



ISSN: 2249-2208

RJPS

RGUHS Journal of Pharmaceutical Sciences

SJIF 2012=3.522

(Scientific Journal Impact Factor Value for 2012)

Indexed in ABC Chemistry, African Index Medicus, Connect Journals, Geneva Foundation for Medical Education and Research (GFMER), Google Scholar, NewJour, OpenJGate, Pharmaceutical Sciences Open Access Resources (PSOAR), Science Central, Ulrichsweb, Indian Science Abstracts, Medicinal and Aromatic Plants Abstracts (MAPA), HINARI, DOAJ [Directory of Open Access Journal], Genamics and Journalseek, Scirus, Primocentral

April - June 2016/ Vol 6 / Issue 2

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ISSN: 2249-2208

R G U H S Journal of Pharmaceutical Sciences

(An Official Publication of RGUHS)

SJIF 2012 = 3.522

(Scientific Journal Impact Factor Value for 2012)



April-June 2016 / Vol 6 / Issue 2

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Quality of Research Publications

This issue of RJPS is my last as Editor-in-Chief. I have pleasant memories of last two and a half year since our team took over the responsibility. Now we are set to lay office and pass on the responsibilities to a new team of enthusiasts. The experience of editing this journal has been fantastic. Therefore, in this farewell editorial I wish to focus on the challenges of scientific publications and quality assurance in scientific journalism.

Research publication is the most authentic means of apprising development in a particular field. Scientific research implies more value in terms of commercialization and service products for community. Whatever we see around us as processed goods or machineries and equipments are the outcome of research done by experts in the field. However, as with all walks of life standards and qualities are deteriorating day by day and me too research/me too paper is becoming order of the day. Pharmaceutical research is also not an exception. Number of research journals is increasing or in other terms opportunities for publication are abundant. Number of published research papers are also increasing steeply, but quality of publication and translation of research into product in market do not match the stride in publication or keep pace with the volume of publication. This is a matter of concern and needs International initiative to monitor quality of scientific papers and journalism.

The scientific value of every published research paper must be assessed and judged to weed out me too papers which are abundant. The quality assurance criteria alone can improve the quality of research articles being published. The system of peer review and impact factor has its own flaws and fails to assure quality in a foolproof manner. This problem has been deeply researched and vented for quite sometime. As a result, papers get published here or there and keep diluting the standard of research as well as quality of communication. The rules for good quality scientific paper should include merit of (i) knowledge gap in the research filed; (ii) appropriateness of approach of research; (iii) authenticity of findings; (iv) justification for the conclusions; (v) scientific/research value of the work; (vi) application/commercial/extension value of the findings. This approach for maintaining standard and quality of research will demand active involvement of retired scientists to scan papers of their field and write critique or letter to Editor so that published paper is no more the last word, rather it is beginning of scientific research communication and only those which withstand postmortem will be creditable paper.

Finally, I must express my deep appreciation to the editorial adviser and editorial board, whose smart, dedicated and able approach had made my task absolutely easy. I also thank all the researchers who opted to publish their research in this journal, the peers who critically reviewed the manuscripts. I had privilege of enjoying the services of numerous experts from India and abroad at various levels in finalizing the manuscripts which helped in maintaining quality of production. My special thanks to all of them. I wish all the best to the new editorial team.

Dr. R. S. Thakur

Editor-in-Chief

DOI:10.5530/rjps.2016.2.1



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Synthesis and Biological Activity of Xanthene Derivatives as Antiasthmatic Agents

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ABSTRACT

Plan: To develop some 1,3-dimethyl-7-[2-(piperazin-1-yl)acetyl]-2,3,6,7-tetrahydro-1H-purine-2,6-dione derivatives for their antiasthmatic activity. **Objective:** Xanthene derivatives are known for their vasodilatory activity. Development of Phosphodiesterase 3 inhibitors is current area of interest for development of anti-asthmatic agents. Many compounds containing xanthene nucleus are also found to possess a number of pharmacological activities. Thus a new series of 1,3-dimethyl-7-(2-(piperazin-1-yl)acetyl)-2,3,6,7-tetrahydro-1H-purine-2,6-dione has been synthesized, characterized and screened for the vasodilator activity. **Methodology:** In our present study the intermediate 27-(chloroacetyl)-1,3-dimethyl-3,7-dihydro-1H-purine-2,6-dione [A] was prepared via reaction of theophylline and chloroacetyl chloride. This compound was treated with Piperazine in presence of methanol followed by hydrazine hydrate to yield the key intermediate 1,3-dimethyl-7-(2-(piperazin-1-yl)acetyl)-2,3,6,7-tetrahydro-1H-purine-2,6-dione (B). This compound was treated with various substituted aromatic amines to get the title compounds [1-12]. The title compounds were characterized by MP, TLC, IR, UV, and NMR & Mass spectrum. The compounds were screened for pulmonary vasodilator activity. **Outcome:** All compounds showed significant activity compared to standard Cilostazol. 7-(2-{4-[1-(3,4-dichlorophenyl)ethyl]piperazin-1-yl}acetyl)-1,3-dimethyl-2,3,6,7-tetrahydro-1H-purine-2,6-dione (8) was most active derivative from the series. 7-(2-{4-[(2,4-dinitrophenyl)methyl]piperazin-1-yl}acetyl)-1,3-dimethyl-2,3,6,7-tetrahydro-1H-purine-2,6-dione (6) and 7-(2-{4-[1-(4-hydroxyphenyl)ethyl]piperazin-1-yl}acetyl)-1,3-dimethyl-2,3,6,7-tetrahydro-1H-purine-2,6-dione (4) showed moderate to mild activity. **Conclusion:** Activity of the derivatives with di-chloro substitution indicated that the compounds with electron withdrawing groups are showing significant activity than other compounds which indicated mechanistic details of all the compounds in near future would lead to potent anti-asthmatic compounds.

Key words: Xanthene, Phosphodiesterase, Bioactivity, Smooth muscle relaxants.

INTRODUCTION

Asthma is a chronic inflammatory condition in which there is reversible blockage of airflow and airway hyper-responsiveness whose cause is not completely understood.¹ Hyper-responsiveness is due to the wide range of stimulus like irritant chemicals, pollen grains, stimulant drugs, pollutants, cold air etc. As per WHO about 100 to 150 million people suffer from asthma world-wide and this number is increasing. It is not just health problem of developed countries but in developing countries also, occurrence of asthma is increasing. In India around 15-20 million patients are asthmatic. It can't be

Received Date : 01/03/2016

Revised Date : 20/05/2016

Accepted Date : 27/05/2016

DOI: 10.5530/rjps.2016.2.2.

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cured completely, but only controlled by proper treatment.² In asthma due to inflammation and hyper-sensitivity the airway passage easily becomes narrow.³⁻⁴ Due to hyper-sensitivity coughing, chest tightness, wheezing, and breathlessness these symptoms are frequently worse in night. Usually narrowing of the airway passage is reversible, but in patients having chronic asthma, sometimes irreversible airflow obstruction occurs which leads to asthmatic attack and finally death. Therefore, there is need of rational drug design as an anti-asthmatic drug. Due to emerging trends in drug design various targets are also identified for anti-asthmatic class of drugs. Now a days research is mainly focused on the Phosphodiesterase (PDE) system because it plays an important role in smooth muscle relaxation.⁵ PDE3 and PDE4 are considered as potential targets for intervention in asthma therapy.⁶ PDE3 inhibitors have subsequently been shown to relax vascular and airway smooth muscle, inhibit platelet aggregation and induce lipolysis, suggesting involvements of PDE3 in the regulation of this physiological and pathophysiological processes.⁷⁻⁹ The xanthene nucleus is important component of various anticancer, antimicrobial, antitussive agents. Here we report development of 12 novel xanthene derivatives as potential antiasthamatic agents.

MATERIALS AND METHODS

Synthesis of 7-(chloroacetyl)-1,3-dimethyl-3,7-dihydro-1H-purine-2,6-dione (A)

In 250 ml round bottom flask (RBF) 1 mol theophylline and 2 mol chloroacetyl chloride were taken and stirred vigorously to get a uniform mixture. The mixture was stirred with heating until the temperature of mixture was raised to 100°. 125 ml of 1.6 N NaOH was added uniformly over a period of 5-8 hrs. The temperature of mixture was maintained at 100°. After completion of reaction, it was filtered to remove precipitated NaCl, and reaction mixture was further concentrated to get compound A.

Synthesis of 1,3-dimethyl-7-[2-(piperazin-1-yl) acetyl]-2,3,6,7-tetrahydro-1H-purine-2,6-dione (B)

1 mol of compound A was taken in RBF. To this 1 mol of piperazine was added and reaction mixture was refluxed for 30 min on water bath. The reaction mixture was then concentrated to get fine crystals of compound B.

Synthesis of 1,3-dimethyl-7-[2-(piperazin-1-yl) acetyl]-2,3,6,7-tetrahydro-1H-purine-2,6-dione derivatives (1-12)

In 250 ml round bottom flask (RBF) 5 ml tetrahydrofuran (THF), 1 mol of compound B and 1 mol of corresponding amine was taken. To this, 1mol sodium borohydride NaBH₄ and 0.5 g silica chloride were added

and reaction mixture was stirred at room temperature. After completion of the reaction, the mixture was filtered and the residue was washed with CH₂Cl₂ to get the different derivatives as shown in Table 1. The scheme of synthesis is presented in Figure 1.

BIOLOGICAL ACTIVITY

Isolated adult goat tracheal tissue was obtained from slaughter house. Trachea was cut into individual rings 2-3 cm long and 1cm wide tracheal muscle was washed in a bath containing standard Krebs-Henseleit solution (Concentration in gm/L; KCl-0.35; CaCl₂-0.3; MgSO₄-0.16; NaHCO₃-32.0; KH₂PO₄-0.164; NaCl-6.9; Glucose-2.0) maintained at 37 ± 0.5°, a stream of oxygen was bubbled through the oxygen tube in organ bath. One end was tied to aerator tube and other thread to force transducer through sigmoid lever to detector of Biopac- M.P. 35 with forced transducer. Tissue was equilibrate in physiological Krebs-Henseleit solution prepared freshly for 45 min in organ bath. During which, the bathing solution was changed at every 15 min. The results were calculated as a concentration of test compounds required for the maximum relaxation using Cilostazol standard.

RESULTS

In the first step of synthetic protocol reaction between theophylline and chloroacetyl chloride leads to formation of aromatic/heterocyclic xanthine derivative. This reaction is Schotten Baumann reaction which was carried out in presence of sodium hydroxide for 5-8 hrs at 100° and stirred until a precipitate was formed. In the second step we synthesized Piperazine derivative of xanthine. In the last step there was formation of polyfunctional xanthine derivatives which involved reductive amination of various aldehydes or ketones yielding substituent's on the piperazine nitrogen. Synthesis of all final compounds was confirmed from the results of chemical, physicochemical, chromatographic and spectral analysis. The biological activity of synthesized compounds was evaluated on goat trachea which showed that compound (8) 7-(2-{4-[1-(3,4-dichlorophenyl)ethyl]piperazin-1-yl} acetyl)-1,3-dimethyl-2,3,6,7-tetrahydro-1H-purine-2,6-dione was the most active derivative from the series of synthesized compounds. Compounds like 6, 1, 4, and 7 showed moderate to mild activity compared with Cilostazol as standard.

1. 1,3-dimethyl-7-(2-{4-[(3-nitrophenyl)methyl]piperazin-1-yl}acetyl)-2,3,6,7-tetrahydro-1H-purine-2,6-dione

M. P.: 190-192°

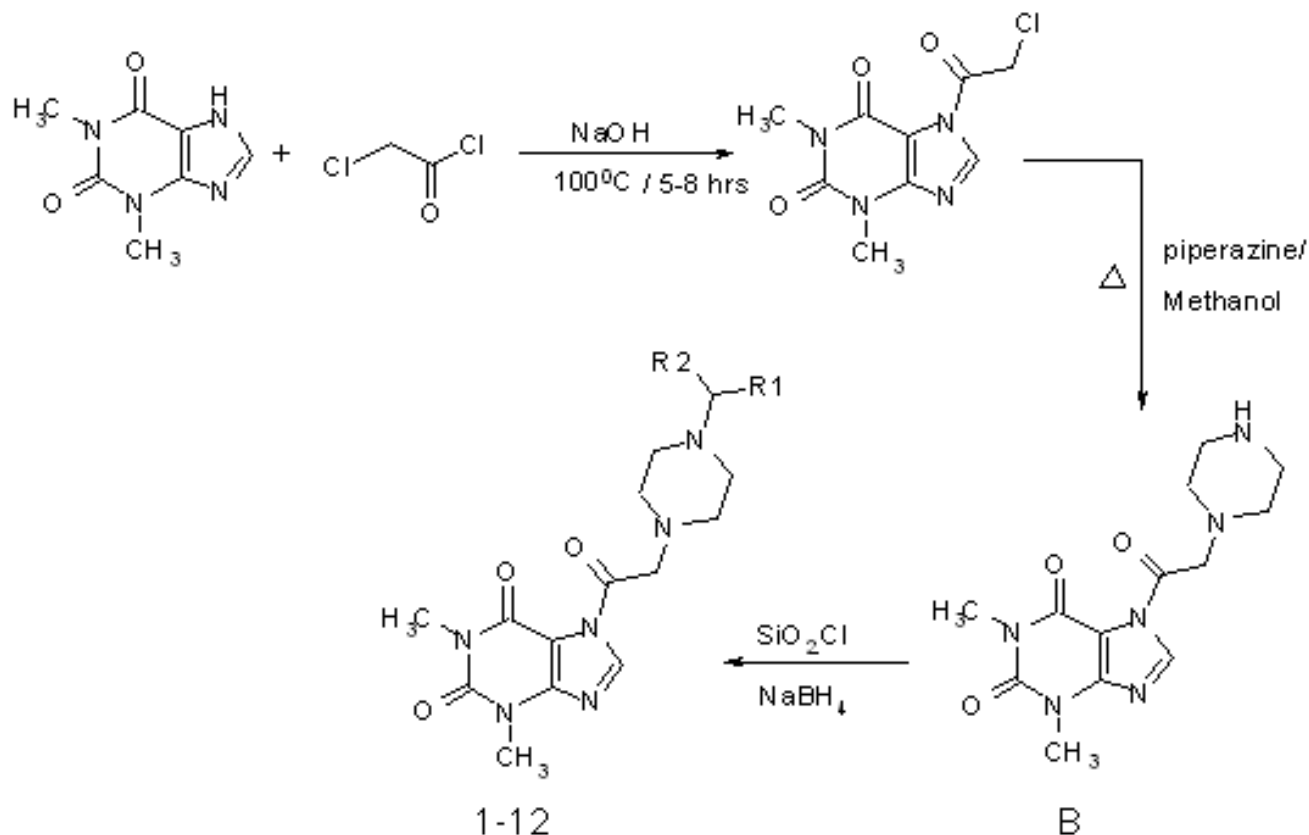


Figure 1: Scheme of Synthesis

Table 1: Various Derivatives Synthesized	
R1	R2
H	3- NO_2 - C_6H_5
CH_3	CH_3
H	4-F- C_6H_5
CH_3	4-OH- C_6H_5
CH_3	4- NH_2 - C_6H_5
H	2,4- NO_2 - C_6H_5
CH_3	2,4-Cl- C_6H_5
CH_3	3,4-Cl- C_6H_5
CH_3	3- NO_2 - C_6H_5
CH_3	4-Br- C_6H_5
CH_3	2,5- OCH_3 - C_6H_5
H	4-Br- C_6H_5

Rf: 0.62 [n-Hexane: ethyl acetate (8.5:1.5)]

UV- 276 nm

IR- (KBr) 1720 cm^{-1} (C-N Str), 1677 cm^{-1} (C=O Str), 1566 cm^{-1} (Ar- NO_2), 1440 cm^{-1} (C-H def), 1107 cm^{-1} (C-H def), 950 cm^{-1} and 885 cm^{-1} (Ar-C=C def)

^1H NMR- (CDCl_3 , 500 MHz), 3.40 (6H, $\text{H}_3\text{C-N}$ at 1 and 3, s), 7.90 (1H, CH s), 3.47 (2H, CH_2 at 2', s), 2.35 (4H, 2' and 6' CH_2 Piperazine, t), 2.48 (4H, 3'

Table 2: Biological Activity of synthesized derivatives			
Sample code	ED ₈₀ (M)	Sample code	ED ₈₀ (M)
1	3×10^{-4}	7	3×10^{-4}
2	4×10^{-4}	8	1×10^{-4}
3	7×10^{-4}	9	4×10^{-4}
4	3×10^{-4}	10	4×10^{-4}
5	4×10^{-4}	11	5×10^{-4}
6	2×10^{-4}	12	5×10^{-4}
		Cilostazol	1×10^{-4}

and 5' CH_2 Piperazine, t), 3.66 (2H, CH_2 at 4' Piperazine, s), 7.8-8.3 (4H, 4H Aromatic 4' Piperazine, m).
MASS- 440.35 (M+1)

2. 1,3-dimethyl-7-{2-[4-(propan-2-yl)piperazin-1-yl]acetyl}-2,3,6,7-tetrahydro-1H-purine-2,6-dione

M. P.: 188-190°

Rf: 0.56 [n-Hexane: ethyl acetate (8.5:1.5)]

UV- 273 nm

IR- (KBr) 3026 cm^{-1} (Ar-CH Str), 2819 cm^{-1} (C-H Str), 1703 cm^{-1} (C=O Ketone), 1654 cm^{-1} (sec. NH), 1560 cm^{-1} (Ar-C=C), 1442 and 1319 cm^{-1} (N-H def).

^1H NMR- (CDCl_3 , 500 MHz): 3.30 (6H, $\text{H}_3\text{C-N}$ at 1 and 3, s), 7.85 (1H, CH s), 3.34 (2H, CH_2 at 2', s),

2.25 (4H, 2' and 6' CH₂ Piperazine, t), 2.28 (4H, 3' and 5' CH₂ Piperazine, t), 3.36 (1H, CH at 4' Piperazine, q), 1.7 (6H, CH₃ at 5'', d).

MASS- 349 (M+1)

3. 7-(2-{4-[(4-fluorophenyl)methyl]piperazin-1-yl}acetyl)-1,3-dimethyl-2,3,6,7-tetrahydro-1H-purine-2,6-dione

M. P.: 210-212°

Rf: 0.71 [n-Hexane: ethyl acetate (8.5:1.5)]

UV- 265 nm

IR- (KBr) 3087 cm⁻¹ (Ar-CH Str), 2789 cm⁻¹ (C-H Str), 1701 cm⁻¹ (C=O Ketone), 1656 cm⁻¹ (sec. NH), 1580 cm⁻¹ (Ar-C=C), 1485 and 1379 cm⁻¹ (N-H def).

¹H NMR- (CDCl₃, 500 MHz): 3.51 (6H, H₃C-N at 1 and 3, s), 7.84 (1H, CH s), 3.49 (2H, CH₂ at 2', s), 2.75 (4H, 2' and 6' CH₂ Piperazine, t), 2.81 (4H, 3' and 5' CH₂ Piperazine, t), 3.56 (2H, CH₂ at 4' Piperazine, s), 7.1-8.2 (4H, Aromatic 4' Piperazine, m).

MASS- 413 (M+1)

4. 7-(2-{4-[1-(4-hydroxyphenyl)ethyl]piperazin-1-yl}acetyl)-1,3-dimethyl-2,3,6,7-tetrahydro-1H-purine-2,6-dione

M. P.: 216-218°

Rf: 0.63 [n-Hexane: ethyl acetate (8.5:1.5)]

UV- 275 nm

IR- (KBr) 3562 cm⁻¹ (Ar-OH), 3245 cm⁻¹ (Ar-NH), 3006 cm⁻¹ (Ar-CH Str), 1677 cm⁻¹ (C=O Str), 1635 cm⁻¹ (C=C str), 1566 cm⁻¹ (Ar-C=C), 1461 cm⁻¹ (CH def), 1440 and 1317 cm⁻¹ (N-H def).

¹H NMR- (CDCl₃, 500 MHz): 3.21 (6H, H₃C-N at 1 and 3, s), 7.90 (1H, CH s), 3.19 (2H, CH₂ at 2', s), 2.65 (4H, 2' and 6' CH₂ Piperazine, t), 2.69 (4H, 3' and 5' CH₂ Piperazine, t), 2.37 (1H, CH at 4' Piperazine, q), 5.25 (1H OH at 7' Phenyl, s), 1.5 (3H, CH₃ at 7', d), 7-8.1 (4H, Aromatic at 7', m).

MASS- 425 (M+1)

5. 7-(2-{4-[1-(4-aminophenyl)ethyl]piperazin-1-yl}acetyl)-1,3-dimethyl-2,3,6,7-tetrahydro-1H-purine-2,6-dione

M. P.: 180-182°

Rf: 0.59 [n-Hexane: ethyl acetate (8.5:1.5)]

UV- 279 nm

IR- (KBr) 3289 cm⁻¹ (Ar-NH), 3746 cm⁻¹ (Ar-CH Str), 2822 cm⁻¹ (C-H Str), 1603 cm⁻¹ (C=O Ketone), 1600 cm⁻¹ (sec. NH), 1560 cm⁻¹ (Ar-C=C), 1441 and 1356 cm⁻¹ (N-H def).

¹H NMR- (CDCl₃, 500 MHz): 3.11 (6H, H₃C-N at 1 and 3, s), 7.92 (1H, CH s), 3.00 (2H, CH₂ at 2', s),

2.55 (4H, 2' and 6' CH₂ Piperazine, t), 2.59 (4H, 3' and 5' CH₂ Piperazine, t), 2.17 (1H, CH at 4' Piperazine, q), 6.25 (2H NH 7' Phenyl, s), 1.4 (3H, CH₃ at 7', d), 7-8.0 (4H, Aromatic at 7', m).

MASS- 426 (M+1)

6. 7-(2-{4-[(2,4-dinitrophenyl)methyl]piperazin-1-yl}acetyl)-1,3-dimethyl-2,3,6,7-tetrahydro-1H-purine-2,6-dione

M. P.: 232-234°

Rf: 0.64 [n-Hexane: ethyl acetate (8.5:1.5)]

UV- 281 nm

IR- (KBr) 3134 cm⁻¹ (Ar-NH), 3006 cm⁻¹ (Ar-CH Str), 2839 cm⁻¹ (C-H Str), 1796 cm⁻¹ (C=O Ketone), 1645 cm⁻¹ (sec. NH), 1560 cm⁻¹ (Ar-C=C), 1478 and 1335 cm⁻¹ (N-H def).

¹H NMR- (CDCl₃, 500 MHz): 3.51 (6H, H₃C-N at 1 and 3, s), 7.96 (1H, CH s), 3.49 (2H, CH₂ at 2', s), 2.45 (4H, 2' and 6' CH₂ Piperazine, t), 2.47 (4H, 3' and 5' CH₂ Piperazine, t), 3.79 (2H, CH₂ at 7', s), 7.9-8.5 (3H, Aromatic at 7', m).

MASS- 485 (M+1)

7. 7-(2-{4-[1-(2,4-dichlorophenyl)ethyl]piperazin-1-yl}acetyl)-1,3-dimethyl-2,3,6,7-tetrahydro-1H-purine-2,6-dione

M. P.: 210-212°

Rf: 0.58 [n-Hexane: ethyl acetate (8.5:1.5)]

UV- 263 nm

IR- (KBr) 3147 cm⁻¹ (Ar-NH), 3082 cm⁻¹ (Ar-CH Str), 2863 cm⁻¹ (C-H Str), 1722 cm⁻¹ (C=O Ketone), 1654 cm⁻¹ (sec. NH), 1541 cm⁻¹ (Ar-C=C), 1489 and 1332 cm⁻¹ (N-H def).

¹H NMR- (CDCl₃, 500 MHz): 3.51 (6H, H₃C-N at 1 and 3, s), 7.91 (1H, CH s), 3.40 (2H, CH₂ at 2', s), 2.41 (4H, 2' and 6' CH₂ Piperazine, t), 2.44 (4H, 3' and 5' CH₂ Piperazine, t), 3.6 (1H, CH at 4' Piperazine, q), 1.6 (3H, CH₃ at 7', d), 7.8-8.2 (3H, Aromatic at 7', m).

MASS- 477 (M+1)

8. 7-(2-{4-[1-(3,4-dichlorophenyl)ethyl]piperazin-1-yl}acetyl)-1,3-dimethyl-2,3,6,7-tetrahydro-1H-purine-2,6-dione

M. P.: 202-204°

Rf: 0.74 [n-Hexane: ethyl acetate (8.5:1.5)]

UV- 262 nm

IR- (KBr) 3247.90 cm⁻¹ (Ar-NH), 1677.90 cm⁻¹ (C=O str), 1633.99 cm⁻¹ (C=C str), 1440.29 cm⁻¹ (C-H def), 1188.07 cm⁻¹ (C-N str), 1170.07 cm⁻¹ (Ar C-H), 950.60 cm⁻¹ (C=C def), 742.58 cm⁻¹ (Ar-Cl).

¹H NMR- (CDCl₃, 500 MHz): 3.54 (6H, H₃C-N at 1 and 3, s), 7.94 (1H, CH s), 3.49 (2H, CH₂ at 2', s), 2.40 (4H, 2' and 6' CH₂ Piperazine, t), 2.43 (4H, 3' and 5' CH₂ Piperazine, t), 3.76 (1H, CH at 4' Piperazine, q), 1.8 (3H, CH₃ at 7', d), 7.9-8.5 (3H, Aromatic at 7', m).
 MASS- 479 (M+1)

9. 1,3-dimethyl-7-(2-{4-[1-(3-nitrophenyl)ethyl]piperazin-1-yl}acetyl)-2,3,6,7-tetrahydro-1H-purine-2,6-dione

M. P.: 196-198°

Rf: 0.59 [n-Hexane: ethyl acetate (8.5:1.5)]

UV- 278 nm

IR- (KBr) 3142 cm⁻¹ (Ar-NH), 3012 cm⁻¹ (Ar-CH Str), 2820 cm⁻¹ (C-H Str), 1736 cm⁻¹ (C=O Ketone), 1658 cm⁻¹ (sec. NH), 1570 cm⁻¹ (Ar-C=C), 1445 and 1322 cm⁻¹ (N-H def).

¹H NMR- (CDCl₃, 500 MHz): 3.62 (6H, S, H₃C-N at 1 and 3, s), 7.89 (1H, CH s), 3.52 (2H, CH₂ at 2', s), 2.47 (4H, 2' and 6' CH₂ Piperazine, t), 2.48 (4H, 3' and 5' CH₂ Piperazine, t), 3.80 (1H, CH at 4' Piperazine, q), 1.9 (3H, CH₃ at 7', d), 8.0-8.8 (4H, Aromatic at 7', m).

MASS- 455 (M+1)

10. 7-(2-{4-[1-(4-bromophenyl)ethyl]piperazin-1-yl}acetyl)-1,3-dimethyl-2,3,6,7-tetrahydro-1H-purine-2,6-dione

M. P.: 182-184°

Rf: 0.75 [n-Hexane: ethyl acetate (8.5:1.5)]

UV- 266 nm

IR- (KBr) 3185 cm⁻¹ (Ar-NH), 3053 cm⁻¹ (Ar-CH Str), 2882 cm⁻¹ (C-H Str), 1710 cm⁻¹ (C=O Ketone), 1632 cm⁻¹ (sec. NH), 1575 cm⁻¹ (Ar-C=C), 1445 and 1300 cm⁻¹ (N-H def).

¹H NMR- (CDCl₃, 500 MHz): 3.4 (6H, S, H₃C-N at 1 and 3, s), 7.96 (1H, CH s), 3.4 (2H, CH₂ at 2', s), 2.3 (4H, 2' and 6' CH₂ Piperazine, t), 2.38 (4H, 3' and 5' CH₂ Piperazine, t), 3.70 (1H, CH at 4' Piperazine, q), 1.91 (3H, CH₃ at 7', d), 7.8-8.1 (4H, Aromatic at 7', m)

MASS- 488 (M+1)

11. 7-(2-{4-[1-(2,5-dimethoxyphenyl)ethyl]piperazin-1-yl}acetyl)-1,3-dimethyl-2,3,6,7-tetrahydro-1H-purine-2,6-dione

M. P.: 190-192°

Rf: 0.65 [n-Hexane: ethyl acetate (8.5:1.5)]

UV- 261 nm

IR- (KBr) 3200 cm⁻¹ (Ar-NH), 3082 cm⁻¹ (Ar-CH Str), 2820 cm⁻¹ (C-H Str), 1701 cm⁻¹ (C=O Ketone), 1651 cm⁻¹ (sec. NH), 1575 cm⁻¹ (Ar-C=C), 1445 and 1309 cm⁻¹ (N-H def).

¹H NMR- (CDCl₃, 500 MHz): 3.6 (6H, S, H₃C-N at 1 and 3, s), 7.91 (1H, CH s), 3.1 (2H, CH₂, s), 2.28 (4H, 2' and 6' CH₂ Piperazine, t), 2.31 (4H, 3' and 5' CH₂ Piperazine, t), 3.64 (1H, CH at 4' Piperazine, q), 3.83 (6H-OCH₃ at 2'' and 5'' phenyl, s), 1.81 (3H, CH₃ at 7', d), 7.6-8.1 (3H, Aromatic at 7', m).

MASS- 471 (M+1)

12. 7-(2-{4-[1-(4-bromophenyl)methyl]piperazin-1-yl}acetyl)-1,3-dimethyl-2,3,6,7-tetrahydro-1H-purine-2,6-dione

M. P.: 216-218°

Rf: 0.52 [n-Hexane: ethyl acetate (8.5:1.5)]

UV- 265 nm

IR- 3274 cm⁻¹ (Ar-NH), 3001 cm⁻¹ (Ar-CH Str), 2809 cm⁻¹ (C-H Str), 1693 cm⁻¹ (C=O Ketone), 1624 cm⁻¹ (sec. NH), 1500 cm⁻¹ (Ar-C=C), 1441 and 1309 cm⁻¹ (N-H def).

¹H NMR- (CDCl₃, 500 MHz): 3.4 (6H, S, H₃C-N at 1 and 3, s), 7.88 (1H, CH s), 3.2 (2H, CH₂, s), 2.30 (4H, 2' and 6' CH₂ Piperazine, t), 2.34 (4H, 3' and 5' CH₂ Piperazine, t), 3.70 (2H, CH₂ at 4' Piperazine, s), 7.7-8.5 (4H, Aromatic, m).

MASS- 474 (M+1)

Biological activity

Results were calculated as ED₈₀ which is the molar dose required producing 80% of the relaxation produced by standard Cilostazol. The tested compounds produced tracheal smooth muscle relaxant activity ranging from 1 x 10⁻⁴ M to 7 x 10⁻⁴ M and standard drug Cilostazol produced 1 x 10⁻⁴ M activity. Compound 8 showed very good activity as compared to the other compounds. Compounds 6, 1, 4 and 7 also had comparable activity, all these compounds have activity that is close and comparable to that produced by the standard drug, Cilostazol as shown in Table 2.

CONCLUSION

Synthesized xanthene derivatives showed profound biological activity which proves therapeutic efficiency of the xanthene nucleus for development of potent anti-asthmatic agents.

Activity of Compound (8) 7-(2-{4-[1-(3,4-dichlorophenyl)ethyl]piperazin-1-yl}acetyl)-1,3-dimethyl-2,3,6,7-tetrahydro-1H-purine-2,6-dione with di-chloro substitution indicating the compounds with electron withdrawing groups showing significant activity than other compounds which indicates mechanistic details of all the synthesized compounds in near future would lead to potent anti-asthmatic compounds. Thus there is need to study these compounds at the molecular level by using different

enzymatic assays to develop potent anti-asthmatic drug like candidate. These studies can confirm identification of novel lead compounds for further investigation

which may produce therapeutic agents for treatment of various respiratory disorders like pulmonary hypertension, asthma etc.

ACKNOWLEDGEMENTS

Authors are thankful to Principal Dr. H. N. More for providing the facilities for the works.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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First Derivative UV Spectrophotometric Method for the Determination of Etodolac in Solid Dosage Forms

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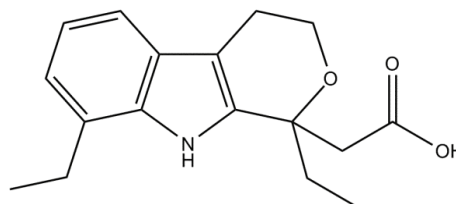
ABSTRACT

Purpose: Simple, accurate, precise and economical spectroscopic method for determination of Etodolac in pure and its tablet dosage form by first order derivative method has been developed for the routine analysis. **Methodology:** Spectroscopic method development for the estimation of Etodolac by first order derivative method was carried out using ethanol as solvent and Shimadzu 1800 Spectronic UV Visible Spectrophotometer. **Findings:** The absorbance maximum in first derivative spectrum was measured at 273 nm and selected as analytical wavelength. Beer's law was obeyed in the concentration range of 10-50 µg/ml. The recovery studies ascertained the accuracy of the proposed method and the results were validated as per ICH guidelines. The results were found satisfactory and reproducible. **Research application:** The method was applied successfully for the estimation of Etodolac in pure drug and tablet dosage form. **Industrial application:** The method can be applied for routine analysis. **Research Value:** The newly developed method is a good alternative for HPLC methods and better than zero orders UV methods. **Conclusion:** The method is simple, rapid, accurate, precise and economic method which can be used without the interference of impurities for the determination of Etodolac in solid dosage forms.

Key words: Etodolac, First derivative method, Spectrophotometric method, UV Estimation, UV determination, Estimation, Tablet assay.

INTRODUCTION

Etodolac is a non-steroidal anti-inflammatory drug (NSAID) with anti-inflammatory and antipyretic properties. Its analgesic effects are due to its ability to inhibit prostaglandin synthesis. It is designed for relief of signs and symptoms of rheumatoid arthritis and osteoarthritis. Chemically it is 2-{1,8-diethyl-1*H*,3*H*,4*H*,9*H*-pyrano[3,4-*b*]indol-1-yl}acetic acid. It is official in Indian Pharmacopoeia.¹ There are few methods so far reported for the determination of Etodolac in its single component formulations and bulk drugs. Four HPLC methods,²⁻⁵ three Colorimetric (Visible Spectrophotometric) methods,⁶⁻⁸ one GC-MS method,⁹ one Capillary Electrophoresis method¹⁰ and one LC-MS method¹¹ are reported so far in literature. No first derivative spectrophotometric method has been reported for routine analysis of Etodolac in its bulk form and formulations. The aim of the present study



Etodolac

was to develop a simple and rapid first derivative spectrophotometric method for the routine determination of Etodolac.

MATERIALS AND METHODS

Materials

Shimadzu 1800 Spectronic double beam UV-visible spectrophotometer with 1 cm matched quartz cells was used for all the measurements. Ethanol (98%) A.R. Grade (Qualigens, Fine Chemicals) was used as the solvent. Commercial brand tablets were obtained from local market.

Received Date : 05/05/2016

Revised Date : 26/05/2016

Accepted Date : 28/05/2016

DOI: 10.5530/rjps.2016.2.3

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Methodology

Preparation of standard stock solution

Standard stock solution of Etodolac was prepared by dissolving accurately weighed quantities (100 mg each) of Etodolac in 40 ml ethanol and transferred into a 100 ml volumetric flask. Volume was made up to mark with ethanol to obtain stock solution of 1000 µg/ml concentration. For obtaining clear solution, further dilutions were made to get the concentration of 100 µg/ml.

Determination of λ_{max}

The standard solution of Etodolac (10 µg/ml) was scanned in the wave length range of 200-300 nm and the spectrum was derivatized in first order at N=5 smoothening factor. Absorption maximum was found to be 273 nm wavelength; where the absorption showed higher intensity than other wavelengths for Etodolac. Therefore analytical wavelength was fixed at 273 nm for the analysis of Etodolac (Figure 1).

Stability of Drug in Selected Solvent

The stability of the drugs in the selected solvent was determined by measuring the absorbance of the drug solution (10 µg/ml) at different time intervals. The absorbance was measured after every 10 min. The solutions were found to be stable. The stability study data is given in Table 1.

Study of Beer-Lambert's law

From the standard stock solution of Etodolac, appropriate aliquots were pipette out in to 25 ml volumetric flasks and dilutions were made with ethanol to obtain working standard solutions of concentrations of 2 µg to 60 µg/ml and the difference in absorbance ($dA/d\lambda$) of Etodolac was measured in the first derivative mode with N=5 smoothening factor of the instrument at 273.0 nm at the interval of 2 µg/ml concentration. The calibration curve of the drugs was plotted. (Figures 2 to 7) The concentration range over which the drugs followed linearity was chosen as an analytical concentration range i.e. 10-50 µg/ml.

Optimum Parameters for the Calibration Curve

The Optimum parameters of the calibration curves are given in Table 2.

Validation of proposed method

Estimation of drug from dosage form

Twenty tablets of Etodolac is weighed, and finely powdered. A quantity of powder sample equivalent to 200 mg of Etodolac was taken in volumetric flask and dissolved in ethanol. Further dilution was made to get concentration of 25 µg/ml. These concentration was

scanned at wavelength 273 nm for in first order derivative mode with N=5 smoothening factor.

The results and statistical parameters for tablet analysis are shown in Table 3.

Accuracy (Recovery Test)

Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of standard solutions to solutions of tablet. The recovery was performed at three levels, 80, 100 and 120% of Etodolac standard concentration. The recovery samples were prepared. Three samples were prepared for each recovery level. The solutions were then analyzed, and the percentage recoveries were calculated using formula:

$$\% \text{ recovery} = \frac{\text{Observed amount of compound in sample}}{\text{Amount of all compound present in sample}} \times 100$$

The recovery values are summarized in Table 4.

Precision Study

The dilution was made to get concentration of 25 µg/ml of Etodolac and scanned at wavelength 273 nm in first order derivative mode by four different analyst using same laboratory and same instrument. The precision data for Etodolac are given in the following Table 5.

RESULTS AND DISCUSSION

The standard solutions of Etodolac in Ethanol (10 µg/ml) subjected to a scan individually at the series of wavelengths of 200 nm to 300 nm at first order derivative mode and the first order derivative spectra was taken at N=5 smoothening factor of the instrument using Shimadzu 1800 spectronic UV Visible spectrophotometer. Absorption maximum of Etodolac was found to be at 273 nm. Therefore, 273 nm was selected as λ_{max} of Etodolac for the present study. The calibration curve of Etodolac was found to be linear in the range of 10-50 µg/ml at 273 nm. Therefore, it was clear that Etodolac can be determined without interference of any irrelevant substance in single component pharmaceutical products.

With the intention of determining the practicability of the developed technique for the assessment of commercially available brands of medicinal formulations, the technique was initially attempted on bulk drugs in their synthetic sample and concentrations were estimated. Then the technique was subjected to the assay of tablets in three marketed dosage brands that is Brand-A, Brand-B and Brand-C and adequate results were attained within

Table 1: Stability Data for Etodolac

Time (min)	Absorbance
10	0.561
20	0.559
30	0.550
40	0.546
50	0.530
60	0.525

Table 2: Optimum Parameters for the Calibration Plot

Parameter	Etodolac
Linearity range (µg/ml)	10-50 µg/ml
Slope	0.0270
Intercept	0.0425
Regression Coefficient(r^2)	0.999
Sandell's Sensitivity (Specific Extinction Coefficient x Concentration of Analyte in µg/L)	0.0294 µg/ml/cm ²
Limit of Detection (LOD) LOD = $3.3\sigma/s$	0.0099 µg/ml
Limit of Quantitation (LOQ) LOQ = $10\sigma/s$	0.0300 µg/ml

* σ = Standard Deviations of Intercepts s = Slope

Table 3: Assay of Etodolac in Tablet formulation Brand- A, B and C

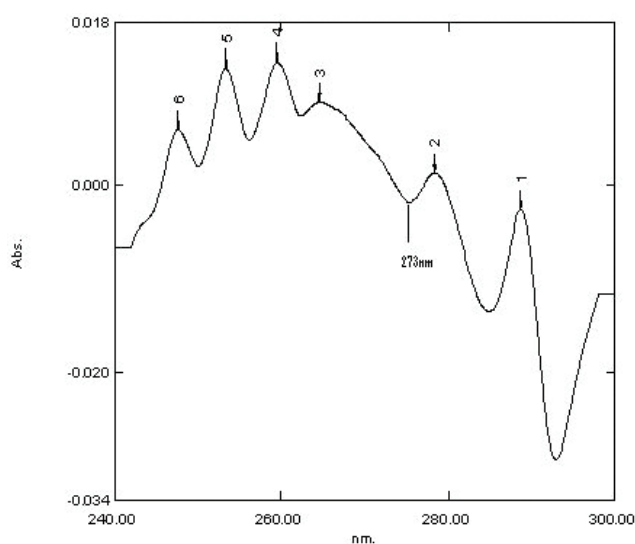
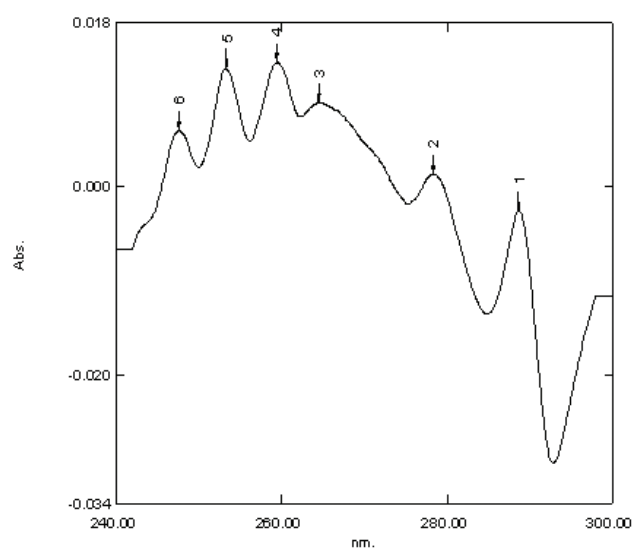
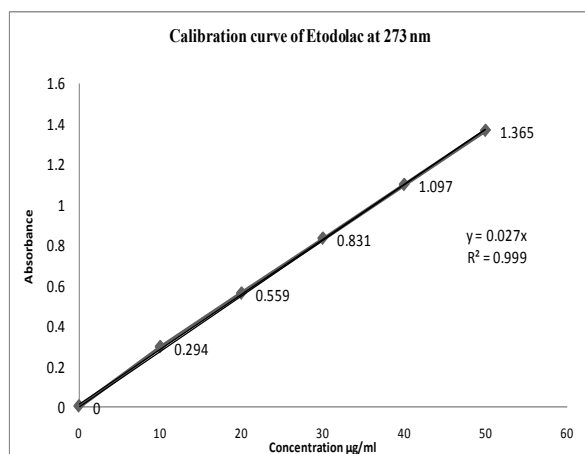
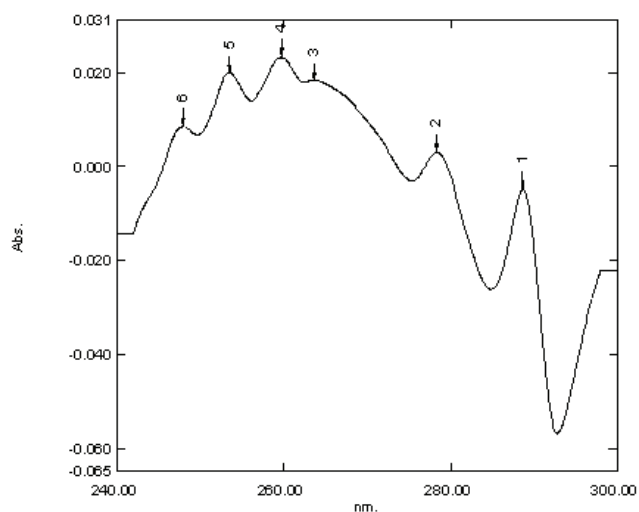
Drug	Label Claim (mg/cap)	Amount Found (mg/cap)	% of Label Claim	Mean %	SD	CV
Etova 200 mg	200	199.78	99.89	100.07	0.6293	0.3960
	200	199.12	99.56			
	200	200.24	100.21			
	200	199.92	99.96			
	200	202.52	101.26			
Etova 300 mg	200	199.16	99.58	99.91	0.2891	0.0835
	300	298.98	99.66			
	300	299.94	99.98			
	300	299.28	99.76			
	300	301.11	100.37			
Etova 400 mg	300	300.27	100.09	100.12	0.2588	0.0670
	300	298.86	99.62			
	400	402.04	100.51			
	400	401.28	100.32			
	400	399.36	99.84			
	400	399.72	99.93			
	400	400.8	100.20			
	400	399.84	99.96			

Table 4: Results of Accuracy (Recovery) parameter of Etodolac

Level of % Recovery	Amount present (µg/ml)	Amount of standard added (µg/ml)	Total amount recovered (µg/ml)	% Recovery	% mean Recovery	SD	CV
80	200	160	361.69	100.47	100.07	0.3464	0.1203
80	200	160	359.38	99.83			
80	200	160	359.71	99.92			
100	200	200	362.12	100.59	100.20	0.3385	0.1146
100	200	200	359.85	99.96			
100	200	200	360.21	100.06			
120	200	240	361.90	100.53	100.12	0.4050	0.1641
120	200	240	360.39	100.11			
120	200	240	358.99	99.72			

Table 5: Determination of Precision of Etodolac

Sample Number	Assay of Artemether as % of Labeled amount			
	Analyst-I	Analyst-II	Analyst-III	Analyst-IV
1	100.36	100.26	99.86	101.18
2	99.79	101.06	100.26	100.30
3	101.31	99.87	101.13	99.82
4	99.56	99.92	101.14	99.89
5	99.62	100.56	99.96	101.24
6	100.20	99.88	100.20	100.39
Mean%	100.14	100.25	100.42	100.47
S.D	0.6557	0.4779	0.5694	0.6150
CV	0.4300	0.2284	0.3243	0.3782

**Figure 1: Absorption Maximum of Etodolac at 273 nm.****Figure 3: First order derivative spectrum of Etodolac 10 µg/ml.****Figure 2: Calibration Plot of Etodolac.****Figure 4: First order derivative spectrum of Etodolac 20 µg/ml.**

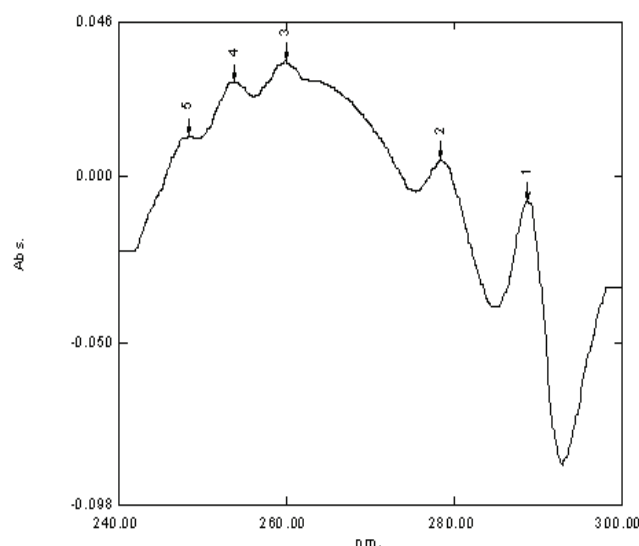


Figure 5: First order derivative spectrum of Etodolac 30 µg/ml.

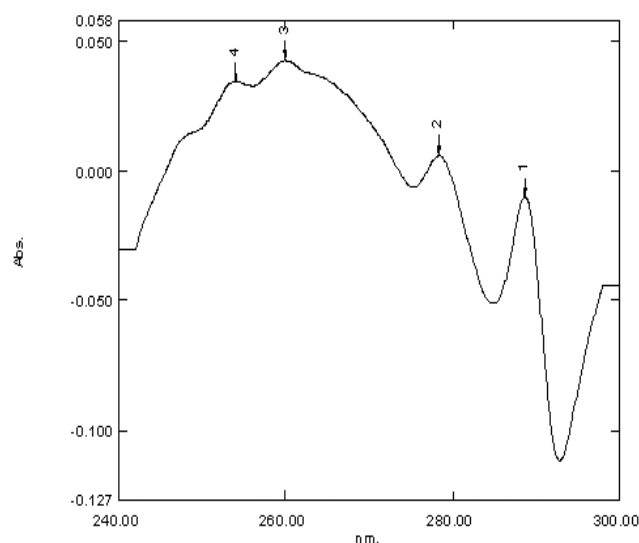


Figure 6: First order derivative spectrum of Etodolac 40 µg/ml.

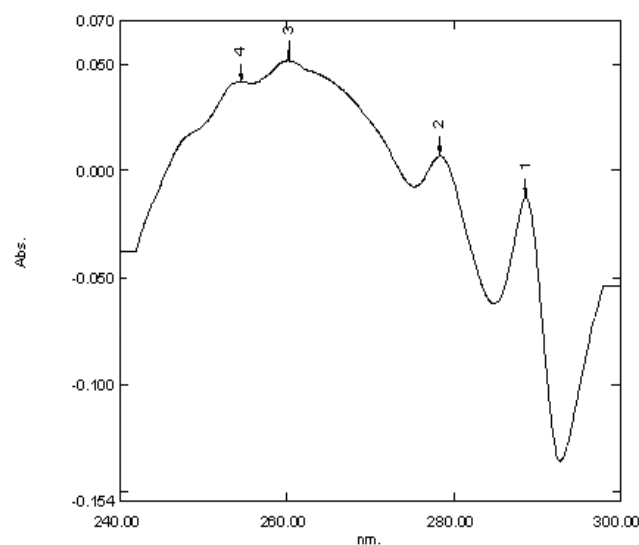


Figure 7: First order derivative spectrum of Etodolac 50 µg/ml.

the acceptable limits as per the content of the label claim for Etodolac.

The recovery experiments were conducted by adding known amounts to tablet. The recovery was performed at three levels, 80, 100 and 120% of three brand of Etodolac standard concentration. Three samples were prepared for each recovery level. The solutions were then analyzed, and the percentage recoveries were found to be satisfactory within the acceptable limits as per the content of the label claim for marketed tablet.

The newly developed method was validated as per the ICH guidelines and parameters. The novel method for the quantitative investigation of Etodolac was subjected to different validation parameters like specificity and selectivity in presence of formulation additives and excipients, studied for Linearity and range at different levels of concentrations and calibration standards where the determination range was optimized, accuracy was proved by recovery studies at different concentration levels, precision for Etodolac was established through the analysis of sample by four different analyst using same instrument and same laboratory. The method was developed successfully for Etodolac in its single component dosage forms by first order derivative method.

CONCLUSION

From the experimental studies it can be concluded that first order derivative method is developed for Etodolac in bulk and single dosage form. The proposed method for the selected drug was found to be accurate and precise. The most striking features of spectrophotometric method are their simplicity and rapidity. Results of validation parameters demonstrate that the analytical procedure is suitable for its intended purpose and meet the criteria defined in ICH Q2A/B. The proposed method can be successfully applied for the routine determination of Etodolac, which is an advantage over HPLC and a better method than colorimetric methods comparing accuracy and interference.

ACKNOWLEDGEMENT

The authors are grateful to the Principal and Vice Principal, BLDEA's SSM College of Pharmacy, Vijaypur for providing necessary lab facilities and help.

CONFLICT OF INTEREST

Nil.

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World Health Organization Indicators for Rational Use of Drugs in a Nigerian Secondary Hospital

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ABSTRACT

Purpose: Prevention of irrational drug use may reduce healthcare costs and potentially save lives. In line with the World Health Organization (WHO) recommendation, retrospective, prospective and cross sectional descriptive studies were conducted to obtain information on patient care, prescribing, and facility indicators in the Outpatient Department of General Hospital, Offa, Kwara State, Nigeria. **Methodology:** A sample of 1,416 prescriptions was randomly selected to determine the prescribing indicators. A total of 472 patients were interviewed to collect information on the drugs being used by the patients. Information on health facility indicators were obtained by assessing sufficient supply of vital drugs, and access to information about these drugs in the hospital. **Findings:** Majority of the patients were females with mean age of 56.2 ± 7.1 years. The average number of drugs per prescription (2.6) was higher than WHO recommendation (1.6-1.8), and most (58.1%) of the drugs prescribed were branded rather than 100% generics. Percentages of antibiotics (23.8%) and injectable drugs (3.4%) prescribed were within WHO cut-off values of 20.0-26.8% and <10.0%, respectively. The Nigerian Essential Drugs List was available in the facility, and a high percentage (99.7%) of drugs was prescribed from the list. The average time used in dispensing drugs (5.26 ± 2.33 minutes) was also adequate and within WHO recommendation (>3 minutes). Appropriate drugs dispensed and adequate labeling were 87.4% and 81.9%, respectively. Hypertension (28.8%) was the most prevalent disease in the community. **Conclusion:** The study concluded that most of the prescribing indicators did not meet WHO standard criteria. **Social Value:** The health facility and patient care indicators are rational. Data obtained in this study can be used to monitor and improve drug prescribing habits of physicians in this facility.

Key words: Irrational drug, Indicators, Antibiotics, World Health Organization.

INTRODUCTION

Irrational use of drugs is a global challenge. The rational use of drugs is defined as patients receiving medication clinical needs in appropriate dosage that meet individual requirements at a low price in an adequate period of time within their community. The use of several drugs per patient (polypharmacy), inappropriate use of antimicrobials, over use of injections, self-medication and prescribing the drug that is inappropriate to clinical guidelines.¹ These requirements would be fulfilled provided the prescribing process are covered by the following steps:

Received Date : 27/04/2016

Revised Date : 25/05/2016

Accepted Date : 31/05/2016

DOI: 10.5530/rjps.2016.2.4

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- (a) defining the patient's problems (diagnosis);
- (b) defining the effective and the safe treatments (non-drug and drug treatments);
- (c) selecting the appropriate drugs, duration and dosage.
- (d) writing a very clear prescription;
- (e) giving the patients appropriate information and counselling as well as planning to evaluate treatment responses.

This is simplified as five rights – the appropriate drug at the appropriate dose by the appropriate route at the appropriate time for the appropriate patient.² Unfortunately, the global trend in the prescribing patterns not always conform with these ideals, and what usually prevails is irrational prescribing.

The irrational use of medicines is widespread and very harmful to individual and that of the population, particularly in chronic disease conditions such as hypertension, mental disorder, diabetes and epilepsy. Other serious challenges involve with irrational drug use are interruption of therapy, development of drug resistance, increase in drug toxicities, organ failure, increase in healthcare costs and sudden death among the patients. Therefore, preventing irrational drug use may influence healthcare costs and potentially save lives.³ Rational use of drugs is one essential element in achieving quality of health and medical care for patients and the community as a whole.⁴ The essence of rational drug use is to avoid inappropriate prescription, problems of under- and over-prescribing, and the use of new, expensive drugs when equally effective, well-tried, safe and cheaper alternatives are available. The misuse, overuse or underuse of medicines usually results to wastage of resources and prevalent health hazards.⁵

Presently, no study has been conducted to measure the use of rational drugs in General Hospital, Offa, to the researchers' knowledge. Hence, the need arise to foster this study to obtain information that will improve drug prescribing habits of physicians and encourage the practice of pharmaceutical care in the facility of study.

METHODS

Setting

General Hospital Offa is a secondary health care facility owned by the State government and run by Kwara State Ministry of Health Hospital in Offa Local Government area of the State. It is located at resource-limited settings of North-Central senatorial district of Nigeria. The facility is a 50-bed hospital and provides in-patient and out-patient health care. The physicians in the facility are general practitioners. The healthcare facility has been providing health services to indigenous people of Offa

and the general public as a whole. The hospital has four pharmacists as well as two pharmacy technicians.

Three hundred and three prescriptions issued to patients attending out-patients' clinics in the facility over a period of three months were examined. Data collection form was designed and used by trained pharmacy technicians to record data and information on the prescribed drugs in the health facility.

Study Design

The study design included a retrospective study of patient medical records and prescription forms for prescribing practice indicators and a prospective, cross-sectional study designed to describe the current WHO drug use Indicators for patient and facility care of General Hospital, Offa.

Data collection

(a) The Prescribing indicators

This study was carried out in the Outpatient Department of General Hospital, Offa, from 17th April, 2015 to 2nd March, 2016. Prescriptions for the patients were spread throughout the year to reduce bias due to seasonal changes. Thirty two was the mean number of prescriptions per day in the facility. A sample of 1,416 prescriptions for the treatment of various diseases both acute and chronic was selected using systematic random sampling to evaluate the prescribing pattern of the facility. Five was the sampling interval used to select the prescriptions for the study.

Prescribing indicator form

Survey form 1 was used to capture information on prescribing manner of the physicians in the facility. The WHO indicators for evaluation of prescribing practices in a healthcare system were used and these include;

Indicator 1: This is average number of drugs per prescription and measured the degree of polypharmacy. It was obtained by dividing the total number of drugs prescribed by the number of prescriptions examined regardless of whether or not the patient received the drug.

Indicator 2: It shows the percentage of drugs prescribed by generic name. This is to assess the tendency to prescribe by generic name. This was obtained mathematically, by dividing the number of drugs prescribed by generic name by the total number of drugs prescribed, multiplied by 100.

Indicator 3: This indicator captured the percentage of prescriptions with an antibiotic prescribed. The essence is to evaluate the overall level of misused of antibiotic. It was calculated by dividing the number of prescriptions

in which antibiotics were prescribed by the total number of prescriptions studied, multiplied by 100.

Indicator 4: This was to determine the percentage of prescriptions with an injection prescribed. It examined the level of overused of injectable. It was achieved by dividing the number of prescriptions in which injections were prescribed by the total number of prescriptions studied, multiplied by 100.

Indicator 5: This determined percentage of drugs that were prescribed from the formulary or the Essential Drug List. It measured the degree to which prescribing practices in the hospital studied conformed with Nigerian Essential Drug List or formulary. It was calculated by dividing the number of drugs prescribed which are listed on the essential drugs list or local formulary by the total number of drugs prescribed multiplied by 100.

(b) The Patient care indicators

This study on patient care indicators was conducted prospectively. Patient care indicator form (Survey form 2) was used to collect information on the drugs being used by the patients. A total of 472 patients were interviewed using Patient Care Form. Patients enrolled into the study were those who attended the General Out-patient Department of the Hospital, those who received treatment, patients who refill their prescriptions in the hospital pharmacy and those who consented to participate in the study. The socio-demographic data of the patients were also recorded. These indicators were as follows:

Indicator 1: This shows the average dispensing time. The average time was attained by dividing the total time taken to dispense drugs to series of patients by the number of patients.

Indicator 2: This measured the percentage of drugs dispensed and computed by dividing number of drugs dispensed by the number of prescribed drugs presented for dispensing.

Indicator 3: The indicator determined the percentage of drugs adequately labelled. This was to measure the degree to which pharmacists provide vital information on the drug packages they dispense. This was achieved by dividing the number of drug packages containing at least patients' name, generic name of the drug, strength, quantity of drugs dispensed and when the drug should be taken were written on the label by the total number of drug packages dispensed, and multiplied by 100.

Indicator 4: This is to assess patient knowledge of correct dosage and measured the effectiveness of information provided to the patients on e dosage schedule of drugs they received from the pharmacists. It also involved discussion with patients on route of administration, duration of drug therapy, intended use of the

drug, expected action, drug interactions, common side-effects or adverse effects, techniques for self-monitoring of drug therapy, proper storage, warnings to keep all medicines out of the reach of children, expiry date, prescription refill information and actions to be taken in the event of a missed dose. Also, the patients knowledge of when, and in what quantity each dispensed drug should be taken as well as instruction on food restriction were examined. Failure of patients to understand this information would result to patients' knowledge being scored as inadequate. It was determined by dividing number of patients effectively reporting the dosage regimen for all drugs, by the total number of patients that were interviewed and multiplied by 100.

(c) The Facility indicators

Information on health facility indicators (Survey form 3) were derived by assessing adequate supply of essential drugs as well as access to information about these drugs in the hospital of study. The five pharmacists in the facility of study were included into the study. The study involved assessment of;

Indicator 1: Availability of copy of Essential Drugs List (EDL). This assessed the extent to which the Nigerian EDL is obtainable at a health facility. It is usually expressed as YES or NO, for such healthcare facility. Essential Drugs have been defined as those drugs that satisfied health care needs of majority of a population. These drugs should therefore be available at all times in adequate amounts and in appropriate dosage forms at all levels of the health care delivery system of the country.

Indicator 2: Availability of essential drugs: This was to measure the availability of key drugs usually recommended for treatment of specific diseases in the hospital. It was computed by the number of drugs actually available in the pharmacy store room divided by the total number of drugs on the EDL list, multiplied by 100.

Ethical consideration

The research was approved by the Institutional Review Board, Kwara State Ministry of Health, Ilorin, Nigeria.

Statistical analysis

The data collected were checked and analyzed using the SAS software program version 9.2.⁶ Descriptive statistics were used in the form of frequency, percentage, mean and standard deviation.

RESULTS

The age composition of the study revealed that 22 (4.7%) were between 14 and 20 years old, 160 (33.9%) were between the age of 21 and 40 years while 290 (61.4%)

Survey form 1: Public Health Facility: Rational Drug Use - Prescribing Indicator Form

Indicators:	Average number of drugs	% patients receiving injection
	% drugs on EDL	% patients receiving antibiotics
Public Health Facility		
Facility # _____ (1-30)		
Facility _____	Date _____	
Location _____	Investigator _____	

Seq. Patient No.	Type (R/P) [A]	Date of Rx	No. of drugs [B]	Antibiotics (yes=1, no=0) [C]	Injections (yes=1, no=0) [D]	No. of drugs on EDL [E]
1.						
2.						
3.						
4.						
5.						
6.						
7.						
8.						
9.						
10.						
11.						
12.						
13.						
14.						
15.						
16.						
17.						
18.						
19.						
20.						
21.						
22.						
23.						
24.						
25.						
26.						
27.						
28.						
29.						
30.						
Total			[B ¹] =	[C ¹] =	[D ¹] =	[E ¹] =
Average			[B ²] =			
Percentage				[C ²] = [C ¹]/No. of cases x 100 =	[D ²] = [D ¹]/No. of cases x 100 =	[E ²] = E ¹ /[B ¹] x 100 =

Notes

- [A] Select 30 outpatients seen within a 12 month period (R=retrospective [from records], P= prospective [those currently being treated]). Sample of cases can be a combination of P and R
- [B] Count number of drugs prescribed for each case ([B¹]=Total no. of drugs). Count as 1 a drug given in different preparations, e.g. paracetamol tablet and injection, two brands of a similar chemical entity/INN/generic name
- [B²] Average no. of drugs prescribed = [B¹]/Number of cases
- [C] Indicate 0 if no antibiotic prescribed and 1 if one or more types of antibiotics were given.
[C¹] = Total cases with antibiotics.
- [D] Indicate 0 if no injection given and 1 if one or more injections were given. [D¹]= Total cases with injection
- [E] From the number of drugs prescribed for the case, count those included on the EDL.
[E¹] = total number of drugs listed in EDL

Survey form 2: Public Health Facility Rational Drug Use - Patient Care Form				
Indicators:		% of drugs dispensed		
		% drugs with adequate label		
		% of patients who know how to take drugs		
Public Health Facility Facility # ____ (1-30)				
Facility _____		Date _____		
Location _____		Investigator _____		
Seq. No.	No. of drugs prescribed [A]	No. of drugs dispensed [B]	No. of drugs adequately labelled [C]	Patient has adequate knowledge (yes=1, no =0) [D]
1.				
2.				
3.				
4.				
5.				
6.				
7.				
8.				
9.				
10.				
11.				
12.				
13.				
14.				
15.				
16.				
17.				
18.				
19.				
20.				
21.				
22.				
23.				
24.				
25.				
26.				
27.				
28.				
29.				
30.				
Total		Total B =	Total C =	Total D =
Average Percentage		[B']=% dispensed = Total [B]/No. prescribed x100 = _____	[C']=% w/adequate label = Total [C]/Total [B] x 100 = _____	[D']=% of patients with adequate knowledge = Total [D]/cases asked x 100 = _____

Notes

[A] Interview 30 patients leaving the dispensing area/pharmacy

[B] Check how many drugs (chemical entity/INN/generic) were given to each patient

[C] Check which are adequately labelled (name of drug, dosage and duration plus any additional criteria specified by country). A drug is adequately labelled only if all criteria are met.

[D] Determine if patient has adequate knowledge about the drugs dispensed.
Ask patient if he/she knows how to take each drug. Indicate:
(1) If patient can correctly give the name of all drugs or state what the drugs are for and how they should be taken plus any additional criteria specified by country.
(0) If the patient cannot give the name of even one drug, cannot state what a drug is for, does not know how to take one of the drugs given, or does not meet any additional criteria specified by country.

Survey form 3: Public Health Facility - Standard Treatment Guidelines/EDL

Indicator:	Availability of STG for common local conditions
	Availability of Essential Drug List (EDL) at the facility
Public Health Facility	
Facility #____ (1-30)	
Facility _____	Date _____
Location _____	Investigator _____

Tick box with correct answer

Standard treatment guidelines available	Yes ¹	No
National STG	☐	☐
STG for URTI	☐	☐
STG for Diarrhoea	☐	☐
STG for Pneumonia	☐	☐
STG for Malaria	☐	☐
STG for Tuberculosis	☐	☐
Others:	☐	☐

EDL available	Yes ¹	No
National EDL	☐	☐
Provincial/District	☐	☐
Primary EDL	☐	☐
Others:	☐	☐

STG is available in this facility ²	Yes ¹ ☐	No ☐
EDL is available in this facility ³	Yes ¹ ☐	No ☐

Notes

1. Mark "yes" only if the facility is able to show you the document.
2. The facility is considered to have an STG if any one of the above STGs is available provided it was developed by an independent group and is not associated with promoting pharmaceutical products.
3. Before the survey, the most up-to-date version of EDL must be identified. The facility is considered to have an EDL if any one of the above EDLs is available.

were for age above 40 years old. The mean age of patients was 56.2 ± 7.1 years. In reference to gender, 322 (68.3%) were females and 150 (31.7%) were males. The occupational status of the patients showed that 314 (66.5%) were traders, public servants 36 (7.6%) and 122 (25.9%) were unemployed (engaged only in activities of daily living such as eating, sleeping and bathing). With regard to educational level of the patients, 225 (47.7%) had no formal education, 53 (11.2%) had primary school certificate, 111 (23.6%) of the patients completed secondary level of education and 83 (17.5%) were graduates (Table 1).

The average number of drugs per prescription was 2.6. Most (58.1%) of the drugs prescribed were branded rather than generics (41.9%). The percentages of antibiotic and injectable prescribed were within WHO cut-off values. Almost all (99.7%) the drugs were prescribed from Nigerian EDL. All the prescriptions, 1,416 (100.0%) were from physicians. (Table 2).

The study revealed that the pharmacists dispensed almost all the drugs prescribed to patients and labeled 81.9% of the prescriptions adequately. Also, majority (91.1%) of the patients had correct knowledge on the drug dosage, time of administration, frequency of administration, food restriction, side effects and duration of treatment. (Table 3).

As shown in Table 4, a copy of EDL was available in the health facility of study to assess information on drugs supplied to patients. Most (99.7%) of the key drugs were available in the pharmacy.

Table 5 presents the morbidity profile in the hospital. Hypertension was the most prevalent disease identified in outpatient department of the hospital accounting for 28.8% of the diseases identified. Co-morbidity with arthritis and infections accounted for 25.1% and 23.8% respectively.

DISCUSSION

In the present study, the average number of drugs per prescription was 2.6, which was higher than WHO standard range of 1.6-1.8. This finding is similar to that of⁷ in India and⁸ in Iran with 2.7 and 3.07, respectively. Polypharmacy is a common problem of prescription in Nigeria like many other developing countries. The percentage of generic drugs prescribed in the present study was 41.9%. This is very low as compared with WHO standard (100%). This corroborates with the findings of⁹ in Nigeria (42.9%). Previous study by¹⁰ in Tanzania found prescribing of generic drugs to be 33%. These findings revealed that prescription of generic drugs is yet to be acknowledged as a routine in African countries as recommended by WHO. The rationale for low

generic drug prescribing in Nigeria might be related to promotion of branded drugs by prescribers for Pharmaceutical representatives as well as the prescribers' believe that branded drugs are more efficacious than generics. This practice is detrimental to the health and pocket of the patients as most of the patients could not afford the exorbitantly priced branded drugs for their long-term disease conditions and eventually leading to non-compliance, irrational drug use and death among others.

The report of this study revealed that the percentage of antibiotics prescribed was 23.8%. This finding is good as compared with WHO standard (20.0-26.8%). The present result was inconsistent with the values obtained in Nigeria by¹¹ who reported 48% and Ethiopia by¹² (58.1%) prescriptions of antibiotics. The results of this study implied that antibiotics were not misused or over-used by the physicians in this facility as study showed that antibiotics are the most frequently prescribed drugs among patients and there are reported concerns about the continuous indiscriminate and excessive use of antimicrobial agents that promote the emergence of antibiotic-resistant organisms.¹³ Rational antibiotic prescription as shown in the present study is the first step for optimum antibiotic use and has the potential impact of reducing resistant micro-organisms generated by excessive use.¹⁴

In this study, the percentage of injectable prescribed was 3.4% and fell within WHO cut-off values of less than 10%. This result was higher than the study of⁷ who reported that the percentage of injections prescribed was found to be 1.6% and lower (41%) as compared to the study of⁸ in Iran. Irrational prescription of injections should be avoided as injections are costly compared to other dosage forms and administration of injection also becomes expensive as well as pose health hazards. It required trained personnel because unhygienic usage of injections can enhance high risk of communicable diseases transmission such as HIV/AIDS, tuberculosis and hepatitis.

Almost all (99.7%) of the drugs were prescribed from Nigerian EDL, which is almost equal with the WHO standard (100%). The result of this study is similar to the findings of⁷ who reported that the percentage of drugs prescribed from EDL was found to be 95.6%. In this study, the average dispensing time was good (5.25 ± 2.33 minutes) as compared to the WHO recommended value of greater than three minutes. These results were higher than the study of¹⁵ in Ethiopia with 1.9 minutes but similar to the study of¹⁶ in India (4 minutes 4 seconds). The present study revealed that the Pharmacists spent more time in interacting with their patients. This was supported by the findings of¹

Table 1: Demographic Profile of Patients for Prospective Study (n=472)

Variables	Frequency	Percentage (%)
Age (Year)		
14-20	22	4.7
21-40	160	33.9
Above 40	290	61.4
Gender		
Female	322	68.3
Male	150	31.7
Educational Level		
No formal Education	225	47.7
Primary Education	53	11.2
Secondary Education	111	23.6
Tertiary Education	83	17.5
Occupation		
Traders	314	66.5
Public Servants	36	7.6
Unemployed	122	25.9

Table 2: WHO Prescribing Indicators

WHO Prescribing Indicators	Current study values	WHO reference values	P-value
Percentage of drugs prescribed in generic	41.9	100%	0.634
Percentage of drugs prescribed from EDL	99.7	100%	0.625
Average number of drugs per prescription	2.6	1.6-1.8	0.958
Percentage of antibiotic prescribed	23.8	20.0-26.8%	0.939
Percentage of injectable prescribed	3.4	<10.0%	0.179

P>0.05 indicates not significantly different at 0.05 level of significance.

Table 3: WHO Patient Care Indicators

WHO Patient Care Indicators	Current study value	WHO value	P-value
Average dispensing time (Minutes)	5.25 ± 2.33	>3.0	0.913
Percentage of drugs actually dispensed	87.4%	100%	0.625
Percentage of drugs adequately labelled	81.9%	100%	0.634
Patient knowledge of correct dosage	91.1%	100%	0.179

P>0.05 indicates not significantly different at 0.05 level of significance.

Table 4: WHO Facility Indicators

WHO Facility Indicators	Current study value	WHO reference value	P-value
Availability of copy of EDL or formulary	(1) Yes	(1) Yes	
Availability of key drugs	100%	99.7%	0.143

P>0.05 indicates not significantly different at 0.05 level of significance.

Table 5: Morbidity profile in the community studied

Disease	Frequency (n=3, 711)	Percentage (%)
Hypertension	1069	28.8
Arthritis	931	25.1
Infection	883	23.8
Anaemia	301	8.1%
Malaria	267	7.2%

who reported that the Pharmacists should spend some time with their patients by explaining the appropriate use of dispensed drugs.

The labeling of drugs (81.9%) in the present study was good as compared to recommended value of 100% by WHO. This is consistent with the result of¹⁷ in Tanzania who reported 87% but higher than the study conducted by¹⁸ where 67% of the drugs were adequately labelled. The adequate labeling of drugs (writing the generic name and strength of the drug, total quantity of drug dispensed, frequency of administration, before/after meals, date medication was dispensed, the name of the patient, the name and address of the pharmacy from which the medication was dispensed and other relevant information/warning such as keep out of reach of children should be indicated) found in this facility will encourage drug adherence and prevent irrational use of drugs. Furthermore, the study shows that 91.1% of patients were able to reiterate the correct dosage regimen of the drugs and pharmaceutical care counseling received; which is comparable with the WHO recommended value of 100%. The rationale for this could be due to availability of pharmacists, pharmaceutical care concept being practiced in the setting of the study, as well as good structural layout of the hospital pharmacy for easy flow of work.

Also, the facility of study had the latest edition of EDL, used as standard treatment guideline. According to the result of present study, most 3,699 (99.7%) of the drugs were prescribed from the latest version of Nigerian EDL. This is in line with the study of¹⁹ with 80.7% and improved as compared to the findings of²⁰ with 64.12%. This uninterrupted supply of drugs in the hospital showed improvement in the quality of health care services rendered to the citizens of Offa and environs. This could be attributed to functional Drug Revolving Fund scheme operating in all State Government hospitals in Kwara State and the appropriate policy about the priorities in supplying drugs by its Project Manager. Globally, more than 50% of all drugs are dispensed wrongly, while 50% of the patients failed to administer them appropriately. Furthermore, one-third of the population in world cannot afford essential medicine. Because of irrational use of drugs, effective drugs

of yesterday becoming ineffective today. Despite accessibility, availability and affordability of essential drugs; it is equally necessary to use the drugs rationally.¹

According to the result of this study, hypertension was however, the most prevalent disease identified in this study. This might be due to the fact that majority of the patients were older (greater than 40), an age category implicated for hypertension. Also, these patients are living a sedentary life style (petty trading and minor household chores with no basic education as to enlighten them on risk factors of hypertension. The study of²¹ was in support of present study that predictors of hypertension include family history, age, race, obesity, physical inactivity, lack of exercise and excessive salt intake.

CONCLUSION

Based on the above findings, most of the prescribing indicators in this health facility are not conforming to the WHO standard criteria. Health facility and patient care indicators are rational. More studies on these indicators are required to identify factors responsible for irrational drug prescription patterns of prescribers in this health facility.

RECOMMENDATION

Public enlightenment of Offa Community on dietary approach to stop hypertension would reduce the scourge in the area of study.

ACKNOWLEDGMENT

The authors are grateful to the Principal Officers and Staff of the General Hospital, Offa, Kwara State, Nigeria, for providing all necessary facilities to carry out the research. Dr. Bello, Omolaran Bashir of the Department of Biological Sciences, Fountain University, Osogbo is also appreciated for the manuscript review and corrections.

CONFLICT OF INTEREST

The authors are hereby declaring no conflict of interest of this study.

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Artesunate Affects Diurnal Variation of Gentamicin Nephrotoxicity in Wistar Rats

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ABSTRACT

Objective: Diurnal variation in gentamicin nephrotoxicity has been previously demonstrated. This study investigated the effect of diurnal variation on gentamicin nephrotoxicity with concurrent artesunate administration in wistar rats. **Methodology:** Gentamicin (120 mgkg^{-1}) was co-administered with artesunate (100 mgkg^{-1}) at 0000 hrs and 1200 hrs being times of least and greatest gentamicin-nephrotoxicity. Renal biomarkers including creatinine, urea, CAT, SOD, MDA, GPx and electrolytes were determined following seven-day co-administration. **Findings:** Gentamicin at 1200 hrs produced significant ($p < 0.05$) elevation in serum urea and creatinine in comparison with controls. Animals that received gentamicin at 0000 hrs had significantly lower creatinine and urea levels compared with the 1200 hrs gentamicin group. Artesunate ameliorated gentamicin-nephrotoxicity at both time points with reduction in serum urea and creatinine values. **Conclusion:** The study showed that artesunate ameliorated gentamicin-induced nephrotoxicity during both periods in rats. **Research Value:** This research suggests that the concurrent administration of both drugs in bacteremia and parasitemia co-infection may offer beneficial effects of alleviating gentamicin induced nephrotoxicity irrespective of rest or activity time administration.

Key words: Artesunate, Gentamicin, Nephrotoxicity, Diurnal Variation, Chronotoxicity.

INTRODUCTION

Malaria is a parasitic infection caused by *Plasmodium* species, and falciparum malaria has been linked with severe complications and mortality.¹ The disease may present in a severe form resulting in death particularly in non immune patients, when diagnosis and treatment are not prompt at the acute phase.² Although many countries have been able to decrease their malaria burden significantly between 2000 and 2010,³ this is not a global success and the treatment and prevention of malaria has continued to undergo reviews and changes in strategies. Current therapy uses artemisinin derivatives in combination with other agents, resulting in rapid clearance, however monotherapy is also deployed for longer duration.⁴ Combination regimens typically combine an artemisinin derivative with a long acting agent,⁵ while life threatening severe malaria is treated with parenteral

artesunate.⁶ Artesunate is largely reported to be safe, although, available data in the literature report conflicting effects on the kidney. These include non association with electrolyte imbalance,⁷ diuretic effect in malaria patients,^{8,9} and renal involvement.^{10,11} Recent data suggests that invasive bacterial infection appears to be associated with severe malaria in children,¹² while previous data reported non typhoidal *Salmonella* with severe malaria in children,¹³⁻¹⁵ the therapy of which often includes gentamicin. Gentamicin is used for treatment of sensitive gram negative bacteria, and its nephrotoxicity¹⁶ limits its clinical use.¹⁷ However its use continues because of efficacy, cost, post antibiotic effect and its synergistic effects with other antimicrobials.¹⁸ The strategies to reduce its toxicity include reduction of dosing frequency, consideration of the circadian rhythm of renal

Received Date : 07/04/2016
Revised Date : 27/06/2016
Accepted Date : 29/06/2016

DOI: 10.5530/rjps.2016.2.5

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function,¹⁹ as well as concurrent administration with antioxidants, and modification of time of administration based on temporal variation in its nephrotoxicity. With concurrent administration of gentamicin and artesunate being a clinical situation in bacteria-falciparum infections, the effect of the concurrent drug therapy on the kidney is important. The study thus investigated the effect of chronomodulated gentamicin-artesunate regimens on nephrotoxicity in wistar rats. Serum biomarkers of nephrotoxicity and relative kidney weights were determined following a seven day concurrent administration of artesunate and gentamicin. The effect of the concurrent drug administration on some markers of oxidative stress was also investigated.

MATERIALS AND METHODS

Animals

Male wistar rats with average weight of 180 g were obtained from the Animal Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria Nigeria. The animals were allowed to acclimatize in their new animal room for about one week before experimental procedures began. The rats were housed in cages of five or six and had wood shavings as beddings which were changed frequently. The animals were placed on Vital Feed and public water supply *ad libitum*. Experiments were performed in accordance with approved institutional Animal Committee guidelines (DAC/IW-OT/66-14).

Experimental design

Animals were divided into six groups for the study with the first group serving as saline control. The second group received 120 mgkg⁻¹ gentamicin, while the third group received artesunate 100 mgkg⁻¹ both at 1200 hrs daily for seven days. Group four received both artesunate and gentamicin also at 1200 hrs daily for seven days. Animals in group five received gentamicin alone at 0000 hrs (midnight) daily for seven days while animals in group six received both gentamicin and artesunate at 0000 hrs similarly for seven days. The 120 mgkg⁻¹ dose of gentamicin was adopted from a previously reported protocol,²⁰ and after an initial pilot study. The dose of artesunate was also based on its established antiplasmodial activity within this dose range.

Preparation of drugs

The drugs used in the study were gentamicin (Gentalek® injection) and artesunate powder. They were obtained via Lek and Tuyil Pharmaceuticals, Nigeria respectively. The drugs were freshly prepared daily for administration

via the intraperitoneal route using suitable syringes and needles.

Serum analysis

At the end of the seven consecutive days of daily treatment, the animals were euthanized with chloroform and the kidneys were excised after dissecting the rats. Kidney weights were determined using a sensitive weighing balance. Blood was also collected from the jugular veins of the rats into anticoagulant free vacutainers. Serum urea, creatinine, electrolytes (sodium, chloride, bicarbonate, potassium) AST, ALT, ALP, total protein, albumin and glucose were determined using a Bayer Automated Analyzer and appropriate kits. Serum oxidative stress markers (GPx, SOD, CAT and MDA) were also determined using Randox® kits.

Data analysis

Data obtained was analyzed using One Way Analysis of Variance followed by Levene's test of equality of variance. Welch Robust test of means was used when significant outcome of Levene's test was obtained. The Hochberg post hoc test was used and a p value of ≤ 0.05 was considered statistically significant for each comparison.

RESULTS

Results from the study show that serum urea and creatinine levels of the group that received gentamicin alone at 1200 hrs was significantly ($p=0.007$) higher than that of the saline treated group. However the creatinine levels in all the other groups that received artesunate or gentamicin alone, or a combination of gentamicin with artesunate at all time points were significantly lower than those of animals that received gentamicin alone at 1200 hrs. A similar result was also obtained for the urea levels (Figures 1 and 2). The serum electrolyte levels were not significantly altered by the different times of administration and combinations with gentamicin and artesunate (Table 1). There was also no significant difference in the group that received artesunate alone when compared with the saline group (Table 1). Of the three markers of hepatic functions (AST, ALT and ALP) only the levels of the ALP for animals TREATED WITH GENTAMICIN at 1200 hrs was significantly higher than that of the normal saline control (Table 2). Data from the study did not show any significant difference in any of the markers of oxidative stress that were investigated (Table 3). Serum levels of total protein, bilirubin and glucose also did not differ significantly (Table 4). The relative left kidney weight of all the groups did not differ significantly from the saline group. However, the group that received artesunate alone at 1200 hrs showed significantly lower relative kidney

Table 1: Effect of chronomodulated gentamicin-artesunate administration on serum electrolytes in wistar rats

Electrolyte levels in mmol/l				
Group	Sodium	Potassium	Chloride	Bicarbonate
Normal Saline 1200 hrs	139.00±0.84	4.28±0.07	98.71±1.30	22.42±0.75
Gent 1200 hrs	139.28±0.89	4.18±0.24	99.00±0.72	23.28±1.08
Art 1200 hrs	137.57±0.78	4.04±0.12	96.85±0.70	22.57±1.10
Art+Gent 1200 hrs	138.00±1.09	4.08±0.18	98.57±1.61	22.42±1.47
Gent+Art 0000 hrs	140.50±1.25	4.20±0.15	100.33±1.17	22.33±1.20
Gent 0000 hrs	139.42±1.02	4.30±0.08	101.14±0.70	21.71±0.80

Data is presented as mean ± SEM (n=6-7) and analyzed using One Way ANOVA. No significant differences were observed following One way ANOVA.

Table 2: Effect of chronomodulated gentamicin-artesunate administration on hepatic biomarkers in wistar rats

Group	ALT IU/l	AST IU/l	ALP IU/l
Normal Saline 1200 hrs	71.28±6.72	65.14±7.12	67.00±7.00
Gent 1200 hrs	74.71±7.40	65.28±7.68	130.57±13.40**
Art 1200 hrs	73.85±4.07	62.42±4.20	72.57±9.48
Art+Gent 1200 hrs	69.85±5.02	60.85±5.76	82.28±13.42
Gent+Art 0000 hrs	70.66±8.56	63.16±7.03	76.33±10.01
Gent 0000 hrs	81.14±6.52	70.71±5.37	85.85±6.34

Data is presented as mean ± SEM (n=6-7) and analyzed using One Way ANOVA followed by Hochberg post hoc test. ** = p<0.01 compared with saline.

Table 3: Effect of chronomodulated gentamicin-artesunate administration on serum markers of oxidative markers in wistar rats

Group	MDA[μmol/l]	SOD[U/l]	CAT[nmol/l]	GPX[U/l]
Normal Saline 1200 hrs	1.07±0.09	2.15±0.11	53.28±1.04	44.57±1.77
Gent 1200 hrs	1.12±0.06	2.00±0.06	53.00±1.04	47.28±1.61
Art 1200 hrs	1.00±0.05	2.27±0.09	48.57±2.10	51.00±1.97
Art+Gent 1200 hrs	2.07±0.82	2.08±0.10	50.57±1.04	48.14±2.89
Gent+Art 0000 hrs	0.93±0.12	2.31±0.11	54.50±1.72	47.00±.93
Gent 0000 hrs	1.11±0.15	1.95±0.10	50.85±2.14	45.28±1.16

Data is presented as mean ± SEM (n=6-7). No statistically significant difference in means following one way ANOVA.

Table 4: Effect of chronomodulated gentamicin-artesunate administration on serum protein and glucose levels in wistar rats

Group	TP[g/dl]	ALB[g/l]	GLUCOSE[mmol/l]
Saline 1200 hrs	64.71±1.08	35.42±1.13	4.51±0.11
Gent 1200 hrs	66.42±1.92	37.14±1.80	4.75±0.26
Art 1200 hrs	66.71±1.40	39.00±1.57	4.75±0.26
Art+Gent 1200 hrs	66.14±0.59	36.71±0.68	4.74±0.09
Gent+Art 0000 hrs	66.50±1.05	36.33±1.30	4.43±0.16
Gent 0000 hrs	63.14±1.12	35.57±1.02	4.78±0.26

Data is presented as mean ± SEM (n=6-7). No statistically significant difference in means following one way ANOVA.

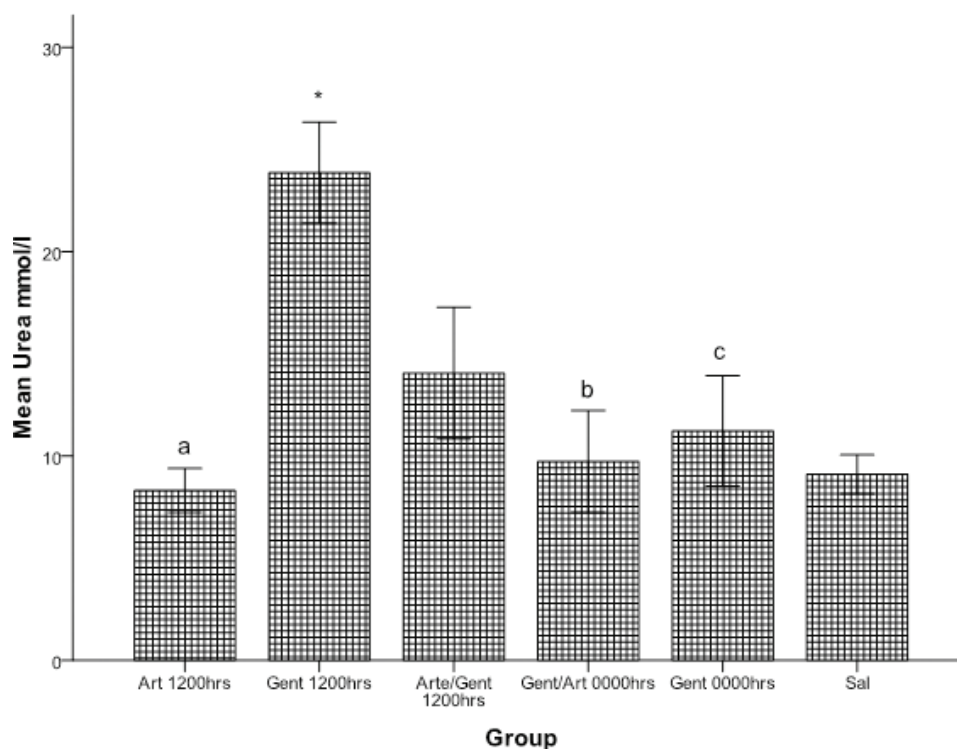


Figure 1: Effect of chronomodulated gentamicin-artesunate administration on serum urea levels in wistar rats.

Data is presented as mean $\pm$ SEM (n=6-7) and analyzed using One Way ANOVA followed by Hochberg post hoc test. *: p = 0.001 in comparison with saline control, a, b, c are p values of .001, .003, .008 for in comparison with gentamicin 1200 hrs

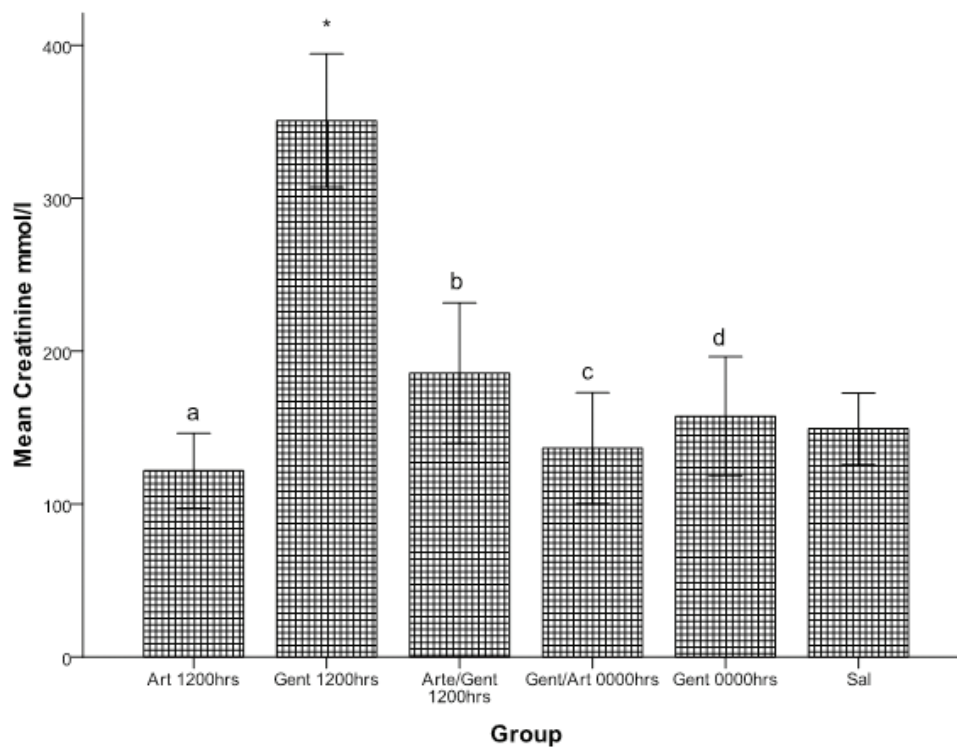


Figure 2: Effect of chronomodulated gentamicin-artesunate administration on serum creatinine levels in wistar rats.

Data is presented as mean $\pm$ SEM (n=6-7) and analyzed using One Way ANOVA followed by Hochberg post hoc test. #=significant difference in comparison to saline; *p=0.007 in comparison with saline control; a, b, c are p values of 0.001, 0.045, 0.005, 0.01 in comparison with gentamicin 1200 hrs

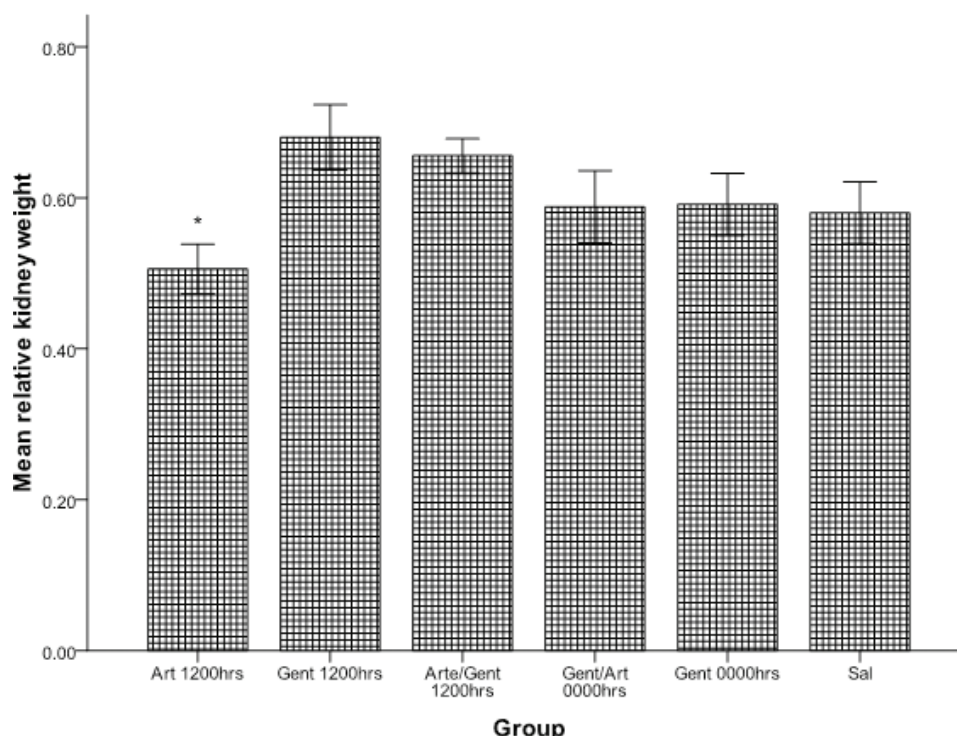


Figure 3: Effect of chronomodulated gentamicin-artesunate administration on relative kidney weight in wistar rats.

Data is presented as mean $\pm$ SEM (n=6-7) and analyzed using One Way ANOVA followed by Hochberg post hoc test.
*p=0.05 compared to Art/Gent 1200 hrs

weight ratio when compared with the gentamicin 1200 hrs group (Figure 3).

DISCUSSION

The current study corroborates previously documented reports²¹ on the temporal variation in gentamicin toxicity. This was evidenced by the high levels of renal biomarkers at the rest period of the rats (1200 hrs) and low levels at the high activity period (0000 hrs). However, the concurrent administration of gentamicin and artesunate at both periods did not result in any observable deleterious effect on the kidney as was also the case with the liver. Temporal variation in gentamicin nephrotoxicity has been previously demonstrated in rats, and the administration of gentamicin during activity period (being 0000 hrs in this study) has been shown to pose a lower risk of nephrotoxicity due to increased glomerular filtration during this period.²² Artesunate has been shown to exhibit some adverse effects on the kidney following a 21-day high dose regimen in mice,²³ and also exhibits reversible renal toxicity at a very high intravenous dose.²⁴ However the concurrent administration of artesunate and gentamicin in this study resulted in amelioration of gentamicin associated nephrotoxicity. Although gentamicin nephrotoxicity is most often oligouric, several diuretics

have been reported to ameliorate the nephrotoxicity of gentamicin. A reduction in the accumulation of gentamicin in the kidney is a major approach to decreasing its nephrotoxicity,²⁵ and the use of single dose and timed administration reduces the quantity and period of contact of the kidney with gentamicin thus reducing nephrotoxicity. Artesunate's diuretic properties have been previously reported.^{8,26} This diuretic effect may have contributed to the clearance of gentamicin and reduced the nephrotoxicity at both 0000 hrs and 1200 hrs. Artesunate, in addition to the diuretic effect had also been shown to increase renal blood flow. This may thus reduce the contact period of the antibiotic with the nephrons thus reducing the toxicity. The diuretic frusemide has been previously reported to reduce gentamicin induced nephrotoxicity.²⁷ Earlier studies also reported that frusemide did not increase the risk of aminoglycoside renal and auditory toxicity.^{28,29} The diuretic effect reported by previous researchers may be a possible mechanism behind the amelioration of the gentamicin induced nephrotoxicity. The night time (0000 hrs) administration of gentamicin results in reduced nephrotoxicity. This period is the activity period in rodents and there is a higher level of glomerular filtration in contrast to the rest period. A reduced clearance during rest periods in man and also possibility that the toxicity of gentami-

cin was more likely due to the duration of contact in the kidney rather than the plasma concentrations has been postulated.³⁰ However, the diuretic effect of artesunate may have equally contributed to the reduction in nephrotoxicity with the 0000 hrs in a similar fashion to that of the 1200 hrs dose which typically exhibits the least toxicity. Thus the available concentration to exert toxicity due to contact is diminished. The kidney sometimes fails acutely as a consequence of malaria or falciparum species infection,³¹ and mortality may be as high as 45% when it occurs.³² Thus the ameliorative effect of artesunate in the face of concurrent malaria and gentamicin sensitive bacteria infection could be advantageous in malaria related kidney failure on artesunate therapy.

CONCLUSION

The nephrotoxicity associated with gentamicin administration was ameliorated in the presence of artesunate both during the active (0000 hrs) and rest (1200 hrs) periods. This could be beneficial in concurrent treatment of bacteria and falciparum co morbid states. Although the exact mechanism by which this occurs remains to be fully elucidated, the diuretic effect of artesunate may be a contributory factor.

ACKNOWLEDGMENT

The authors acknowledge the assistance of Mr. Olu Ayegbusi of the Department of Chemical Pathology, Ahmadu Bello Teaching Hospital, Zaria-Nigeria.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Haematological Profile of Patients on Antiretroviral Therapy in a Nigerian Teaching Hospital

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ABSTRACT

Background: Haematological parameters are important monitoring tool for assessing prognosis and treatment in Human Immunodeficiency Virus (HIV) infected patients. Therefore, examining these parameters for abnormalities are absolutely imperative. **Objective:** This research was conducted to examine the effects of ARV drugs on haematological indices of HIV-infected patients under treatment. **Research approach:** The study was conducted in President's Emergency Plan for AIDS Relief Clinic of the University of Benin Teaching Hospital, Benin City, Nigeria between October, 2012 and April, 2013. The blood samples of 275 HIV-infected patients on antiretroviral (ARV) drugs and 109 patients yet to receive ARVs were analysed for haematological parameters with automated blood analyser. **Findings:** A higher incidence of anaemia, thrombocytopenia and lymphocytopenia were observed in those patients that were yet to commence ARV drugs. Leucopenia and neutropenia were prominent among HIV-infected patients on ARV drugs. Microcytic anaemia was more frequent in patients that were about to start drugs, while macrocytic anaemia was identified among HIV-infected patients on ARV drugs. **Conclusion:** All types of anaemia except life threatening were found in patients receiving ARV drugs. The anaemia, leucopenia and neutropenia found in patients on ARV drugs were associated with first-line Tenofovir-based regimen. **Research Value:** Further research is needed to ascertain the safety of Tenofovir-based therapy in managing HIV-infected patients.

Key words: Human immunodeficiency virus, Anaemia, Leucopenia, Neutropenia, Cytopenia, Antiretroviral drugs.

INTRODUCTION

Human Immunodeficiency Virus (HIV)/Acquired Immune Deficiency Syndrome (AIDS), continues to be a major global public health concern,¹ with a prevalence of about 36.9 million in 2014. In terms of HIV burden however, Nigeria is second to South Africa worldwide with 3.4 million people living with the disease.² Haematological parameters (such as haemoglobin, white blood cells, red blood cells, neutrophils, lymphocytes and platelets) are important monitoring parameters for assessing treatment and prognosis in HIV/AIDS.^{3,4} Antiretroviral (ARV) drugs represent major advancement in managing HIV infection. However, the use of these drugs could either negatively or positively affect haematological parameters, depending on the choice of combinations. In the early 2000s, adverse

reactions began to appear and started to challenge the goals of ARVs.⁵ Although many drugs used for the treatment of HIV-related disorders are myelosuppressive, severe cytopenia is most often associated to the utilization of zidovudine.⁶ These complications are generally marked with cytopenias involving anaemia, neutropenia, leucopenia, lymphocytopenia and thrombocytopenia.⁷ The prevalence of the cytopenia especially anaemia majorly serves as a predictor of advancement to AIDS or death.⁸ The study⁹ reported decline in the haematocrit values of HIV-infected patients on ARV drugs and those that were yet to receive ARVs. A previous research conducted in the United Kingdom also showed that cytopenia is a prevalent abnormality of HIV infection, and more than 70% of the

Received Date : 24/05/2016

Revised Date : 27/06/2016

Accepted Date : 30/06/2016

DOI: 10.5530/rjps.2016.2.6

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patients developed anaemia, sometimes requiring transfusion.¹⁰ Anaemia, ranging between 1.3% and 95% is the first frequent haematological complications in the HIV-infected patients on ARV drugs. This is followed by thrombocytopenia, the second most frequent haematological complication of HIV infection which affected 3% to 40% of individuals with the infection. Neutropenia is also common in HIV-infected individuals and may occur in 10% to 30% of the patients.^{11,12}

A study¹³ in Ethiopia revealed that prevalence of anemia was of great magnitude in those patients who are yet to start ARV drugs, while leucopenia and neutropenia were prominent in patients on ARV drugs. Study in Ghana¹⁴ reported the incidence of 63% anaemia, and 16.7% lymphocytopenia in those patients that were yet to start ARV drugs, while 46% anaemia and 5.3% lymphocytopenia were recorded for those patients on ARV drugs. Furthermore, the findings in New Guinea¹⁵ showed that anaemia, leucopenia, eosinophilia, thrombocytopenia, neutropenia and monocytosis were prevalent among HIV-infected patients. These different reports provide a framework for conducting further studies on haematological profile of HIV-infected patients on antiretroviral therapy and those that are yet to commence ARV drugs in President's Emergency Plan for AIDS Relief (PEPFAR) Clinic at University of Benin Teaching Hospital, Benin City, Nigeria.

MATERIALS AND METHOD

Study site

The study was conducted in the PEPFAR Clinic, University of Benin Teaching Hospital (UBTH), Benin City, Nigeria which provides medical services to over three million citizens of Edo State of Nigeria. Ethical approval to conduct the study was obtained from Ethics and Research Committee of UBTH before the enrolment of subjects (Protocol Number: ADM 22/A/VOL.VII/833 on 10th September, 2012). Permission to work with the patients was obtained from the Consultant and Coordinator in charge of the Clinic.

Population of study

The population of study were HIV-infected patients diagnosed and established to be positive, using the Determine HIV 1 and 2 Test Kit (Alere Medical Company Limited, Chiba, Japan) and STAT-PAK HIV 1 and 2 Test Kit (Chembio Diagnostic System Inc, USA).

Experimental design

The experimental design was a cross-sectional, prospective, randomized controlled study. The study time spanned between October, 2012 and April, 2013. Adult

HIV-infected patients attending the PEPFAR Clinic, were included in the study, who have been on a combination therapy of ARV drugs for at least one year, and those that were yet to receive ARV drugs. Patients excluded were children, pregnant women and cigarette smokers. A total of 384 HIV-infected patients who met the inclusion criteria were randomly selected among the population of 14,610 HIV-infected patients receiving care in the facility of study. The 384 patients recruited were divided into two equal groups that were well-matched in sex and age.

Group 1: 275 HIV-infected patients on ARV drugs;

Group 2: 109 HIV-infected patients yet to be placed on ARV drugs (control group).

Haematological analysis

Written informed consent was obtained from the patients before blood sample collection. Three millilitre of whole blood was obtained from each patient for haematological assay, using automated blood analyser—sysmex KX-21N (Sysmex Corporation, Kohe, Japan). The blood samples collected were evaluated within 24 hours for haemoglobin (Hgb), White Blood Cell count (WBC), Total Lymphocyte Count (TLC), Red Blood Cell count (RBC), neutrophils count, Mean Cell Volume (MCV), platelet count, Mean Cell Haemoglobin Concentration (MCHC), and Mean Cell Haemoglobin (MCH). The blood collected were mixed and fed to the automated machine which aspirated 50 µl of the blood. The reagents supplied by the manufacturer were used for the analysis. The machine displayed the analysis results of the haematological parameters on the Liquid Crystal Display (LCD) screen.

Haematological complications in HIV patients

Haematological disorders were determined in the patients using World Health Organization criteria. Anaemia was defined when Hgb concentration is less than 12.0 g/dl and 13.8 g/dl in females and males respectively. Leucopenia was considered as WBC count less than 2.75×10^3 cells/µl, lymphocytopenia as lymphocyte count of $< 0.8 \times 10^3$ cells/µl, neutropenia as neutrophils count $< 1.0 \times 10^3$ cells/µl and thrombocytopenia as platelet count $< 125 \times 10^3$ cells/µl for females and $< 156 \times 10^3$ cells/µl for males.¹⁶

Statistical analysis

Descriptive analysis and Student's 't' test were performed using the SAS software program version 9.2.¹⁷ Results are presented as means $\pm$ Standard deviation (SD), while $P \leq 0.05$ was considered significant.

RESULTS

It was observed that the patients were receiving a combination of three to four ARV drugs. Almost all the patients (91.5%) were on first line regimen (Table 1). The most widely used first-line regimen was lamivudine + zidovudine + nevirapine combination. Also, less than 10% of the patients were on second-line combination therapy, which includes lamivudine + tenofovir + lopinavir/ritonavir and lamivudine + zidovudine + lopinavir/ritonavir. The zidovudine backbone regimen (82%) constituted the main drugs of choice and the rest (18%) were on tenofovir backbone. Furthermore, 79.7% of the patients were on ARV drugs for duration of three to five years. The average time on ARV drugs was 3.7 years (range from 1-5 years) (Table 1). A higher incidence of thrombocytopenia (14.3%), anaemia (26.6%) and lymphocytopenia (6.6%) were found in patients who were yet to commence ARV drugs as compared to patients on drugs (6.6%; 17.5% and 2.7% respectively) (Figure 1). Higher frequency of leucopenia (27.6%) and neutropenia (13.5%) were also observed in patients on ARV drugs as compared to those that were about to start ARV drugs (21.4% and 6.3% respectively).

There were statistically significant ($P < 0.05$) differences in all the haematological parameters except the lymphocyte and platelet counts of the patients on ARV drugs, as compared with those that were yet to receive medication (Table 2). The levels of Hgb, RBC, MCV, MCH and MCHC of patients on ARV drugs however, were higher relative to those who were not on drug therapy. The values of WBC, neutrophil and platelet counts of patients that were about to commence drugs were higher than those on drugs. Anaemia was more prominent (26.6%) in patients that were yet to start drugs compared with 17.5% observed in patients on therapy between 1 and 5 years. All grades of anaemia were found in patients receiving ARV drugs but none was life threatening. Less severe forms of anaemia (mild and moderate) occurred in those that were yet to commence therapy (Table 3).

As shown in Table 4, microcytic anaemia occurred most frequently in HIV-infected patients not yet on treatment, while macrocytic anaemia was found in patients on treatment. Table 5 shows the haematological profile of patients in relation to their ARV drugs. A significant difference in neutrophil levels of patients on CPE and those on TRN was observed. Also, there were statistically significant variations in the values of RBC, MCV and MCH of those on TRA and patients managed with five other combination regimens. Among the six ARV drug combination therapy used in the management of these patients, TRN; Lamivudine + Tenofovir + Nevirapine regimen was found to have lowest values of platelets, neutrophils, WBC, lymphocytes, RBC as well

as Hgb. Furthermore, those patients on Zidovudine based regimens (CPN, CPE, and CPA) were found to have higher values of haemoglobin concentration of greater than 12 g/dl. However, patients receiving Tenofovir back bone regimens (TRA, TRE and TRN) were observed to have haemoglobin concentrations below normal reference value of 12 g/dl for females and 13.8 g/dl for males.

DISCUSSION

It has been observed that Lamivudine + Zidovudine + Efavirenz combination recommended by World Health Organization as first line therapy was commonly used for treating HIV patients in the facility of this study. This regimen is tolerable, feasible, less toxic and less pill burden. This result is in line with¹⁸ in Cameroon who reported that 95% of patients were on first line regimen which includes Nevirapine, Lamivudine, Efavirenz, Stavudine and Zidovudine, while very few (5%) were on second line ARV regimen.

The values of haematological parameters obtained for the patients (yet to start treatment) were also similar to those observed by¹⁹ in Thailand. Contrarily, previous findings²⁰ in Cameroon, in Osogbo,²¹ Nigeria and Italy²² revealed that therapeutic combination of Lamivudine + Zidovudine + Efavirenz is less prescribed and rather used as a second line therapy. The higher incidence of anaemia was also found in patients that were yet to receive drugs as compared to patients on ARV drugs was similar to findings⁹ in Benin City, Nigeria, where the occurrence of anaemia was higher among ARV drugs-naïve patients than patients on ARV drugs. The rationale for the decrease in incidence of anaemia in patients on ARV drugs as compared to those that were yet to receive medication indicated the efficacy of ARV drugs in managing HIV infection. ART has also been shown to promote blood cells production.⁹

Anaemia which was the commonest haematological disorder among patients that were yet to be on drugs may be due to several factors ranging from nutritional deficiencies, opportunistic infections and chronic nature of the disease.^{23,24} HIV infection causes decrease in the production of RBCs by suppressing the Erythroid Colony Forming Unit (CFU-E).²⁴ Again, a diminished production of erythropoietin has been observed in HIV-infected patients that were yet to be placed on drugs.²⁵ Decreased value of MCV found in patients that were yet to start ARV drugs as compared to patients on ARV drugs was in line with the findings of,²⁶ who reported that the majority of the patients about to start ARV drugs presented with microcytic hypochromic anaemia, common in most chronic diseases. The microcytic

Table 1: Treatment variables of HIV-infected patients on ARV drugs

Variables	Frequency (n=275)	(%)
ARV drugs combination		
Lamivudine + Zidovudine + Nevirapine (CPN)	194	70.6
Lamivudine + Zidovudine + Efavirenz (CPE)	19	6.9
Lamivudine + Zidovudine + Lopinavir/Ritonavir (CPA)	12	4.5
Lamivudine + Tenofovir + Efavirenz (TRE)	24	8.5
Lamivudine + Tenofovir + Nevirapine (TRN)	15	5.5
Lamivudine + Tenofovir + Lopinavir/Ritonavir (TRA)	11	4.0
Duration of ARV therapy (Years)		
1-2	55	20.3
3-4	81	29.3
5	139	50.4

Table 2: Haematological parameters of HIV-infected patients

Parameter	Patients on ARV drugs Mean $\pm$ SD	Patients not yet on ARV drugs Mean $\pm$ SD	P-value
Hgb (g/dl)	11.75 $\pm$ 1.80	10.94 $\pm$ 2.06	0.001
WBC ($10^3/\mu$ l)	4.78 $\pm$ 1.32	5.13 $\pm$ 1.53	0.049
Lymphocyte count ($10^3/\mu$ l)	2.16 $\pm$ 0.77	2.16 $\pm$ 0.87	0.970
Neutrophil count ($10^3/\mu$ l)	1.93 $\pm$ 0.93	2.37 $\pm$ 1.15	0.001
RBC ($10^6/\mu$ l)	4.21 $\pm$ 0.79	3.75 $\pm$ 0.62	0.001
MCV (fl)	95.02 $\pm$ 10.18	83.48 $\pm$ 7.67	0.001
MCH (pg)	31.72 $\pm$ 4.55	26.06 $\pm$ 3.46	0.001
MCHC (g/dl)	33.26 $\pm$ 1.71	31.27 $\pm$ 2.11	0.001
PLT ($10^3/\mu$ l)	230.60 $\pm$ 67.67	238.58 $\pm$ 102.25	0.414

Table 3: Anaemia among HIV-infected patients based on haemoglobin concentration

Haemoglobin g/dl	Patients on ARV drugs with anaemia n=48 (17.5%)	Patients not yet on ARV drugs with anaemia n=29 (26.6%)
Mild (9.5-11.9)	40 (82.3)	22 (77.7)
Moderate (8.0-9.4)	6 (12.8)	7 (22.3)
Severe (6.5-7.9)	2 (4.9)	0
Life threatening (<6.5)	0	0

Table 4: Types of anaemia in HIV-infected patients by mean cell volume

Mean cell volume (Femtoliter)	Patients on ARV drugs 48 (17.5%)	Patients not yet on drugs 29 (26.6%)
Microcytic anaemia (<80)	13 (27.8 %)	22 (77.3%)
Macrocytic anaemia (>100)	35 (72.2%)	7 (22.7%)

hypochromic anaemia was caused by the infections of the gastrointestinal tract that may be responsible for chronic blood loss, with eventual iron deficiency anaemia.²⁷

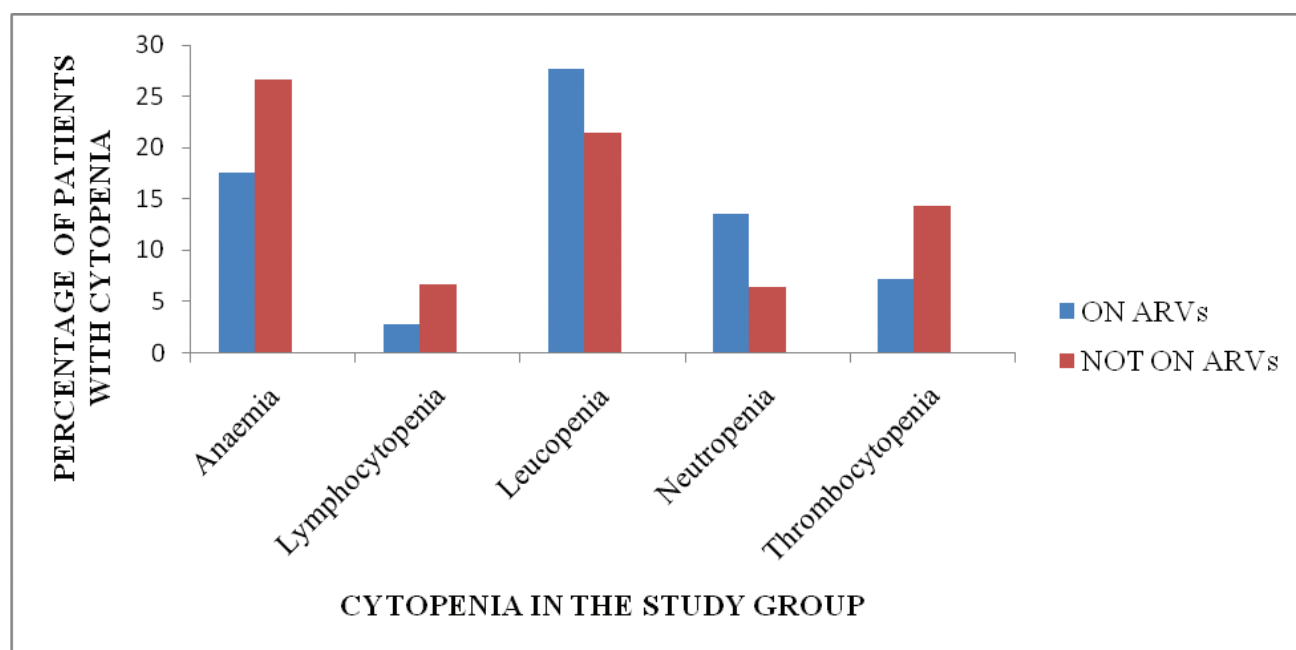
The incidence of anaemia among patients on ARV drugs (17.5%) in this study was high as compared to the findings in Cameroon¹⁸ which reported 3.8% among HIV-infected patients on ARV drugs for the median

onset of five months. The difference in the results could be attributed to the fact that the patients in present study were on ARV drugs for a median time of 3.4 years (range 1-5 years). However, the present study corroborates with earlier reports.²⁸⁻³⁰ In the present study, mild to severe anaemia was found in a few patients on ARV drugs especially among those on Tenofovir

Table 5: Haematological parameters and antiretroviral drugs of patients on ARV drugs

Parameter	CPE	CPN	CPA	TRA	TRE	TRN	P-value
Haemoglobin (g/dl)	12.03 (a)	12.14 (a)	12.06 (a)	11.89 (a)	11.66 (a)	10.77 (a)	0.392
White blood cells ($10^3/\mu\text{l}$)	4.89 (a)	4.69 (a)	5.35 (a)	5.34 (a)	4.88 (a)	4.50 (a)	0.203
Lymphocyte count ($10^3/\mu\text{l}$)	2.10 (a)	2.18 (a)	2.08 (a)	2.29 (a)	2.15 (a)	1.99 (a)	0.893
Neutrophil count ($10^3/\mu\text{l}$)	2.02 (ab)	1.85 (ab)	2.48 (b)	2.42 (b)	2.07(ab)	1.51 (a)	0.013*
Red blood cells ($10^6/\mu\text{l}$)	3.94 (ab)	3.67 (a)	3.85 (ab)	4.18 (b)	3.83 (ab)	3.65 (a)	0.005*
Mean Cell Volume fl	93.55 (ab)	96.26 (b)	93.59 (ab)	88.15 (a)	94.23 (ab)	91.70 (ab)	0.017*
Mean Cell Haemoglobin (pg)	30.96 (ab)	32.28 (b)	31.56 (ab)	28.81 (a)	31.12 (ab)	30.02 (ab)	0.017*
Mean Cell Haemoglobin Concentration (%)	32.95 (a)	33.43 (a)	33.67 (a)	32.58 (a)	32.84 (a)	32.60 (a)	0.086
Platelets ($10^3/\mu\text{l}$)	236.14 (a)	228.87 (a)	241.33 (a)	233.22 (a)	237.60 (a)	220.23(a)	0.948

NB: Means having the same letter(s) are not significantly different at 0.05 level of significance. Values with different letter(s) indicates significant difference at $P < 0.05$; C; Zidovudine, P; Lamivudine, N; Nevirapine, E; Efavirenz, R; Lamivudine, A; Lopinavir/ Ritonavir, T; Tenofovir.

**Figure 1: Haematological abnormalities in HIV/AIDS patients on ARV drugs and not yet on ARV drugs.**

based regimen. This was contrary to the findings^{14,31} which reported that anaemia in HIV infected patients was caused by zidovudine based regimen. Leucopenia and neutropenia were the most prominent haematological abnormalities among patients on ARV drugs. These were similar to previous reports.^{32,33} The likely cause of these haematological disorders in this study could be due to the use of Tenofovir + Emtricitabine + Nevirapine combination.

The findings in Sokoto,³⁴ Nigeria also revealed that Stavudine + Lamivudine + Nevirapine regimen was responsible for leucopenia and neutropenia.²⁹ It was also reported that the primary culprit in drug-induced neutropenia was Zidovudine. Furthermore, lymphopenia was less common in patients on ARV drugs as compared to those that were yet to be on the drugs,

this was attributed to the ability of these drugs to boost the immune system and reduce the risk of opportunistic infections. Also, the study³⁵ reported that treatment with ARV drugs in HIV-infected patients usually resulted in sustained platelet increases, this was contrary to the present study whereby the platelet level of patients on therapy was lower than the patients not yet on drugs.

CONCLUSION

Anaemia, leucopenia and neutropenia were found in a few patients on ARV drugs for one to five years, and this was associated with first-line Tenofovir-based regimen. Further research is needed to determine the safety of Tenofovir-based therapy in managing HIV-infected patients.

ACKNOWLEDGMENT

The author is thankful to the Principal, Officers and Staff of the University of Benin Teaching Hospital (UBTH), Benin City, Nigeria, for providing all necessary facilities to carry out the research. Dr. Bello, Omolaran Bashir of the Department of Biological Sciences, Fountain

University, Osogbo, Nigeria is also appreciated for the manuscript review with appropriate corrections.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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Example: 1. Canada. Environmental Health Directorate. Radiation protection in dentistry: recommended safety procedures for the use of dental x-ray equipment. Safety Code 30. Ottawa: Ministry of Health; 2000.

Chapter in a book

Example: 1. Alexander RG. Considerations in creating a beautiful smile. In: Romano R, editor. The art of the smile. London: Quintessence Publishing; 2005. p. 187-210.

E-book

Example: 1. Irfan A. Protocols for predictable aesthetic dental restorations [Internet]. Oxford: Blackwell Munksgaard; 2006 [cited 2009 May 21]. Available from Netlibrary: <http://cclsw2.vcc.ca:2048/login?url=http://www.netLibrary.com/urlapi.asp?action=summary&v=1&bookid=181691>

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Example: 1. Medline Plus [Internet]. Bethesda (MD): U.S. National Library of Medicine; c2009. Dental health; 2009 May 06 [cited 2009 Jun 16]; [about 7 screens]. Available from: <http://www.nlm.nih.gov/medlineplus/dentalhealth.html>

Conference proceedings: **Example:** 1. Kimura J, Shibasaki H, editors. Recent advances in clinical neurophysiology. Proceedings of the 10th International Congress of EMG and Clinical Neurophysiology; 1995 Oct 15-19; Kyoto, Japan. Amsterdam: Elsevier; 1996.

Dissertation: **Example:** 1. Kaplan SJ. Post-hospital home health care: the elderly's access and utilization [dissertation]. St. Louis (MO): Washington Univ.; 1995.

Patent: **Example:** 1. Larsen CE, Trip R, Johnson CR, inventors; Novoste Corporation, assignee. Methods for procedures related to the electrophysiology of the heart. US patent 5529 067. 1995 Jun 25.

Abbreviations for Journals For More information on medline indexed journals : Download list of medlinejournals: ftp://ftp.ncbi.nih.gov/pubmed/J_Medline.zip

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British Journal of Pharmacology and Chemotherapy- (**Brit J Pharmacol**)

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