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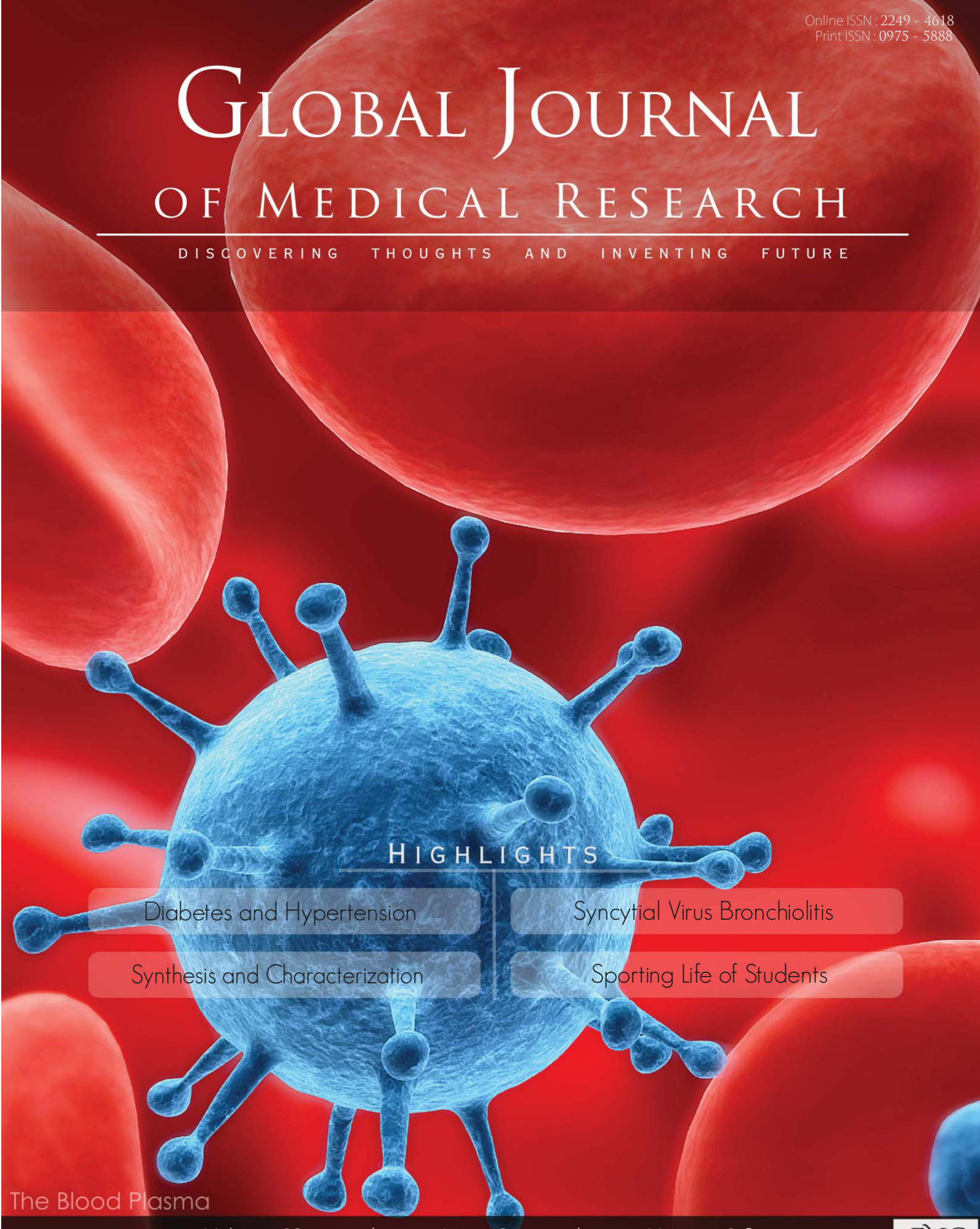

Highlights

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In Vitro and In-Vivo Studies of Tolmetin Release From Natural Gel Base Extracted From Okra Seed (Abelmoschus Esculentus) That Cultivated In Egypt

By S. Abd El Rasoul, Sayed H. Auda & Alaa M. Nafady
Al-Azhar University, Assiut, Egypt

Abstract – Tolmetin is a non-steroidal anti-inflammatory drug commonly used for the treatment of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis and periarticular disorders. In this work, we prepared and evaluated tolmetin release from mucilage extracted from Okra (Abelmoschus esculentus L) as a natural gel base. The in vitro release of tolmetin from natural gel base was studied using Franz diffusion cells with cellophane membrane placed between the donor and the receptor compartments. Possibility of solid state changes of Tolmetin with Okra seed mucilage (OSM) was studied using differential scanning calorimetery (DSC). The anti- inflammatory activity of tolmetin from natural gel base was evaluated using the carrageenan induced rat paw edema method. The results revealed that the in-vitro release of tolmetin from OSM without any additives was about 75 % after 180 minutes. The drug was transformed from solid state to amorphous one indicating that there is a physical interaction between tolmetin and OSM in gel form. At the same time there is no interaction was observed in case of physical mixture. Finally, tolmetin from OSM gel base gave a significant anti-inflammatory activity when compared with reference.

Keywords : *Okra seed, mucilage, Natural Gel base, Tolmetin, In-vitro and In-vivo studies.*

GJMR-E Classification : *NLMC Code: WQ 208*

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S. Abd El Rasoul [α], Sayed H. Auda [σ] & Alaa M. Nafady [ρ]

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Keywords : Okra seed, mucilage, Natural Gel base, Tolmetin, In-vitro and In-vivo studies.

I. Introduction

Tolmetin is a pyrrole, acetic acid derivative, non-steroidal anti-inflammatory drug commonly used for the treatment of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis and periarticular disorders. It inhibits cyclooxygenase activity with a reduction in the tissue production of prostaglandins.[1,2] A topical preparation of tolmetin would allow the administration of tolmetin in those patients who cannot tolerate the drug orally because of its adverse GIT effects. Chemical structure of Tolmetin is shown in figure 1.

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It is well known that transdermal gels are more popular among all topical preparations due to ease of application and better percutaneous absorption than other semisolid dosage forms. Although many researches concerning the topical application of non-steroidal anti-inflammatory drugs are existing in the literature, a few records are available regarding the release study of tolmetin from gel bases.[3]

The synthetic polymers have certain disadvantages such as high cost, toxicity, environmental pollution during synthesis, side effects, less patient compliance.[4] At the same time, the advantages of natural plant based materials include low cost, natural origin, renewable source, environmental-friendly processing, local availability (especially in developing countries), better patient tolerance as well as public acceptance, from edible sources.[5]

Mucilage is soluble hydrophilic polysaccharides and complex polymers of carbohydrate with branched structures.[6] It consists of polyuronides and galacturonides that chemically resemble the pectic compounds; upon hydrolysis, arabinose, galactose, glucose, mannose, xylose and various uronic acids are the most frequently observed components.[7] High concentration of hydroxyl groups in the polysaccharide has high water binding capacity. The complex polysaccharide is a part of dietary fibers which can absorb a large amount of water. It can dissolve and disperse, and can form viscous or gelatinous colloids. [8] Moreover, mucilage is, being used for their binding, thickening, stabilizing and humidifying properties in medicine.[9] Okra seed mucilage (OSM) is extracted from the seeds of Abelmoschus esculentus L. which is a common herbaceous annual occurring weed throughout Egypt.

Up to date, no research articles have dealt with formulation and evaluation of tolmetin from natural gel bases. The aim of this work was to prepare topical gel of tolmetin using natural gel base extracted from seeds of Abelmoschus esculentus. The prepared gel formulation was evaluated for in vitro release study and anti-

inflammatory activity using the carrageenan induced rat paw edema method.

II. Experimental

a) Materials

Tolmetin sodium salt was kindly provided by Minapharm Co. for pharmaceuticals (10th Ramadan, Cairo, Egypt). Standard cellophane membrane of molecular cut of rang = 1200, carrageenan, urethane (Sigma Chem. Co., USA). Male albino rats weighing 90-110 gm (Animal house, Assiut University, Assiut, Egypt). Okra plant (Abelmoschus esculentus L) was cultivated on the Experimental station, Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Assiut, Egypt. A voucher specimen was kept on Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Assiut, Egypt.

b) Extraction of Okra Mucilage

Seeds of Abelmoschus esculentus L. (250 g) was powdered and defatted by soxhlet extraction using petroleum ether as a solvent (1.5 L, five times) at temperature 60-70°C this was repeatedly extracted with stirring using double distilled water and boiled for 5hrs till the complete mucilage was extracted and a slurry was formed. The slurry was cooled and kept in refrigerator overnight for settling protein and fibers so that the undisclosed portion was settled out. The mucilaginous solution was then filtered off and concentrated at 60oC on water bath until the volume reduced to one third of its original volume. Solution was cooled down to room temperature and was poured into thrice the volume of acetone by contentious stirring. The precipitate was washed repeatedly with acetone, absolute ethanol, diethyl ether and petroleum ether and dried at 50°C under vacuum. The dried material was powdered, sieved and kept in desiccators.[10,11,12,13,14]

c) Preparation of Tolmetin Free Acid

Tolmetin free acid was prepared from its sodium salt by dissolving a weighed quantity of the salt in deionized water and precipitating out the free acid with an excess of concentrated hydrochloric acid. The precipitate was then washed with copious amounts of deionized water to remove unreacted hydrochloric acid. The tolmetin free acid was dried under vacuum at 40oC, to a constant weight. The free acid was obtained and then examined by melting point method, UV assay and infrared spectrum.

d) Preparation of Topical Gel

Gel was prepared by cold mechanical method.[15] Briefly, required quantity of OSM (20 % w/w) was weighed and slowly sprinkled on the surface of purified water for 2 hours. After which it was continuously stirred by mechanical stirrer till become completely soaked in water. Triethanolamine was added with continuous stirring to neutralize the gel. Finally tolmetin was added to the natural gel with continuous stirring until the drug completely dispersed in the gel base. The prepared gel base was packed in wide mouth screw capped glass container and kept in dark and cool place.

e) Drug Content Determination

Drug content of medicated gel was determined by dissolving accurately weighed (1gm) of gel in phosphate buffer of pH 5.5. After suitable dilution, absorbance was recorded at 324 nm.[16]

f) In-Vitro Release Studies

The release of tolmetin from gel bases were studied using Franz diffusion cells with cellophane membrane placed between the donor and the receptor compartments. The membrane was soaked in sorensen's buffer overnight and then washed before use. A known amount (1 gm) of gel was added to the donor side and a known volume of Sorensen's buffer was added to the receptor side. The receptor compartments were maintained at 32oC ± 0.5 throughout the experiment. At predetermined time intervals, a sample was removed from the receptor compartments and analyzed spectrophotometrically at 324 nm. The withdrawn samples were replaced immediately with an equal volume of fresh buffer. A control experiment was carried out using gel formulation without drug in order to ascertain any interference in the analysis by either the formulation or the membrane.

g) Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) was recorded using T.A. 501 Differential scanning calorimeter (Shimadzu Co., Japan). Samples of about 5 mg were accurately weighed and encapsulated into flat-bottomed aluminum pans with crimped-on lids. The scanning speed of 10°C/min from 0°C to 200°C was used in presence of nitrogen at flow rate of 40 ml/min.

h) Preparation of Samples for DSC

The prepared plain and medicated gel base (5%) were dried in a desiccator for one week. After complete dryness, the co-precipitate was scratched, powdered and stored in capped amber-glass containers until use. For preparation of physical mixture, Samples having the same composition of the medicated gel base were prepared by simply mixing the triturated powdered drug and the tested mucilage powder in a porcelain mortar. The mixtures were then sieved, and the particles below 420 μm were collected and stored in capped amber-glass containers until use. The samples were analyzed within one week after their preparation.

i) Anti-Inflammatory Effects of OSM Gel Containing Tolmetin on Carrageenan-Induced Paw Edema

The experiment was conducted on male albino rates weighing 90-110 g divided into four groups each group consisting of 5 rats. Group 1: control, injected

with carrageenan.Group 2: received plain natural gel base (without drug).Group 3: received medicated natural gel base (5%). Group 4: received commercial indomethacin synthetic gel base (Indotopic®, Ramida Pharmaceuticals Co. Cairo, Egypt). Paw edema was induced by subcutaneous injection of 0.1 ml of 1% carrageenan in physiological solution in the plantar surface of rat hind paw.[17] The rats were fasted for 16 hrs before the experiment with free access to water. The rats were anaesthetized by urethane (0.5 ml intraperitoneal) then 100 mg of each topical preparation were applied to the right hind of the rat. The thickness of rat hind was determined before and immediately after injection of carrageenan. Subsequent measurements for resulted edema were carried out at 0.5, 1, 2, 3, 4, 5 and 6 hrs after induction of edema. The anti-inflammatory effect was expressed as percentage inhibition of edema thickness compared with control according to the following equation; % inhibition of edema = $(T_o - T_t) / T_o$ x 100. Where To is the edema thickness in control group, T_t is the edema thickness in treated group.[18,19]

III. Results and Discussion

a) *In-Vitro Release Studies*

The in vitro release study of tolmetin from natural gel base was investigated in table (1) and graphically represented in figure (1). The results indicated that tolmetin released freely from natural gel base without any addition of penetration enhancers (about 75% after 3 hrs). This suggests that tolmetin free acid forms a hydrophobic complex with TEA, whereas, the OSM is hydrophilic. Hence, an increase in the aqueous content of the gels may have possibly increased the escape tendency of the relatively hydrophobic drug-TEA complex.[3]

b) *Differential Scanning Calorimetry DSC*

In order to shed a light on the possibility of solid state changes of tolmetin free acid, when dispersed in mucilage gel base extracted from Okra, DSC were performed on 5% medicated gel after complete dryness, its corresponding physical mixture and plain gel base after its dryness as well as the individual solid components. The DSC scan of untreated tolmetin free acid showed an endothermic peak (Figure 3A), at 160°C which is corresponding to tolmetin free acid melting point. DSC tracing of OSM in solid powdr and gel state exhibited a broad shallow peak at about 78°C (Figure 3B and C). Concerning the corresponding physical mixture (Figure 3D), characteristic endothermic peak of tolmetin were seen again at 158 °C but with low intensities. Upon scanning the DSC thermogram of tolmetin - OSM 5%, a complete disappearance of the drug fusion peaks (Figure 3E), suggesting a homogeneous dissolution of the drug in the polymer matrix.[20,21] Any abrupt or drastic change in the thermal behavior of rather the drug or excepient may indicate a possible drug-polymer interaction.[22] The endothermic peak of pure drug was observed at about 160° (figure 3A). However in the thermogram of the medicated gel there was no endothermic peak of the drug melting, suggesting the amorphous state of the drug in the microparticles. Pignatello et al.[23] studied the thermal behavior of Diflunisal-Eudragit RS100 and Eudragit RL100 coprecipitates (1:5). They found that a blend of the drug with polymers resulted in the disappearance of such a fusion peak, replaced by broad endothermic signals exhibiting a reduced melting endotherm and a lowering of the peak temperatures. These findings suggest that Diflunisal is able to dissolve in the polymer to a certain degree to form a solid solution.

c) *Anti-Inflammatory Activity*

The effect of topical formulation on the anti-inflammatory activity of the drug was studied. The percent inhibition of carrageenan induced edema by 5% tolmetin in natural gel base and commercial indomethacin synthetic gel base is graphically represented in Fig. (4). the edema swelling was inhibited in all groups of rats either treated with the medicated natural gel base or in the group received topical indomethacin as standard drug. Three hours after carrageenan injection the percent edema inhibition was 43.30 and 48.70 % for medicated natural gel base and standard indomethacin respectively. The slight increased inhibition effect in case of reference group may be attributed to the incorporation of penetration enhancers. The group treated with natural gel base showed the highest inhibitory effect at 5 hrs after injection of carrageenan (percentage of edema inhibition was 58.59%). These results were nearly equal to inhibitory effect that obtained in group treated with commercial indomethacin gel as a reference (percentage of edema inhibition was 64.04 %). The results of this study show the possibility of commercial formulating tolmetin in natural gel topical formulation, which can produce a desirable local anti-inflammatory effect. It has been reported that topical application of NSAID containing formulations in animals can markedly attenuate inflammation pain and related behaviors.[24,25,26]

IV. Conclusion

Tolmetin gel is prepared by using Okra seed mucilage (OSM) as gel base. The prepared gels are evaluated for in vitro release, possibility of interaction between the drug and base and the anti-inflammatory activity. From the results, it has been observed that, the in-vitro release of tolmetin from OSM without any additives was 75 % after 180 minutes. The drug was transformed from solid state to amorphous one indicating that there is a physical interaction between tolmetin and OSM in gel form. At the same time there is no interaction was observed in case of physical mixture.

Tolmetin from OSM gel base gave a significant anti-inflammatory activity when compared with reference. It can be concluded that tolmetin-natural mucilage gel for use as an anti-inflammatory dosage form is possible and may be applicable in future.

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Table (1) : Release data of tolmetin from OM gel base in phosphate buffer of pH 5.5 at 32°C.

Time (minutes)	Percentage of tolmetin released from natural gel base (± SD)
15	18.68 ± 2.90
30	22.50 ± 1.91
45	34.27 ± 1.97
60	41.25 ± 2.00
90	52.15 ± 2.03
120	61.67 ± 2.97
150	68.88 ± 3.31
180	75.02 ± 2.58

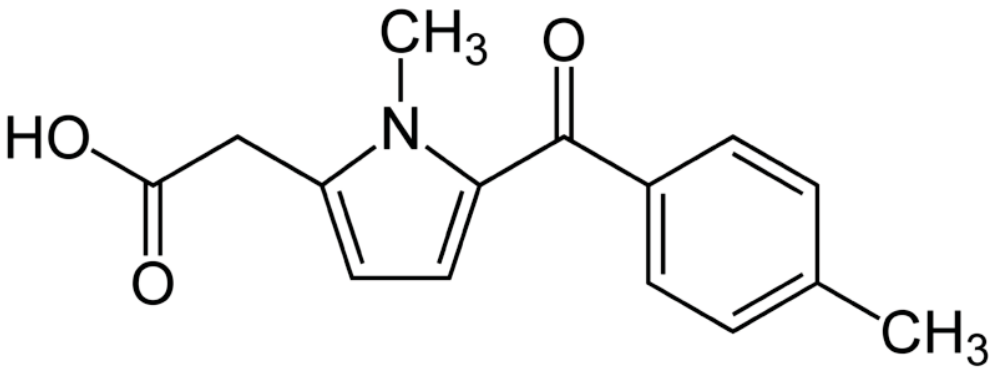

Figure 1. Tolmetin free acid

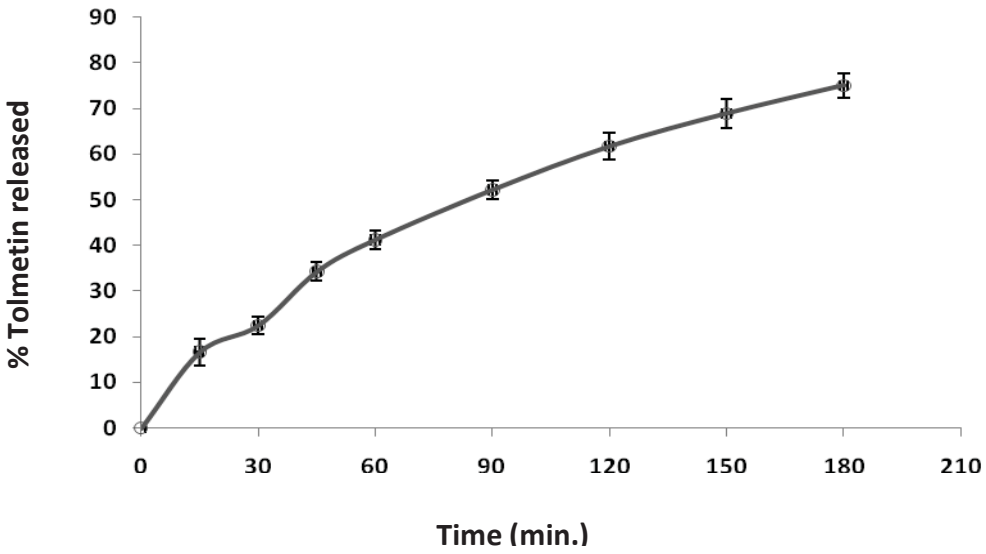

Figure (2) : Release profile of tolmetin from natural gel base

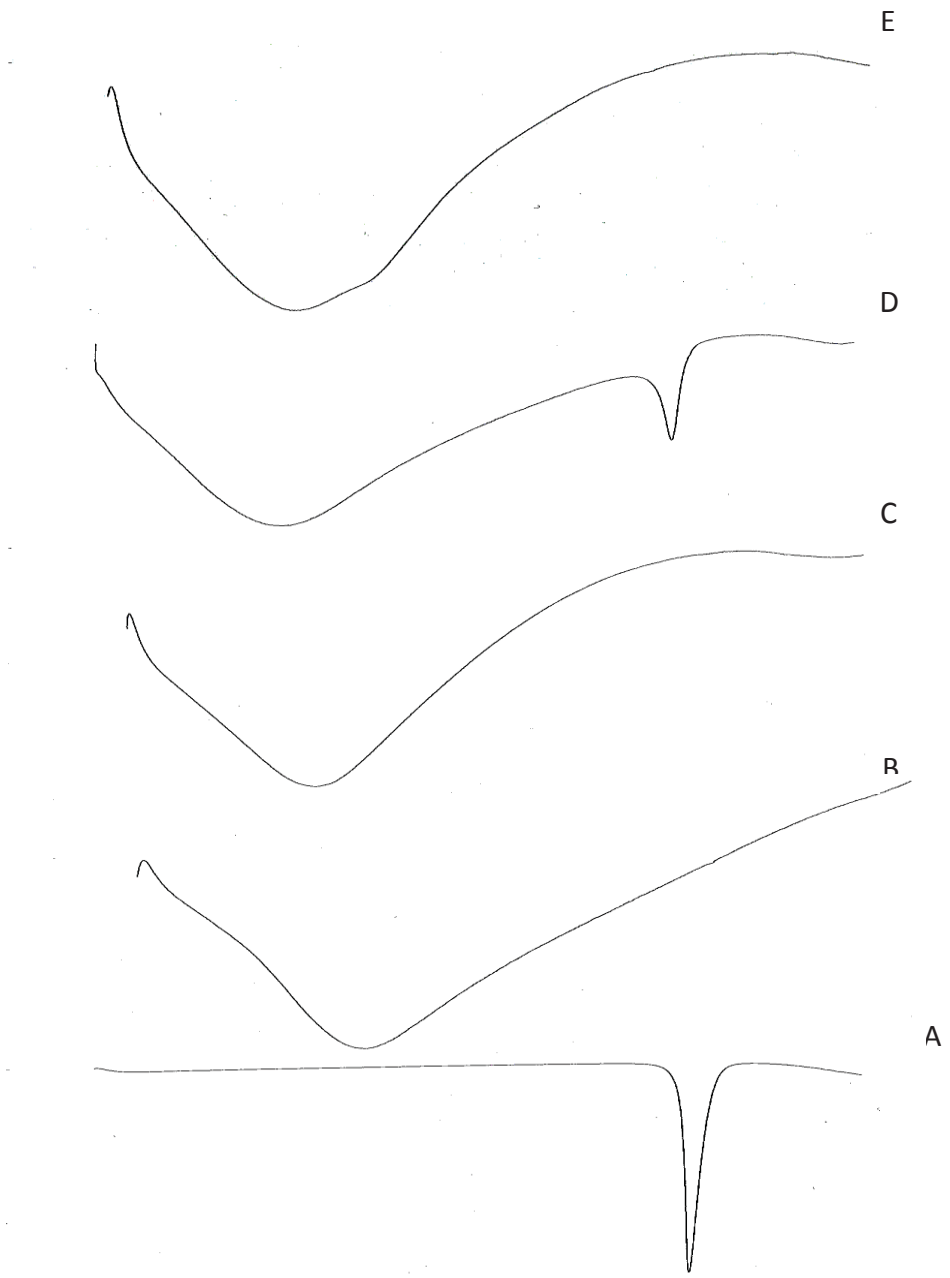

Figure 2: DSC spectra of A) untreated tolmetin, B) powdered OM, C) dried gel of plain OM, D) tolmetin –OM 5% physical mixture and E) tolmetin –OM 5% gel.

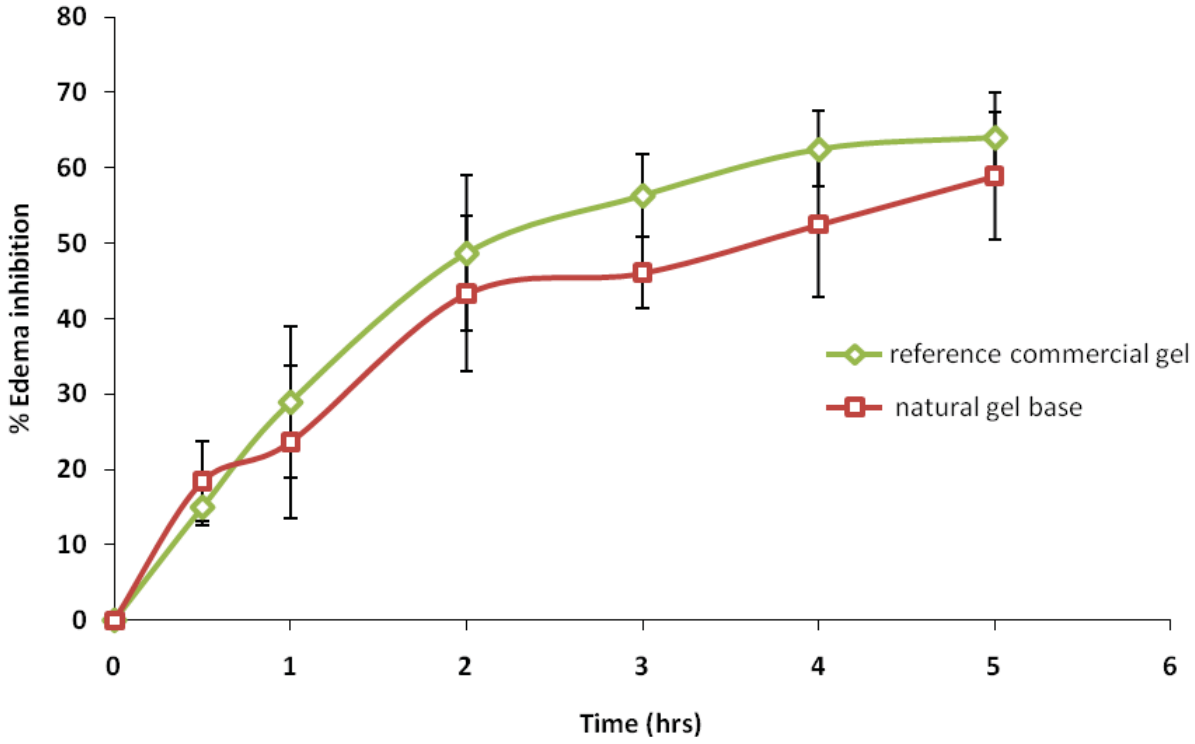

Fig : Effect of Tolmetin gel formulation of natural origin on the inhibition of rat hind paw edema induced by carrageenan.

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Professionalization of Sports Management: Antidote to Dwindling Fortunes in Nigerian Sports

By E.J. Jeroh
Delta State University Abraka, Delta State, Nigeria

Abstract – The paper sees management as the greatest challenge confronting Nigerian sports. Sports management is in the hands of non-professionals who know little or nothing about sports. This has led to the dismal performances of national teams in world sports. A way out of this imbroglio is to professionalize sports management and the Nigeria Association for Physical, Health Education, Recreation, Sports and Dance (NAPHER-SD) as a professional sports body is called upon to play a dominant role in this regard. It is recommended that Nigerian universities should mount post – graduate programmes in physical education to train well baked professionals in all areas of sports so as to complement the efforts of The National Institute for Sports in Lagos.

Keywords : *All–comers, Benchmark, Fire brigade approach, Specialized knowledge, Undeniable conclusion.*

GJMR-E Classification : *NLMC Code: QT 261,QV 601, QT 255*

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Professionalization of Sports Management: Antidote to Dwindling Fortunes in Nigerian Sports

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Abstract - The paper sees management as the greatest challenge confronting Nigerian sports. Sports management is in the hands of non-professionals who know little or nothing about sports. This has led to the dismal performances of national teams in world sports. A way out of this imbroglio is to professionalize sports management and the Nigeria Association for Physical, Health Education, Recreation, Sports and Dance (NAPHER-SD) as a professional sports body is called upon to play a dominant role in this regard. It is recommended that Nigerian universities should mount post – graduate programmes in physical education to train well baked professionals in all areas of sports so as to complement the efforts of The National Institute for Sports in Lagos.

Keywords : All–comers, Benchmark, Fire brigade approach, Specialized knowledge, Undeniable conclusion.

I. Introduction

Events across the globe especially in business circles have underscored the need for changes in tactics and management approach to survive in the 21st century. Change, according to Fasan (2002), has become a prevailing culture which every organization, be it private or public cannot underestimate. The philosophy behind change hinges on the fact that managerial approaches and strategies of the 20th century may not be able to sustain the trends in the 21st century. Sport is no exception.

Management of sports in Nigeria has become an all comers affair. While engineers are allowed to manage and direct engineering companies and institutions, doctors to direct and manage the medical institutions, everybody has been allowed to manage sports and sporting institutions (Abdulkadir, 2002). This clearly shows why Nigeria sports is in shambles. The sports environment is becoming polarized and some of the operators do not have the prerequisites to do the job but they merely learn on the job. Some have even been appointed on benevolence.

The desire to professionalize sports management has become a welcome idea following the trauma Nigerian sports has brought to stakeholders. A cursory look at the Nigerian Olympic Committee in time past revealed that physical educators were in the Committee, but now it will be a surprise if any is a member. What goes on in the NOC is replicated in the various Sports Associations or Federations.

Traditionally, the physical education profession particularly in Nigeria was viewed basically as that of teaching responsibilities (Mgbor, 2002). Other professionals in the field like Parks and Zanger (1990) have, however, identified other occupational roles of the physical education personnel to include those of sports coaches, sports marketers, sports programme manager, sports journalists, fitness programme manager, recreation services directors etc.

Before progressing in the discourse, there is the need to know the meanings of the terms "sports management" and "profession."

a) Sports Management

Fasan (2002) sees sports management as an academic discipline, a field of endeavour which encompasses other disciplines utilized to bring sports programmes nearer to the people, provide heroic healthy struggle, graceful balance and harmony. It can also be seen as the application of management theories and techniques to sports programmes, programming and execution.

Desensi and Rosenberg (1996) see sports management as a field interested in the organization and administration of specific sports – related areas. It is a field of endeavour involving leadership, decision making process, staging of athletic events and marketing sports. Chelladurai (1983) sees sports management as the activities of personnel in an organisation from different disciplines working with limited resources with the aim of accomplishing the goals of the organisation.

Udoh (2002) sees many specialized areas in sports management and goes on to suggest that the professional in sports management in Nigeria, apart from the basic educational qualification should be exposed to series of training courses, seminars and workshops in the various areas of the sports enterprise after the initial training. To him, a bachelor degree in sports science (physical education) is a pre – requisite, a necessary platform from where to start on a life long training and experiences on how to manage sports.

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Understanding the concept of sports management via its multifarious disciplines is a guide towards professionalism (Fasan, 2002).

b) Profession Defined

There are many definitions of the word "profession." A thorough examination of the various definitions clearly reveals almost the same idea(s) (Ladani, 2002). Hodge and Johnson (1970) define a profession as "a vocation requiring some significant body of knowledge that is applied with high degree of consistency in the service of a segment of society." Frost (1975) posits that a profession is "a calling or occupation requiring specialized knowledge, intensive education, a philosophy of commitment and service and high standards of conduct and achievement." Cratty (1971) identifies different criteria to define profession as follows:

a) A profession provides a service to improve the capabilities of members;
b) Membership requires academic credentials of rather high level;
c) Professions constantly seek to improve the capabilities of their members and continually engage in self – examination and evaluation; and
d) Practices carried out within a profession are based upon a valid body of knowledge which has been established as a result of a scientifically sound research programme.

Having perused the meanings of profession, there will also be the need to know the characteristics of a profession. According to Ladani (2002) the nature of a profession is quite different from that of a trade union, business concern or entertainment group. A true profession has certain characteristics as propounded by Hodge and Johnson (1970), Singer (1976), Baley and Field (1976), Resick and Seidel (1978) to include:

(a) A rigorous and lengthy period of educational preparation;
(b) A relevant body of unified knowledge;
(c) Mandatory qualifying examination;
(d) A code of ethics with potential elimination from practice for those violating the code;
(e) A life career and permanent membership;
(f) A profession sets up its own standard;
(g) A profession exalts service above all personal gain;
(h) A strong closely knit and cohesive professional organisation;
(i) Members accept social responsibility in the society served by the profession;
(j) A profession demands continuation in – service growth.

In some well known professions like law, architecture, accountancy, medicine or even in the armed forces, there are prescribed lists of dos and dont's which serve as guidelines to the practitioners and which must be strictly adhered to before a member is regarded as belonging to the particular profession (Udoh, 2002). The implication of a profession here is that there is an occupation or vocation which demands special education programme or training. Thus a professional is an individual who is highly trained and makes a living by the practice of that for which he has been trained. In sports such a person could be an athlete, performing in the sport he specializes in, a coach with expertise in the sport he coaches, or an individual who has specialized training in sports management; a term which is the core of this discourse.

Using the above characteristics as benchmark to assess our sports management, the undeniable conclusion is that Nigeria has not started and except there are concerted efforts by stakeholders in sports, the situation will continue to be bleak for us in the realm of success in our sports endeavour. The United States cannot be regarded as a failure in world sports because sports management is a recognized profession and its services according to Fasan (2002) are requested for in sports and other allied business. The schools, the media houses, fitness centres, clubs, recreational centres, private organizations, marketers, sporting goods manufacturing companies need them. This, however, is not the case in Nigeria because our career orientation and outlet are limited.

Sports have many divisions such as archery, athletics, basketball, power sports, baseball and other ball games, gymnastics, equestrian sports, aquatic sports, cycling, motor bike sports, motor racing and miscellaneous sports including traditional sports. Each of the sports or class of sports has its own peculiar nature, characteristics, body of knowledge, facilities, equipment, rules and regulations which a new entrant must know and understand to succeed.

II. Rationale For Professionalization of Sport Management In Nigeria

Nigeria has of recent failed woefully in sports. In the just concluded All Africa Games in Maputo our performance was dismal. Because soccer is about the most popular sport in the country, Nigerians cry out anytime we fail to qualify for any major international competition. A case in point is the 2012 Olympic qualifier in soccer for our U – 23 team in Morocco. The performance expectation of sports operators is very high and anytime we fail the blame is on the coach(es) as if other sports are performing magic. The sickness is Nigerian soccer is contagious with other sports. The major problem with Nigerian sports is management.

While it takes some countries like the United States and Germany four or more years to prepare for any major competition like the Olympics, Nigeria will

normally wait for one month to the competition before embarking on a fire brigade approach and we are expected to perform miracles. Adequate preparation is a desideratum if we are to achieve appreciable results. Any sport manager worth his salt should be able to plan, direct, organize, budget, delegate duties, control, report, co-ordinate, motivate and innovate as the case may be. These are all duties of a professional manager.

Scientific and technological advancement have compartmentalized sports into many areas of specialization thus creating a myriad of professional positions. For anybody to specialize in sports administration and management, therefore, he or she must among other things study the nature of the particular sport and know very well their body of knowledge as well as the practice of the sport (Ajiduah, 2002). He concludes that sport is so specific that it may be necessary to study separately the administration and management of football, swimming, basketball, tennis, etc because their characteristics and nature are quite different. This is not so in Nigeria where enthusiasts are appointed to run sports and until we professionalize sports management sport will continue to nosedive in the country.

III. Conclusion

Each of the positions of sports operators (managers) from the Honourable Minister/Commissioners for Sport through Chairmen of Sports Federations down to organizing secretaries and coaches is a profession under the Nigeria Association for Physical, Health Education, Recreation, Sports and Dance (NAPHER-SD) but unfortunately most of these positions are not occupied by NAPHER-SD professionals. The specific functions in each of the positions may be different but the main characteristic of these professional practices is that they manage people, materials and funds. Each of these operators is supposed to be well grounded in a body of knowledge from the academic discipline of sports administration and management.

We have seen that the all – comers and fire brigade approaches to sport in Nigeria have not yielded good dividends. Like some other well known professions like law, medicine and engineering, physical educators through NAPHER – SD need to rekindle the flame of sports management by being involved and their success will definitely remove sports administration and management from the hands of enthusiasts and opportunists. One needs to train to acquire the skills of competence from a body of knowledge that will make him / her a success in his / her chosen field of human endeavour. Professionalizing sports management will remove obscurity which physical education is currently suffering from and bring practitioners to prominence.

IV. Recommendations

To professionalize sport management in Nigeria, physical education has a great role to play. The pedagogical nature of the subject as it is now in our universities and colleges of education need radical changes. The curriculum has to be redesigned to put in practice the multi – faceted nature of sports. The universities through their departments of physical and health education need to mount post – graduate programmes in specific areas of physical education tilted towards sports management to compliment what goes on at the National Institute for Sports in Lagos.

For now, there should be a gradual replacement of non professionals from managing sports. Alternatively they should be sponsored to study courses that will make their services relevant to sports.

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Knowledge of Disease and Adherence to Drug Therapy in Persons with Type 2 Diabetes and Hypertension

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Abstract – This study was carried out to determine the level of knowledge of disease and adherence to drug therapy among patients with Type 2 diabetes and Hypertension. One hundred and seventy-seven (177) patients attending cardiology and endocrinology clinics at University College Hospital (UCH) Ibadan, in Nigeria participated in the study. Socio-demographic characteristics, patients' knowledge of diabetes and hypertension and adherence to drug therapy were determined with the use of pre-tested questionnaires. Anthropometric measurements and blood pressure were taken with fasting blood glucose.

Exactly 45.2% of the study population were males and 54.8% were females. Patients who had Type 2 diabetes alone were 23.0% and 20.3% had hypertension alone while 55.9% had type 2 diabetes with hypertension combined. The mean age was 63.2 ± 10.7 years. Exactly 64.0% of the study population had good knowledge of diabetes and hypertension while 58.8% were adherent to drug therapy by self-reported methods. Blood pressure control was poor among patients who had hypertension as only 42.9% of hypertensives had a systolic blood pressure of less than 140mmHg and diastolic blood pressure of less than 90mmHg. In patients with Type 2 diabetes combined with hypertension, blood pressure control was poor as only 41.9% had a systolic blood pressure of < 140mmHg and a diastolic blood pressure of < 90mmHg. Exactly 36.0% of patients with Type 2 diabetes alone and 40% of patients with type 2 diabetes with hypertension combined had good glycemic control. Descriptive statistics, Chi-square tests and Pearson correlation were used in evaluating the data obtained.

Knowledge of diabetes and hypertension among these patients was above average however adherence to drug therapy was poor. This calls for increase in awareness the importance of adherence to drug therapy among patients with type 2 diabetes complicated with hypertension which will involve a concerted effort by all members of the healthcare team.

Keywords : *Type 2 Diabetes mellitus, Hypertension, Adherence, Knowledge Running title: Knowledge of Disease by Diabetics and Hypertensives.*

GJMR-E Classification : *NLMC Code: WK 815, WK 820, WK 835,WG 106, WG 340*

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I. Introduction

Diabetes and Hypertension are chronic illness which requires a life-long management. Hypertension is common in patients with type 2 diabetes with a prevalence of 40-60% over the age range of 45-75 (Turner et al, 1998). The interrelationship of the dual diagnosis of hypertension and diabetes is significant with diabetes diagnosed 2.5 times more in hypertensive patients (Grass TW et al, 2000). The incorporation of the patient in the management of his disease condition is very vital in the management of persons with Diabetes and Hypertension because the management of such chronic illnesses, the likelihood for non-adherence to medication may increase in patient. Some patients are not aware of the chronic nature of their conditions and therefore believe a short term treatment will totally cure them of the disease. This has led to abrupt discontinuation of medications among patients resulting in an exacerbation of their conditions. (Diabetes control and complications Trial Research group 1993)

Consistent control of blood pressure, consistent control of blood glucose, adherence to medication and dietary regiments are very important in patients with hypertension and diabetes mellitus (Haffner SM, et al 1998, Stern MP 1998). Patients' poor understanding of the disease, poor understanding of proper use of the medications as well as the benefits and risks of treatment have been identified as some of the patient-related barriers to adherence (Osterberg and Blaschke, 2005). Adequate knowledge of the disease and of the benefit and risk of treatment will therefore be required in the management of patients with chronic conditions such as diabetes combined with hypertension.

Patients with type 2 diabetes and hypertension see the pharmacists often. The pharmacists are therefore in a good position to have a significant impact on the quality of care of such patients by providing adequate counselling about the disease conditions and the medication used in their management. (Brian Cross 2006, Stephen M Setter, et al 2006)

The main objective of this study is therefore to determine the level of knowledge of patients with hypertension complicated with type 2 diabetes about their disease condition and the level of their adherence

to recommended drug therapy with the goal of providing and promoting pharmaceutical care.

II. Patients and Methods

This study was carried out among patients attending the endocrine and cardiology clinics of the University College Hospital, Ibadan. One hundred and seventy –seven (177) patients comprising patients with type 2 diabetes alone, patients with hypertension alone, and patients with type 2 diabetes coexisting with hypertension were involved in the study.

The study was carried out within a period of nine weeks of between August 4th to October 5th 2010. Informed consent was obtained from all patients with structured questionnaires covering demographic data, duration of disease diagnosis, disease knowledge and self-reported medication adherence.

Type 1 diabetes patients, pregnant patients, immune-compromised patients and mentally retarded patients were excluded from the study. Stratified random sampling was used in sampling population for the study. Stratification was based on sex and both sexes were fairly represented in the sample population.

The study was cross-sectional and consisted of a well structured questionnaire which was interviewed-administered. The study was carried out every Monday and early before the usual clinic time of 10.00am. The interviewers were research assistants recruited and trained for this purpose. The non- English speaking patients were interviewed by the interviewers who interpreted the contents of the questionnaires into local yoruba language.

The questionnaire consisted of seventeen relevant knowledge questions, eleven of which were strictly on hypertension, five on diabetes and one on both diabetes and hypertension. These questions were drawn from standard knowledge test on diabetes and hypertension. The median score (50th percentile) which was eleven was chosen as the cut-off value. Patients with eleven points and above had good knowledge while patients with scores below eleven points had poor knowledge.

The data obtained from each questionnaire were entered using Epi info. Analysis was done using the Statistical Package for Social Sciences (SPSS) Version XV (15). Results were presented in frequencies, percentages, means and standard deviations. Two categorical variables were compared using the Chi-square test and two unrelated variables were compared using Pearson correlation. Statistical significance was decided at the 5% level ($p<0.05$).

Ethical clearance for this study was obtained from the University of Ibadan/ University College Hospital (UI/UCH) Ethical Committee of the Institute of Advanced Medical Research and Training with Assigned Number UI/EC/10/0119.

III. Results

A total of one hundred and seventy-seven (177) patients who met the inclusion criteria were included in the study. Eighty (80) (45.2%) of the patients were males while 97 (54.8%) were females. Exactly 24% of these patients had type 2 diabetes alone, 20% had hypertension alone and 56% had type 2 diabetes co-existing with hypertension. The age ranges from 33years to 87 years with a mean of 63.2years. Most of the patients studied were traders (35.6%). Others were Civil servants (13.6%), Retired (10.2%), Businessmen (6.8%), Artisans (5.6%), Professionals (3.4%), Clergy (3.4%) with 6.2% being unemployed while 15.3% did not specify their occupation. Exactly 27.1% had no formal education, 28.2% had primary education, 14.1 % had secondary education, and 6.2 % had vocational education while 24.1 % had tertiary education. Majority of the study population (80.8%) were married while 19.2% were either widowed or divorced.

The details of the socio-demographic characteristics of the study population are presented in Table 1. Seventeen questions were asked to test patient's knowledge on diabetes mellitus and hypertension. Exactly 63 (35.6%) patients knew their blood pressure within five days prior to clinic visit and 65 (36.7%) patients knew the optimum blood pressure for patients with both diabetes and hypertension while 140 (79.1%) knew high blood pressure could cause heart attack. Exactly 161(91%) knew high blood pressure could cause stroke and 107 (60.1%) patients knew that diabetes could cause kidney failure while 98 (55.4%) knew that high blood pressure could cause kidney failure. Exactly 119 (67.2%) believed a blood pressure of 140/90mmHg was normal while 132 (74.6%) believed a blood pressure of 160/90mmHg was high. Questions asked and responses are summarized in the Table 2.

The level of knowledge on diabetes and hypertension was determined from relevant questions with a maximum of 17 points and the median (representing the 50th percentile, 11points) was used as the cut-off point to categorise knowledge as already described in the methodology section. A total of 112 (63.3%) had good knowledge while 65(36.7%) had poor knowledge. This is summarised in Table 3.

Males in this study had more basic formal education than females as 16.3% of the males had no formal education while the percentage of those without formal education among the females (36.1%) double that of males (16.3%). Exactly 31.3% males had a tertiary education while this was only 18.6% in females. A statistically significant association exists between sex and education among the study population ($p<0.05$). This is shown on Table 4.

Patients who failed to take medication on purpose gave various reasons why they did so. Exactly 10.2% said they did so when their medication finished,

6.1% claimed financial constraint as reason why they deliberately discontinued medications, 6.1% claimed forgetfulness; others failed to adhere when fasting (2%), when busy (2%), when sick of fever (4.1%), when they travelled (4.1) , inconvenience of the doses (4.1%), based on faith (2%), polypharmacy (2%), felt better (2%) while greater proportion (55.1%) did not give any response when asked why they failed take medication on purpose. These are summarized on Table 5

Questions on adherence were used to assess patients' adherence to drug therapy. Patient with 80% scores and above were regarded as adherent to drug therapy. Table 6 summarizes the degree of adherence among the study population.

Adherence was determined among the different disease groups. Exactly 54.8% of patients with type 2 diabetes alone reported adherence to medications, 69.4% of those with hypertension alone were adherent and 56.6% of patients with both diabetes and hypertension combined reported adherence to drug therapy. The association between adherence and disease type was however not statistically significant ($p<0.05$). This is summarized on Table 7

IV. Discussion

Older adults who are above 45 years of age are more affected by hypertension than the younger adults below the age of 45 years (Brian Cross et al 2006). Diabetes in people older than 20 years account for 90%,while diabetes in people below 20 years account for only 10% of all cases. Half (50%) of all cases of diabetes occur in adults over the age of 55 and approximately 55 years. About 18% of the older population who are above 60 years have diabetes (Stephen M. Setter et al 2006). The inter relationship of the dual diagnosis of hypertension and diabetes is significant with diabetes being diagnosed 2.5 times more often in hypertensive patients (Stephen M Setter et al 2006). The prevalence of the metabolic syndrome which combines hypertension and diabetes is highly age dependent. The disease is more common in older patients above the age of 50 years and the prevalence of this metabolic disease increases with age (Chobanian et al 2003). One of this condition predisposes to other (Turner et al 1998, Grass TW et al 2000).This study clearly indicated that hypertension alone is a disease of the older adults and diabetes is also more pronounced in older adults than younger adults. It follows that hypertension and diabetes combined is a disease of older adults above the age of 50 years. The result from this study was in agreement with above findings in that among the patients 18 (10.2%) with age group less than 50 years, 7 (38.9%) had diabetes alone, 3 (16.7%) had hypertension alone and 8 (44.4%) had hypertension combined with type 2 diabetes. There were less percentage of patients with hypertension and type 2 diabetes as separate disease when compared with patients that combine hypertension and diabetes. The result also showed that 19 (54.3%) of patients between 50 and 59 years had hypertension compared with diabetes while only 9 (25.7%) and 7 (20.0%) had diabetes and hypertension alone respectively. Among the age of between 60 and 69 years, there were more patients 46 (61.3%) with hypertension combined with diabetes when compared with 14(18.7%) and 15(20.0%) of those having diabetes alone and hypertension alone respectively. (Table1).

Among patients who were above the age of 70 years, 26 (53.1%) had combined disease of hypertension and type 2 diabetes while 12 (24.5%) and 11 (22.4%) patients had diseases separately and respectively.The result also indicated that irrespective of other socio-demographic characteristics such as sex, occupation, educational level and marital status, there were more patients with hypertension and diabetes combined than patients with type 2 diabetes alone and hypertension alone (Table1).

Table 2 indicates that majority of the studied population 101 (57.1%) did not know their blood pressure within the last five days of this study. However, the majority of the studied population were well aware of the complications such as heart attack, stroke and kidney failure that could result in patients having diabetes complicated by hypertension while majority of the population were not aware of the fact that cancer could result from either hypertension and diabetes. The awareness of majority 148 (83.6%) that patients with hypertension and diabetes should take their medicine could be due to universal education provided through the Joint National Committee (JNC) on the Detection, Evaluation and Treatment of high blood pressure in their four (4) reports comprising JNC IV (1992), JNC V (1996), JNC VI (2000) and JNC VII (2006) (Robert T. Weibert (1992), Robert T. Weibert (1996), Robert T. Weibert (2000) and L. Brain Cross 2006). The JNC reports set forth recommendations to help health care providers improve the assessment and management of patients with hypertension and its complications which includes diabetes. Awareness of hypertension has improved from 50% during the period 1976-1980 to 70% during the period 1999-2000. Likewise, the percentage of hypertensive patients receiving therapy and the percentage of those receiving therapy actually reaching recommended BP goals have increased from 31% to 59% and 10% to 34%, respectively, during the same time period. Death from stoke and coronary heart disease (CHD) has decreased by approximately 50% since 1972. These numbers represent significant improvements resulting from increased public and medical community awareness (Brian Cross, 2006). Education provided by the Diabetes Control and Complication Trial (DCCT, 1998) along with twenty years United Kingdom Prospective Study (UKPDS) is also

beneficial as it confirmed the effect of the benefit of strict glycemic control in patients with type 2 diabetes and its complications which includes hypertension. UKPDS thus provides additional education to help health care professionals to treat diabetes and hypertension. Results of the two landmark studies have shown that there was a 41% reduction in risk of macrovascular diseases according to DCCT research group in 1998.The following results according to UKPDS showed 25% reduction in macrovascular diseases with intensive blood glucose control with sulfonyl urea and insulin, 37% reduction in macrovascular disease with tight blood pressure control (<150/85mmHg) in hypertensive patients. Atenolol (betablocker) and captopril (ACE inhibitor) in risk reduction of microvascular and macrovascular complications showed that both agents (atenolol and captopril) are equally effective in maintaining blood glucose control and that their was no difference in risk of macrovascular and microvascular diseases between atenolol and captopril (4 reports of UKPDS group,1998, Davis M, Mellus H et al 1999).

Table 3 shows the levels of knowledge of diabetes and hypertension across socio-demographics of the studied population. According to this table, the level of good knowledge of diabetes and hypertension was higher in males 59 (73.8%) than females 53 (54.6%).The association between general knowledge and sex was statistically significant ($p=0.009$) ($p<0.05$). Knowledge was evenly distributed across the age group as over 60% of the studied population had good knowledge of diabetes and hypertension. Studies (Aviles et al, 2007, Sanne et al, 2008) had shown that being younger was not a factor in having good knowledge of diabetes and hypertension. However, there was no significant association between knowledge and age ($p=0.991$) ($p>0.05$).

Knowledge of diabetes and hypertension generally increased across levels of education with patients with no formal education having lesser knowledge than those with primary, secondary and tertiary education. This result depicted the fact that patients with higher education are more knowledgeable about their disease conditions (Sanne et al, 2008). There was a significant association between educational level and knowledge ($p=0.00$) ($p<0.05$).

Over 90% of patients who are civil servants and retired had good knowledge of hypertension and diabetes.Patients who are civil servants and those retired are likely to be most educated among the studied population. There was a significant association between occupations and knowledge ($p=0.007$) ($p<0.05$) (Table 3).

A study (Nisar et al, 2008) indicated that males were more knowledgeable than females on diabetes in contrast to another study on hypertension (Busari et al, 2010) where females were found to be more knowledgeable than males. This study showed that males were more knowledgeable in the combination of hypertension and diabetes as a disease probably because males were more educated than females in the studied population. There were 13 (16.4%) males with no formal education in comparison with 35 (36.1%) females with no formal education. The association between gender and educational level is statistically significant ($p=0.04$) ($p<0.05$) (Table 4).

A little under 60% of the study population were adherent to drug therapy as measured by self-reported methods. This was very low. Patients who missed doses of their medications gave various reasons for doing so, with 27.7% of the study population missed their medications on purpose, 24.9% did so when they forgot, 8.5% and 6.8% missing their medication when they felt worse or better respectively. Forgetfulness was the major singular reason why medications were missed and it has been implicated as one of the major reasons why doses of medications are missed in patients with type 2 diabetes (Adisa et al, 2009) and hypertension (Omole et al, 2008; Al-Mehza et al, 2009). Among patients who gave reasons for deliberately missing doses of their medications, those who stopped medication when drugs were exhausted comprised a greater percentage. Other reasons given were financial constraints, when fasting, inconvenience of doses, poly-pharmacy and some also 'used faith'. (Table 5)

Age groups 60-69years and 70 years and above had the highest percentage (65.3%) of patients who were adherent respectively each and there is a statistical significance association between self-reported medication adherence and age group ($p=0.037$) ($p<0.05$) (Table 6) The higher degree of medication adherence in the older age groups could be explained by the fact that patients in this age groups comprised 72.8% of those with diabetes co-existing with hypertension and have learnt the importance of using their medication overtime; This is contrary to report from another study which reported high level of medication non-adherence among the elderly (Sweileh et al, 2005). Patients with hypertension alone had adherence rate of 69.4% which is higher than adherence in patients with diabetes alone and in patients with diabetes and hypertension combined. This reported rate of medication adherence in patients with hypertension is higher than that seen in other reports (Omole et al, 2010, Sweileh et al, 2005). About 66.7% of patients with no formal education adhered to their medication and this was higher than adherence in any of the other educational levels which suggests that these patients knew the clinical importance of their medication regardless of their low educational level; and this could be as a result of provision made for patients education in local language in this centre to ensure better understanding of diabetes and hypertension by the non-English speaking population. Contrary to this, other studies (Omole et al, 2010; Sweileh et al, 2005) showed

a least compliance in patients who were illiterate. There was no significant association between self- reported medication adherence and educational level. (p=0.683) (p=0.05) (Table 6). Although 54.8% of patients with type 2 diabetes reported adherence to drug therapy, which was lower than that for patients with hypertension being 69.4%; however, 56.6% of the total number of patients who were reported adherence to medication had type 2 diabetes co-existing with hypertension. There was no significant association between the disease group and adherence (p=0.34) (p>0.05). (Table 7)

V. Conclusion

Although, this study revealed a higher than average level of disease knowledge among all the patients, patients who had type 2 diabetes were less knowledgeable about their disease conditions than those with hypertension. There is therefore the need to increase patients' education when diabetes is complicated with Hypertension. This requires the concerted effort of all members of the healthcare team.

VI. Acknowledgement

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Table 1. Socio-demographic characteristics of patients studied

	Diabetes alone N(%)	Hypertension alone N(%)	Hypertension and Diabetes N(%)	TOTAL N(%)
Sex				
Male	18(22.5)	15(18.8)	47(58.8)	80(45.2)
Female	24(24.7)	21(21.7)	52(53.6)	97(54.8)
Age group				
<50	7(38.9)	3(16.7)	8(44.4)	18(10.2)
50-59	9(25.7)	7(20.0)	19 (54.3)	35(19.8)
60-69	14(18.7)	15(20.0)	46(61.3)	75 (42.8)
70 +	12(24.5)	11(22.4)	26(53.1)	49(27.7)
Occupation				
Artisan	3(30.0)	3(30.0)	4(40.0)	10(5.6)
Civil servant	49(16.7)	6(25.0)	14(58.3)	24(13.6)
Trader	15(23.8)	13(20.6)	35(55.6)	63(35.6)
Businessman	3(50.0)	0(0)	3 (50.0)	6(3.4)
Professional	4(33.3)	4(33.3)	4 (33.3)	12(6.8)
Retired	3(15.7)	1(5.6)	14(77.8)	18(6.8)
Clergy	1(16.7)	0(0)	5(83.3)	6(3.4)
Unemployed	2(18.2)	2(18.2)	7(63.6)	11(6.2)
Not specified	7(25.9)	7(25.9)	13(48.1)	27(15.3)
Educational Level				
No Formal	11(22.9)	13(27.1)	24(50.0)	48(27.1)
Primary	13(26.0)	13(26.0)	24(48.0)	50(28.2)
Secondary	8(32.0)	3(12.0)	14(56.0)	25(24.1)
Vocational	2(18.2)	2(18.2)	7(63.60	11(6.2)
Tertiary	8(18.6)	5(11.6)	30(69.8)	43(24.1)
Marital Status				
Married	34(23.8)	26(18.2)	83(58.0)	143(80.8)
Divorced/Widowed	8(23.5)	10(29.4)	16(47.1)	34(19.2)
Total	**42(23.7)**	**36(20.3)**	**99(55.9)**	**177(100.0)**

Table 2. General Knowledge on Diabetes and Hypertension

Questions	Responses N(%)		
	Yes	**No**	**Not Sure**
Do you know your BP within the last five days.	63(35.5)	101(57.1)	13(7.35)
Do you know the optimum BP for a person with both DM and HTN?	65(36.7)	71(40.1)	41((23.2)
High BP can cause Heart attack	140(79.1)	14(7.9)	23(13.0)
High BP can cause stroke	161(91.0)	5(2.8)	11(6.2)
High BP can cause cancer	27(15.3)	55(31.0)	59 (33.3)
DM can cause kidney failure	107(60.5)	11(6.2)	95(53.7)
High BP can cause kidney failure	98(55.4)	23(13.0)	56(31.6)
DM can cause can cause cancer	29(16.4)	48(27.1)	99(55.9)
	High	**Low**	**Normal**
If someone‟s BP is 120/80mmHg, it is	15(19.5)	48(27.1)	99(55.9)
If someone‟s BP is 160/100mmHg, it is	132(74.6)	1(0.6)	4(2.3)

Table 3. (Continued).General Knowledge on Diabetes and Hypertension

Questions	Responses N(%)			
	A few years	**5-10years**	**the rest of their lives**	**Don'tknow**
Once someone has DM, it usually lasts	1 (0.6)	5 (2.8)	125 (70.6)	8 (4.5)
Once someone has HTN, it usually lasts	10 (5.6)	2 (1.1)	136 (76.8)	25 (14.1)
People with high BP and DM should take their medicines	3 (1.7)	2 (1.1)	148 (83.6)	20 (11.3)
	Go up	**Go down**	**Stay the same**	**Don't know**
Eating less salt usually makes BP	12 (6.7)	132 (74.6)	6 (3.4)	22 (12.4)
Losing weight usually makes BP	15 (8.5)	105 (59.3)	10 (5.7)	39 (22.0)
		Lowers it	**Raises i**	**Has no effect**
For person with good control, what effect does exercise have on blood glucose		125 (70.6)	5 (2.8)	56 (31.6)
		Urine testing	**Blood testing**	**Both**
Which is the best method for testing blood glucose		2 (1.1)	79 (44.6)	71 (40.1)

Table 4. Level of Knowledge of Diabetes and Hypertension among the Study Population

	Good Knowledge	Poor knowledge	Chi square	P Value N(%)
Sex				
Male	59 (73.8)	21 (26.3)	6.891	0.009
Female	53 (54.6)	44 (45.4)		
Age Group				
<50	12 (66.7)	6 (33.3)	0.104	0.991
50-59	22 (62.9)	13 (37.1)		
60-69	47 (62.7)	28 (37.3)		
70+	31 (63.3)	18 (36.7)		
Married				
Married	92 (64.3)	51 (35.7)	0.359	0.549
Divorced/Widowed	20 (58.8)	14 (41.2)		
Educational Level				
No Formal	13 (27.1)	35 (72.9)	45.609	0.00
Primary	32 (64.0)	18 (36.0)		
Secondary	19 (76.0)	6 (24.0)		
Vocational	8 (72.7)	3 (27.3)		
Tertiary	40 (93.0)	3 (7.0)		
Occupation				
Artisan	7 (70.0)	3 (30.0)	28.677	0.00
Civil Servant/Retired	38 (90.5)	4 (9.5)		
Trader/Businessman	30 (43.5)	39 (56.5)		
Professional/Clergy	15 (83.3)	3 (16.7)		
Unemployed	7 (64.7)	4 (35.3)		

Table 5. Association between Sex and Education among the study population

	SEX N (%)			
	Male	Female	Chi-Square	P value
Educational Level				
No formal	13 (16.3)	35 (36.1)		
Primary	24 (30.0)	26 (26.8)		
Secondary	12 (15.0)	13 (13.4)	9.892	0.04
.Vocational	6 (7.5)	5 (5.2)		
Tertiary	25 (31.3)	18 (18.6)		

Table 6. Reasons why medication was failed on purpose

Reasons	Frequency N (%)
When drugs are finished	5 (10.2)
Forgetfulness	3 (6.1)
Financial constraints	3 (6.1)
Inconvenience of doses	2 (4.1)
Travelled	2 (4.1)
Sick/not feeling too good	2 (4.1)
Busy	1 (2.0)
Fasting	1 (2.0)
"Using faith"	1 (2.0)
Felt better	1 (2.0)
Polypharmacy	1 (2.0)
No response	27 (55.1)
Total	**49 (100)**

Table 7. Self-Reported Medication Adherence among the Study Population

	Yes (Adherent)	No (Nonadherent)	Chi Square	P value N(%)
Sex				
Male	46 (57.5)	34 (42.5)	0.095	0.758
Female	58 (59.8)	39 (40.2)		
Age Group				
< 50	6 (33.3)	12 (66.7)	8.505	0.037
50-59	17 (48.6)	18 (51.4)		
60-69	49 (65.3)	26 (34.7)		
70+	32 (65.3)	17 (34.7)		
Marital Status				
Married	84 (58.7)	59 (41.3)	0.00	0.993
Divorced⁄Widowed	20 (58.8)	14 (41.2)		
Educational Level				
No formal Education	32 (66.7)	16 (33.3)	2.286	0.683
Primary	29 (58.0)	21 (42.0)		
Secondary	14 (56.0)	11 (44.4)		
Vocational	5 (45.5)	6 (54.5)		
Tertiary	24 (55.8)	19 (44.2)		
Occupation				
Artisan	6 (60.0)	4 (40.0)	0.171	0.997
Civil servant/Retired	25 (59.5)	17 (40.5)		
Trader/Businessman	42 (60.9)	27 (39.1)		
Professional/ Clergy	11 (61.1)	7 (38.9)		
Unemployed	6 (54.5)	5 (45.5)		

Table 8. Association between Self-reported medication adherence and disease conditions

	Self-reported medication Adherence N (%)		Chi Square	p value
	Adherence	Non-adherence		
Disease group				
Diabetes	23 (54.8)	19 (45.2)		
Hypertension	25 (69.4)	11 (30.6)	2.17	0.34
Diabetes and Hypertension	56 (56.6)	43 (43.4)		

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Respiratory Syncytial Virus Bronchiolitis: Iga & LT-E4 Responses and Effects of Host Factors in Two Iraqi Pediatric Hospitals

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Abstract – Background : Respiratory syncytial virus (RSV) is a leading cause of lower respiratory tract disease in infants and young children. Both the magnitude, intensity of infection and the host response to RSV infection determine the severity and intensity of disease. Objective : Our goal was to evaluate the effect of immune response (RSV IgA) and inflammatory mediators (LT-E4), in addition to the influence of host factors on the severity of the disease. Methods : This was a randomized, prospective study in two Iraqi pediatric hospitals. Sixty infants (mean age: 6.99±0.62 ,35 boys & 25 girls) ,with a first episode of acute bronchiolitis were randomly divided into four treatment groups: oxygen plus intravenous fluid, montelukast pediatric chewable tablet,salbutamol given in combination as oral plus nebulized salbutamol, and dexamethasone IV injection. Control infants with non respiratory diseases were also studied for comparisons. The measured parameters was RSV IgA titer, LT-E4 titer, and a variety of environmental and host factors that may contribute to the severity of RSV bronchiolitis.Severity of bronchiolitis was based on the quantization of lowest O2 saturation and the length of hospital stay. Results : There were significant increase in RSV IgA values in patients (1.58 ± 0.24 U/mL) compare to the control (0.36 ± 0,03 U/mL);also there were a significant increase in the leukotriene E 4 values in patients (2.66 ± 0.52 ng/ml) compared to the control infants(0.15 ± 0.007 ng/ml). Age was found to be a significant factor in the severity of infection. The younger an infant was, the more severe the infection tended to be as measured by the lowest oxygen (O2) saturation. We also found that infants exposed to postnatal cigarette smoke from the mother had a lower O2 saturation than those not exposed. Although a history of maternal atopy seemed to be protective. Conclusion : Secretory IgA antibodies level was found to be a good indicator to respiratory syncytial virus infection as seen by significantly higher levels in patients compared to the control infants. The severity of RSV bronchiolitis early in life seems modified by postnatal maternal cigarette smoke exposure, atopy and age of the infants.

Keywords : *RSV, Respiratory Syncytial Virus IgA (RSV-IgA); Leukotriene E-4(LT-E4), Bronchiolitis.*

GJMR-E Classification : *NLMC Code: WC 518, WC 505,WF 546*

Strictly as per the compliance and regulations of:

Respiratory Syncytial Virus Bronchiolitis: Iga & LT-E4 Responses and Effects of Host Factors in Two Iraqi Pediatric Hospitals

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Abstract - Background : Respiratory syncytial virus (RSV) is a leading cause of lower respiratory tract disease in infants and young children. Both the magnitude, intensity of infection and the host response to RSV infection determine the severity and intensity of disease.

Objective : Our goal was to evaluate the effect of immune response (RSV IgA) and inflammatory mediators (LT-E4), in addition to the influence of host factors on the severity of the disease.

Methods : This was a randomized, prospective study in two Iraqi pediatric hospitals. Sixty infants (mean age: 6.99±0.62 ,35 boys & 25 girls) ,with a first episode of acute bronchiolitis were randomly divided into four treatment groups: oxygen plus intravenous fluid, montelukast pediatric chewable tablet,salbutamol given in combination as oral plus nebulized salbutamol, and dexamethasone IV injection. Control infants with non respiratory diseases were also studied for comparisons. The measured parameters was RSV IgA titer, LT-E4 titer, and a variety of environmental and host factors that may contribute to the severity of RSV bronchiolitis.Severity of bronchiolitis was based on the quantization of lowest O2 saturation and the length of hospital stay.

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Conclusion : Secretory IgA antibodies level was found to be a good indicator to respiratory syncytial virus infection as seen by significantly higher levels in patients compared to the control infants. The severity of RSV bronchiolitis early in life seems modified by postnatal maternal cigarette smoke exposure, atopy and age of the infants.

Keywords : RSV, Respiratory Syncytial Virus IgA (RSV-IgA); Leukotriene E-4(LT-E4), Bronchiolitis.

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I. Introduction

Respiratory syncytial virus (RSV) is the leading cause of serious respiratory tract infections in infants and young children throughout the world (1). RSV replicates for 1–3 days before producing lower respiratory tract symptoms affecting almost 60% of infants and up to 25% of toddlers and preschoolers. Current treatment approaches for severe RSV induced disease are ineffective. Therefore, prevention of disease is a high priority .Immunoglobulin A(IgA) is the most abundant immunoglobulin in mammals. Unlike other antibody isotypes, IgA is targeted to mucosal tissues, and virus-specific IgA in mucosal secretions has been shown to protect from reinfection. IgA, unlike IgG, is able to bind and neutralize viral proteins intracellularly at the site of initial replication in epithelial cells .Therefore; mucosal IgA may be of particular importance in immunity against RSV, which is a mucosally restricted pathogen (2,3). Inflammatory mechanisms in bronchiolitis have been documented recently, including increased airway secretion, mucosal edema, and infiltration of inflammatory cells. Cysteinyl leukotrienes (CysLTs) are released during respiratory syncytial virus (RSV) airway infection in infants, and their levels are significantly elevated. CysLTs are known to cause bronchial obstruction, mucosal edema, and infiltration of eosinophilic granulocytes and to increase bronchial responsiveness (4). CysLTE4 (LTE4), one of the terminal CysLT metabolites, is significantly increased in the infants hospitalized with RSV bronchiolitis (5). The risk of severe RSV disease is increased by factors that compromise the ability to control and withstand a respiratory tract infection. Therefore; environmental factors also play a role, including ones that affect lung function (e.g., household tobacco use) or that increase exposure to infection (e.g., day care, hospitalization, multiple siblings, crowding) (6,7). The objective of the

present randomized, prospective study was to evaluate the effects of immune response, inflammatory mediators, host and environmental factors on the severity of the acute viral bronchiolitis.

II. Materials & Methods

This prospective study was conducted in two Iraqi pediatric hospitals. Baghdad Health Office/Karkh, Child's Central Teaching Hospital & Karbala Health Office, Karbala Pediatric Teaching Hospital. Inclusion criteria were infants' patients aged >8 weeks and <2 years with a respiratory symptom duration of<4 days. Additional inclusion criteria included first episode of wheezing or shortness of breath, randomization within 12 hours of admission and informed consent. Exclusion criteria were any previous hospital admissions with respiratory illnesses, had ever been treated with anti-asthma medications before the current illness, corticosteroids treatment in any form during current illness, and underlying cardiopulmonary disease. Gender, age, weight, height , body temperature ,family history in (first-degree relatives), of asthma ,atopy, tobacco smoking, usage of kerosene heater ,type of feeding ,duration of exclusive breast feeding, concurrent diseases, and concomitant medications, were recorded for each infants .A total number of 60 patients mean age: 6.99±0.62 with mild to moderate bronchiolitis were divided randomly into four treatment groups:

Group A: Ten infants' patients had received oxygen + intravenous fluid. Group B: Ten infants' patients had received study treatment, montelukast pediatric chewable tablet 4mg once daily, if vomiting occurred one additional dose was given. Group C: Twenty infants' patients had received salbutamol given in combination as syrup & by nebulization, (oral salbutamol 0.1-0.3mg/kg/dose q8 hour+salbutamol nebulizer 0.01-0.02 mg/kg /dose q6hour). Group D: Twenty infants' patients had received dexamethasone ampoule (4mg/1ml), with a dose of, 0.25 -0.5 mg/kg/dose q 12 hours intravenously.

From all enrolled infants, blood samples were taken and try to measure both (RSV IgA) & LT-E4, antibody to RSV & inflammatory mediators that release during RSV acute bronchiolitis, respectively. These parameters were measured by the enzyme linked immunoassays (ELISA), to investigate the etiology of acute respiratory infections in hospitalized infants .The test was explained to the parents and they signed the informed consent form. The obtained optical density (OD) of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method [8,9,10].

Other type of samples that taken from the patients that put on the study treatment, montelukast pediatric chewable 4mg tablets, was the nasal swab. In the present study, we prospectively tried to examine the association between the presence of nasal eosinophils and severity of acute bronchiolitis and the effect of montelukast on nasal eosinophil. In this study we tried to quantify the number of neutrophils and eosinophils in nasal secretions by utilizing the semiquantitative nasal cytology grading score by Meltzer[11,12].

III. Results

Table (1) : Demographic data and baseline characters of patients and control infants. The data were expressed as number (n), and percentage (%).

	Patients (n_{total} =60)	Control Infants (n_{total} =20)
Characteristics	(n,%)	(n,%)
Age <6months.	40, (59)	12, (60)
Age >6months.	20, (41)	8,(40)
Male.	35 ,(58)	14, (70)
Female.	25 ,(42)	6 ,(30)
Family history of asthma.	30, (52)	8 ,(40)
Family history of atopy.	38 ,(63)	12, (60)
History of passive tobacco smoking.	44,(74)	16, (80)
Family history of kerosene heating.	46 ,(77)	14, (70)
Presence of pets at house.	26, (45)	10, (50)
Breast feeding.	33 ,(54)	2, (10)
Bottle feeding.	18,(30)	14 ,(70)
Mixed feeding.	8,(13)	4, (20)
< 5 Member.	8,(13)	4,(20)
>5 Member.	52,(87)	16(80)
Mean weight, kg.	7.2 ± 0.79	9.3 ± 1.14
Duration of exclusive breast feeding, months.	4.53± 0.303	8 ± 2.68
The values of weight,& duration of exclusive were expressed as mean ± standard error of mean (SEM).		

Table (1), demonstrated that, there were no significant differences between the groups in terms of demographic variables.

The table (2) showed the RSV IgA values for infants' patients with acute viral bronchiolitis, together with RSV IgA values for the control infants. There was a significant increase in RSV IgA values in patients compared to the control infants. There was a significant relationsihips between titer of the antibody against RSV(RSV IgA) and family history of atopy,tobacco smoking ,and the ages of infants patients.

Table (2): Relationships between host factors and RSV IgA titer for infants' patients with acute viral bronchiolitis and for the control infants. Data were expressed as mean ± standard error of mean (SEM), number (n) and percent (%).

		RSV IgA		RSV IgA
	Patients	U/ml	Control	U/ml
	n ,(%)	Patient	n ,(%)	Control
	total = 45		total = 20	
RSV IgA high titer (mean ± SEM)		1.58* ± 0.24		0.36 ± 0.03
RSV IgA low titer (mean ± SEM)		0.39 ± 0.02		
Age <1year.	34 (75.6)	1.24* ± 0.28	14,(70)	0.31 ± 0.03
Age >1 year.	11 (24.4)	2.63 * ± 0.39 †	6,(30)	0.36 ± 0.06
Male.	34 (75.6)	1.37 * ± 0.19	14,(70)	0.35 ± 0.03
Female.	11 (24.4)	2.23* ± 0.78	6,(30)	0.29 ± 0.03
Positive family history of asthma.	21,(46.6)	1.21* ± 0.21	6,(30)	0.36 ± 0.06
Negative family history of asthma.	24(53.3)	1.91 * ± 0.41	14,(70)	0.32 ± 0.03
Positive family history of atopy.	33 (73.3)	1.18* ± 0.15	12,(60)	0.33 ± 0.03
Negative family history of atopy.	12 (26.6)	2.69 *± 0.74 †	8,(40)	0.33 ± 0.05
Positive history of passive tobacco smoking.	35 (77.7)	1.79* ± 0.30	16,(80)	0.32 ± 0.03
Negative history of passive tobacco smoking.	10(22.2)	0.86* ± 0.13 †	4,(20)	0.36 ± 0.09
Positive family history of kerosene heating.	38 (84.4)	1.49 *± 0.19	14,(70)	0.32 ±0.03
Negative family history of kerosene heating.	7 (15.6)	2.21* ± 1.19	6,(30)	0.36 ± 0.001
Positive presence of animal in the house.	20(44.4)	1.26* ± 0.22	6, (30)	0.30 ± 0.029
Negative Presence of animal in the house.	25 (55.5)	1.84* ± 0.39	14,(70)	0.35 ± 0.04
Positive breast feeding.	22(48.8)	1.73* ± 0.43	6,(30)	0.26 ± 0.006
Negative breast feeding.	23 (51.1)	1.44 * ± 0.25	14,(70)	0.36 ± 0.03 †
Positive bottle feeding.	21,(46.6)	1.41* ± 0.25	14,(70)	0.35 ± 0.03
Negative bottle feeding.	24,(53.3)	1.73* ± 0.4	6,(30)	0.26 ± 0.006
Number of family member > 5.	36 ,(80)	1.5 * ± 0.28	16 ,(80)	0.35 ± 0.03
Number of family member < 5.	9 ,(20)	1.91* ± 0.53	4 ,(20)	0.26 ± 0.009 †

*P<0.05: Significant difference between patients and control group.
†P<0.05: Significant difference from preceding value.
RSV IgA = Respiratory Syncytial Virus Immunoglobulin A.
Control :infants with non respiratory illness

Table (3): Relationships between host factors and leukotriene -E4 for infants' patients with acute viral bronchiolitis and for the control infants. Data were expressed as mean ± standard error of mean (SEM), number (n) and percent (%).

	Patients	Patients	Control	Control
	n ,(%)	LTE4	n ,(%)	LTE4
	total = 48	ng / ml	total = 20	ng / ml
LT-E4 High Titer.		2.66 * ± 0.52		0.15 ± 0.007
LT-E4 Low Titer.		0.142±0.004		
Age <1year.	36 (75)	2.46* ± 0.6	14,(70)	0.14 ± 0.006
Age >1 year.	12 (25)	3.28 * ± 1.1	6,(30)	0.15 ± 0.02
Male.	34,(70.8)	2.10* ± 0.56	14,(70)	0.14 ± 0.01
Female.	14,(29.1)	4.25 * ± 1.07 †	6,(30)	0.15 ± 0.01
Positive Family history of Asthma.	23,(47.9)	1.93* ± 0.60	6,(30)	0.15 ± 0.02
Negative Family history of Asthma.	25 (52.1)	3.31 * ± 0.84	14,(70)	0.14 ± 0.006
Positive Family history of Atopy.	34 (70.8)	2.47 *± 0.65	12,(60)	0.15 ± 0.01
Negative Family history of Atopy.	14 (29.1)	3.11* ± 0.91	8,(40)	0.14 ± 0.008
Positive History Of Passive Tobacco Smoking.	38 (79.2)	2.87 *± 0.63	16,(80)	0.15 ± 0.008
Negative History Of Passive Tobacco Smoking.	10(20.8)	1.84* ± 0.77 †	4,(20)	0.15 ± 0.02
Positive Family history of Kerosene Heating.	40 (83.3)	2.68 *± 0.60	14,(70)	0.15 ± 0.01
Negative Family history of Kerosene Heating.	8 (16.6)	2.64* ± 1.04	6, (30)	0.13 ± 0.005
Positive Presence of Animal in the house.	20(41.6)	2.33 *± 0.63	10, (50)	0.15 ± 0.01
Negative Presence of Animal in the house.	28 (58.3)	2.89 * ± 0.78	10,(50)	0.14 ± 0.006
Positive Breast Feeding.	29 (60.4)	3.26* ± 0.74	6,(30)	0.15 ± 0.01
Negative Breast Feeding.	19 (39.6)	1.74* ± 0.66	14,(70)	0.15 ± 0.01
Positive Bottle feeding.	20 (41.6)	2.09 *± 0.71	14,(70)	0.15 ± 0.01
Negative Bottle feeding.	28 (58.3)	3.06* ± 0.74	6,(30)	0.15 ± 0.01
Number Of Family Member > 5.	36 ,(75)	2.45* ± 0.59	16,(80)	0.15 ± 0.009
Number Of Family Member < 5.	12 ,(25)	3.43* ± 1.31	4,(20)	0.14 ± 0.002
*P<0.05: Significant difference between patients and control group.				
†P<0.05 : significant difference from the preceding value				
LT-E4= Leukotriene E 4				
Control :infants with non respiratory illness				

Table (3) showed the leukotriene E4 values in infants patients with acute bronchiolitis, together with leukotriene values of the control infants. There was a significant increase in the leukotriene E4 values in patients compared to the control infants As the table shown, only the gender and family history of tobacco smoke showed significant differences.

Table (4): Effects of host factors on length of stay (LOS) and oxygen saturation (S_PO2) for the infants patients with acute viral bronchiolitis .Data were expressed as mean ± standard error of mean (SEM) ,number (n) and percent (%).

	Patients n ,(%) total = 60	Patients LOS (day)	Patient S_PO2
Male.	41 (68.3)	2.9 ± 0.18	94.14 * ± 0.34
Female.	19 (31.7)	3.3 ± 0.32	93.84 * ± 0.39
Age > 1 year.	14 (23.3)	2.86 ± 0.38	94.8 * ± 0.64
Age < 1 year.	46 (76.7)	3.11 ± 0.17	93.7 * ± 0.28
Positive family history of asthma.	27 (45)	3.18 ± 0.24	94.41 * ± 0.35
Negative family history of asthma.	33 (55)	3.2 ± 0.20	93.75 * ± 0.38
Positive family history of atopy.	42 (70)	2.5 ± 0.23	94.16 * ± 0.31
Negative family history of atopy.	18 (30)	3.16 ± 0.21 †	93.77 *± 0.51
Positive history of passive tobacco smoking.	46 (76.7)	3.26 ± 0.19	94.04 * ± 0.29
Negative history of passive tobacco smoking.	14 (23.3)	3.25 ± 0.29	94.07 * ± 0.61
Positive family history of kerosene heating.	51 (85)	3.22 ± 0.19	93.90 * ± 0.28
Negative family history of kerosene heating.	9 (15)	2.83 ± 0.18	94.88 * ± 0.69
Positive presence of animal in the house.	27 (45)	3.22 ± 0.26	94.29 * ± 0.34
Negative presence of animal in the house.	33 (55)	3.19 ± 0.21	94.36 * ± 0.32
Positive breast feeding.	31 (51.6)	2.86 ± 0.23	94.58 * ± 0.38
Negative breast feeding.	29 (48.3)	3.09 ± 0.23 †	93.54 * ± 0.34 †
Positive bottle feeding.	30 (50)	3.17 ± 0.28	93.83 *± 0.35
Negative bottle feeding.	30 (50)	2.8 ± 0.18	94.26 * ±0.39
Number of family member > 5.	48 (80)	3.18 ± 0.19	94.12 * ± 0.28
Number of family member < 5.	12 (20)	3.13 ± 0.27	93.7 * ± 0.7
RSV IgA high titer (mean ± SEM).	1.58 * ± 0.24	2.9 ± 0.14	94.2 ± 0.29
RSV IgA low (mean ± SEM).	0.39 ± 0.02	3.9 ± 0.47 †	93.06 *± 0.58 †
LTE4 high titer (mean ± SEM).	2.66* ± 0.52	3.26 ± 0.20	94.16 ± 0.27
LTE4 low titer (mean ± SEM).	0.142 ± 0.004	2.79 ± 0.18 †	93.58 * ± 0.72

*P<0.05: Significant difference between patients and control group.
†P<0.05: Significant difference from the preceding value.
RSV IgA : Respiratory Syncytial Virus Immunoglobulin A.
LT-E4: Leukotriene E4.
LOS: Length of hospital stay; S_PaO2= blood oxygen saturation.

Table (4) showed the effects of host factors on the length of hospital stay (LOS) and oxygen saturation (S_PO_2) in infants patients with acute viral bronchiolitis. As the table shown, only the host factors of family history of atopy and breast feeding of infants showed significant effects on duration of hospital stay and oxygen saturation of blood.

Concerning nasal swab from infants' patients with acute viral bronchiolitis before and after treatment with montelukast chewable 4 mg tablets once daily; according to Meltzer grading there was a significant differences in the count of eosinophils –neutrophils before and after treatment with montelukast ; 1.6 ± 0.32 versus 0.33 ± 0.16 respectively. This could indicate eosinophil-recruiting chemokines were strongly produced and released from bronchial epithelial cells after *in vivo* stimulation with RSV.

IV. Discussion

RSV is a highly infectious and prevalent virus. More than other respiratory viruses, RSV infection can occur very early in life despite maternal antibodies, and

reinfection can readily occur throughout life without significant antigenic change .The relative contribution of viral versus various host factors to RSV pathogenesis remains controversial (6). The immune response to primary RSV infection is generally inefficient and consequently subsequent reinfections are common throughout life. In RSV infection, innate and adaptive immunity are out of balance (13).

Comparing the risk factors with RSV IgA values of infants' patients, only the age, history of atopy and passive tobacco smoking showed significant differences (14). In the age category older infants' patients (over 1 year) had significantly higher RSV IgA value compared to younger patients (below 1 year) . Patients with negative family history of atopy had significantly higher RSV IgA value compared to patients with positive history of atopy. On the other hand patients with positive history of passive tobacco smoking had significantly higher RSV IgA value compared with those of negative history of passive tobacco smoking. This could indicate that, parental smoking did not inhibit the production of anti-microbial IgA, suggesting that other factors are responsible for the increased susceptibility to infection in these infants. Infants who lived in tobacco smoking environments had increased severity of disease, as results of Th2 predominance, with decreased expression of Th1 cytokines [(15)] , and IgA titer was less effective for protecting against RSV infection [(2)] . Lanari *et al*. (2002) [(14)], demonstrated that exposure to cigarette smoke, in general, seems to worsen the severity of the viral bronchiolitis.

Comparing the risk factors with LTE4 values, only the gender and family history of tobacco smoke showed significant difference. Concerning the gender, the value in female babies was significantly higher than male babies. This could indicate that the females infants had more sever RSV infections compared to male infants; this has been attributed to the tendency of parents to bring sick male babies to the hospital earlier than female babies [(3)].CysLT increased in infants who exposed to the tobacco smoke. This could indicated that, the exposure to the tobacco smoke increases the severity of RSV bronchiolitis, which was described here by the increased level of LTE4 in the infants who lived in tobacco smoking environments [(16,17,18).]

Comparing the effects of host factors (age, sex, family history of asthma, atopy, tobacco smoking, kerosene heating, presence of pets at home, breast or bottle feeding and number of family members) on the length of hospital stay and oxygen saturation in infants with acute viral bronchiolitis; only the host factors of family history of atopy and breast feeding of infants showed a significant effects on duration of hospital stay and oxygen saturation of blood [(19)] . Infants with a positive family of atopy showed a shorter duration of hospital stay and a higher value of blood oxygen saturation compared to infants with acute viral bronchiolitis and have no family history of atopy. Breast feeding of infants with acute viral bronchiolitis showed a significant effect on the blood oxygen saturation and length of hospital stay. Breast feeding is protective, through either transfer of maternal antibody or enhancement of virus-specific lymphocyte transformation activity. Infants with breast feeding have a shorter length of stay and higher value of blood oxygen saturation relative to infants without having breast feeding and have bottle fed. This finding is substantiated further by the fact that infants with a higher O2 saturation spent less time in the hospital than infants with a lower O2 saturation[(14)].

Regarding to the effects of RSV IgA level on the length of hospital stay and patients oxygen saturation, there were a significant effects on both length of hospital stay and patient oxygen saturation. Infants with low titer of RSV IgA showed longer period of hospital stay & lower values of oxygen saturation compared to the patients with a high titer of RSV IgA , which could indicated effects of immune response of the patients on the resolution of symptoms and the time at which patients were fit to the discharge [(7,20)]. Regarding to the effects of inflammatory mediators' cysteinyl leukotriene and its metabolite LTE4 on the period of hospital stay and oxygen saturation of infants patients with acute viral bronchiolitis, there were significant effects. High titers of LTE4 associated with prolong hospital stay and lower value of blood oxygen saturation .Female, younger infants, negative family history of atopy, and absence of breast feeding, showed longer period of hospital admission & lower value of blood oxygen saturation.

According to Meltzer grading there were a significant differences in the counts of eosinophils – neutrophils before and after treatment with montelukast tablet for the infants patients with acute viral bronchiolitis. This could indicated that eosinophil-recruiting chemokines are strongly produced and released from bronchial epithelial cells after stimulation with RSV[(12)] ;and montelukast treatment has been shown to reduce eosinophils in nasal mucosa of infants [(21)].

V. Conclusion

The relationships between risk factors and RSV IgA titer in infants with viral bronchiolitis, only age, family history of atopy and tobacco smoking showed significant effects. Patients with low titer of RSV IgA showed longer period of hospital stay & lower values of oxygen saturation comparing to the patients with a high titer of RSV IgA. Concerning the relationships between risk factors of infants with bronchiolitis and leukotriene E4 level, only the gender and family history of tobacco smoke showed significant difference. There were a significant effects of high level of LTE4 on the period of hospital stay compared to the low level of LTE4.

Host factors of family history of atopy and breast feeding of infants showed significant effects on duration of hospital stay and oxygen saturation of blood. Infants exposed to postnatal cigarette smoke from the mother had a lower O_2 saturation than those not exposed. Infants with a family history of atopy especially a maternal history of asthma had a higher O_2 saturation. Infants with highest blood oxygen saturation, have shorter length of hospital stay.

There were significant differences in the count of eosinophils –neutrophils before and after treatment with montelukst,which could indicated that, there was a correlation between nasal eosinophil and severity of viral bronchiolitis .

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Adherence and generic substitution among hypertensive patients in a specialist hospital, Nigeria

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Abstract – Hypertension is the commonest non-communicable disease in Nigeria with a prevalence of about 20-25 per cent in adult Nigerians. This research work examined generic drug substitution and its impact on drug adherence among hypertensive patients attending the Consultant Out-Patient Department of Sobi Specialist Hospital, Ilorin, Nigeria. Self-report adherence, personal interview, structured questionnaires and patients' prescriptions were used to determine drug adherence and treatment outcomes of 167 hypertensive patients on antihypertensive drugs. Adults between the ages of 40 and 80 years were mostly affected, and women were more vulnerable to the disease. The clinical signs and symptoms mostly reported by the patients include severe headache, chest pain, numbness of extremities and general body weakness. The widely utilized generic antihypertensive agents in the studied hospital include Amlodipine, Methyldopa, Nifedipine, Lisinopril, and constituted 91.6% of all the prescriptions. The high rate of generic drug prescriptions in this hospital coupled with the consistent drug counselling offered to the patients by the pharmacist had greatly improved adherence rate to 67.7%. Generic drug substitution should be encouraged in all tiers of healthcare system to improve drug adherence and stem the tide of hypertension in this society. Also, public enlightenment and education should be strengthened to increase population awareness on the symptoms, risk factors, lifestyle modifications and complications of this silent killer disease.

Keywords : *Hypertension, drug adherence, generic, prescriptions, substitution, Nigeria.*

GJMR-E Classification : *NLMC Code: WG 340,*

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Adherence and generic substitution among hypertensive patients in a specialist hospital, Nigeria

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Abstract - Hypertension is the commonest non-communicable disease in Nigeria with a prevalence of about 20-25 per cent in adult Nigerians. This research work examined generic drug substitution and its impact on drug adherence among hypertensive patients attending the Consultant Out-Patient Department of Sobi Specialist Hospital, Ilorin, Nigeria. Self-report adherence, personal interview, structured questionnaires and patients' prescriptions were used to determine drug adherence and treatment outcomes of 167 hypertensive patients on antihypertensive drugs. Adults between the ages of 40 and 80 years were mostly affected, and women were more vulnerable to the disease. The clinical signs and symptoms mostly reported by the patients include severe headache, chest pain, numbness of extremities and general body weakness. The widely utilized generic antihypertensive agents in the studied hospital include Amlodipine, Methyldopa, Nifedipine, Lisinopril, and constituted 91.6% of all the prescriptions. The high rate of generic drug prescriptions in this hospital coupled with the consistent drug counselling offered to the patients by the pharmacist had greatly improved adherence rate to 67.7%. Generic drug substitution should be encouraged in all tiers of healthcare system to improve drug adherence and stem the tide of hypertension in this society. Also, public enlightenment and education should be strengthened to increase population awareness on the symptoms, risk factors, lifestyle modifications and complications of this silent killer disease.

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I. Introduction

High blood pressure (hypertension) is one of the major cardiovascular diseases which ranked third as a cause of disability-adjusted life-years worldwide. It has affected millions of people in both the developed and developing world, and the problem is likely to increase dramatically over the next 15 years (Kearney et al., 2005). Hypertension is the commonest non-communicable disease in Nigeria with a prevalence of about 20-25 per cent in adult Nigerians (Alebiosu, 2010). The silent nature of hypertension often encouraged the tendency of patients to be non-adherent. The reasons for non-adherence are complex which include; ambivalence about taking drugs, concerns over side effects and complexity of treatment regimen (Ekwunife et al., 2010). Numerous studies have demonstrated that patients' adherence with antihypertensive medications is poor (Inkster et al., 2006). One retrospective study reported just 36% of patients hypertension were adherent with their antihypertensive therapy 12 months after initiating the medications (Cardinal et al., 2004; Chapman et al., 2005; Agarwal et al., 2009). In Ibadan, Nigeria, Yusuff and Alabi (2007) reported adherence level of 49%, while Ekwunife et al. (2010) observed 70.7% adherence level in Nsukka. However, tackling the widespread failure to take medication correctly could lead to a major reduction in stroke and heart disease. Non-adherence to drug treatment is an established major obstacle in health improvement especially chronic and symptom-free conditions like hypertension (Burnier, 2003). A long term reduction of blood pressure would lead to a reduction in stroke of 56% and a reduction in chronic heart disease of 37% suggesting that adherence therapy would likely be a cost-effective intervention (Fadwa, 2011).

The ultimate economic goal of hypertension management is to balance costs and benefits, but defining these entities may be difficult. The overall cost of treating high blood pressure includes direct costs, such as drug acquisition, physician fees, laboratory and diagnostic tests as well as management of side effects. Indirect costs are inadequate blood pressure control, non-adherence with therapy, and loss to follow up. Generics are a class of lower cost medications prescribed by the physician, and the patient expected same therapeutic effects as the brand-name. The United States Food and Drug Administration (FDA) considered generic and branded drugs to be therapeutically equivalent if they were pharmaceutically equivalent and bioequivalent (Duh et al., 2009). In an era of continuously rising health care costs, the increased use of generic prescription drugs as alternatives to more expensive brand-name products is encouraged by health authorities worldwide. Promotion of cheaper generics, either by generic prescribing or generic substitution, has led to substantial savings in the health care sector in many countries (Duh et al., 2007). When generic substitution takes place, concerns about drug adherence tend to occur. In this respect, drug adherence is defined as the extent to which a person's

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medication-taking behaviour corresponds with agreed recommendations from a health care provider. The use of brand names may lead to increased cost of drugs for these patients. This means that prescribers were not complying fully with World Health Organization (WHO) recommendation that drugs should be prescribed using their international non -proprietary names. Factors that may be responsible for this trend include the influence of drug promotional activities, pressures of pharmaceutical representatives (detail men), lack of continuing education on the principles of rational prescribing and non-familiarity with generic names among the prescribers. Since generic drugs have the same therapeutic effect as the original formulation but at generally lower costs, their use should be more heavily promoted (Berg, 2007). However, a considerable number of barriers to their wider use have been observed in many countries. Generic substitution has been associated with notable monetary savings for society in several settings and represents one of several strategies aimed at curb pharmaceutical expenditure (Kramer et al., 2007). Generic drugs which contain the same therapeutic substance as the original formulation, become available once the patent protection granted to the brand name drug has expired, leading to greater market competition and lower prices (Jobst and Holmes, 2004). There are, however, different barriers to the wider use of generic drugs. The first is the concern of patients. Secondly, generic substitution is generally met with skepticism by health professionals despite a lack of proven differences in the clinical outcomes of generics and original formulations (Crawford et al., 2006). Physicians who play a central role in the prescription decision have their individual prescribing habits (Danish agency) and tend to prescribe by brand name, generally ignoring drug prices (Nielsen et al., 2008).Thirdly, Pharmacies may also influence the choice of medication by informing patients of the costs or by adopting procedures that increase generic use. Finally, economic and regulatory conditions play a major role on the drugs market, with financial incentives for all parties (prescribers, pharmacists, and patients) being an important factor (Kramer et al., 2007). One major barrier to drug adherence in hypertensive patients is cost of their medications. Blood pressure in un-medicated patients adversely affects cognitive function and social activity with a deterioration of the sense of well being, as the duration of illness increases. To prevent patients' from being trapped in this vicious circle, it is important to consider generic drug substitution (affordable option) along with drug adherence of 90% in achieving blood pressure control. However, little effort has been put into studying generic substitution specifically. The aim of this study was therefore to investigate whether, and in what way, generic substitution might have on drug adherence in hypertensive patients, using personal interviews, drug prescriptions and self report adherence.

II. Materials and Methods

a) *Setting*

This study was conducted at a Specialist Hospital, Sobi, Ilorin, Kwara State, located in the North Central of Nigeria. The hospital is a tertiary health facility offering health services to the residents of Kwara State and neighbouring States. The hospital was established by the Kwara State Government in April, 1985. The hospital receives its drug supply from Essential Drug Project (EDP) in the Kwara State Ministry of Health. The EDP is a Central Medical Store which distributes quality and affordable drugs to all the hospitals in the State under the Drug Revol ving Fund Scheme. The drug supply to this hospital is very consistent and in accordance with Physicians' requests. Generic drugs were encouraged in line with WHO recommendations except in generic drug-resistant hypertensive patients who may require branded drugs. Hypertension is classified as "resistant" if medications do not reduce blood pressure to normal levels.

b) *Population sample*

One hundred and sixty-seven patients made up of 47 males and 120 females diagnosed to have High Blood Pressure (using Sphygmomanometer and Stethoscope) and on antihypertensive agents therapy between February, 2011 and October, 2011 were selected for the study. Inclusion criteria were outpatients diagnosed and confirmed to be hypertensive, between ages of 30 – 80 years, attending the Consultant Outpatient Department and refilling their prescriptions in the hospital Pharmacy Department within the study period. The patients who keep to appointments at the hospital and using their drugs for upwards of 2 months prior to the study were included. The benefits, confidentiality and voluntary participation features of the study were explained and written informed consent were obtained from the patients. Three classes of antihypertensive agents considered in the study include the use of Centrally acting drugs (Methyldopa), Angiotensin Converting Enzyme Inhibitors (Lisinopril, Enalapril, Ramipril) Inhibitors and Calcium Channel Blockers (e.g Nifedipine, Amlodipine). Patients excluded were adults below age of 30 years, Psychiatric patients, Prisoners and patients with history of HIV/AIDS.

c) *Study design*

Ethical approval was sought from the management of the hospital and informed consent from all the patients participating in this study at the time of enrollment. All data collected were obtained from the structured questionnaires, drug prescriptions and personal interview with the patients. The patients were asked to show their prescriptions and drugs dispensed to them during interview with the Pharmacist. Within the study period of eight months, 6,122 prescriptions were dispensed of which 91.6% were generic drugs. Also, the

different classes of antihypertensive drugs utilized by the patients were recorded. The interview was carried out in local language (Yoruba) which was the main spoken language in the area of study.The importance of the study was duly highlighted to the patients by the researcher. Learned patients themselves completed a paper format questionnaire, which was explained in details prior to completion. Patients who had no formal education or had primary education were interviewed by the pharmacist using the survey forms. Generic drug counseling for each patient was usually carried out at the counseling room of the hospital Pharmacy using standard procedures whenever visit is made to refill their prescriptions.

d) Adherence assessment

Self-reporting method was used to determine hypertension treatment medication adherence at the end of each month during drug refilled for eight months. In the self-reporting patients' adherence method, the patients were interviewed on adherence by asking them to recall how they administered drugs at home during refill of prescription. The Special Projects of National Significance (SPNS) Adherence Initiative self-report questions were used to determine adherence rate among these patients. The number that corresponded with the answer to the questions were added together to get their scores. Scoring greater than 10 equals to good adherence while less than 10 symbolizes poor adherence.

e) Statistical analysis

Data generated from the structured questionnaires, drug prescriptions and personal interview were keyed into Genstat statistical package (Genstat, 1995) and analysed for frequencies and percentages.

Table 1: Socio-demographic features of the patients

Variable	Description	Number of patients	Percentages
Gender	Male	47	28.1
	Female	120	71.9
Age (years)	30 – 39	21	12.3
	40 – 60	105	63.1
	61 – 80	41	24.6
Education	None	137	82.4
	Primary	19	11.1
	Secondary	11	6.5
	Tertiary	0	0
Marital status	Married	131	78.4
	Single	2	1.2
	Widowed	28	16.8
	Divorced	6	3.6
Occupation	Traders	85	50.9
	Civil Servants	25	15.0
	Professionals	18	10.7
	Farmers	27	16.2
	Retired workers	12	7.2

Table 2 : Periods of initiation of therapy

Months since initiating anti-hypertensive drug treatment	Number of patients	Percentage (%)
2 – 4 months	84	50.2
5 – 6 months	32	19.1
7 – 9 months	17	10.2
10 months and above	34	20.5

Table 3 : Profile of clinical conditions among hypertensive patients

Symptoms	Number of patients	Percentage (%)
Severe headache	73	43.8
Chest tightness	34	20.2
Numbness of hands and legs	19	11.3
Fatigue	18	11.1
Breathing difficulty	17	10.3
Pounding in the head, neck or ears	6	3.3

Table 4 : Frequency distribution of generic antihypertensive agents prescribed

Prescribed Drugs	Number of patients	Percentage (%)
Amlodipine	61	36.5
Methyldopa	38	22.7
Nifedipine	35	21.0
Lisinopril	30	18.0
Ramipril	1	0.6
Enalapril	2	1.2

Table 5 : Outcomes of hypertensive patients at Sobi Specialist Hospital

Parameters Studied	Classification	Number of patients	Percentage (%)
Patients knowledge about disease condition	Patients with knowledge	34	20.1
	Patients without knowledge	133	79.9
Fear about medication use	Show no fear	151	90.4
	Showing fear	16	9.6
Drug counselling received from Pharmacist	Well counselled	154	92.2
	Not well counselled	13	7.8
Prescription studied	Generic drugs	5, 608 (prescriptions)	91.6
	Brand drugs	514 (prescriptions)	8.4
Classification of hypertension	Stage 1	59	35.3
	Stage 2	83	49.7
	Isolated systolic	25	15.0
Cost of antihypertensive drugs per month	Generic drugs	₦480 ($3)	
	Brand drugs	₦2, 220 ($14)	

Table 6 : Patients' assessment of adherence

Self-report patient assessment of adherence	Number of patients	Percentage (%)
Good Adherent	113	67.7%
Poor Adherent	54	32.3%

III. Results

a) Demographic and socio economic characteristics of hypertensive patients at Sobi Specialist, Ilorin.

In the present study one hundred and sixty-seven patients met the inclusion criteria. Female patients dominates with 120 (71.9%) while 47 (28.1%) were males. Twenty-one (12.3%) of them were of the age range of 30-39 years, one hundred and five patients (63.1%) were between 40-60 years old while forty-one (24.6%) of the patients ranged between 61-80 years. On the marital status, most of the patients interviewed were married with one hundred and thirty one (78.4%), while single, widowed and divorced were 2(1.2%), 28(16.8%) and 6(3.6%) respectively. Based on educational career, one hundred and thirty seven (82.4%) had no formal education, nineteen (11.1%) received primary schooling, eleven (6.5%) patients possessed secondary certificates while none had tertiary training. As many as eighty five (50.9%) were traders, the rest were civil servants 25 (15.0%), professionals 18 (10.6%), farmers 27(16.2%) and retired workers 12 (7.2%) (Table 1).

b) Therapy initiation period

Most of the patients eighty four (50.2%) were in the early stage of therapy of 2-4 months, followed by thirty two (19.1%) for 5-6months, seventeen (10.2%) for 7-9 months, while 10 months and above with thirty four (20.5%) also constituted some parts (Table 2).

c) Clinical symptoms of patients

The clinical conditions experienced by the patients were indicated in Table 3. Patients with severe headaches; seventy three (43.8%) rank first among other symptoms. Chest tightness; thirty-four (20.2%), numbness of extremities; nineteen (11.33%), fatigue; eighteen (11.1%), breathing difficulty; seventeen (10.3%) and six (3.3%) pounding in the head, neck and ears were other conditions that brought the patients to the hospital.

d) Antihypertensive drugs prescribed for patients at Sobi Specialist Hospital

The most commonly prescribed antihypertensive drugs in the hospital being studied were as follows, sixty-one (36.5%) were on Amlodipine, thirty-eight (22.7%) patients were on methyldopa, thirty-five(21.0%) were placed on Nifedipine. Only thirty (18.0%) remained on lisinopril. Rarely use antihypertensive drugs were Ramipril 1(0.7%) and Enalapril 2(1.2%) (Table 4).

e) Outcomes of hypertensive patients at Sobi Specialist Hospital, Ilorin

Majority of these patients, one hundred and thirty three (79.9%) had no knowledge about their disease state while only 34(20.1%) had an idea about the disease condition being treated for. Most patients, one hundred and fifty-four (92.2%) received comprehensive drug counselling from the pharmacist during prescription refills and very few, thirteen (7.8%) could not benefit from the counselling. A total of six thousand one hundred and twenty-two prescriptions were seen during the study period, of which (91.6%) constituted generic drugs and only (8.4%) were branded drugs. Many patients, one hundred and fifty-one (90.4%) showed no concern or fear for the generic drugs offered to them at the Pharmacy, while minority, sixteen (9.6) patients were uneasy with the generic drugs. On the classification of hypertension, fifty-nine (35.3%) were in stage 1 while majority; eighty-three fell into stage 2, and only twenty-five (15.0%) had Isolated systolic hypertension. In comparison with cost of drugs for a patient per month, generic drug cost four hundred and eighty Naira ($3) while for branded counterpart was two thousand two hundred and twenty Naira ($14) (Table 5)

f) Adherence

In the present study, based on patients' self-report adherence rating (Table 6), one hundred and twenty-three (67.7%) of the patients adhered strictly to their medications while only forty-four (32.3%) were poor adherents.

IV. Discussion

If a generic drug is deemed to be bioequivalent and has the same active ingredient as the branded drug with the same dose availability and routes of administration, the drug is approved as therapeutically equivalent and substitution is allowed without risk of toxicity or diminished efficacy (Jobst, and Holmes, 2004; Feely et al., 2005; Crawford et al., 2006; Liow et al., 2007). Therefore it is advised that physicians stress the importance of patient compliance and drug adherence to patients and caregivers as it is paramount to treatment success whether they are taking brand, generics, old or new hypertensive agents (Hakonsen, 2009). In this study, about two-third of the patients were females same as reported by Cenedese, (2006) in Minnesota and Degl' Innocent (2004) in UK. Contrary to this was the studies of Helle et al. (2009) in Norway whereby 56.9% were males and study of Alebiosu, (2010) in Nigeria that risk factor for essential hypertension include male. The rational for large proportion of women in this study was that women visit hospital more often during their reproductive years (antenatal) whereby they were exposed to different medical tests and received comprehensive counselling on various health diseases including high blood pressure. As soon as they perceived medical ailment, they tend to seek advice from healthcare providers. This contributed to better life expectancy of 48 years for women compared with 46 years for men (CIA World Factbook, 2010).

More than three quarter of the patients receiving treatment for hypertension were within the age range of 40 years and above. This finding is synonymous to study of Alebiosu, (2010), in Nigeria that age greater than 40 years is a risk factor for essential hypertension. This is attributed to the aetiology of hypertension whereby blood pressure generally tends to rise with age. This same patients were victims of rheumatoid arthritis consuming a substantial amount of Nonsteroidal anti-inflammatory drugs (NSAIDs) that produce increases in blood pressure averaging 5 mm of mercury (Chobanian et al., 2003). In this study, less than one fifth of the patients were literate while the rest of the patients could not read nor write. This is the consequence of low literacy level of Nigeria citizens compared with developed countries whereby the minimum years of education received by the patients was12 years in Norway (Helle et al, 2009). The high level of ignorance among the population in this country has deprived them of information on how to prevent and manage hypertension as publicised on radio, television and papers. In support of this research work is The World Health Organization. The World Hypertension League (WHL) recognized that more than 50% of the hypertensive populations worldwide are unaware of their condition. To address this problem, the WHL initiated a global awareness campaign on hypertension in 2005 and dedicated May 17 of each year as World Hypertension Day (WHD). In 2007, there was record participation from 47 member countries of the WHL. During the week of WHD, all these countries – in partnership with their local governments, professional societies, nongovernmental organizations and private industries, – promoted hypertension awareness among the public through several media and public rallies. Using mass media such as Internet and television, the message reached more than 250 million people. As the momentum picks up year after year, the WHL is confident that almost all the estimated 1.5 billion people affected by elevated blood pressure can be reached (Chockalingam; 2007, 2008)

In the present study, most of the patients were traders. This is in line with study of Enwere et al., (2006) in Ibadan, Nigeria. In the origin of hypertension, stress is a contributory factor to the development of hypertension. Stress which involves physical exertion, psychological disturbance and mental arithmetic stimulate sympathetic nervous system resulting in acceleration of heart rate. These effects of sympathetic stimulation serve to elevate blood pressure (Aguwa, 2004). This reflected in the present study as more than half of the patients were traders. These patients when interviewed were able to give information on their daily schedule has been rigorous accompany by sleepless nights (recalling the day's sales).This has proned them to severe high blood pressure. Almost half of the patients were new on treatment and showed up within a short period of 4 months to reduce cases of complications. Dispensing of generic drugs to these patients raise little concern as they are new in the therapy and can be easily convinced through drug counselling by the Pharmacist. This improves medication adherence among this category of patients in this setting. Hypertension, being a silent killer, as it is asymptomatic in nature; most patients could not discover the disease at early stage. Almost half of the patients showed up at stage two of the disease. There are generally no symptoms of high blood pressure, people do not feel it. When the blood pressure is extremely high, signs and symptoms now emerged.

In the present research work, severe headache, numbness of the extremities and breathing difficulty were the common symptoms that prompt people to visit primary healthcare providers for treatment. The asymptomatic nature of hypertension tends to subject patients to sudden death as a consequence of non-adherence to their medications. However, patients with such symptoms usually recognize the severity of hypertension and have higher tendency to adhere to their medications. The choice of antihypertensive drug will depend on the relevant indications or contra-indications for the individual patients. In this institution, Amlodipine, a dihydropyridine calcium channel blocker is mostly prescribed compared to other antihypertensive agents. Reason being that acceptability of Amlodipine among hypertensive patients is high because of its milder side effects, availability, affordability and simple dosing of once daily regimen. Also, it is the drug of choice in isolated systolic hypertension in the elderly (BNF, 2007).

Systolic hypertension is common among the elderly who constituted the greater percentage of patients receiving treatment for hypertension. Lisinopril, an Angiotensin Converting Enzyme Inhibitor is widely prescribed for patients because of its renoprotective effect. This is an advantage for hypertensive patients with diabetes mellitus. The side effects of dry cough which irritate and inconveniencing patients is a setback to its use. The dry cough could be alleviated by the use of Cromoglycate Sodium. Methyldopa is a centrally acting antihypertensive drug preferred by some physicians because of its cheapness, availability and safety in pregnancy. The side effects of decreased libido and severe depression restricted its acceptability by some patients. However, these side effects could be minimised if the daily dose is kept below 1g (BNF, 2007). In this study, Nifedipine is valuable in the management of hypertension because of its cost effectiveness and accessibility but the disadvantages of palpitations and chest pain experienced by few patients limited its use. Generic Enalapril and Ramipril were scantily available in the market except the branded types which seems to be out of pocket for most patients. In support of this work is the generic prescribing pattern of antihypertensive agents by the study of Jan and Eoin (2007) in Belgium and Enwere et al. (2006) in Ibadan, Nigeria. Furthermore, in agreement with present study is the trials involving beta-blockers, diuretics, calcium channel blockers, anti-platelet agents, statins, angiotensin-converting enzyme inhibitors, and alpha-blockers, no evidence of superiority of brand-name drugs against generics was found (Pawel and Przemyslaw, 2010). Patients taking generic drugs appear to be more willing to continue therapy than those taking brand-name medications. Lower co-pays are a key factor. In one recent study of patients with hypercholesterolemia or diabetes, those taking generics had greater adherence compared with patients receiving brand-name drugs (Pawel and Przemyslaw, 2010). The high cost of medications and the large number of prescribed drugs were the common reasons given by patients for non-adherence to prescribed drugs. This further emphasizes the need to reduce the cost of medications to patients through increased prescription of drugs in their generic names and rational drug prescription without a fall in treatment standards (Enwere et al., 2006).

In this setting, generic drugs were encouraged with almost all the prescriptions from the physicians carrying generic anti hypertensive drugs as recommended by World Health Organisation that drugs should be prescribed using their international non-proprietary names. This contributed to the better drug adherence of 67.7% recorded in this study, since the patients were able to afford the cost of the drugs compared to brand drugs which were almost five times the price of generic drugs. It is a better adherence because the average, adherence to antihypertensive drug therapy is 50% (Berg, 2007), and this is an important factor in why only half of the patients on these drugs achieve adequate blood pressure control (Chobanian et al., 2003, Hajjar and Kotchen, 2003). Furthermore, in Ibadan, Yusuff and Alabi (2007)

reported adherence level of 49%, while Ekwunife et al. (2010) observed 70.7% adherence level in Nsukka. Also, the consistent and thorough drug counselling for these patients received from the Pharmacist during drug refill contributed immensely to the promotion of generic drugs and drug adherence. The mutual relationship existing between Pharmacist and patients allay the fear / concern on generic drugs that the patients might have experienced during therapy leading to loyalty in their medications. In line to this was the study of Hakonsen et al. (2009) who reported that reduced adherence was as a result of insufficient information on generic substitution. Although, ninety percent level of adherence is required to achieve a well controlled blood pressure in this group of patients (Youssef and Moubarak, 2002). Lack of comprehensive knowledge about the disease such as causes, symptoms, risk factors as well as complications of hypertension is an obstacle to 100% drug adherence. In this research work, it is a contributory factor to those few non-adherent patients since they were ignorant of the ailment. To keep the public abreast of hypertension, at every entry point to the hospital, all patients should be screened for hypertension and well equipped with relevant information on the understanding of high blood pressure and its complications. This could reduce morbidity and mortality accruing from this silent killer disease. Cost of drug is a setback in drug adherence especially hypertension.

Cost-containment measures in healthcare provision include the implementation of therapeutic and generic drug substitution strategies in patients whose condition is already well controlled with pharmacotherapy. Treatment for hypertension is frequently targeted for such measures (Johnston and Stergiou, 2010). This is reflected in the present study whereby the small number of patients (fifty-four non-adherent patients) could not meet the expenses of branded drugs, hence influencing adherence negatively. The use of brand names may lead to increased cost of drugs for these clients. Factors that may be responsible for this trend include the influence of drug promotional activities, demands of pharmaceutical detail medical representatives, lack of continuing education on the principles of rational prescribing and non-familiarity with generic names among the prescribers. Since generic drugs have the same therapeutic effect as the original formulation but at generally lower costs, their use should be more heavily promoted (Duh et al., 2007). A patient experiencing financial hardship may find it difficult to spend money on a drug particularly if it does not result in an immediate change in health status or the benefit of the drug, is not properly understood (Crawford et al., 2006). Quality healthcare outcomes depend upon patient adherence to recommended treatment regimen. Patients' non-adherence cannot only be a pervasive threat to health, but also carry an appreciable burden as well as human well being (Martin et al., 2005).

V. Conclusion

Adults between the ages of 40 and 80 years were mostly affected by the hypertension, and women were more vulnerable to the disease. The clinical signs and symptoms mostly reported by the patients include severe headache, chest pain, numbness of extremities and general body weakness. The widely utilized generic antihypertensive agents in the studied hospital include Amlodipine, Methyldopa, Nifedipine, Lisinopril, which constituted 91.6% of all the prescriptions. The high rate of generic drug prescriptions in this hospital coupled with the consistent drug counselling offered to the patients by the pharmacist had greatly improved adherence rate to 67.7%.

VI. Recommendations

Generic drug substitution should be encouraged in all tiers of healthcare system to improve drug adherence and stem the tide of hypertension in the society. Also, public enlightenment and education should be created on media to keep the populace abreast of symptoms, risk factors, lifestyle modifications and complications of this silent killer disease.

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Microbial Synthesis and characterization of silver nanoparticles using the Endophytic bacterium Bacillus cereus: A novel source in the benign synthesis

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Abstract – The influx of nanoparticles into the living systems especially for medical purposes has opened up a new challenge of synthesizing them in a benign fashion. Green synthesis of nanoparticles is looked upon as an alternative to the existing physical and chemical methods of syntheses as they are associated with undeniable disadvantages. This initiated the biogenic synthesis of nanoparticles by using various microorganisms and plants. In this study we report the use of endophytic bacterium Bacillus cereus isolated from the Adhatoda beddomei to synthesize the silver nanoparticles (AgNPs). The AgNPs were synthesized by reduction of silver nitrate (AgNO3) solution by the endophytic bacterium after incubation for 3 days at room temperature. The synthesis was initially observed by colour change from pale white to brown which was further confirmed by UV - Vis spectroscopy. The AgNPs were characterized using FTIR, SEM – EDAX and TEM. The synthesized nanoparticles were found to be spherical and uniformly distributed with the size in the range of 11-16 nm. The energy-dispersive spectroscopy of the nanoparticle dispersion confirmed the presence of elemental silver. The AgNPs were found to have reasonable antibacterial activity against a few pathogenic bacteria like Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus. Determining the minimum inhibitory concentration leading to inhibition of bacterial growth is still under way.

Keywords : *Endophytic bacteria, Bacillus cereus, silver nanoparticles, UV -Vis Spectra, TEM, antibacterial activity.*

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Microbial Synthesis and Characterization of Silver Nanoparticles Using the Endophytic Bacterium Bacillus Cereus: A Novel Source in the Benign Synthesis

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Abstract - The influx of nanoparticles into the living systems especially for medical purposes has opened up a new challenge of synthesizing them in a benign fashion. Green synthesis of nanoparticles is looked upon as an alternative to the existing physical and chemical methods of syntheses as they are associated with undeniable disadvantages. This initiated the biogenic synthesis of nanoparticles by using various microorganisms and plants. In this study we report the use of endophytic bacterium *Bacillus cereus* isolated from the *Adhatoda beddomei* to synthesize the silver nanoparticles (AgNPs). The AgNPs were synthesized by reduction of silver nitrate ($AgNO_3$) solution by the endophytic bacterium after incubation for 3 days at room temperature. The synthesis was initially observed by colour change from pale white to brown which was further confirmed by UV - Vis spectroscopy. The AgNPs were characterized using FTIR, SEM – EDAX and TEM. The synthesized nanoparticles were found to be spherical and uniformly distributed with the size in the range of 11-16 nm. The energy-dispersive spectroscopy of the nanoparticle dispersion confirmed the presence of elemental silver. The AgNPs were found to have reasonable antibacterial activity against a few pathogenic bacteria like *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus.* Determining the minimum inhibitory concentration leading to inhibition of bacterial growth is still under way.

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I. Introduction

The last decade had witnessed an enormous focus on nanoparticles and nanomaterials because of their unique size dependent physical and chemical properties. Their widespread uses in various fields had made their study more challenging. Nanoparticles are of great scientific interest as they bridge the gap between bulk materials and atomic or molecular structures as they deal with materials at nanoscale levels (Saifuddin 2009). Some of the physical properties exhibited by nanomaterials are due to large surface atom, large surface energy and spatial confinement and reduced imperfections. The applications of nanoparticles are innumerable ranging from fluorescent biological labels (Bruchez 1998; Chan 1998; Wang 2002) to drug and gene delivery (Mah 2000; Panatarotto 2003), bio detection of pathogens (Edelstein 2000), detection of proteins (Nam 2003), probing of DNA structure (Mahtab 1995), tissue engineering (Ma 2003; De La 2003), tumor destruction via heating (hyperthermia) (Yoshida 1999), separation and purification of biological molecules and cells (Molday 1982), MRI contrast enhancement (Weissleder1990), phagokinetic studies (Parak 2002) makes their synthesis an important area of research.

Owing to the growing usability of nanoparticles in biological systems especially as drug delivery vehicles into the cellular world, questions concerning the development of rapid, reliable and nature friendly experimental protocols is on the rise. A wide variety of physical and chemical methods to synthesize nanoparticles are in practice but their inherent flaws that include contamination from precursor chemicals, use of toxic solvents and generation of hazardous by-products (Thakkar 2010) that makes their use inappropriate in biological systems. These disadvantages demanded the development of nanoparticles using novel and well refined methods in experimental processes. This paved the way to explore for new benign "green" routes for synthesizing high-yielding, low cost, non-toxic and environment friendly nanoparticles. Nature by itself has offered an answer by being a store house of diverse biological species including plant and plant products, algae, fungi, yeast, bacteria and viruses that could be employed in the biosynthesis of nanoparticles. This has been earlier confirmed by various reports that advocate the production of intra-cellular or extracellular organic material by unicellular and multicellular organisms (Mann 1996).

The biosynthesis of nanoparticles emerging as an intersection between nanotechnology and biotechnology has been receiving increasing attention in the recent past. First evidence of biosynthesis was reported using *Pseudomonas stulzeri* (Klaus 1999) where the nanoparticles were deposited on the cell

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membrane. This study was followed by various other reports that demonstrated the use of different microorganisms that include Bacillus *licheniformis* (Kalimuthu 2008), *Lactobacillus* strains (Nair 2002), *Bacillus subtilis* (Saifuddin 2009), *Cornybacterium* sp. (Zhang 2005), *E. coli* (Gurunathan 2009a; Gurunathan 2009b) in the extra and intracellular synthesis of nanoparticles. Minaeian and coworkers have reported the synthesis of silver nanoparticles in the size range 50-100 nm *Klebsiella pheumoniae, Escherichia coli, Enterobacterdoacae* (Minaeian 2008). Certain investigations also revealed that formation of nanoparticles using fungi like *Fusarium oxysporium* (Ahmad 2003) and *Aspergillus fumigatus* (Bhainsa 2006).

While a number of reports are available on the biological synthesis of silver nanoparticles, the potential of endophytic microorganisms - microbes that colonize living internal tissues of plants without causing any immediate, overt negative effects (Bacon 2000) has not yet been tapped. Very few reports are available where in endophytic fungi were used for the synthesis of nanoparticles. One such study employed an endophytic fungus (*Colletotrichum* sp.) isolated from geranium leaves (*Pelargonium graveolens*) for the extracellular synthesis of gold nanoparticles (Shiv Shankar 2003). Another study revealed the use of *Aspergillus clavatus* (AzS-275), an endophytic fungus isolated from sterilized stem tissues of *Azadirachta indica* and reported about the antibacterial effect of silver nanoparticles synthesised by it (Vijay C Verma 2010).

To the best of our knowledge, there were no reports on the synthesis of silver nanoparticles using endophytic bacteria. The present investigation was carried out to synthesize silver nanoparticles from endophytic bacterium that is identified as *Bacillus cereus* isolated from *Adathoda beddomei*. The potential antibacterial activity of the nanoparticles has also been evaluated.

II. Materials and Methods

The medicinal plant *Adhatoda beddomei,* under study, was obtained from Siddha Institute, Chennai, India. This is an evergreen herb used in Ayurveda where the leaves, seeds and the roots are administered for treatment of cough and asthma. Silver nitrate was obtained from SISCO Research Laboratories, India. The test organisms used for antibacterial assay were *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923.

a) Isolation of Endophytic Bacteria

Leaf samples of *Adhatoda beddomei* were cleaned under running tap water to remove debris and then air dried and processed within 5 hrs of collection. From each leaf sample, 4 segments of 1 cm length were separated and treated as replicates. Surface sterilization was carried out by submerging them in 75% ethanol for 2 min. The explants were further sterilized sequentially in 5.3% sodium hypochlorite (NaOCl) solution for 5 min and 75% ethanol for 0.5 min (Ravi Raja 2006). Samples were allowed to dry on paper towel in a laminar air flow chamber. Four segments per plant were placed horizontally on separate Petri dishes containing Nutrient Agar. After incubation at 32°C for three days, the endophytic bacteria was collected and placed onto nutrient agar and incubated for 3 days and checked for culture purity. Eventually, pure cultures were transferred to nutrient agar slant tubes and subcultured regularly.

b) Molecular Characterization of Endophytic Bacteria

The sequence of the 16s rRNA gene has been widely used as a phylogenetic marker to study genetic relationships between different strains of bacteria. The analysis of this gene can therefore be considered as a standard method for the identification of bacteria at the family, genus and species levels (Woese 1987; Weisburg 1991), and has infact been included in the latest edition of **Bergey's Manual of Systematic Bacteriology** (Garrity 2005). Genomic DNA was isolated from the pure culture pellet and the approximately 1.4 kb fragments corresponding to 16s rRNA was amplified using universal primers, high – fidelity PCR polymerase. The PCR product was sequenced bi-directionally using the forward, reverse primers. This sequence was compared with the 16s rDNA sequence data from strains available at the public databases (Genbank, EMBL and DDBJ) using BLASTN sequence match routines (Procópio 2009). The sequences are aligned using CLUSTALW2 program and phylogenetic and molecular evolutionary analysis were conducted.

c) Culture Conditions

The endophytic bacterial culture was maintained on nutrient agar slants by subculturing at monthly intervals. 100 mL of Luria Broth medium was prepared, sterilized and inoculated with 12 hr old cultures of the endophytic bacterium. The culture flasks were incubated for 36 hrs at 37°C with shaking at 150 rpm. After incubation period, the bacterial cell pellet was collected by centrifugation at 10,000 rpm for 10 min. This was used as the starting material for the synthesis of nanoparticles.

d) Synthesis of Silver Nanoparticles

After 36 hrs of incubation, the biomass is separated from the medium by centrifugation and was washed three times in sterile distilled water to remove any adhering nutrient media that might interact with the silver ions. The bacterial biomass obtained, about 1g wet weight was then resuspended into 20 mL of 1mM Silver nitrate solution and incubated for 72 -120 hrs at room temperature (Shiying 2007).

e) Characterization Techniques

The formation of AgNPs was followed by visual observation of color change from pale white to brown and was further confirmed by the sharp peaks given by the AgNPs in the visible region from UV – vis spectrum of the reacting solution using Perkin-Elmer Lamda-45 spectrophotometer, in a 1cm path quartz cell at a resolution of 1 nm from 250 to 800 nm. The studies on morphology, size, composition and the distribution of nanoparticles were performed by Transmission Electron Microscopic (TEM) analysis using a TEM, JEM- 1200EX, JEOL Ltd., Japan, Scanning Electron Microscope (SEM) using Hitachi S-4500 SEM and energy dispersive spectroscopy (EDAX) as an attachment on SEM. The probable biomolecules involved in the synthesis and stabilization of nanoparticles was recorded by FTIR spectrum using FTIR Nicolet Avatar 660 (Nicolet, USA).

f) Antibacterial Screening

Though different types of nanomaterials have come up, silver nanoparticles have proved to be the most effective antimicrobial agents. Hence the potential of the synthesized silver nanoparticles was determined, using the agar well diffusion assay method (Perez 1990). The test organisms used were gram negative bacteria *Escherichia coli* ATCC 35218*, Pseudomonas aeruginosa* ATCC 27853 and gram positive bacteria *Staphylococcus aureus* ATCC 25923. Two replicas of respective test organisms were prepared by spreading 100 µL of revived culture on the nutrient agar plate. Wells were cut with the help of a sterilized stainless steel cork borer into which 100 µL of AgNP solution was loaded and incubated at 37°C. The plates were examined for evidence of zones of inhibition, which appear as a clear area around the wells. The diameter of such zones of inhibition was measured for each organism and expressed in millimeter.

III. Results and Discussion

The intersection of nanotechnology and biology referred to as nanobiotechnology is a recently emerging field. The applications of this merger have spread across widely, extending its arms into the biological world at a rapid pace. This technical approach to biology allows the scientists to imagine and create systems that can be used for biological research. Nanoparticles that form the crux of nanotechnology have the innate ability to penetrate into the living systems owing to their size and properties. This insists a need to develop a greener route of synthesizing them thus making the process facile and ecofriendly. Biological systems possess unique ability to be self-organized and to synthesize molecules that have highly selective properties. This opened a new possibility of using microorganisms as the nanoparticle factories. This study demonstrates the capability of endophytic bacteria to synthesize silver nanoparticles in a more environment friendly manner.

a) Isolation and Molecular Characterization of Endophytic Bacteria

One endophytic bacterium was isolated from surfaced sterilized leaf fragments of *Adhatoda beddomei* after 24 hrs of incubation and appreciable growth was noticed after 48 hrs. Basic microbiological and biochemical characteristics of the endophytic bacteria identified the organism to be gram positive *Bacilli sp*. This was further confirmed by 16s rDNA analysis. 16S rRNA gene sequences contain hypervariable regions that can provide species-specific signature sequences useful for bacterial identification. As a result, 16S rRNA gene sequencing has become prevalent in medical microbiology as a rapid, accurate alternative to phenotypic methods of bacterial identification (Procópio 2009).

The endophytic bacterial DNA was isolated and the 16s rDNA sequence was amplified and sequenced and has been deposited in GENBANK with the accession number HM998898.1. The 16s rDNA sequence of the endophytic bacterium obtained was compared with the non-redundant BLAST database to obtain the sequences that displayed maximum similarity. All the sequences reported by BLAST revealed that the endophytic bacterial species showed a very high percentage of similarity (99%) with the sequence of *Bacillus cereus*, with a reasonably high score and e-value being zero. The sequences showing the maximum similarity were used for alignment using CLUSTAL W2 to arrive at phylogentic relationship represented by a phylogenetic tree (Fig.1) showing the evolutionary relationship that was constructed from the alignment using the neighbor-joining algorithm.

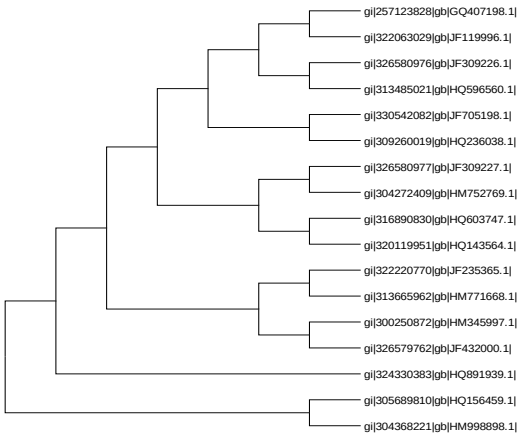

Fig. 1. Phylogenetic relationship of the endophytic bacterial 16s rDNA sequence (HM998898.1) derived by Neighbourning method using CLUSTALW.

There exists a clear evolutionary relation between all the 16s rDNA sequences as this is a highly conserved sequence. The tree derived by distance based, neighbor joining method is an unrooted tree inferring that the sequences do not come from a common ancestor. But they exhibit cladistic relationship which could be due to the similarities within the sequences. All the taxa under comparison belong to the genera *Bacillus* and species *cereus* except for a few sequences whose species has not yet been identified. The sequence of the endophytic bacterium HM998898.1 was shown to be related to HQ156459 *Bacillus cereus* to form a clade as they exhibit a very high similarity (99%) and very low e-value indicating its closest resemblance to the sister-group.

b) Synthesis and Characterization of Nanoparticles

The biomass obtained after centrifugation was challenged with silver nitrate solution and the formation of nanoparticles was observed from the colour change of the treated solution from pale white to brown that indicated the reduction reaction. (Fig 2.A) (Kalimuthu 2008; Gurunathan 2009b; Minaeian 2008; Sadowski 2008). The characteristic brown color arises due to excitation of surface plasmon vibrations in the silver metal nanoparticles (Minaeian 2008; Sadowski 2008). This bioreduction of Silver nitrate ions was followed by UV-vis spectroscopy. The spectrum showed a strong surface plasmon absorption band at around 425 nm (Fig.2B) indicating the presence of spherical or roughly spherical AgNPs that remained the same throughout the reaction period, suggesting that the particles are dispersed in the aqueous solution with no evidence for aggregation (Saifuddin 2009). Observation of this sharp clear peak, assigned to a surface plasmon, was well documented for various metal nanoparticles with sizes ranging from 2 to 100 nm (Kowshik 2003; Henglein 1993). A long tailing on the large-wavelength side may be due to small amount of particle aggregation (Minaeian 2008).

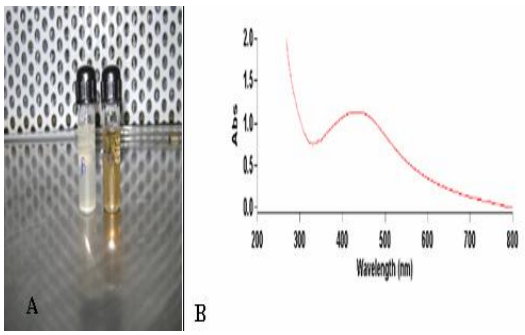

Fig 2. A) Picture of tubes containing the bacterial biomass *Bacillus cereus* biomass from *Adhatoda beddomei* before and after 120hrs incubation in an aqueous of $AgNo_3$ solution at neutral pH; B) UV–Vis absorption spectra of Silver nanoparticles after the reaction with 10^{-3} M aqueous solution $AgNo_3$ at neutral pH with the endophytic bacteria *Bacillus cereus.*

The same process when repeated with culture supernatant was unable to show any color change that states that the bacterial biomass was responsible for the bioreduction of $AgNO_3$. Controls (organism and reagent) showed no change in color when incubated under the same conditions indicating the role of the bacteria in the reduction of silver (Saifuddin 2009). When tested for stability, the silver nanoparticle solution was stable for two months which is evident from UV – Vis spectra after which the particles started to show aggregation (data not shown).

The stability of the AgNPs may be conferred by the proteins that may be involved in their synthesis. This is evident from the FTIR spectrum of AgNPs (Fig.3) which gave peaks at 3442 cm^{-1} corresponding to the OH stretch of carboxylic acid and the peak at 2350 cm^{-1} corresponding to aldehydic C–H stretching and 1641 cm^{-1} corresponding to N-H bending of primary amines amide I bonds of proteins that may arise due to carboxyl stretch and N–H deformation vibrations (Sathyavati 2010; Mann 1996).

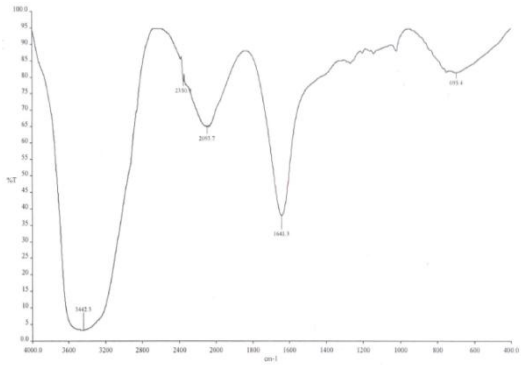

Fig. 3. FTIR spectrum of the silver nanoparticles synthesised by the endophytic bacteria *Bacillus cereus.*

The proteins function as capping agents as the carbonyl group from the aminoacid residues show stronger ability to bind to metals (Sathyavati 2010). It has already been reported that the biological molecules perform dual functions of formation and stabilization of silver nanoparticles in the aqueous medium (Mallikarjuna 2011).

The morphology, size and the distribution of nanoparticles was observed through the SEM and TEM micrographs (Fig. 4A & B). The SEM micrographs recorded showed comparatively spherical of roughly spherical nanoparticles which were observed to be uniformly distributed. This was further confirmed by the representative TEM images recorded from the drop-coated film of the silver nanoparticles that exposed spherical silver nanoparticles that were distributed on the surface and were uniformly dispersed without much traces of aggregation. The size of the silver nanoparticles ranged from 11 – 16 nm.

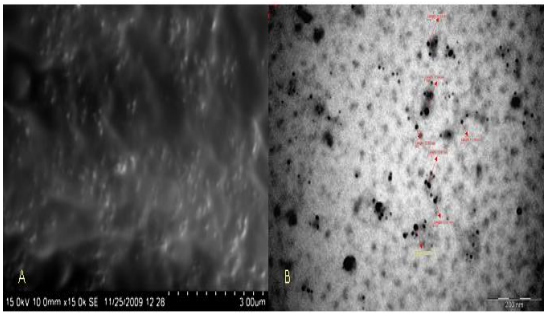

Fig. 4. (A) SEM micrograph recorded for silver nanoparticles (B) TEM image of the silver nanoparticles

The presence of elemental silver in the biologically synthesised nanoparticle solution was confirmed by EDX analysis (Fig. 5) where strong optical absorption peaks were observed approximately at 3 keV, which is typical for the absorption of metallic silver nanocrystallites (28%) due to surface plasmon resonance (Mouxing 2006). Few weaker signals from C, O and N were also recorded which may be due to X-ray emissions from the organism (Mouxing 2006).

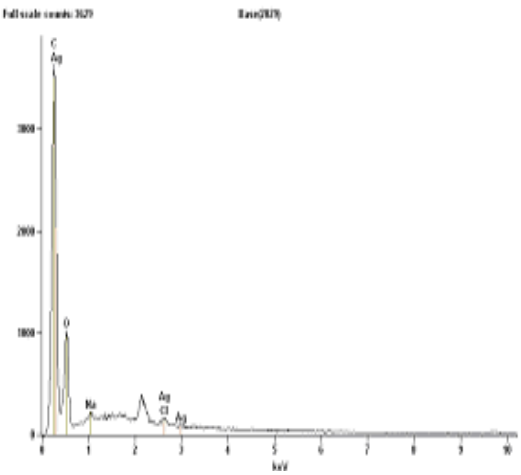

Fig. 5. EDX spectrum of silver nanoparticles synthesized by endophytic *Bacillus cereus.*

c) Antibacterial Activity

For quite some time, silver was known to possess disinfecting effect and was found to have an important place in traditional medicine. But the discovery of penicillin minimized the use of silver for the treatment of bacterial infections. But the proposition by Moyer et al in 1965 that silver nitrate doesnot interfere with epidermal proliferation and posess antibacterial activity against *Staphylococcous aureus, Pseudomonas aeruginosa* and *E. coli* has given silver and its derivatives a comeback. Thus, it is appropriate to investigate the antibacterial property of nanoparticles (Mahendra Rai 2009).

The bactericidal activity of AgNPs was studied using the pathogenic strains of bacteria namely gram negative *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853 and gram positive bacteria *Staphylococcus aureus* ATCC 25923 using agar well diffusion method. After the incubation time, clear zones were observed against all the test organisms by AgNPs and were recorded in millimetres (Table 1).

Test Organism	Zone of Inhibition (mm)	
	Sample	Ofloxacin
Pseudomonas aeruginosa	17	12
Escherichia coli	15	13
Staphylococcus aureus	12	15

Table 1: Zones of inhibition by the nanoparticles against the test organisms

The efficacy of silver nanoparticles can be attributed to the fact that their larger surface area enables them a better contact with the microorganisms. This is further supported by the revelation that size dependent interaction of silver nanoparticles with bacteria leads to its antibacterial activity (Pal 2007).

The toxicity of silver ions, though not very clearly understood, could be by their adhesion to the cell membrane and further penetration inside or by interaction with phosphorus containing compounds like DNA disturbing the replication process or preferably by their attack on the respiratory chain. It has also been suggested that a strong reaction takes place between the silver ions and thiol groups of vital enzymes thus inactivating them. Some studies reported that the attachment of the nanoparticles on to the surface of the cell membrane disturbs the permeability and respiration functions of the cell. Experimental evidence advocated the loss of replication ability by the DNA when treated with silver ions (Mahendra Rai 2009). The effect of the silver nanoparticles was observed to be more in gram negative bacteria than gram positive bacteria which are attributed to the fact that the relative abundance of negative charges on gram negative bacteria facilitated the interaction between the nanoparticles and the cell wall (Siddhartha 2007).

IV. Conclusion

A novel approach for the green synthesis of silver nanoparticles was carried out using the endophytic bacteria, bacillus cereus isolated from adathoda beddomei. the ability of the bacteria to reduce ag+ to ag0 was harnessed with the size of nanoparticles ranging between 11-16 nm. they were found to be spherical and uniformly distributed and extracellularly synthesised. additionally the agnps were found to have antibacterial activity against the test organisms, more profound against gram negative

bacteria compared to that of gram positive organisms. the present study contributes to the possibility of using endophytes in the biosynthesis of silver nanoparticles which was further confirmed using material analysis techniques.

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Attitude of University Community towards Sporting Life of Students in Nigeria

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Abstract – Purpose : This research ascertained the attitude of University Communities towards the sporting lives of students in Nigeria. Methods & Materials: Participants included sports officials (153) and students (270) from nine universities. A validated self-structured questionnaire with a test- retest reliability coefficient of 0.90 was the instrument used. Frequency counts, simple percentages and chi-square (x2) were the descriptive and inferential statistics used to analyse the data obtained at 0.05 level of significance. Results: The computed chi-square value (5.59) is less than the table value (7.82) suggesting that the attitude of a University community does not play any significant role in student sports life. Conclusion & Applications: There is need for cooperation between the academic staff and Sports Council staff as their functions complement each other.

Keywords : *Elite athletes, Motivation, Mercenaries Beneficiaries, Antithetical*

GJMR-E Classification : *NLMC Code: QT 255, QT 250, QT 275*

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Attitude of University Community towards Sporting Life of Students in Nigeria

Jeroh Eruteyan Joseph Ph.D.

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I. Introduction

In recent years, Nigerian universities have been blamed for failing Nigeria in producing elite athletes for international sports competitions This recrimination stems from the fact that most successful sporting nations of the world like the United States, Russia, and Germany recruit most of their athletes from among the universities. Since Nigeria's appearance and participation at the World University Games (F.I.S.U), it was only in 1975 that a home.-based athlete in the person of Late Major Taiwo Ogunjobi won a bronze medal in 400 metres hurdles in Rome. Subsequent medals in 1983 and 1985 were won by foreign-based athletes (Andah, 1986; Obajinmi, 1996; Akiga, 2002). Where athletes are produced from the universities, evidence abounds that such nations invest a lot of money in providing standard facilities and equipment and athletic scholarships and other forms of motivation are at the disposal of the student athletes (Amuchie, 1984). In Nigeria the story is quite different generally. Most star athletes who have basic qualifications for university admissions are frustrated by the Joint Admissions Matriculation Board (JAMB). The alternative at their disposal is to seek greener pastures overseas where they not only perform well academically but in addition have their athletic abilities developed for national and international sports competitions. Only very few Nigerian universities give recognition to the athletic performance of their students. Very few Nigerian universities possess adequate standard sporting facilities and equipment that can be used in producing Olympic materials. There seems to be a little or no relationship between the State Sports Councils coaches, Ministry of Sports officials and the university efforts to produce star athletes. Yet the universities are expected to perform miracles at international sports meets with all these glaring examples of shortfalls. Serious motivation strategies are either absent or not properly utilized by university coaches and sports administrators (Jeroh, 2005). One major problem facing sports development in Nigerian universities today is the uncompromising attitude of the universities to sport. Attitude as used here refers to the lack of sports and admission policies as well as negative disposition of many academic staff towards sports (Jeroh, 2005). The lack of functional sports policy, according to Mshelia (2002) also affects the development of sports in the universities and thus affects the performance of the students. Ojeme (1998) asserted that there is no well thought out and documented policy for university sports. As a result, he concluded that sports development is not seen as a statutory duty of the universities. Mgbor and Obiyemi (2001) criticized the present status of sports in Nigerian universities as being grossly under funded, lacking adequate facilities and equipment, lacking adequate and competent staff, improper training programmes for athletes and lack of incentives to athletes and coaches. Sport according to them, is regarded as an area for the never-do-well in our tertiary institutions.

Quoting from Williams Committee Report of 1983 on the attitude of universities to sports. Egborge (1986) said as follows:

"On the whole it can be said with a great measure of truth that at the moment, the universities of our land have not evolved any policies, and have not been able to evolve any policies to meet the problems (p. 160)

On the attitude of the university students, the report says:

"There have been occasions when university students who have been called to camp refused to show up. Usually, the reason for such refusal is connected with lectures and examinations (p. 160)"

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The pressure on the universities to fulfill the expectations of producing the nation's sportsmen and women has been on the increase. The universities have consistently come under sharper criticism for failing in this regard. The accusations have centred mainly on over concentration on academics with little or no cognizance for physical development through sports (Ikulayo, 1986). In a speech at the University of Benin Sports Dinner in 1984, Group Captain Emeka Omeruah, the then Federal Minister of Information, Social Development, Youth, Sports and Culture chastised Nigerian universities for not fulfilling the role expected of them on national sports development effort. Lamenting the position. Ekpeyong (1986) posited that our academics do not allocate any period for sports in their curricula. He argued that in other countries, university undergraduates are made to register for a certain number of courses in one form of sports or another. Though some Nigerian Universities Senates have decided to allocate some afternoons as lecture-free to enable students participate and excel in sports, such decisions are never implemented. These are all pointers to the fact that the universities have no articulated policy for sports development. In all these, the undergraduate athlete's effort at improving himself/herself sports wise is stultified.

Another great criticism against the universities is on the attitude of lecturers and other university staff towards sports. Many lecturers have been known to discourage sportsmen and women from participating in sporting activities. Ekpeyong (1986) said that "in Nigeria, there are many retrogressive academics who believe that those students who take part in sports are not serious students. Some of these teachers go an extra length of threatening students involved in sports". Evidence abounds that many athletes are occasionally victimized in examinations because of their participation in sports (Amuchie, 1986).

A contemptuous issue that arises is the rivalry that exists between academics in the Departments of Physical Education or Human Kinetics and their professional colleagues in the University Sports Council. This attitude, according to Salau (1996) has contributed greatly to the poor results recorded by Nigerian university undergraduates. Lamenting her ordeal. Miss Saidat Onanuga of the University of Ilorin, and an international 400 metres hurdler was quoted as saying "I missed my examinations because I went for a competition and nobody could assist me". How then can the students be interested in sports participation? Many university athletes have not been able to put in their best because they are torn between two loves-academics and sports. Though sports are of the moment, their educational qualification is a lasting effort which they won't forgo.

One other important problem identified with the universities' inability to develop sports concerns the recruitment of athletes with a view to training them to produce star and winning teams that can bring prestige and honour to the nation. Nigerian universities, according to Kamiyole 1986), have rigid rules on admission that make many university sports administrators helpless. As a result of this handicap, many universities nowadays result to the use of "mercenaries" and athletes that do not make admission requirements in an attempt to win medals (Adio, 1996).

Universities in the United States are famous for recruiting sporting talents from all over the world with their admission policies relaxed. Both admission and visa requirements are eased by the institution seeking to admit the athlete. Ijirigho, Porbeni, Obasogie. Egbunike. etc. are Nigerian beneficiaries of this gesture. We are all aware of the performances of these illustrious sons in international sports competitions donning Nigerian colours. Nigerian universities have not fully embraced this practice which has seen the cream of Nigerian. athletes migrating to the United States from where they have come to represent the country in major international sports competitions.

There are many cases where a Nigerian has been considered academically unfit for admission into a Nigerian university only for him to get admission abroad, get a higher degree, and then come home and be employed as a lecturer in the same university which had rejected him (Egborge, 1986). What an irony? Nigerian universities must find ways and means of encouraging athletes with their admission policies.

In a goodwill message to the 13th NUGA games in Calabar in 1990, Air Commodore Anthony Ikhazoboh appealed to university authorities to help design a curriculum and programme to enable them admit potential sportsmen and women who could combine their academic work with their sports training programme. This, according to him, is the position in the United States among others from where NUGA has had to "borrow" Nigerian students for FISU (World University Games). Unless this is embarked upon, there will be little or no progress in the nation's march to sports excellence.

While supporting a relaxation in the admission policy in favour of talented athletes, Ikulayo (1986) cautioned on the inherent danger of waiving admission requirements for athletes. She said that one of such dangers is that of' harassment by their counterparts within the same university. The comment such as "if not for sports you would not have been here" could be demoralizing for a sportsman or woman. So long as athletes and some lecturers perceive sports as antithetical to academics so long will the best sportsmen and women withdraw from active sports participation and competition (Amuchie, 1986).

This study was therefore embarked upon to examine if the attitude of the university community has any effect in sports performance among Nigerian university students.

II. Materials & Methods

The study employed the ex-post facto study design.

a) Participants

The balloting technique was used to select nine universities from the three geo-political zones in Southern Nigeria. The sample consisted of student captains (male and female) of fifteen NUGA sports, directors of sports and their assistants full time and honorary coaches who are full time staff of the universities (three from each zone) that were selected from thirty one universities in Southern Nigeria. There were 153 sports officials and 270 students.

b) Instrumentation

A self-developed structured questionnaire with a modified four-point Likert scale was used for data collection. The questionnaire consisted of two sections, "A" and "B". Section A sought information on demographic data of respondents which included name of institution, sport participated in and the status of respondents Section B sought information on the independent variable (attitude of the university community) of the study. The instrument was validated by colleagues and the test-retest method was used to establish the reliability using 30 students and 4 staff of Delta State University, Abraka who were not part of the study. The correlation of the scores yielded a correlation coefficient of 0.90 which was considered high enough for the study.

c) Procedure

The researcher personally administered the questionnaire with assistance from the directors of sports of the universities used and retrieved them resulting in a 100% return rate.

d) Statistical Analysis

Five items were used to test the null hypothesis. The responses for each of "strongly agree" and "agree" and those for "strongly disagree" and "disagree" were added for the two categories of respondents (athletes and officials) after which simple statistical tools of frequencies and percentages were used. The non-parametric tool of chi-square (X^2) was used to test the hypothesis at 0.05 level of significance.

III. Results

The data analysis is based on the hypothesis formulated to guide the study. The hypothesis was tested based on the responses of both athletes and sports officials.

Ho : There is no significant role played by the attitude of the university community in sports performance among Nigerian university students.

Table 1: Frequency and Chi-square (X^2) Analysis of Attitude of University Community and Sports Performance Among Nigerian University Students.

Respondents	SA	A	% SA + A	D	SD	% D+ SD	TOTAL
Officials	39 (31.47)	26 (22.79)	42.5	41 (48.17)	47 (50.64)	57.5	**153**
Athletes	48 (55.53)	37 (40.21)	31.5	92 (84.89)	93 (89.36)	68.5	**270**
Total	**87**	**63**	**35.5**	**133**	**140**	**64.5**	**423**

Source : Field work.

NB: Expected frequencies are in parentheses
Calculated X^2 value = 5.59
Degree of freedom (df) = 3
Level of significance = 0.05
Critical Value = 7.82
Decision = Hypothesis Accepted.

The table above shows the frequency, percentages and chi-square computation of responses on the role played by the attitude of university community in sports performance among Nigerian university students. The combined percentage responses for "strongly agree" and "agree" for officials was 42.5% while those of "disagree" and "strongly disagree" was 57.5%. The combined percentage of "strongly agree" and "agree" for athletes was 31.5% while that for "disagree" and "strongly disagree" 68.5%. A combination of both categories of respondents resulted in 37% for "strongly agree" and "agree" whereas, "disagree" and "strongly disagree" amounted to 63%.

To determine the significance of role, the calculated chi-square value of *5.59* was matched against the critical or table value of 7.82 at 0.05 level of significance. Since the calculated chi-square value of *5.59* is less than the table value of 7.82, the null hypothesis was accepted.

IV. Discussion

The data presented in Table 1 returned a verdict no significance on the role played by the attitude of the university community in sports performance among Nigerian university students. This position is disturbing when experience has shown that students who participate in sports have been victimized and scorned by lecturers. The fear of lecturers has led many athletes in the university to shun sports. The threat of victimization by lecturers in the Nigerian scene has been widely reported in the literature. (Amuchie 1986; Ekpeyong, 1986; Kamiyole, 1986; Adio. 1996: Salau. 1996).

The admission policies in the universities have made many of our talented athletes to go to the United States of America and Europe where conditions are better. Universities abroad are known to recruit talented athletes into their institutions through the offer of' scholarships and other incentives. Sportsmen there are well catered for and extra lectures/tutorials are organized for athletes who miss lectures while representing their institutions. All these opportunities are non-existent in Nigerian universities, Athletes who were denied admission in Nigerian universities on the basis of their basic qualifications went abroad, qualified and came back to lecture in the universities that denied them admission (Egborge, 1986; Ijirigho, l986). From the foregoing, one can conveniently conclude that Nigerian academics do not encourage and support student athletes. The researcher strongly feels that our academics should treat university athletes as their wards if not as their children by counseling and encouraging them instead of deriding them.

V. Conclusion

Based on the finding, the conclusion is that the attitude of the university community is not antithetical to sports performance among Nigerian university students.

VI. Recommendations/ Implications/Applications

From the study, the following recommendations are made. It is the ardent belief' of the researcher that if' they are properly and meticulously implemented, Nigerian universities would wake up from their lethargy to produce star athletes for the nation.

1. There should be a co-operative deal between the academic staff and staff of the Universities Sports Councils as their functions complement each other in the overall development of the students. University ego and pride are boosted by sports and all hands must be on deck to encourage those who bring such honour.
2. Extra lectures/tutorials should be organized for athletes who miss lectures as result of competitions. There is need for attitudinal change by lecturers who must encourage rather than discourage athletes who they should regard as their children/wards.
3. Special consideration should be given for the admission of talented athletes as done in the United States and Europe. While not supporting the admission of recluse in the name of sports, it will be sufficient if the prospective student who is good in sports has relevant credits in his area of interest rather than demanding compulsory credits in English Language and Mathematics.
4. University management should enforce the lecture free afternoons to enable athletes have the good opportunity to train in their respective sports. Engagement in sports could lead to the eradication/elimination of anti-social behaviour on campus.
5. Physical Education and Sports should be made compulsory in our universities and domiciled in the General Studies (GST) or General Education Studies (GES) Department. This provision will also serve the purpose of talent hunt, discovery and grooming.
6. There should be a policy that clearly defines the position of sports in the universities. The situation as it exists now where Universities sports are left to the whims and caprices of each university should be discontinued. A directive where each university is requested to make appropriate budgetary allocation to sports should emanate from the National Universities Commission (NUC) to the Vice Chancellors.
7. Sports in the universities should be run by a Committee/Council under the Vice Chancellor's office with membership drawn from the various Faculties/Colleges. This will bring the much desired co-operation between the sports office and lecturers as well as students.

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Spectroscopic Estimation of Pioglitazone Hydrochloride

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Abstract – A simple, sensitive, accurate, precise, reproducible and cost effective UV spectroscopic method has been developed for the estimation of Pioglitazone hydrochloride in bulk and tablet dosage form. Pioglitazone hydrochloride shows maximum absorption at 269 nm with molar absorptivity of 9.6013 × 104 l/mol.cm. Beer's law was obeyed in the concentration range of 10-70 µg/ml. The method was validated for linearity, precision, accuracy, sensitivity and specificity. The data obtained was treated with the statistical approach. The proposed method was found to be accurate and precise for estimation of Pioglitazone hydrochloride in bulk and tablet dosage form.

Keywords : *Pioglitazone hydrochloride, UV spectrophotometeric, validation, dissolution test, quality control test.*

GJMR-E Classification : *NLMC Code: QV 25, QV 38,*

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A. P. Kulkarni [α], Shahnawaz Mohd [σ], Zahid Zaheer [σ] & M. H. Dehghan [σ]

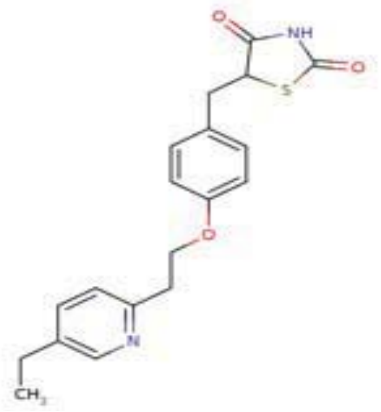
Abstract - A simple, sensitive, accurate, precise, reproducible and cost effective UV spectroscopic method has been developed for the estimation of Pioglitazone hydrochloride in bulk and tablet dosage form. Pioglitazone hydrochloride shows maximum absorption at 269 nm with molar absorptivity of 9.6013 × 104 l/mol.cm. Beer's law was obeyed in the concentration range of 10-70 μg/ml. The method was validated for linearity, precision, accuracy, sensitivity and specificity. The data obtained was treated with the statistical approach. The proposed method was found to be accurate and precise for estimation of Pioglitazone hydrochloride in bulk and tablet dosage form.

Keywords : Pioglitazone hydrochloride, UV spectrophotometeric, validation, dissolution test, quality control test

I. Introduction

Analysis of the drug is the most important aspect of formulation development. A suitable method is essential for the estimation of bulk drug, of the drug in formulation, in dissolution studies and in biological samples.

Chemically Pioglitazone hydrochloride (PH) is 5-(4-[2-(5-ethylpyridin-2- yl) ethoxy] benzyl) thiazolidine -2, 4-Dione. (Figure 1) [1] It belongs to the Class II of the BCS system and is practically insoluble in water but soluble in organic solvents like methanol, dimethyl sulfoxide and dimethylformamide. [1] It selectively stimulates the nuclear peroxisome proliferator-activated gamma receptor (PPAR-γ) and to a lesser extent peroxisome proliferator-activated alpha receptor (PPAR-α). It is primarily used in the treatment of diabetic conditions in combination or singly. It modulates the transcription of the insulin-sensitive genes involved in the control of glucose and lipid metabolism in the muscle, adipose tissue and the liver. As a result, it reduces insulin resistance in the liver and peripheral tissues, increases the expense of insulin-dependent glucose, decreases withdrawal of glucose from the liver, and reduces quantity of glucose. [2]



.HCl

Figure 1: Chemical Structure of Pioglitazone hydrochloride

According to literature review, a HPLC method for the estimation of Pioglitazone hydrochloride is available. [3] The method is relatively complex and expensive. The UV method for estimation of Pioglitazone hydrochloride in methanol: water: hydrochloric acid (250:250:1) system [4] and in 0.2 M sulphuric acid [5] have been reported. However, quantitative estimation of PH in other media has not been reported. This is essential in drug release study. The objective of the study was to develop a simple, accurate, precise, cost effective and reproducible UV method for estimation of PH in 0.1N hydrochloric acid as per ICH guidelines. [6]

II. Materials and Methods

Shimadzu UV/Visible double beam spectrophotometer and a Jasco V-630 instrument with 1 cm matched quartz cells were used for the spectral measurement. Shimadzu AX200 analytical balance was used for the weighing purpose. The reference standard of PH was obtained as a gift sample from Aarti Drugs, Thane (India) with 99.8% assay value. PH tablets (Piomed, 15 mg) were obtained from the market and utilized for the study. All other chemicals were of analytical grade.

a) Selection of The Media

The criterion for selection of the medium was the solubility and the stability, i.e. PH should be soluble

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and stable in the medium for sufficient time during the study. Methanol: water: hydrochloric acid (250:250:1) was used in the reported method. Dissolution studies recommend 0.1N hydrochloric acid. Hence 0.1N hydrochloric acid was selected as the analytical medium for the present work.

b) Preparation of Standard Solution

Standard solution of PH was prepared by dissolving 100 mg of drug in 100 ml of 0.1N hydrochloric acid (Solution A, 1000 µg/ml). Further 10 ml of the solution A was diluted to 100 ml with 0.1N hydrochloric acid (Solution B, 100 µg/ml). Solution B was used as the standard stock solution.

c) Preparation of Calibration Curve

Aliquots of 1 ml to 7 ml of the standard solution B were transferred into a series of calibrated 10 ml standard volumetric flasks and the final volume was made up using 0.1N hydrochloric acid. The solutions were scanned in the range of 200-400 nm against blank (0.1N hydrochloric acid). The absorption maximum was found to be at 269 nm. (Figure 2)The absorbance of the solutions were measured at 269 nm against the blank (Table 1) and the calibration curve was constructed. (Figure 3)

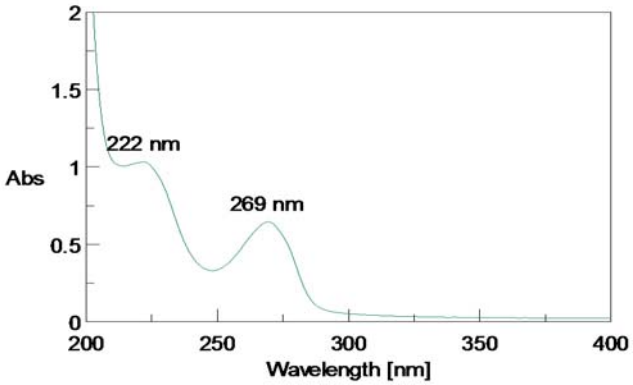

Figure 2 : Pioglitazone hydrochloride absorbance spectrum

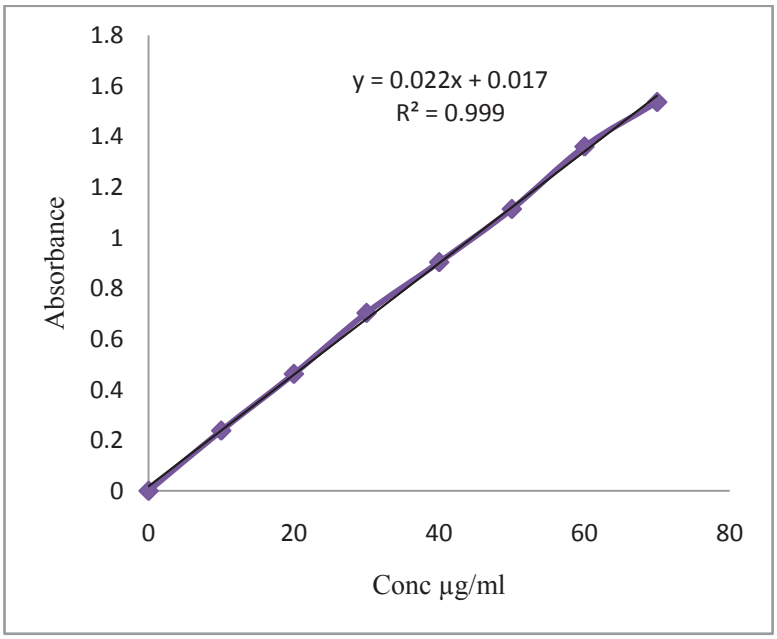

Figure 3 : Calibration Curve of Pioglitazone hydrochloride

Table 1: Calibration Curve of Pioglitazone hydrochloride

Sr. no	Concentration (µg/ml)	Absorbance	Standard deviation
1	0	0	0
2	10	0.2380	± 0.003551
3	20	0.4620	± 0.003404
4	30	0.7385	± 0.003593
5	40	0.9034	± 0.002524
6	50	1.1134	± 0.000917
7	60	1.3600	± 0.001000
8	70	1.5359	± 0.002571

The optical characteristics were summarized. (Table 2)

Table 2 : Validation Parameters

Sr no	Parameter	Result
1.	Absorption maxima	269 nm
2.	Linearity range	10-70 μg/ml
3.	Standard Regression Equation	y = 0.022x + 0.017
4.	Correlation coefficient (r^2)	0.999
5.	Molar Absorptivity	9.6013×10^4 l/mol.cm
6.	A (1%, 1 cm)	244.328 dl/gm/cm
7.	Accuracy (% recovery ± S.D)	99.3233 ± 0.7026
8.	Specificity	A 20 μg/ml of drug in 0.1 N HCl at UV detection wavelength of 269 nm shows an absorbance value of 0.4620 ± 0.003404
9.	LOD (μg/ml))	0.03
10.	LOQ (μg/ml)	0.10

d) Preparation of Sample Solution

The proposed method was applied to marketed PH tablets (Piomed, 15 mg). Twenty tablets of PH were weighed and powdered in a glass mortar. Powder equivalent to 100 mg of the drug was weighed accurately and transferred to a 100 ml standard volumetric flask. It was dissolved in about 50 ml of 0.1N hydrochloric acid and the volume was made up with 0.1N hydrochloric acid so that the concentration was 1000 μg/ml (Solution P). Ten ml of the solution P was transferred to a 100 ml standard volumetric flask and the volume was adjusted with 0.1N hydrochloric acid (Solution Q). The solution was filtered through Whatmann filter paper no. 41. The filtrate was diluted suitably with 0.1N hydrochloric acid to obtain a sample solution (20μg/ml). The absorbance of the sample solution was measured at 269 nm and the amount of PH was determined from the calibration curve. The method was studied for accuracy and precision.

III. Results and Discussion

a) Linearity

Pioglitazone hydrochloride exhibited maximum absorption at 269 nm and obeyed Beer's Law in the range of 10-70 μg/ml. [8, 10] Linear regression of absorbance Vs concentration yielded equation y= 0.022x + 0.017 with a correlation coefficient of 0.999.

b) Accuracy

To determine the suitability and reproducibility of the proposed method, recovery studies were carried out by adding known amount of standard PH (80%, 100%, and 120%) to the tablet solution P and analyzing the mixtures by the proposed method. Three samples were prepared for each recovery level. The percentage recovery of PH was found to be 99.3233 ± 0.7026 (Table 3) indicating that there is no interference by the excipients in the method. According to ICH guidelines, an acceptance criterion for the % recovery is 98-102%.

Table 3 : Accuracy by recovery method

Ingredient	Pioglitazone hydrochloride		
Tablet amount (μg/ml)	20	20	20
Level of addition (%)	80	100	120
Amount added (μg/ml)	16	20	24
Amount recovered (μg/ml)	35.748	39.574	44.1288
% Recovery	99.3000	98.9350	100.2927
Average % recovery	99.3233 ± 0.7026		

c) Precision

Precision of the method was demonstrated by intra-day and inter-day variation studies. For intra-day precision, six sample solutions of Pioglitazone hydrochloride of same concentration (20μg/ml) were analyzed three times in a day. The result is indicated by % RSD in Table 4.

During the intermediate precision (inter-day precision), six sample solutions of the same concentration (20μg/ml) were analyzed on three consecutive days and by two different analysts in same laboratory. The results are indicated by % RSD in Table 5 and 6.

For intra- day precision, the % drug content and the relative standard deviation (RSD) were found to be 99.958 ± 0.7874, 99.928 ± 1.104, 99.297 ± 1.114 and 1.0087 respectively; whereas for inter- day precision, the % drug content and the relative standard deviation (RSD) values 99.59722 ± 0.4722, 100.7488 ± 0.4522, 100.4226 ± 0.5617 and 0.4940 respectively. When the analyst was changed the RSD values were 0.48225 and 0.4662. According to ICH guidelines, an acceptance criterion for the precision is RSD ≤ 2%.

Table 4 : Intra- day Precision for Pioglitazone hydrochloride

Sample Number	Analysis of Pioglitazone hydrochloride as percent of drug content		
	10:00 am	2:00 pm	6:00 pm
1	101.214	98.979	98.569
2	99.458	99.568	100.598
3	99.587	99.259	99.454
4	100.254	101.871	100.598
5	98.979	99.298	97.995
6	100.256	100.598	98.568
Mean ± SD	99.958 ± 0.7874	99.928 ± 1.104	99.297 ± 1.114
Average ± RSD	99.7276 ± 1.00875 1.0087		

Table 5 : Inter-day precision for Pioglitazone hydrochloride

Sample number	Analysis of Pioglitazone hydrochloride as percent of labeled content		
	DAY-1	DAY-2	DAY-3
1	99.8467	100.725	100.053
2	100.0230	101.146	99.8792
3	99.4538	99.9875	99.9103
4	98.9985	100.5473	101.163
5	99.1356	100.856	100.5409
6	100.1257	101.231	100.9892
Mean ± SD	99.59722 ± 0.4722	100.7488 ± 0.4522	100.4226 ± 0.5617
Average ± RSD	100.2562 ± 0.4940 0.4940		

Table 6 : Determination of Precision, different analysts (Intra-day precision)

Sample number	Analysis of Pioglitazone hydrochloride as percent of labeled amount	
	Analyst-I	Analyst-II
1	100.2346	99.1035
2	100.9812	99.1418
3	99.8754	98.7460
4	100.0213	99.2435
5	99.5381	97.9924
6	100.1509	98.6356
Mean	100.1335	98.8149
Std. Deviation	0.48225	0.4662

d) Robustness

When the analysis was carried on two different instruments, the RSD values were 0.5297 and 0.5213.

Table 7 : Determination of Robustness (Different Instruments)

Sample number	Analysis of Pioglitazone hydrochloride as percent of labeled content	
	Shimadzu	Jasco
1	99.184	100.231
2	98.793	101.104
3	99.862	100.863
4	100.021	99.982
5	98.795	101.016
6	99.568	99.989
Mean	99.3705	100.5308
Std Deviation	0.5297	0.5213

e) Limit Of Detection (LOD) And Limit of Quantitation (LOQ)

The LOD and LOQ of PH were determined by using standard deviation of the response and the slope approach as defined in the ICH Guidelines [6]. The LOD and LOQ were found to be 0.03μg/ml and 0.1μg/ml respectively. The proposed method showed molar absorptivity of 9.6013 × 104 l/mol.cm. (Table 2)

IV. Conclusion

The developed method was found to be simple, accurate, precise, reproducible and can be used for dissolution studies and routine quality control analysis of PH in bulk and in tablet form.

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Machiavellian Orientation among Medical Representatives in Pharmaceutical Industry

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Abstract – Machiavellianism is an essential personality attribute for medical representatives in the pharmaceutical industry. The purpose of the study is to find out the Machiavellian orientation among gender and domestic or multinational pharmaceutical companies. Data is collected using a simple random and cluster sampling through a structured questionnaire using Mach IV scale (n=300). The research design is hypothesis testing and it is a cross-sectional study. Results indicate that male and multinational company medical reps are low Machiavellians compared to female and domestic company medical representatives. If the pharmaceutical companies can determine Machiavellianism of medical representatives at the time of their selection itself this can be a litmus test to determine their potential as productive medical representative. Machiavellian orientation will be more beneficial in the short term, and may damage the long term relationship with wholesalers and the medical fraternity. Hence, there is a need of more studies on long term relationships.

Keywords : *Medical representatives, Machiavellianism, pharmaceutical industry.*

GJMR-E Classification : *NLMC Code: QV 737,QV 744, QV 785,*

Strictly as per the compliance and regulations of:

Machiavellian Orientation among Medical Representatives in Pharmaceutical Industry

Molugulu Nagashekhara [α] & Ravindran Ramasamy [σ]

Abstract - Machiavellianism is an essential personality attribute for medical representatives in the pharmaceutical industry. The purpose of the study is to find out the Machiavellian orientation among gender and domestic or multinational pharmaceutical companies. Data is collected using a simple random and cluster sampling through a structured questionnaire using Mach IV scale (n=300). The research design is hypothesis testing and it is a cross-sectional study. Results indicate that male and multinational company medical reps are low Machiavellians compared to female and domestic company medical representatives. If the pharmaceutical companies can determine Machiavellianism of medical representatives at the time of their selection itself this can be a litmus test to determine their potential as productive medical representative. Machiavellian orientation will be more beneficial in the short term, and may damage the long term relationship with wholesalers and the medical fraternity. Hence, there is a need of more studies on long term relationships.

Keywords : Medical representatives, Machiavellianism, pharmaceutical industry

I. Introduction

The word Machiavellianism derived from the Niccolo Machiavelli (1469-1527) who wrote the "The Prince" advising others on how to acquire and maintain power. Christie and Geis (1970) were the first psychologists to study Machiavellianism. Machiavellianism is defined as "a process by which the manipulator gets more of some kind of reward than he/she would get without manipulating, while someone else gets less, at least within the immediate context" (Christie and Geis, 1970). Machiavellianism (MACH) used as a personality trait or dimension to classify people for several decades. The core of this personality type explains in terms of manipulative, exploitation, expediency, deviousness and is devoid of the traditional virtues of trust (Tang T L P and Yuh-Jia Chen 2008). Individuals having a Machiavellian personality trait or style may extol the virtues of using guile if necessary to achieve their objectives and they are self-oriented as far as personal goals concerned. A twentieth century Machiavellian may employ aggressive, manipulative, exploiting and devious behavior to achieve personal and organizational goals (Jamal A A et al 2007; Tang T L P and Yuh-Jia Chen 2008). In addition to this, it is also proved that high mach's employ aggressive and devious methods to achieve goals without regard for feelings, rights and needs of other people (Shapiro et al, 1995; Wilson et al., (1996).

Numerous studies have been carried out by many researchers on this personality concept in the west. In addition, a couple of studies are also available in the Arab world (Mostafa M M, 2007). A small number of studies have been carried out in the Indian scenario, but hardly any studies on medical representatives in the pharmaceutical industry. The present study fills the gap and tries to study the reliability and gender comparisons and type of company of Mach IV scale in Indian scenario, especially among medical representatives in the pharmaceutical industry.

Firstly, the main objective of the study is to find out the Machiavellianism orientation among medical representatives in the pharmaceutical industry. There is a need to study this concept especially in the medical representatives as they are dealing with business minded wholesalers and retailers and professionals like doctors on the other side. Sales targets and pressure from the managers to achieve the sales quotas place the medical representatives under significant pressure to perform. For example, medical representatives are likely to exaggerate the indications, side effects, availability of the drugs that they sell. In addition to this, some medical representatives, are becoming so desperate that they will stop at nothing, from backstabbing representatives on their own team to running bogus deals through accounting (Ya-Hui Hsu et al, 2008). This type of manipulative, exploitive, aggressive and devious tactics and war-type of sales environment has become progressively worse in the last few years causing conflicts among them and also adversely affect the society at large (Stewart, 2003; Marchetti, 1997; Ya-Hui Hsu et al, 2008; Gulhati C M, 2004). Presently, in the competitive promotion of pharmaceutical products, the medical representatives are led to depend on the tried and trusted 3C's: *Convince* if possible, *confuse* if necessary and *corrupt* if nothing else works (Gulhati C M, 2004). Hence, the present study tries to fill the gap in examining the reliability of the Mach IV scale in an Indian scenario, especially among medical representatives in pharmaceutical industry.

Secondly, the Mach IV scale developed by Christie and Geis (1970) is the best known instrument to

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measure the Machiavellianism in an individual. This standard instrument contains 20 items with a seven point Likert scale. Many studies reported a reliability coefficient below the acceptable Cronbach's alpha values, for example, studies showed 0.54, 0.36 and even 0.31 among undergraduate students (Whitney, 1976; Zook, 1985; Mudrack and Mason, 1995). Reliability studies are very important in empirical research as it provides confidence that the empirical findings accurately reflect the proposed constructs and also it provides consistent results in repeated uses (Gatewood and Field, 1990).

Hence, the research objective of the study is to investigate the Machiavellianism orientation among male and female medical representatives in the pharmaceutical industry in India. In addition to this, to test the reliability of the Mach IV scale in Indian scenario especially among medical representatives in the pharmaceutical industry.

Various studies offers evidence of differences in the Machiavellian orientation associated with demographic descriptions such as gender, age, education background and religiosity. Number of studies argues that females have high Machiavellian scores compare to their counterparts (Rayburn and Rayburn, 1996, Bolino and Turnley, 2003; Webster and Harmon, 2002). In contradiction to this, a study found that males are high Machiavellians compare to females (Hegarty and Sims 1978) and another study identified Machiavellian orientation of females and males were significantly different (Chonko, 1982). Research says that young managers are more Machiavellians than older ones (Hunt and Chonko, 1984). Research also says that there is no significant relationship between religiosity and Machiavellianism (Quah, C H et.al, 2008).

Hypothesis

Against this background, the present study hypothesizes Machiavellianism concept as:

H1. Male medical representatives will exhibit higher Machiavellian scores than female medical representatives in the pharmaceutical industry.

H2. Domestic pharmaceutical company medical representatives have high Machiavellian scores compare to Multinational pharmaceutical company medical representatives.

II. Methodology

This study uses the hypothesis statistical testing. It utilizes both descriptive and inferential statistics to test the hypothesis. There are two important hypotheses based on the demographic description of the medical representatives. The extent of researcher interference with the study is minimal because the researcher collects data from medical representatives (through a structured questionnaire). Beyond that the researcher has not interfered with the normal activities or the work of medical representatives. It is a correlational study and is conducted in non-contrived settings. The data is collected only once in the study for a period of 12 weeks and hence, it is a cross-sectional study (Sekaran U, 2003). The purview of this research focuses on pharmaceutical industries and products related to allopathic formulations. Medical representatives working for domestic and multinational pharmaceutical companies in Allopathic formulations with an at least one year experience are selected in the study's population. Data is collected among 300 medical representatives using simple random and cluster sampling method. The questionnaire consisted of four demographic description items of respondents and 19 items of Mach IV scale with a five point Likert scale from strongly disagree (one) to strongly agree (five). The item called "P.T. Barnum was wrong when he said that there's a sucker born every minute" is removed from the Mach IV scale after the pilot study, because many respondents were not convinced with the language used in the item.

III. Results and Discussion

The population for the present study consists of all medical representatives working for domestic and multinational companies in Karnataka State, India. The sample in the study covers all parts of the Karnataka state like north, south, central and coastal areas. Major cities involved are Bangalore, Mangalore, Manipal, Mysore, Bellary, Tumkur, Davanagere, Hubli, and Belgaum. Data is gathered through a structured questionnaire and personal visits.

Table 1: Demographic description of medical representatives

Gender	Male	213	71.0
	Female	87	29.0
Company type	Domestic	214	71.0
	Multinational	86	29.0

Among the 300 respondents, 71% are males and 29.0% are females. Similarly, 214 (71%) are working for domestic pharmaceutical companies whereas 86 (29%) respondents are working for multinational companies.

Table 2: Comparison of reliability studies of the past with the current study

Past studies	Sample size	Cronbach's alpha value
Al-Khatib et al., 1995	318	0.57
Ashton et al., 2000	610	0.72
Boon, 2002	451	0.76
Christie and Geis, 1970	1477	0.79
Corzine et al., 1999	183	0.62
Deluga, 2001	39	0.85
Dion and Banting, 1988	302	0.64
Ghosh and Grain, 1996	54	0.74
Hunt and Chonko, 1984	1076	0.76
Macrosson and Hemphill, 2001	50	0.71
O'Connor and Morrison, 2001	501	0.70
Shackleton et al., 1990	102	0.64
Singhapakdi, 1993	367	0.75
Siu and Tam, 1995	50	0.61
Tan, 2002	451	0.76
Topol and Gable, 1990	212	0.70
Tziner et al., 1996	51	0.69
Vitell et al., 1991	394	0.62
Wastell and Booth, 2003	100	0.51
Webster and Harmon, 2002	280	0.68
Mostafa M M, 2007	482	0.63
Current study coefficient alpha value	300	0.80

The Mach IV survey instrument is measured for the reliability test which consisted of 19 items. The squared multiple correlations in the items range of 0.5 to 0.853. Maximum scale mean if item deleted is 62.573 and maximum scale variance if item deleted is 100.726.

The average Cronbach's Alpha value for the Machiavellianism scale is 0.801. Some of the reliability studies are mentioned and compared with the present study in the table 2 which is adopted from Mostafa M M, 2007.

Table 3: Normality distribution of Mach IV scale

Mach IV	Mean	Std. Deviation	Skewness		Kurtosis	
Items	Statistic	Statistic	Statistic	Std. Error	Statistic	Std. Error
MACH 1	3.500	1.023	-0.368	0.141	-0.970	0.281
MACH 2	3.263	1.142	-0.042	0.141	-1.293	0.281
MACH 3	3.287	1.093	-0.047	0.141	-0.572	0.281
MACH 4	2.857	1.292	0.307	0.141	-0.936	0.281
MACH 5	3.237	1.222	-0.085	0.141	-0.978	0.281
MACH 6	3.900	1.178	-0.818	0.141	-0.447	0.281
MACH 7	3.863	1.147	-0.734	0.141	-0.344	0.281
MACH 8	3.933	1.110	-0.664	0.141	-0.768	0.281
MACH 9	4.070	0.974	-0.885	0.141	0.008	0.281
MACH 10	3.800	1.130	-0.719	0.141	-0.507	0.281
MACH 11	3.930	1.072	-0.844	0.141	-0.155	0.281
MACH 12	3.487	1.003	-1.234	0.141	0.647	0.281
MACH 13	2.827	1.175	-0.107	0.141	-1.185	0.281
MACH 14	2.580	1.201	0.061	0.141	-1.298	0.281
MACH 15	3.477	0.909	-0.846	0.141	0.326	0.281
MACH 16	3.757	1.010	-1.044	0.141	0.932	0.281
MACH 17	3.413	1.222	-0.533	0.141	-0.803	0.281
MACH 18	3.393	1.308	-0.722	0.141	-0.747	0.281
MACH 19	2.393	1.179	0.664	0.141	-0.522	0.281

Normality tests were carried out for all the items of Mach IV scale. In the table 3, all z values of skewness and kurtosis are within the range of -2.58 to +2.58. However, item number 12 shows a negative skewness of more than one (-1.234). This item need not be transformed to natural log as it is close to standard one. Only abnormal skewness of two or three needs transformation (Bajgier, S M., & Aggarwal, L K, 1991;

Barnett, V., & Lewis, T., 1996). Thus, it is evident that all the items have relatively normally distribution.

Table 4: Male and female Mach IV scores

	Gender	N	Mean	Std. Deviation	Std. Error Mean
Machiavellianism	Male	213	64.498	9.727	0.666
	Female	87	66.115	10.717	1.149

The average mean of Machiavellianism score for male is 64.498 while female is 66.115 with a standard deviation of 10.717, which imply that the female medical representatives in the sales force are high Machiavellians compared to male counterparts.

Table 5: Mean Differences between Male and Female Respondents

Variable	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Diff.
Machiavellianism	2.059	0.152	-1.268	298	0.206	-1.617	1.275

In the Table 5, there is a difference in both variance and in t test results. The groups behavior is not only different but also there is a small gap between the two groups (mean difference is -1.617). The results support the study of Mostafa, 2007 stating that females are more Machiavellian compared to their male counterparts. Women in India, have little control over many aspects in their lives. For example, especially after marriage, women are pressurized to quit their jobs and become full time mothers to take care of the children. In addition, there is a strong belief that, women are suitable for domestic activities. Even the parents in the families, educate their sons rather than their daughters on the assumption that boys are greater economic asset than girls (El-Ghannam, 2002). The researcher strongly believes that, women may view all these constraints as personal and try to overcome these constraints and this may influence them to become high Machiavellians compare to male (Mostafa M M, 2007). Hence, based on the findings, H1 cannot be accepted.

Table 6: Mach IV scores of domestic and multinational company's respondents

	Company type	N	Mean	Std. Deviation	Std. Error Mean
Machiavellianism	Domestic	214	67.215	6.761	0.462
	Multinational	86	59.372	13.970	1.506

In the Table 6, Domestic pharmaceutical company's medical representative's Machiavellianism score is compared with the multinational company's medical representatives by using independent sample t-test which compares the two means for their significant differences. The average mean of Machiavellianism score for domestic pharmaceutical companies medial representatives is 67.215 while multinational pharmaceutical companies medical representatives is 59.372, which imply that the domestic pharmaceutical companies medical representatives in the sales force are high Machiavellians compared to multinational pharmaceutical companies medical representatives.

Table 7: Mean difference between domestic and multinational medical representatives

	F	Sig.	T	Df	Sig. (2-tailed)	Mean Diff.	Std. Error Diff.
Machiavellianism	124.635	0.000	6.536	298	0.000	7.843	1.200

Machiavellianism score in the multinational pharmaceutical companies have significant difference when compared to domestic pharmaceutical companies, the mean difference is very high (7.843). It may be due to the high sales targets or pressure from the managers to perform or the presence of commission structure or loose behavior control by their superiors or boundaries may conform to their own beliefs in making any decisions (Ya-Hui Hsu et al., 2008). It states that, additional attention by some domestic pharmaceutical companies is needed to adopt and adapt to the standards and guidelines of professional bodies like

IDMA (Indian Drug Manufacturers Association), OPPI (Organization of Pharmaceutical Producers of India) or IFPMA (International Federation of Pharmaceutical Manufacturers Association). In addition to this, medical representatives should not be ignored or given tacit approval by these managers even if the sales goals are not met (Manthan D J et al., 2007). Exposure by multinational companies to advanced induction programs or capsule training and periodical instructions by the managers may influence these medical representatives to score low in the Machiavellianism scores. Hence, based on the findings H2 is accepted.

It is a cross-sectional study, so data limits the type of inferences. The future researchers may be able to adopt a longitudinal study to track the respondents, because, initially the medical representatives follow the standards and guidelines of the company and later because of many organizational and external variables they become high Machiavellians. This study was conducted amongst the medical representatives of pharmaceutical companies based in Karnataka state only and the results cannot be considered as being the same for the entire industry in India.

There a need to compare Machiavellian orientation with the job satisfaction, as many medical representatives are under the dual pressures of their managers and the physician demands on the other side which is further proved by high turnover of these medical representatives. In addition to this, some medical representatives, may recognize that the presence of company imposed policies anticipate irregularities and questionable behavior and may encourage or discourage these activities which results in the sales performance (Ya-Hui Hsu et al., 2008).

Medical representatives with high mach's can be better performers compare to low mach's. If the pharmaceutical companies can determine Machiavellianism of medical representatives at the time of their selection itself this can be a litmus test to determine their potential as productive medical representative and they can be effective in selecting the medical representatives. However, this Machiavellian orientation will be more beneficial in the short term, and may damage the long term relationship with wholesalers and the medical fraternity. Hence, there is a need of more studies on Machiavellian orientation effect on long term basis.

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- Insertion a title at the foot of a page with the subsequent text on the next page

- Separating a table/chart or figure - impound each figure/table to a single page
- Submitting a manuscript with pages out of sequence

In every sections of your document

· Use standard writing style including articles ("a", "the," etc.)

· Keep on paying attention on the research topic of the paper

· Use paragraphs to split each significant point (excluding for the abstract)

· Align the primary line of each section

· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

· Shun familiar wording, don't address the reviewer directly, and don't use slang, slang language, or superlatives

· Shun use of extra pictures - include only those figures essential to presenting results

Title Page:

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

Abstract:

The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript--must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing 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 approach 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. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than 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 maintain the initial two items to no more than one ruling each.

- Reason of the study - 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 quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
- As a outline of job done, it is always written in past tense
- A conceptual should situate on its own, and not submit to any other part of the paper such as a form or table
- Center on shortening results - bound background information to a verdict or two, if completely necessary
- What you account in an conceptual must be regular with what you reported in the manuscript
- Exact spelling, clearness 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 to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

- Explain the value (significance) of the study
- Shield the model - why did you employ this particular system or method? What is its compensation? You strength remark on its appropriateness from a abstract point of vision as well as point out sensible reasons for using it.
- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled 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 with every section. If you make the four points listed above, you will need a least of four paragraphs.
- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
- Shape the theory/purpose 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 sound written Procedures segment allows a capable scientist to replacement 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 for the least amount of information that would permit another capable scientist to spare 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 object, mention the specific item describing a way but draw the basic

principle while stating the situation. The purpose is to text 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:

- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

Methods:

- Report the method (not 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 - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in 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 a 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. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise 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 in manuscript form.

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.

- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to 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 part.

Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
- Despite of position, each figure must be numbered one after the other and complete with subtitle
- In spite of position, each table must be titled, numbered one after the other and complete with heading
- All figure and table must be adequately complete that it could situate on its own, divide from text

Discussion:

The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a 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 implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly 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 with prospect, and let it drop at that.

- Make a decision if each premise is supported, 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 the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One 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 available information
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.

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

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