PCO2 gap and end point of resuscitation

Time Point	Point Biserial Correlation (Rpb)	Probability (P) Value
0 hour	-0.206	0.121
2 hours	-0.206	0.011
4 hours	-0.350	0.010
6 hours	-0.380	0.007
24 hours	-0.398	0.007

It was also observed that the pco2 gap at 0h,2h,4h, 6hours had a statistically significant positive correlation with lactate clearance.(Table 4)

Table 5: Correlation between the PCO2 gap and lactate clearance

Time point	Point biserial correlation (rpb)	Probability (p) value
0 hour	0.390	0.001
2 hours	0.362	0.002
4 hours	0.318	0.009
6 hours	0.311	0.018
24 hours	0.311	0.068

IX. Discussion

The association of lactate accumulation and oxygen debt during shock states has been described for decades15. Throughout the years, there has been continued interest in refining resuscitation triggers, and response to therapy. Lactate clearance as an endpoint of resuscitation is supported by at least two multi-center studie[16,17]. However, lactate clearance has disadvantages as lactates can sometimes be normal in septic shock[18], lactate elevation not solely due to oxygen delivery- consumption mismatch and it has different prognostic implications based on the initial value.

It was recognized in sepsis that pCO2 gap (or its mathematical derivatives) outperformed other markers in detecting tissue hypoperfusion[13,19-21]. The arterial carbon dioxide is dependent on the pulmonary gas exchange and the venous carbon dioxide is dependent on the blood flow to the tissue[22]. So, when the flow reduces in low cardiac output states like shock, the difference between the venous and arterial carbon dioxide increases. It has been demonstrated that the pCO2 gap increases in various types of shock.[2]

In our study we found a statistically significant correlation of pCO2 gap at 0 hour and and 2nd hour of resuscitation and mortality in patients. It shows that high pCO2 gap on initial presentation can be used as a predictor of outcomes in patients with shock. Ospina-Tascón, G.A. et al.,[24] found that the persistence of high PCO2gap during the early resuscitation of septic shock was associated with higher 28 day mortality.

We also found that there was a statistically significant correlation between end point of resuscitation and pCO2 gap at 2h,4h, 6h and 24 hours. Hence, pco2 gap can be used as an endpoint of resuscitation in patients with shock. This was similar to the findings of Vallet B et al.,[11] who found that determining the gap during resuscitation of critically ill patients is useful when deciding when to stop resuscitation.

Our analysis also showed that PCO2 gap at various time points had positive correlation with lactate clearance. This was similar to a study done by Shyam M et al.,[25] who showed that the PcvCO2–PaCO2/CaO2–CcvO2 ratio and lactate are positively correlated during the first 24 hours of active resuscitation from sepsis-induced hypotension,

Pco2 gap is not inferior to lactate levels as a hemodynamic marker. It can be substituted in place of lactate levels to predict outcomes in patients presenting with shock. It can also be used as a guide for therapy to achieve endpoint of resuscitation.

X. Limitations

Our study has its limitations. It is a descriptive study without randomization of the patients. Also some technical aspects should be kept in mind when these indices are used in clinical practice. First, some errors in the PCO2 gap measurements may occur when sampling the venous blood: incorrect sample container, contaminated sample by air or venous blood or catheter fluid. Second, a too long delay of transport of blood sampling may significantly change the blood gas content at the venous and the arterial site.

XI. Summary and Conclusion

The PCO2 gap can be used a marker of the adequacy of the cardiac output in patients with shock. Using pCO2 gap has potential to avoid administration of unnecessary fluids and inotropes in patients, who have lactate elevated in the absence of tissue hypo perfusion. We suggest using pCO2 gap as a complementary tool to evaluate the adequacy of blood flow to global metabolic demand. A high pCO2 gap on initial presentation was associated with high mortality rates. So it can be used as a predictor of outcomes in patients with shock.

List of abbreviations

ICU - Intensive care unit

MAP- Mean arterial pressure

CO2- carbon dioxide

PCO2- Partial pressure of carbon dioxide

EtCO2- End tidal concentration of carbon dioxide

CVP- Central venous pressure

SCVO2- Central venous oxygen saturation

VO2- Oxygen consumption

VCO2- Carbon dioxide output

CaCO2- Carbon dioxide content in the blood

K pa- Kilo pascal

SOFA- Sequential organ failure assessment

Mm Hg- millimeters of mercury.

VBG- Venous blood gas

ABG- Arterial blood gas.

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Appendices

Proforma

PCO2 Gap – AS AN ENDPOINT OF RESUSCITATION AND PREDICTOR OF MORTALITY IN PATIENTS WITH SHOCK.

1. DATE OF ADMISSION :
2. AGE : ___ YEARS
3. SEX : MALE / FEMALE
4. COMORBIDITIES : DIABETES / HYPERTENSION/ IHD/ CKD/THYROID DISORDERS/ OTHERS __
5. PROVISIONAL DIAGNOSIS :
6. TYPE OF SHOCK : CARDIOGENIC/HYPOVOLEMIC/DISTRIBUTIVE/ANEMIC OR HYPOXEMIC/CYTOPATHIC
7. SOFA SCORE :
8. MEAN ARTERIAL PRESSURE (ON ARRIVAL TO ER) :
9. FLUID BOLUS : YES/ NO
   SPECIFY DETAILS –
10. VASOPRESSOR : YES/ NO, if YES specify the drug _____
11. DOBUTAMINE SUPPORT : YES/ NO
12. ENDPOINT OF RESUSCITATION:
13. FINAL OUTCOME OF PATIENT :

TIME	LACTATE mmol/L	P(cv-a)CO2 mmHg	SCVO 2 %	END POINT OF RESUSCITATION		
				MAP	IVC COLLAPSIBILITY	PULMONARY EDEMA
ARRIVAL						
2 HOURS						
4 HOURS						
6 HOURS						
24 HOURS						

Informed Consent and patient information sheet

Dr. Prabhu,
Emergency medicine department,
Narayana health.

This Informed Consent Form is for men and women who come to the emergency department in state of shock- with low blood pressure not responding to IV fluids, and who we are inviting to participate in research. The title of our research project is PCO2 Gap – AS AN ENDPOINT OF RESUSCITATION AND PREDICTOR OF MORTALITY IN PATIENTS WITH SHOCK: A PROSPECTIVE OBSERVATIONAL STUDY.

This Informed Consent Form has two parts:
Information Sheet (to share information about the research with you)
Certificate of Consent (for signatures if you agree to take part)

PART I: Information Sheet

Introduction

I am Dr. Prabhu. We are doing research on patients presenting with shock to the emergency room, which can occur due to various causes like blood loss, cardiac failure, infection, anemia. I am going to give you information and invite

you to be part of this research. Before you decide, you can talk to anyone you feel comfortable with about the research.

There may be some words that you do not understand. Please ask me to stop as we go through the information and I will take time to explain. If you have questions later, you can ask them of me, or the staff.

pCO2 gap is the difference between the venous and arterial carbon dioxide. When a patient presents with shock, they will be treated with IV fluids or medication to increase blood pressure (inotropes) by constriction of blood vessels depending upon the cause of the shock. To know when the shock has resolved, we are going to compare pCO2 gap to other parameters which have been previously established.

Purpose of the research

To evaluate if pCO2 gap can be used to predict mortality and marker for end point of resuscitation

Participant selection

We are inviting all adults with shock to participate in the research on pCO2 gap.

Voluntary Participation

Your participation in this research is entirely voluntary. It is your choice whether to participate or not. Whether you choose to participate or not, all the services you receive at the hospital will continue and nothing will change. You may change your mind later and stop participating even if you agreed earlier.

Procedures and Protocol

Once you understand the study and give consent, your pCO2 gap will be measured on presentation, 2nd hour, 4th hour, 6th hour and at 24th hour. Patients presenting with shock will have an arterial line for invasive blood pressure measurement and a central line for administration of inotropes to treat the shock. Blood samples from these lines will help us to measure pCO2 gap. Treatment will be given for the shock as per standard guidelines and hospital protocol according to the patient's condition. Other parameters such as mean arterial pressure, IVC collapsibility, lactates will be compared to find out if pCO2 gap has a good correlation for endpoint of resuscitation (resolution of shock)

Duration

The research takes place over the course of 1 year. You will be followed up for12 to 24 hours depending upon your clinical condition.

Side Effects

No new intervention or procedure is done for the study. You will already have lines from which blood samples will be taken. Hence there are no side effects for the study.

Risks

No additional risks and discomfort will be caused during this study.

Benefits

The findings of this study can change the views of using pCO2 gap as an endpoint of resuscitation.

Confidentiality

The information that we collect from this research project will be kept confidential. Information about you that will be collected during the research will be put away and no-one but the researchers will be able to see it. Any information about you will have a number on it instead of your name. Only the researchers will know what your number is and we will lock that information up with a lock and key.

Right to Refuse or Withdraw

You do not have to take part in this research if you do not wish to do so and refusing to participate will not affect your treatment at this hospital in any way. You may stop participating in the research at any time that you wish without losing any of your rights as a patient here. Your treatment at this hospital will not be affected in any way.

Whom to contact

If you have any questions you may ask them now or later, even after the study has started. If you wish to ask questions later, you may contact any of the following:

Contact the principal investigator
Name: Dr. Prabhu
Address: Narayana Health City, Bangalore
Contact No. 7358248887
Email: prabhu.adms@gmail.com

This proposal has been reviewed and approved by Narayana Health Academic ethical committee, which is a committee whose task it is to make sure that research participants are protected from harm. If you wish to find about more about the IRB, contact Narayana Health Academic Ethics Committee. Name: Dr. Sanjay Rao
Designation: Member Secretary
Contact No. 9538008940;
Email: nhaec@narayanahealth.org

PART II: Certificate of Consent

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this research.

Print Name of Participant_____

Signature of Participant _____

Date _____
Day/month/year

Statement by the researcher/person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands the objectives of the research.

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this ICF has been provided to the participant.

Print Name of Researcher/person taking the consent_____

Signature of Researcher /person taking the consent_____

Date _____ Day/month/year

Print name of the impartial witness in capitals_____

Signature of impartial witness_____

Date _____ Day/month/year

Deferred Consent for Research Participation

Title of Project: PCO2 Gap – AS AN ENDPOINT OF RESUSCITATION AND PREDICTOR OF MORTALITY IN PATIENTS WITH SHOCK: A PROSPECTIVE OBSERVATIONAL STUDY.

Principal Investigator: Dr. Prabhu
Emergency medicine department,
Narayana health,Phone Number: 7358248887

The patient named below is being enrolled in this research study by deferred consent. The process of obtaining written informed consent will be deferred until after the patient is able to understand and has capacity to give consent. Written informed consent will be obtained to continue data collection after resuscitation from the patient or, if the patient lacked capacity, a legal representative.

Patient's Name: _____

Date/time assessed for enrolment: _____/_____/_____ (dd/mm/yyyy) at ____ : _____ (time)

Reason(s) deferred consent process is used (check all that apply):

____ The patient is unconscious or lacks capacity to understand the risks, methods and purposes of the research study.

____ No next of kin/substitute decision maker is available to provide consent, or attempts to contact them have been unsuccessful despite diligent and documented efforts.

____ A substitute decision maker _____ (name and relationship) has been contacted by telephone, and the purpose, methods and risks of participation in this study have been explained to the third party. While the substitute decision maker has given verbal consent for participation, written consent must be still be obtained.

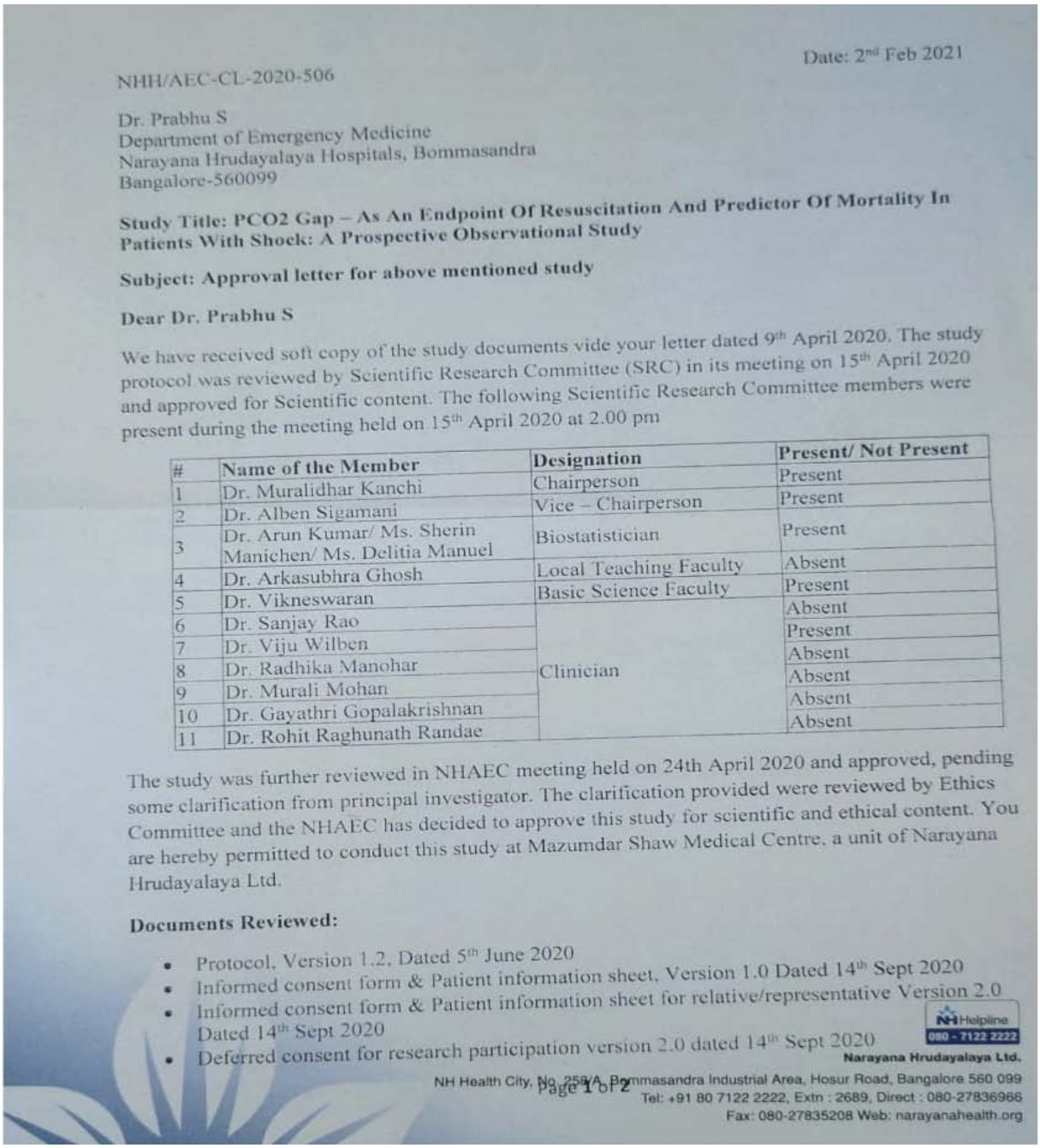
____ No relevant prior directive by the patient is known to exist.

____ Other: _____

_____ _____

Signature of investigator Date and Time

Annexure 1



Date: 2nd Feb 2021

NHH/AEC-CL-2020-506

Dr. Prabhu S
Department of Emergency Medicine
Narayana Hrudayalaya Hospitals, Bommasandra
Bangalore-560099

Study Title: PCO2 Gap – As An Endpoint Of Resuscitation And Predictor Of Mortality In Patients With Shock: A Prospective Observational Study

Subject: Approval letter for above mentioned study

Dear Dr. Prabhu S

We have received soft copy of the study documents vide your letter dated 9th April 2020. The study protocol was reviewed by Scientific Research Committee (SRC) in its meeting on 15th April 2020 and approved for Scientific content. The following Scientific Research Committee members were present during the meeting held on 15th April 2020 at 2.00 pm

#	Name of the Member	Designation	Present/ Not Present
1	Dr. Muralidhar Kanchi	Chairperson	Present
2	Dr. Alben Sigamani	Vice – Chairperson	Present
3	Dr. Arun Kumar/ Ms. Sherin Manichen/ Ms. Delitia Manuel	Biostatistician	Present
4	Dr. Arkasubhra Ghosh	Local Teaching Faculty	Absent
5	Dr. Vikneswaran	Basic Science Faculty	Present
6	Dr. Sanjay Rao	Clinician	Absent
7	Dr. Viju Wilben		Present
8	Dr. Radhika Manohar		Absent
9	Dr. Murali Mohan		Absent
10	Dr. Gayathri Gopalakrishnan		Absent
11	Dr. Rohit Raghunath Randae		Absent

The study was further reviewed in NHAEC meeting held on 24th April 2020 and approved, pending some clarification from principal investigator. The clarification provided were reviewed by Ethics Committee and the NHAEC has decided to approve this study for scientific and ethical content. You are hereby permitted to conduct this study at Mazumdar Shaw Medical Centre, a unit of Narayana Hrudayalaya Ltd.

Documents Reviewed:

- Protocol, Version 1.2, Dated 5th June 2020
- Informed consent form & Patient information sheet, Version 1.0 Dated 14th Sept 2020
- Informed consent form & Patient information sheet for relative/representative Version 2.0 Dated 14th Sept 2020
- Deferred consent for research participation version 2.0 dated 14th Sept 2020

NH Helpline 080 - 7122 2222

Narayana Hrudayalaya Ltd.
NH Health City, No. 258/A, Bommasandra Industrial Area, Hosur Road, Bangalore 560 099
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Fax: 080-27835208 Web: narayanahealth.org

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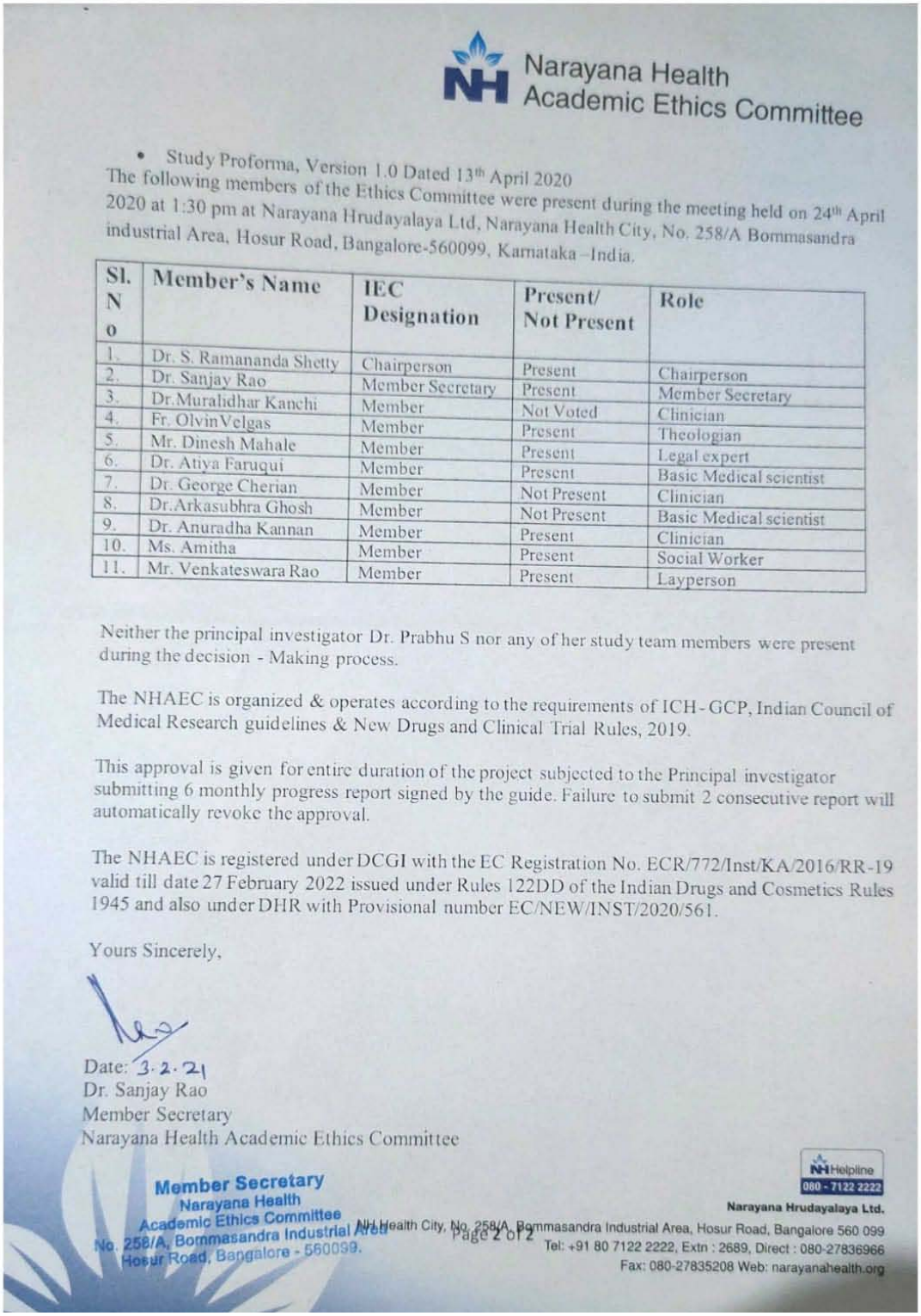
Annexure ii



Narayana Health
Academic Ethics Committee

- Study Proforma, Version 1.0 Dated 13th April 2020

The following members of the Ethics Committee were present during the meeting held on 24th April 2020 at 1:30 pm at Narayana Hrudayalaya Ltd, Narayana Health City, No. 258/A Bommasandra industrial Area, Hosur Road, Bangalore-560099, Karnataka –India.

Sl. No	Member's Name	IEC Designation	Present/ Not Present	Role
1.	Dr. S. Ramananda Shetty	Chairperson	Present	Chairperson
2.	Dr. Sanjay Rao	Member Secretary	Present	Member Secretary
3.	Dr.Muralidhar Kanchi	Member	Not Voted	Clinician
4.	Fr. Olvin Velgas	Member	Present	Theologian
5.	Mr. Dinesh Mahale	Member	Present	Legal expert
6.	Dr. Atiya Faruqui	Member	Present	Basic Medical scientist
7.	Dr. George Cherian	Member	Not Present	Clinician
8.	Dr.Arkasubhra Ghosh	Member	Not Present	Basic Medical scientist
9.	Dr. Anuradha Kannan	Member	Present	Clinician
10.	Ms. Amitha	Member	Present	Social Worker
11.	Mr. Venkateswara Rao	Member	Present	Layperson

Neither the principal investigator Dr. Prabhu S nor any of her study team members were present during the decision - Making process.

The NHAEC is organized & operates according to the requirements of ICH-GCP, Indian Council of Medical Research guidelines & New Drugs and Clinical Trial Rules, 2019.

This approval is given for entire duration of the project subjected to the Principal investigator submitting 6 monthly progress report signed by the guide. Failure to submit 2 consecutive report will automatically revoke the approval.

The NHAEC is registered under DCGI with the EC Registration No. ECR/772/Inst/KA/2016/RR-19 valid till date 27 February 2022 issued under Rules 122DD of the Indian Drugs and Cosmetics Rules 1945 and also under DHR with Provisional number EC/NEW/INST/2020/561.

Yours Sincerely,

Date: 3.2.21
Dr. Sanjay Rao
Member Secretary
Narayana Health Academic Ethics Committee

Member Secretary
Narayana Health
Academic Ethics Committee
No. 258/A, Bommasandra Industrial Area
Hosur Road, Bangalore - 560099.

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Potential Effects of a Herbal Remedy "DD3" on Free Radical Inhibition and Glucose Tolerance in Orally Hyperglycemic Wistar Rats

By Kporou Kouassi Elisée, Gbogbo Moussa, Ouattara Sitapha, Yao Akissi Nadège, Tanoh Aya Honorine, Traoré Aboubakar, Kroa Ehoulé & Djaman Allico Joseph

Université Jean Lorougnon Guédé Daloa

Abstract- This study aimed to evaluate antihyperglycemic and antioxidant activities of an herbal medicinal product codified "DD3". From dry aqueous extract, the differents tests were carried out. Phytochemical screening showed presence of alkaloids, polyphenols, flavonoids, saponosides, sterols and terpens. Regarding antioxidant activities, DPPH test showed that DD3 remedy (IC50= 59.39 ± 0.69 µg/mL) had a lower antioxidant potential than vitamin C (IC50= 7.93 ± 0.45 µg/ mL), and that was identical for ABTS test for which IC50 = 94.34 ± 0.73 µg/mL for the Remedy against IC50= 25.87 ± 0.46 µg/mL for Gallic acid. About antihyperglycaemic test, unlike glibenclamide which induced hypoglycaemia after 3 hours, the remedy at 500 mg/kg bw brings blood sugar levels back to normal values after this time. The DD3 remedy could be used as a blood sugar regulator.

Keywords: *activity, antioxidant, antihyperglycemic, medicine, plant.*

GJMR-B Classification: *NLMC Code: QT 162.S8*

Strictly as per the compliance and regulations of:

Potential Effects of a Herbal Remedy "DD3" on Free Radical Inhibition and Glucose Tolerance in Orally Hyperglycemic Wistar Rats

Effets Potentiels D'un Remède A Base De Plantes « DD3 » Sur L'inhibition Des Radicaux Libres Et Sur La Tolérance Au Glucose Chez Des Rats Wistar Rendus Hyperglycémiques Par Voie Orale

Kporou Kouassi Elisée [1], Gbogbo Moussa [2], Ouattara Sitapha [3], Yao Akissi Nadège [4], Tanoh Aya Honorine [5], Traoré Aboubakar [6], Kroa Ehoulé [7] & Djaman Allico Joseph [8]

Résumé- Cette étude visait à évaluer les activités antihyperglycémiantes et antioxydantes d'un médicament à base de plantes codifié « DD3 ». A partir d'extrait aqueux sec, les différents tests ont été réalisés. Le criblage phytochimique a montré la présence d'alcaloïdes, de polyphénols, de flavonoïdes, de saponosides, de stérols et de terpènes. En ce qui concerne les activités antioxydantes, le test DPPH a montré que le remède DD3 (CI_{50}= 59, 39 ± 0,69 µg/mL) avait un potentiel antioxydant inférieur à la vitamine C (CI_{50}= 7,93 ± 0,45 µg/mL), et cela était identique pour le test ABTS pour lequel CI_{50} = 94,34 ± 0,73 µg/mL pour le remède contre CI_{50}= 25,87 ± 0,46 µg/mL pour l'acide gallique. Concernant le test antihyperglycémiant, contrairement au glibenclamide qui induit une hypoglycémie au bout de 3 heures, le remède à 500 mg/kg pc ramène la glycémie à des valeurs normales après ce temps. Le remède DD3 pourrait être utilisé comme régulateur de la glycémie.

Mots clés: *activité, antioxydant, anti hyperglycémie, médicament, plante.*

Abstract- This study aimed to evaluate antihyperglycemic and antioxidant activities of an herbal medicinal product codified "DD3". From dry aqueous extract, the differents tests were carried out. Phytochemical screening showed presence of alkaloids, polyphenols, flavonoids, saponosides, sterols and terpens. Regarding antioxidant activities, DPPH test showed that DD3 remedy (IC50= 59.39 ± 0.69 µg/mL) had a lower antioxidant potential than vitamin C (IC50= 7.93 ± 0.45 µg/mL), and that was identical for ABTS test for which IC50 = 94.34 ± 0.73 µg/mL for the Remedy against IC50= 25.87 ± 0.46 µg/mL for Gallic acid. About antihyperglycaemic test, unlike glibenclamide which induced hypoglycaemia after 3 hours, the remedy at 500 mg/kg bw brings blood sugar levels back to normal values after this time. The DD3 remedy could be used as a blood sugar regulator.

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Keywords: *activity, antioxidant, antihyperglycemic, medicine, plant.*

I. Introduction

Le diabète est une maladie chronique qui apparait lorsque le pancréas ne produit pas suffisamment de l'insuline ou que l'organisme n'utilise pas correctement l'insuline qu'il produit (OMS, 2015). Actuellement considéré comme une pandémie par l'Organisation Mondiale de la Santé (OMS), le diabète est l'une des maladies non transmissibles les plus répandues dans le monde, avec près de 463 millions de personnes atteintes en 2019 (FID, 2019). En 2010, il était estimé que près de 438 millions de personnes dans le monde seraient atteintes de diabète en 2025. Cependant, cette prévision a déjà explosé (soit 25 millions de cas en plus). Selon les estimations de la Fédération Internationale du Diabète (FID), 578 millions d'adultes seront atteints de diabète d'ici 2030, et 700 millions d'ici 2045 (FID, 2019). Par conséquent, le diabète pourrait être la septième cause de décès dans le monde d'ici 2030, au vu des prévisions de l'OMS (AIP, 2019). Les dépenses allouées au diabète sont énormes en Afrique, et représenteraient environ 23 % du budget total de la santé (FID, 2019). Près de 9,5 milliards USD a été dépensés pour le diabète sur le continent africain en 2019. En Côte d'Ivoire, le diabète représente un problème majeur de santé publique de par sa prévalence élevée (6,2 %), soit 700 000 personnes atteintes dans la population (AIP, 2019).

Malgré d'importants progrès réalisés dans le traitement de cette maladie, des recherches sur de nouveaux médicaments contre le diabète continuent car plusieurs des médicaments de synthèse existant ont montré leurs limites. Parmi les solutions préconisées, il y a la phytothérapie antidiabétique. Cette approche offre à ce jour, une alternative intéressante du fait de la découverte de plus en plus croissante d'extraits de

plantes efficaces dans le traitement du diabète du type 2 (Zhang *et al*.,2000 ; Katemo *et al*., 2012).

Selon plusieurs études effectuées sur des modèles *in vitro* et *in vivo,* il a été constaté que les conditions d'hyperglycémie pouvaient provoquer l'installation d'une auto-oxydation du glucose, d'une phosphorylation oxydative et d'une toxicité du glucose conduisant à la formation des radicaux libres impliquant un stress oxydatif (Riserus *et al*., 2009; Pitocco *et al.,* 2014). A ce sujet, les polyphénols (anthocyanes, flavonoïdes, leucoanthocyanes and tanins ont montré des résultats intéressants à la fois comme hypoglycémiants et antioxydants. En effet, ces résultats suscitent de plus en plus de l'intérêt pour la prévention, et le traitement de différentes maladies dont les cancers, les maladies inflammatoires, cardiovasculaires et neuro dégénératives (Lacopini *et al.,* 2008). Les coûts prohibitifs pour les populations des pays pauvres, qui accèdent difficilement aux médicaments modernes, contribuent à orienter les patients vers les remèdes traditionnels. Dans cette dynamique, l'OMS encourage l'intensification de la recherche des pistes incluant également celles qui ont recours aux traitements traditionnels à base de plantes médicinales.

Dans la ville de Daloa, au Centre-Ouest de la Côte d'Ivoire, un remède codé « DD3 » à base de plantes, produit par un centre de médecine traditionnel, est proposé aux patients souffrant de diabète. Selon ces fabricants, ce remède est supposé avoir des effets dans la régulation de la glycémie. C'est dans l'élan de la recherche de nouveaux composés antidiabétiques que cette présente étude se propose comme objectif général d'évaluer les potentialités antihyperglycémiques du remède traditionnel « DD3 » utilisé par certains patients souffrant de Diabète. De façon spécifique il s'agira de: (1) caractériser les métabolites secondaires du remède; (2) évaluer le potentiel antioxydant du remède; (3) étudier l'activité antihyperglycémique du remède « DD3 » chez des rats en hyperglycémie provoquée par voie orale.

II. Matériels et Méthodes

a) *Matériel végétal*

Le produit à tester est un remède liquide à base d'extraits de plantes de couleur marron foncé codifiée « DD3 » fabriqué par l'ONG le Daoutra Santé, un centre de médecine traditionnelle reconnu par le Programme National de Promotion de Médecine Traditionnelle (PNPMT). Ce centre est situé dans la ville de Daloa au Centre-Ouest de la Côte d'Ivoire. Dans le cadre de cette étude, la poudre issue de l'extrait sec du remède « DD3 » a été utilisé pour les tests.

La composition du remède DD3 est consignée dans le tableau I.

Tableau I: Composition du remède « DD3 »

Noms scientifiques	Familles botaniques	Noms vernaculaires (Baoulé)
Nauclea lotifolia	*Rubiaceae*	Alobogna
Cnestis ferruginea	*Connaraceae*	Blakassa
Zingiber officinale	*Zingiberaceae*	Sah
Anogeissus leiocarpus	*Combretaceae*	N'galama

b) *Matériel animal*

Au total douze (12) rats de l'espèce *Ratus norvegicus*, de souche Wistar (Figure 1), âgés de 6 à 8 semaines, et pesant en moyenne 110 g ont été utilisés pour les tests. Tous les animaux ont été soumis à une température de 25 °C ± 2 et à une alternance de 12 heures de lumière et 12 heures d'obscurité.

c) *Screening phytochimique*

Le screening phytochimique permet d'avoir une idée générale sur les différentes familles de métabolites secondaires présentes dans les plantes sans toutefois renseigner sur la structure d'une molécule bien déterminée. A partir de l'extrait aqueux du lyophilisat du remède « DD3 » nous avons recherché les groupes chimiques tels que les alcaloïdes, les polyphénols totaux, les flavonoïdes, les quinones, les tanins, les saponosides et les polyterpènes grâce aux méthodes décrites par Trease & Evans (2002).

i. *Recherche des alcaloïdes:* Un échantillon de 6 mL de solution aqueuse obtenue à partir du lyophilisat de la solution soignante filtrée ont été évaporés à sec dans une capsule en porcelaine au bain de sable. Le résidu est repris dans 6 mL d'éthanol (60°). La solution ainsi obtenue a été répartie dans deux tubes à essai (A et B).

- Dans le tube A, deux gouttes de réactif de DRAGENDORFF ont été additionnées.
- Dans le tube B, deux gouttes de réactif de BOUCHARDAT ont été aussi additionnées.

ii. *Recherche des polyphénols:* À 2 mL d'extrait aqueux obtenu à partir du lyophilisat de la solution soignante est ajouté une goutte d'une solution alcoolique de chlorure ferrique à 2 % (Bonga *et al*., 1995).

iii. *Recherche des tanins:* 5 mL du remède sont évaporés à sec dans une capsule en porcelaine au bain de sable ; à ce résidu ont été ajoutés 15 mL du réactif de Stiasny (réaction au formol chlorhydrique). L'ensemble a été porté au bain-marie à 80 °C pendant 30 minutes.

Par la suite, la solution obtenue précédemment (après le test des tanins cathéchiques) a été filtrée et saturé à l'acétate de sodium. On n'y a additionné 3 gouttes de $FeCl_3$ à 2 %.

iv. *Recherche des flavonoïdes:* 2 mL d'extrait aqueux obtenue à partir du lyphilisat de la solution soignante ont été évaporés à sec dans une capsule en porcelaine au bain de sable. Le résidu est repris après refroidissement dans 5 mL d'alcool chlorhydrique au demi (dilué de moitié 1/2). La solution obtenue a été renversée dans un tube à essai. En ajoutant quelques coqeaux de magnésium, il y a un dégagement de chaleur puis apparition d'une coloration rose-orangée ou violacée. L'addition de 3 gouttes d'alcool isoamylique permet d'intensifier cette coloration qui confirme la présence de flavonoïdes.

v. *Recherche des saponosides:* Un échantillon de 2 mL d'extrait sec du remède est repris à l'eau bouillante (20 mL), refroidi et filtré. Dix millilitres (10 mL) du filtrat sont ensuite introduits dans un tube à essai. Le tube est ensuite agité verticalement pendant environ 15 secondes et laissé au repos pendant 15 minutes. La hauteur de la mousse formée est mesurée (Bonga *et al*.,1995).

vi. *Recherche des quinones:* Dans une capsule en porcelaine, 2 mL du remède liquide ont été évaporés à sec au bain de sable, puis triturés avec 5 mL d'acide chlorhydrique dilué au 1/5. L'ensemble est porté au bain-marie brouillant pendant 30 minutes. Après refroidissement, la solution a été additionnée à 20 mL de chloroforme dans un tube à essai. La phase chloroformique est ensuite saturée avec 0,5 mL d'ammoniaque dilué au demi.

vii. *Recherche des stérols et polyterpènes:* 5 mL du remède ont été évaporés à sec dans une capsule en porcelaine au bain de sable. Le résidu a été dissout à chaud dans 1 mL d'anhydride acétique. L'ensemble est renversé dans un tube à essai auquel on ajoute 0,5 mL d'acide sulfurique concentré.

d) Evaluation du Potentiel antioxydant du remède

- Test DPPH (2,2-diphényl-1-1-picryl-hydrazyl)

L'activité de piégeage des radicaux libres a été mesurée par la méthode de DPPH (2,2-diphényl-1-picrylhydrazyl) selon les travaux de (Hammoudi, 2015). La solution mère a été préparée par la dissolution de 24 mg de DPPH dans 100 mL de méthanol. La solution obtenue possède une absorbance d'environ 0,98 ± 0,021 à 517 nm en utilisant le spectrophotomètre. 1,68 mL de cette solution a été mélangée avec 1600 µL de l'échantillon à diverses concentrations (3,125 à 100 µg/mL). Le mélange réactionnel a été bien agité et incubé dans l'obscurité pendant 30 min à température ambiante. Ensuite, l'absorbance a été mesurée à 517 nm. Le contrôle a été préparé comme ci-dessus, sans aucun échantillon.

Chaque test a été répété trois fois, les résultats ont été présentés par la moyenne des trois essais

Le pourcentage de piégeage du radical DPPH a été calculé selon l'équation suivante Torres (2005):

$$\boldsymbol{I}\,(\%) = \frac{\boldsymbol{DO\ témoin - DO\ échantillon}}{\boldsymbol{DO\ témoin}} \times \mathbf{100}$$

Les CI_{50} ont été déterminées graphiquement par les régressions linéaires des graphes tracés; pourcentages d'inhibition en fonction des différentes concentrations des fractions testées. Plus la CI_{50} est faible plus l'activité antioxydante est importante.

- *Test de l'ABTS « acide 2,2'-azino-bis- (3-éthylbenzothiazoline-6-sulfonique)*

La méthode utilisée a été celle décrite par Leong et Shui (2002) Une quantité de 38,40 mg de ABTS a été préalablement dissoute dans 10 mL d'eau avant ajout de 6,75 mg de persulfate de potassium. Le mélange obtenu a été conservé à l'obscurité et à température ambiante pendant 12h avant usage. Il a été par la suite dilué avec de l'éthanol afin d'obtenir une absorbance de l'ordre de 0,7 à 734 nm. L'activité antioxydante a été mesurée en additionnant 2 mL d'une solution éthanolique du lyophilisat de l'extrait aqueux du remède à tester à 2 mL de la solution de ABTS+•. Les extraits ont été testés aux concentrations suivantes : 2,5; 10; 100 et 200 µg/mL. L'acide gallique, utilisé comme antioxydant de référence a été testé aux mêmes concentrations. La lecture de l'absorbance a été faite au bout de 2 minutes au spectrophotomètre à 734 nm en utilisant l'éthanol comme blanc. Trois mesures de l'absorbance ont été effectuées pour chaque concentration testée. L'expression des résultats s'est faite comme précédemment (test DPPH), c'est-à-dire le calcul des pourcentages d'inhibition et des concentrations inhibitrices à 50 % (CI_{50}).

e) Activité antihyperglycémiante de l'extrait de DD3

Douze (12) animaux repartis en 4 lots de 3 rats chacun ont été utilisés. Tous les animaux ont été mis à jeun depuis la veille. Un prélèvement sanguin a été effectué 30 minutes avant de rendre hyperglycémiques par voie orale tous les rats grâce à une solution de glucose anhydre (250 mg/mL) (Lawson-Evi & Gadegbeku, 1997). Un prélèvement sanguin a été réalisé 30 min après l'induction de l'hyperglycémie provoquée puis, les animaux ont reçu immédiatement après, 2 mL pour 100 g de poids corporel un traitement.

Ainsi, le lot 1 ou lot témoin négatif était composé de rats qui ont reçu uniquement de l'eau distillée par gavage; les rats du lot 2 et 3 ont été traités avec l'extrait aqueux de « DD3 » aux doses respectives de 100 et 500 mg/kg de poids corporel. Le lot 4 ou témoin positif a été traité avec du glibenclamide (Daonil 5mg), la substance hypoglycémiante de référence. Un prélèvement sanguin a été effectué aux temps 90 min (T90), 150 min (T150) et 210 min (T210) pour évaluer l'effet des différents traitements sur l'hyperglycémie. Aussi le glucose a été dosé directement à partir du sang total à l'aide d'un glucomètre de marque Accu-check® (Roche Diagnostics) selon la méthode de glucose oxydase (Tietz, 1987).

f) Analyse statistique

L'étude statistique a été réalisée grâce au logiciel informatique d'analyses statistiques XLSTAT-PRO 7.1. Les résultats ont été analysés à l'aide d'une ANOVA à un facteur. Les valeurs sont données sous forme de moyenne suivie de l'erreur standard sur la moyenne. Certains résultats ont été présentés sous forme de proportions et leur analyse a été effectuée à l'aide du test paramétrique de comparaison de k proportions (Test G).

Pour l'étude de l'activité antihyperglycémiante de l'extrait, nous avons utilisés le rapport suivant (Begbin *et al.,* 2021):

$$\text{TEG} = \frac{\text{Glycémie finale} - \text{Glycémie initiale}}{\text{Glycémie initiale}} \times 100$$

Ces tests nous donnent le degré de significativité pour $p < 0,05$.

III. Résultat

a) Screening phytochimique

Le tri phytochimique a permis de mettre en évidence la présence des principaux groupes chimiques dans l'extrait total aqueux. Il a révélé la présence des alcaloïdes, des polyphénols, des flavonoïdes, des Saponosides, des tanins, galliques et catéchiques, des quinones, des Stérols et terpènes. Les résultats sont consignés dans le Tableau II.

Tableau II: Composition phytochimique de « DD3 ».

Métabolites secondaires	Résultats
Alcaloïdes	+
Polyphénols	+
Flavonoïdes	+
Tanins galliques	-
Saponosides	+
Quinones	-
Stérols et terpènes	+
Tanins catéchiques	-

+ : Présence ; - : Absence

- Test au DPPH (2,2-diphényl-1 picrylhydrazyle)

Les résultats de l'activité antioxydante de la vitamine C et du remède sur le radical libre au DPPH sont représentés sur les figures 1 et 2. Dans la gamme de concentrations fixées, ces résultats ont indiqué que la concentration inhibitrice du radical DPPH par la vitamine C tendait vers 100% soit un CI_{50} égale à 7,84 μg/mL alors que celle du remède n'a pas pu atteindre les 70 % avec une CI_{50} égale à 59,39 μg/mL. L'analyse des figures 1 et 2 a montré que la vitamine C a une activité antioxydante plus élevée que celle du remède.

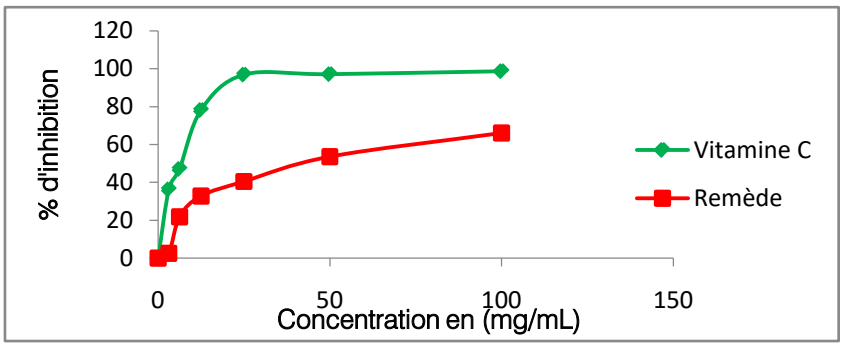

Figure 1: Évolution des pourcentages d'inhibition du DPPH par le remède par le test de DPPH

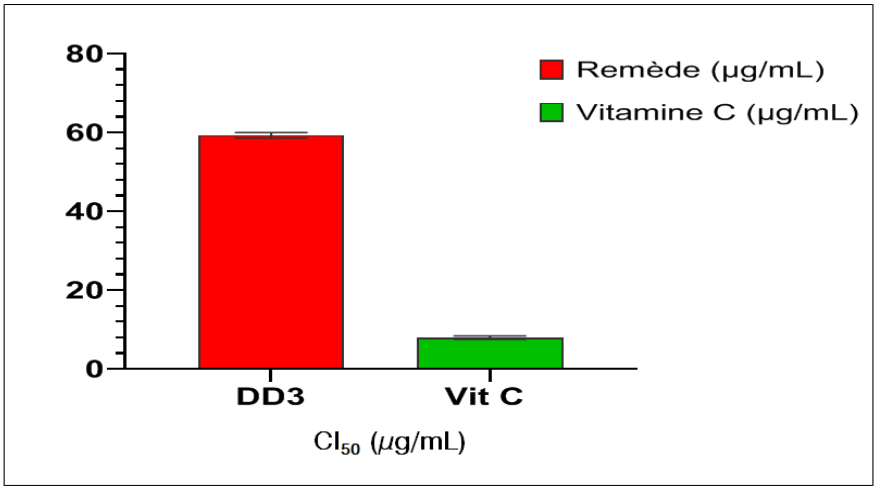

Figure 2: Histogramme des valeurs CI_{50} du remède « DD3 » et la vitamine C par le test DDPH

- Test ABTS

Les résultats de l'activité antioxydante de l'acide gallique et du remède sur le radical libre ABTS sont représentés par les figures 3 et 4. Les résultats indiquaient que dans la gamme de concentrations fixées, l'évolution de l'inhibition de l'ABTS par l'acide gallique tendait vers 100 % et qu' une CI_{50} égale à 25,72 µg/mL a été obtenue, alors que celle du remède n'a pas pu atteindre les 70 % et une CI50 égale à 94,67 µg/mL a été déterminée. L'analyse des figures 3 et 4 montre que l'acide gallique a une activité antioxydante meilleure que celle du remède.

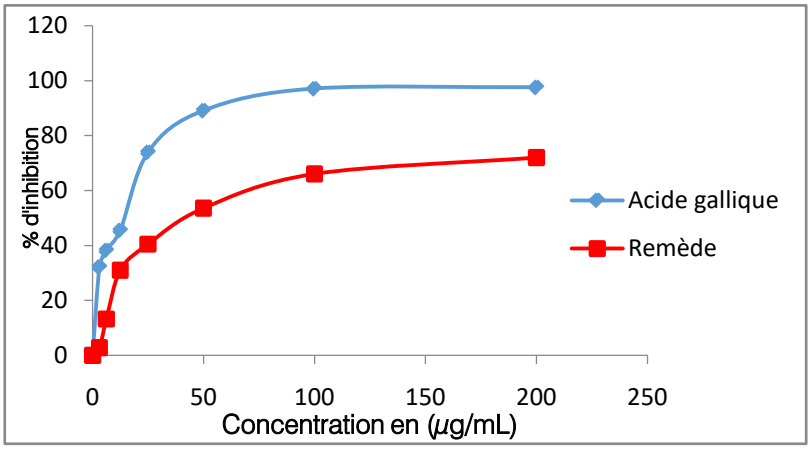

Figure 3: Évolution des pourcentages d'inhibition ABTS par le remède et l'acide gallique

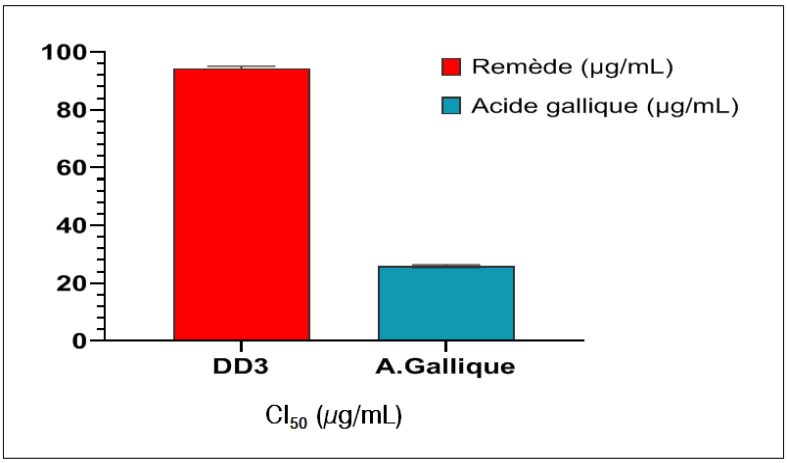

Figure 4: Histogramme des valeurs des CI_{50} du remède « DD3 » et de l'acide gallique par le test ABTS

L'évolution des valeurs des glycémies des rats au cours de cette étude sont consignées dans le Tableau III. Ainsi à T_0, c'est-à-dire avant traitement des rats, la glycémie à jeun n'a pas indiqué de différence significative entre les quatre lots expérimentaux. En revanche, à T_{30} c'est-à-dire 30 min après traitement avec le glucose anhydre, une hyperglycémie a été observée dans l'ensemble des lots des animaux.

Par ailleurs, après traitement des lots expérimentaux avec le remède « DD3 » et le glibenclamide notamment aux temps T_{90} et T_{150}, une baisse significative et très significative de la glycémie a été observée dans ces lots comparativement au lot témoin ayant reçu l'eau distillée. Ainsi, des diminutions de -26,58% et -37,07% ; -26,25% et -32,20% et -22,79% et -25,30% ont été constatées respectivement chez les rats traités avec le glibenclamide, et ceux ayant reçu les doses de 100 et 500 mg/kg de poids corporel du remède comparativement au lot témoin négatif (Tableau III). En revanche, à T_{210}, une baisse significative de la glycémie chez les rats traités avec le glibenclamide a été observée par rapport au lot témoin négatif alors qu'avec les extraits, la glycémie revient presqu'à sa valeur initiale.

Tableau III: Evolution de la glycémie chez les rats traités et témoin

Lots	Temps								
	T_0	T_{30}		T_{90}		T_{150}		T_{210}	
	Glycémie (g/L)	Glycémie (g/L)	TEG (%)	Glycémie (g/L)	TEG (%)	Glycémie (g/L)	TEG (%)	Glycémie (g/L)	TEG (%)
Lot1 Témoin Négatif (Eau distillée)	0,98±0,091	1,75±0,102	+78,57	1,60±0,102	-8,57	1,35±0,102	-15,62	1,01±0,102	-25,18
Lot 2 Extrait (100 mg/kg de pc)	0,91±0,091	1,60±0,102	+75,82	1,18±0,102	-26,25[a]	0,80±0,102	-32,20[b]	0,79±0,102	-10,25
Lot 3 Extrait (500mg/kg de pc)	1,12±0,091	2,15±0,102	+91,96	1,66±0,102	-22,79[a]	1,24±0,102	-25,30[a]	0,90±0,102	-21,05
Lot 4 Témoin Positif (Glibenclamide)	0,84±0,091	1,58±0,102	+88,09	1,16±0,102	-26,58[a]	0,73±0,102	-37,07[b]	0,37±0,102	- 49,31[b]

T_0: Temps initial au début des expérimentations; T30: Temps 30 minutes après l'induction de l'hyperglycémie: T90; T150 et T210: Temps 90, 150 et 210 minutes après traitement des rats avec le Glibenclamide et le remède « DD3 » aux doses de 100 et 500 mg/kg. TEG: Taux d'évolution de la glycémie. a : baisse significative ($p < 0,05$) de la glycémie par rapport au témoin négatif; b: baisse très significative ($p<0,01$) de la glycémie par rapport au témoin négatif.

IV. Discussion

La présente étude avait pour objectif de déterminer grâce à un screening phytochimique, la présence des métabolites secondaires dans le remède « DD3 », et d'en évaluer les potentiels anti-radicalaires et anti-hyperglycémiants. Les résultats ont mis en évidence différentes molécules bioactives telles que les polyphénols, les flavonoïdes, les saponosides, les alcaloïdes, les stérols et terpènes qui ont été retrouvés dans le remède « DD3 ». Ces résultats sont similaires à ceux obtenus par Choho *et al.,* (2022) qui ont montré qu'un remède à base de plantes denommé « Daoutra Epigastro » utilisé dans le traitement des gastrites contenait également les polyphénols, les flavonoïdes, les saponosides, et les stérols/polyterpènes. Les phytocomposés retrouvés dans le remède « DD3 » pourraient suggérer des activités pharmacologiques intéressantes. En effet, les flavonoïdes sont souvent présentés comme des anti-inflammatoires, hépato-protecteurs (Bruneton, 2009). On leur revendique aussi des propriétés antioxydantes, vasculo-protectrices (oedèmes, antihémorroïdaires), antihépatotoxiques, antiallergiques, antiulcéreuses et même anti-tumorales significatives (Elliott *et al.,* 2000; Fenglin et *al*., 2004). Les saponosides sont fongicides, molluscicides, anti-inflammatoires, anti-oedémateuses, analgésiques, spermicides, anti-tussives et expectorants mais peuvent également faciliter l'absorption des éléments nutritifs (Bruneton, 1999; Nacoulma, 1996).

Le test de piégeage des radicaux libres DPPH est un modèle largement utilisé pour évaluer la capacité antioxydante de divers composés. Comparativement au remède avec une CI50= 59,39 ± 0,69 g/mL, la vitamine C (avec une CI50 plus base de 7,84 ± 0,45µg/mL) a présenté une meilleure activité de piégeage des radicaux libres. En comparant nos résultats à ceux de (Koua, 2018) qui a obtenu pour le même test de piégeage des radicaux libres DPPH, les CI50 suivantes: la vitamine C (CI50 de 6,052 µg/mL), l'extrait aqueux (2740 µg/mL) de *Crinum scillifolium* (Amaryllidaceae). Il ressort que le remède « DD3 » a présenté un meilleur

profil antioxydant que *Crinum scillifolium*. En outre, Comparativement à l'extrait acétate d'éthyle de *Albertisia cordifolia* étudié par Diomandé *et al* '2018) pour lequel la (CI50 était de 20 µg/mL), le remède « DD3 » a un profil antioxydant moins intéressant (Diomandé *et al.,* 2018).

Les résultats du deuxième test, basé sur la capacité de piégeage du proton par le radical cationique ABTS°+ viennent corroborer ceux déjà obtenus avec le test de la DPPH sur l'aptitude antioxydante du remède. En effet, en analysant les concentrations efficaces à 50% (CI_{50}) obtenues, il ressort que l'acide gallique (CI_{50} = 25,87 ± 0,46 g/mL) et le remède (CI_{50} = 94,34±0,73g/mL) inhibent tous le radical cationique ABTS°+mais le remède à un degré moindre. Les composés phénoliques (polyphenols, flavonoïdes, tanins) contenus dans ce remède pourraient être un groupe majeur de composés qui agissent comme antioxydants primaires de radicaux libres (Ayoola *et al.,* 2008). Ce qui confirme le potentiel antioxydant du remède.

S'agissant de l'effet des extraits du remède DD3 sur l'hyperglycémie induite par voie orale chez les rats, les résultats ont indiqué une baisse significative de la glycémie aux temps T_{90}, T_{150} et T_{210} respectivement au 100 et 500 mg / kg de poids corporel chez l'ensemble des lots expérimentaux. Ces résultats sont similaires à ceux obtenus par Begbin *et al.,* (2021). En effet, ces auteurs ont observé une diminution significative de la glycémie chez les souris rendues hyperglycémiques et traités avec l'extrait aqueux de feuilles de *Cnestis Ferruginea* Vahl ex DC. (Connaraceae) aux doses de 100, et 500 mg/kg de poids corporel. Ce résultat est rassurant puisque les feuilles de *C. ferruginea* contenues dans le remède « DD3 » exerceraient un contrôle antihyperglycémique modéré conduisant à éviter les symptômes d'hypoglycémie selon Kouassi *et al*., (2021). En effet, les composés chimiques autres que les flavonoïdes, les saponines, les alcaloïdes et autres contenus dans le remède « DD3 » apporteraient un supplément d'activité antihyperglycémique. L'usage du remède « DD3 » pourrait être une alternative dans la prise en charge d'une pathologie telle qu'une hyperglycémie.

Le glibenclamide a exercé une activité hypoglycémique par rapport au remède « DD3 » durant l'e xpérience. L'usage du remède « DD3 » pourrait être une alternative dans la prise en charge d'une pathologie telle qu'une hyperglycémique. Au terme de l'expérience, le glibenclamide a exercé une importante activité hypoglycémique par rapport au remède « DD3 » qui a ramené la glycémie à sensiblement égales aux valeurs initiales. Le remède « DD3 » aurait une action régulatrice de la glycémie ce qui constituerait une importante alternative dans la prise en charge d'une pathologie telle qu'une hyperglycémique.

V. Conclusion

La présente étude avait pour objectif d'étudier les activités antioxydantes et antihyperglycémique du remède codé « DD3 ». Le screening phytochimique a permis de montrer que ce remède contient des stérols, des terpènes, des flavonoïdes, des polyphénols, des alcaloïdes et des saponosides. La présence de ces composés confère à ce remède un potentiel antioxydant réel. Toutefois, avec des valeurs de CI_{50} plus élevées, ce potentiel est moins fort que celui de la vitamine C dans la gamme de concentrations évaluées. L'évaluation du potentiel antihyperglycémique du remède chez les rats a montré une très bonne activité sur la tolérance orale au glucose comparativement au témoin négatif. En d'autres termes, l'utilisation de ce remède contribuerait à ramener la glycémie à sa valeur normale au bout de 3h30 en cas d'hyperglycémie par voie alimentaire là où le glibenclamide ramènerait cette valeur à la normale au bout 3h mais avec un effet sous-jacent d'hypoglycémie par la suite.

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Evaluation of the Quality Indicators of the Drug "Bralekord" Solution for Infusions

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Keywords: quality indicators, standardization, quality control, injection solution, pharmacopoeia, normative document.

GJMR-B Classification: NLM: QV 778

Strictly as per the compliance and regulations of:

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Abstract- **The results of standardization and quality control of the drug "Bralecord" solution for infusions are given, according to such quality indicators as: description, authenticity, transparency, color, pH, mechanical inclusions, impurities, osmolality.**

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I. Introduction

Processes of globalization of the pharmaceutical market; a high level of competition, as a result of which there is a shift in competitiveness factors from the level of the product to the level of the organization as a whole; an increase in the number of interactions in the process of drug circulation; social responsibility of business and its customer orientation; the characteristics of pharmaceutical products, as well as the requirements of regulatory legal acts, are the main reasons for the development and implementation of quality assurance systems or, at a higher level of development, quality management in the implementation of pharmaceutical activities.

An essential condition for the functioning of the sphere of drug circulation, as well as one of the main mechanisms for ensuring the required level of quality and safety of pharmaceutical products and services in the interests of the consumer, is the standardization procedure [1-2].

Standardization is the activity of establishing rules and characteristics for the purpose of their voluntary reuse, aimed at achieving orderliness in the areas of production and circulation of products and increasing competitiveness.

To date, one of the pressing issues of healthcare in the Republic of Uzbekistan is the provision of vital drugs that meet the high requirements of modern medicine. The quality of infusion solutions must meet the stringent requirements of modern standards. Only in the conditions of pharmaceutical production, it is possible to eliminate the influence of the human factor as much as possible and introduce multi-stage quality control. The control of the main indicators of the quality of the finished product and the parameters of the technological process plays an important role in obtaining drugs of guaranteed quality. An increased risk in the parenteral route of administration of large volume solutions (100 ml or more) or infusion solutions causes high requirements for their quality [3].

Quality standards for medicines should ensure the development of a high quality, effective and safe medicine, and should be revised in a timely manner, taking into account new achievements in medical, pharmaceutical and other sciences and the requirements of leading foreign pharmacopoeias.

An objective assessment of the quality of medicines depends not only on the merit of the methods, but also on the fact that their use in different laboratories allows obtaining identical results. This is ensured by the standardization of quality assessment methods, the preparation of solutions, reagents and indicators, the standardization of instruments, etc. The standard as ND establishes a set of norms or requirements for the object of standardization [4].

Bralecord is a combined drug containing in its composition: sodium citicoline, L-arginine hydrochloride, levocarnitine, used as a nootropic and metabolic agent.

Purpose of the study. The purpose of these studies is to develop methods for assessing the quality and establishing indicators of the quality of the combined drug "Bralecord" solution for infusion.

II. Experimental Part

a) Materials and research methods

As objects of study, 5 series of pilot samples of the drug "Bralecord" solution for infusions were used. During the study, solvents, reagents and consumables from MERCK (Germany) were used. The following auxiliary equipment was also used in the tests: magnetic stirrers, BP-310S electronic analytical balance from Sartorius (Germany), HS 32 AC sterilizer with automation, Seven Easy pH meters from Mettler Toledo (Switzerland) and 766 Calimatic Knick (Germany), Julabo water thermostat (Germany), Osmomat 010 type osmometer, Gonotek (Germany), PAMAS SVSS liquid particle counters (Germany).

b) Results and its discussion

The evaluation of the quality indicators of the study drug was carried out in accordance with the modern requirements of the national and foreign pharmacopoeias [5-7], as well as in accordance with the general technical regulation on the safety of medicines [8] in terms of such indicators as:

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description, authenticity, transparency, color, pH, mechanical inclusions, impurities, osmolality, sterility, bacterial endotoxins, abnormal toxicity, quantitation.

The initial stage of work is organoleptic control, which is a mandatory type of control and consists in checking the medicinal product in terms of appearance, smell, mixing uniformity, and the absence of mechanical impurities in liquid dosage forms.

c) Description

The drug should be a clear, colorless or slightly yellowish solution (visual method). All studied five series of the drug meet the requirements of the pharmacopoeia.

d) Authenticity

Identification was carried out by a chemical method using qualitative reactions to the main components of the drug: sodium citicoline, arginine, levocarnitine and chlorides.

Sodium citicoline was determined by the spectrophotometric method at an absorption maximum at 280 nm. 1.0 ml of the drug was placed in a volumetric flask with a capacity of 100 ml, the volume of the solution was brought to the mark with a 0.01 mol/l solution of hydrochloric acid and mixed. 10 ml of the resulting solution was placed in a volumetric flask with a capacity of 50 ml, the volume of the solution was brought to the mark with a 0.01 mol/l solution of hydrochloric acid and stirred (test solution).

The optical density of the resulting solution was measured on a spectrophotometer at the absorption maximum at a wavelength of 280 nm in a cuvette with a layer thickness of 10 mm, using a 0.01 mol/l hydrochloric acid solution as a reference solution. In parallel, the optical density of the RSO solution of sodium citicoline was measured at the same wavelength.

Arginine. 15 ml of water was added to 5 ml of the preparation. To 2 ml of the resulting solution was acidified 1 ml of α-naphthol solution and 2 ml of a mixture of equal volumes of 3% sodium hypochlorite solution and water; a red color is formed.

Levocarnitine. 2 ml of the drug was transferred into a test tube, 5 ml of 1 mol/l hydrochloric acid solution and a few drops of ammonium rheinecate solution were added, and a red-violet precipitate formed.

Preparation of ammonium rheinecate solution: about 500 mg of ammonium rheinecate was mixed with 20 ml of water, shaken periodically for 1 hour and filtered. The solution is used within 2 days.

Chlorides. 2.0 ml of the drug was acidified with dilute nitric acid, 0.4 ml of a silver nitrate solution was added, and a white curdled precipitate (chlorides) was formed upon standing.

All studied five batches of the drug confirmed the presence of citicoline sodium, arginine, levocarnitine and chlorides in the solution of the study drug.

Transparency. The solution of the drug should be transparent compared to water for injection.

Chromaticity was determined in accordance with the European or British Pharmacopoeia. It was found that the color of the preparation should not be more intense than the reference solution Y7.

The pH was set potentiometrically. It was found that the pH of the drug solution should be in the range from 5.0 to 7.5.

All studied five batches of the drug met the requirements of pharmacopoeias in terms of mandatory indicators: transparency, color, pH.

Foreign impurities

Citicoline sodium. High performance liquid chromatography method.

Chromatographic system and determination conditions

Instrument liquid chromatograph "ShimadzuLC-6A"

Steel column, size 30 cm x 4.0 mm

Octadecylsilane filler chemically bonded to porous silica gel or ceramic microparticles 10 μm in diameter (LI, USP)

Flow rate 2 ml/min

Detection 275 nm

Injection volume 20 μl

A column filled with octadecylsilane silica gel and phosphate buffer solution was used (equal volumes of 0.1 mol/l potassium dihydrogen phosphate and tetrabutylammonium phosphate solution were mixed, the pH value of 0.01 mol/l tetrabutylammonium hydroxide was adjusted to 4.5 with phosphoric acid), methanol (95 : 5) as mobile phase.

1 ml of the drug was carefully transferred into a 10 ml volumetric flask and diluted with water to the mark (test solution).

Dissolve the required amount of citicoline sodium RSO and levocarnitine RSO in water to obtain a solution of 1.045 mg/ml sodium citicoline and 2.0 mg/ml levocarnitine (control solution).

Transfer 1.25 ml of the control solution to a 100 ml volumetric flask. Dilute to the mark with water and mix well (system suitability solution).

Introduced 20 μl of reference solution 1 into the column. The dilutions were adjusted so that the main maximum in the chromatogram was 20-25% of the full scale of the chart. Add 20 μl of each test solution, system suitability solution, and control solution separately to the column. Chromatography was continued at 2.5 times the retention time of the citicoline peak.

Calculations:

The content of an individual non-identifying or identifying impurity, in percent:

$$X = \frac{AT \times 100\%}{As}$$

where: AT = Peak area of an individual impurity in the test solution

As = Sum of all peak areas in control solution

At the same time, it was established that the individual impurity should be no more than 0.5%, and the total impurity - no more than 2.0%. All five batches of the study drug met the established norm.

Substances detected by ninhydrin. The determinations are carried out by thin layer chromatography.

System suitability solution: 0.4mg/ml each of arginine hydrochloride and L-lysine hydrochloride with water

Sample solution: 10 mg/ml RSO arginine hydrochloride with water

Standard solution: dilute 1 ml of sample solution with water to 100 ml. Dilute 5 ml of the resulting solution with water to 10 ml (0.05 mg/ml)

Note: The concentration of the solution is approximately 0.5% of this sample solution.

Test solution: 100 µl of the drug is added to 320 µl of water (10 mg/ml)

Chromatography System Mode: TLC

Adsorbent: 0.25 mm layer of chromatographic silica gel mixture

Applied sample volume: 5 µl

Mobile solvent system: isopropyl alcohol and ammonium hydroxide (70:30)

Nebulizer: 2 mg/ml ninhydrin in butyl alcohol and 2N acetic acid (95:5)

Drying: at a temperature from 100 °C to 105 °C until complete removal of ammonia. The plate is sprayed with a solution of 2 mg/ml ninhydrin in a mixture of butyl alcohol and 2 N acetic acid (95:5) and heated at 100°C to 105°C for 15 minutes. Examine the plate under daylight. The system suitability chromatogram shows two completely separated spots.

Norm: any spot, except for the main one, should not be more intense than the main spot on the chromatogram of the standard solution (0.5%). Individual impurities: no more than 0.5%, total impurities: no more than 2.0%.

The results of the analysis are considered reliable if two clearly separated spots appear on the chromatogram of the system suitability solution.

All studied five series of the drug corresponded to the established norm.

Levocarnitine. High performance liquid chromatography method.

Reagents: 2M sodium hydroxide solution. Solution A: 6.81 g of KH_2PO_4 are dissolved in 800 ml of water, 2M sodium hydroxide solution is added to obtain a pH of 4.7, the volume of the solution is adjusted to the mark with water and mixed; Acetonitrile.

Chromatography conditions. Column: Aminopropyl methylsilangel (USP L8), 250x4.6mm, 5µm; Column temperature: 30^0C+1^0C; UV detector: 205nm. Mobile phase: A mixture of 35 volumes of solution A and 65 volumes of acetonitrile; flow rate: 1.0ml/min; injection volume: 25µl.

Standard solutions and test solution are introduced into the chromatograph and chromatograms are recorded within 20 minutes. The sensitivity of the system is determined by the height of the main peak on the chromatogram of the standard solution (c), which is at least 20% of the full scale of the recording device. The test is considered invalid if the resolution between the peaks of levocarnitine and levocarnitine impurity A in the chromatogram of the standard solution (c) is 0.9. On the chromatogram of the test solution: the area of any peak of levocarnitine impurity A is not more than the main peak of the chromatogram of the standard solution (c) (1%); the area of any peak, except for the main peak and the peak of levocarnitine impurity A, does not exceed the area of the main peak in the chromatogram of the standard solution a (0.2%); the sum of the areas of all peaks, except for the main peak and the peak of impurity A, does not exceed 2.5 times the area of the main peak in the chromatogram of standard solution a (0.5%).

Preparation of standard solution "a". 100 mg of the drug solution is transferred into a 100 ml volumetric flask, the volume is adjusted to the mark with solution A. 1 ml of the resulting solution is diluted to 10 ml with the same solvent (0.2%).

Preparation of standard solution "c". 20.0 mg of the working standard sample of levocarnitine impurity A is dissolved in water, the solution is adjusted with water to 100 ml. 2.5 ml of the resulting solution is diluted to 10 ml with solution A (1.0%).

Preparation of standard solution "c". 10 mg of the working standard sample of levocarnitine impurity A is dissolved in water and diluted with water to 25 ml and 2.5 ml of the resulting solution is diluted to 10 ml with solution A.

Preparation of standard solution "d". Dissolve 100 mg of the working standard of levocarnitine in 10 ml of standard solution C.

Preparation of the test solution. 2.5 ml of the preparation solution is transferred into a volumetric flask with a capacity of 100 ml and the volume is brought to the mark with solution A.

Norm: impurities A should be no more than 1.0%; other impurities - no more than 0.2%; the sum of impurities, except for impurity A - no more than 0.5%.

According to the results of the studies, all five series of the drug under study corresponded to the established norm.

Osmolality. The determination of the osmolality of the solution is carried out by the cryoscopic method using a Beckmann thermometer. The determination of the freezing point is carried out on the installation shown in Figure 1.

The main part of the setup is a test tube with a side branch. Its upper opening is tightly closed with a cork through which the Beckmann thermometer and a wire stirrer pass, one end of which is bent in the form of a ring freely covering the lower part of the thermometer. This test tube is inserted into a wider test tube, which acts as an air jacket that prevents the liquid from cooling too quickly. The assembled apparatus is placed in a Bunsen beaker, which is filled with a cooling mixture before the experiment.

The role of the cooling mixture is performed by ice chips, to which crystalline sodium chloride is added to reduce the temperature. A stirrer is used to stir the cooling mixture. The temperature of the cooling mixture should be (4-5)°C below the freezing point of the test liquid.

The zero point of the instrument is set to water for injection. The instrument is calibrated using standard sodium chloride solutions. The determination is carried out three times and the average value is taken.

As needed, prepare standard solutions based on the data in Table 1.

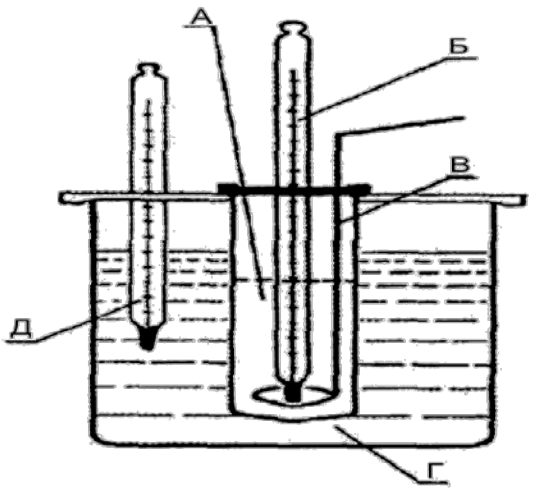
To determine the freezing point of a solvent or a test solution, 28-30 g of liquid is placed into an inner small test tube through a side hole, a stirrer and a Beckmann thermometer are also placed here. This tube is placed through an air jacket into the cooled mixture and the test liquid is evenly mixed by raising and lowering the stirrer. When determining the freezing point of a liquid, the mercury column in the thermometer begins to fall as the liquid cools. Usually, before freezing, the liquid is supercooled, and the temperature of the liquid drops below the freezing point. As soon as the crystallization process begins, the temperature of the solution rises beyond the freezing point.

Table 1: Data for osmometer instrument

Mass of sodium chloride, in grams per 1 kg of water	Actual osmolarity (mosm/kg)	Theoretical osmolality (ideal)	Molar osmotic coefficient (mosm/kg)	Freezing temperature drop ΔTzam.
3,087	100	105,67	0,9463	0,186
6,260	200	214,20	0,9337	0,372
9,463	300	323,83	0,9264	0,558
12,684	400	437,07	0,9215	0,744
15,916	500	544,66	0,9180	0,930
19,147	600	655,24	0,9157	1,116
22,380	700	765,86	0,9140	1,302

The increase in temperature occurs due to the release of open heat of solidification.

After that, the test tube is removed from the liquid, the crystals are melted by heating the test tube with the hand, and the determination is repeated again. The experiment is carried out three times. The discrepancy between the definitions should be no more than 0.01°C. In case of hypothermia, it is necessary to add a solvent crystal to the liquid. The accuracy of determination with this method is +5%.



The device of the Beckman device: A - a vessel for the test solution; B - Beckman thermometer; B - stirrer; G - container with coolant; D - thermometer for measuring the temperature of the cooling mixture.

Before each measurement, the tube with the lateral process is rinsed with the solution intended for the study, and the measurement is also made with the test samples.

The instrument is ready to measure the test product if the value obtained for the calibration solutions is within two values of the calibration scale. To reduce the error and check the reproducibility, it is recommended to repeat measurements with several samples from the same sample, averaging the results. The measurement error should not exceed ±2%.

The calculation of osmolality is carried out according to the following formula:

$$S_{osm}, \text{ mOsmol/kg (mOsmol / l)} = \frac{(T2\text{-}T1) \times 1000}{1.858},$$

where: 1.858 is the molar cryoscopic constant of water, corresponding to the decrease in freezing point that occurs as a result of dissolution

1 mole of a substance in 1 kg of water;
1000 - Conversion factor osm/kg to mosm/kg;
T_2 - freezing point of pure solvent (0C);
T_1 is the freezing point of the solution (0C).

Based on the measurements carried out, it was found that the osmolality should be in the range from 410 mOsmol/kg to 590 mOsmol/kg. All studied five series of the drug corresponded to the established norm.

Mechanical inclusions. Mechanical inclusions in dosage forms for parenteral use are extraneous mobile insoluble particles, with the exception of gas bubbles, accidentally present in drug solutions. The test for the presence of visible and invisible particulate matter is intended for visual evaluation of liquid parenteral dosage forms, including infusion solutions, and is a mandatory pharmacopoeial quality indicator.

Based on the foregoing, as a result of the research, visible and invisible mechanical particles were determined. To detect visible particles in accordance with the SP RUz., Eur.F., 2.9.20, OFS 42 Uz-0006-3341-2018, during visual inspection, the test preparation should not contain visible particles (visible particles should be absent).

To detect invisible particles in accordance with the SP RUz., Eur.F., 2.9.19, OFS 42 Uz-0005-3340-2018, invisible particles per 50 ml and 100 ml bottle for particles ≥ 25 microns should be no more than 600 pieces, for particles≥10 microns should be no more than 6000 pieces (Ev.F., 2.9.19, OFS 42 Uz-0005-3340-2018).

For 200 ml vial:

Particlesize	Number of particles in 1 ml
10 micronsormore	Not more than 25 h/ml
25 micronsormore	Not more than 3 h/ml

All studied five series of the drug corresponded to the established norm in terms of "mechanical inclusions".

The results of studies on the quality indicators of the drug "Bralecord" solution for infusion are presented in table 2.

Table 2: Research results on some quality indicators drug "Bralecord" solution for infusion

The name of indicators	Norms	Series 1	Series 2	Series 3	Series 4	Series 5
Description	Clear, colorless or slightly yellowish solution.	Corresponds	corresponds	corresponds	corresponds	corresponds
Authenticity	SF-metry, qualitative reactions	confirmed	confirmed	confirmed	confirmed	confirmed
Transparency	The drug must be transparent	transparent	transparent	transparent	transparent	transparent
Chroma	The color of the preparation should not be more intense than the reference solution Y_7	no more intense than reference solution Y_7	no more intense than reference solution Y_7	no more intense than reference solution Y_7	no more intense than reference solution Y_7	no more intense than reference solution Y_7
pH	5,0-7,5	6,05	6,10	6,08	6,12	6,02

Foreign matter:						
Citicoline sodium:	no more than 0,5%	no more than 0,5%	no more than 0,5%	no more than 0,5%	no more than 0,5%	no more than 0,5%
Individual impurity total impurity	no more than 2,0%	no more than 2,0%	no more than 2,0%	no more than 2,0%	no more than 2,0%	no more than 2,0%
Levocarnitine admixture A: others: the sum of impurities, except for impurity A:	no more than 1,0% no more than 0,2% no more than0,5%	no more than 1,0% no more than 0,2% no more than0,5%	no more than 1,0% no more than 0,2% no more than0,5%	no more than 1,0% no more than 0,2% no more than0,5%	no more than 1,0% no more than 0,2% no more than0,5%	no more than 1,0% no more than 0,2% no more than0,5%
Substancesdetectedbyninhydrin	Any spot, except for the main one, should not be more intense than the spot on the chromatogram of the standard solution (0.5%)	no more than0,5%	no more than0,5%	no more than0,5%	no more than0,5%	no more than0,5%
Osmolality	410-590 mosmol/l	475mosmol/l	478 mosmol/l	476 mosmol/l	477 mosmol/l	480 mosmol/l
Mechanicalinclusions (visible)	Visible particles must be absent	missing	missing	missing	missing	missing
Mechanicalinclusions (invisible)	One vial for particles ≥ 25 microns should contain no more than 600 pieces; Particles ≥ 10 microns should be no more than 6000 pieces	corresponds	corresponds	corresponds	corresponds	corresponds

III. Conclusion

On the basis of the conducted experimental studies, the main indicators of the quality of the drug "Bralecord" solution for infusions were established in terms of: description, authenticity, transparency, color, pH, impurities, osmolality, mechanical inclusions. The limits of their normalization in accordance with the pharmacopoeias have been established. Approved quality indicators are included in the regulatory documentation for the study drug.

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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.

The Administration Rules

Administration Rules to Be Strictly Followed before Submitting Your Research Paper to Global Journals Inc.

Please read the following rules and regulations carefully before submitting your research paper to Global Journals Inc. to avoid rejection.

Segment draft and final research paper: You have to strictly follow the template of a research paper, failing which your paper may get rejected. You are expected to write each part of the paper wholly on your own. The peer reviewers need to identify your own perspective of the concepts in your own terms. Please do not extract straight from any other source, and do not rephrase someone else's analysis. Do not allow anyone else to proofread your manuscript.

Written material: You may discuss this with your guides and key sources. Do not copy anyone else's paper, even if this is only imitation, otherwise it will be rejected on the grounds of plagiarism, which is illegal. Various methods to avoid plagiarism are strictly applied by us to every paper, and, if found guilty, you may be blacklisted, which could affect your career adversely. To guard yourself and others from possible illegal use, please do not permit anyone to use or even read your paper and file.

Criterion for Grading a Research Paper (Compilation) by Global Journals

Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals.

Topics	Grades		
	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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