Assessment of Vitamin B12, Folate Levels and some Haematological Parameters among Females on De-Deon syrup in Port Harcourt, Nigeria

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Received: February 07, 2022, **Accepted:** April 21, 2022, **Published:** May 10, 2022

ABSTRACT

Introduction: There is a disturbing trend with intake of un-prescribed doses of multivitamin syrup as a recovering medication after illness since it is believed to boost blood blood cell production. This study aims to assess the effect of De-Deon haemoglobin syrup on Vitamin B_{12}, Folate Levels and some Haematological Parameters among Females in Port Harcourt, Nigeria.

Methods: Ten (10) apparently healthy female subjects between the ages of 20 to 30 years were recruited for the study; blood samples were collected via standard vein-puncture technique before administration of the drug; on the fourth (4th) day, after day 3 of drug administration; and after day 6 of drug administration. Vitamin B_{12}, and folate were analyzed using ELISA method; while haematological parameters were determined using Sysmex autoanalyzer. Data were analyzed using Statistical Package for Social Sciences computer database (Version 10.0; SPSS Inc., Chicago, IL, USA) for mean, standard deviation, analysis of variance and correlation, p-value of <0.05 was considered statistically significant.

Results: showed no significant change ($p>0.05$) in vitamin B_{12}, folate, red blood cells (RBC), white blood cells (WBC), haemoglobin (Hb), haematocrit (HCT) and platelet count before treatment (Day 1), during treatment (day 3) and after treatment (day 6) of De-Deon syrup administration. Correlation analysis of Vitamin B_{12} against haematological parameters for day, 1, 3, and after day 6 showed significant negative correlation against haemoglobin level ($r = 0.6731$; $p = 0.0373$) and statistically significant positive correlation against platelets ($r = 0.6731$; $p = 0.0165$) after day 1. Statistically significant positive correlation of vitamin B_{12} against platelets ($r = 0.6795$; $p = 0.0153$) was observed after day 3; no significant statistically significant correlation in levels of vitamin B_{12} against other haematological parameters were observed. No significant correlation was also observed for folate and haematological parameters after day 1, 3, and 6.

Conclusion: The study has therefore revealed that De-Deon syrup of haemoglobin when taken within a short period of time (acute

administration), showed no statistically significant effect on vitamin B_{12}, folate and haematological parameters. However, vitamin B_{12} was increased albeit without any significance. Although no significance was observed in the study, the significant positive correlation between vitamin B_{12} and platelet count points out the relevance of vitamin B_{12} supplementation as it is required in the metabolism of every cell of the human body and also a cofactor in DNA synthesis in both fatty acid and amino acid metabolism.

Keywords: *De-Deon's Syrup of Haemoglobin; Vitamin B_{12}; Folate; Haematotological Parameters*

INTRODUCTION

Multivitamin syrups and/or drugs are sold over the counter in medicine shops and pharmacies without prescription and are mostly abused by consumers here in Nigeria. Most persons at the slightest observation in body weight drop or immediately after recovering from illness visits the pharmacy shop and buy multivitamin syrups (blood tonic).

De-Deon of Haemoglobin Syrup is one of such blood tonics sold in Nigerian Drug Market. It is a vitamin B_{12} and haemolobin supplement used for correcting such conditions as; anaemia, vitamin B_{12} deficiencies, liver diseases, chronic fatigue syndrome, liver function, liver damage, muscle development, addisonian anaemia, strength and physical endurance, and other conditions. De-Deon Syrup of Haemoglobin vitamin B_{12} enriches the blood not only with haemoglobin but also with the chief amino acids which are the basic elements indispensable in the process of blood regeneration. Vitamin B_{12}, an antipernicious factor accelerates the synthesis of proteins and favours the maturation of red globules. De-Deon of Haemoglobin Vitamin B_{12} Syrup contains cyanocobalamin, haemoglobin and liver extract as active ingredients. It works by carrying oxygen from the lungs to the body's tissues; increasing the number of liver cells; normalizing the formation of red blood cells and nerve tissues. De-Deon of Haemoglobin Vitamin B12 Syrup is composed of the following active ingredients (salts); haemoglobin - 16%W/V, liver extract - 3.1%W/V and cyanocobalamin - 0.0001%W/V. [1].

The use of dietary supplement is increasing daily around the globe [2]. An estimated 30–50% increase has occurred in the rate of energy drinks and dietary supplements intake in 2010 [3]. Vitamin supplementation impact significant benefits in terms of disease prevention and treatments; and has been accepted as a measure of controlling micro nutrient deficiencies [4].

Haemoglobin is a complex ferruginous molecule whose main function is to absorb oxygen from the lungs and then distribute it to all body cells of human [5]. Lack of adequate haemoglobin in the blood is one of the major causes of anaemia [6]. Haemoglobin is the main protein found within red blood cells. It consists of two components haem and globin. Haem, an iron and porphyrin compound is 4% and globin (amino acids) is 96%. Haemoglobin's chemical composition allows it to bind oxygen coming into the lungs for transport to the body's tissues. From there, haemoglobin binds carbon dioxide, the main waste product of the cell's metabolism, and releases it into the lungs, so it can be exhale out of the body. Haemoglobin gives blood its red colour [7].

Vitamin B_{12}, generally called cyanocobalamin, is a porphyrin like ring compound with central cobalt atom attached to a nucleotide. Its

anti-anaemic function has been known for years [6]. Cyanocobalamin is a manufactured form of vitamin B_{12} used to treat vitamin B_{12} deficiency [7]. A low concentration of vitamin B_{12} is associated with complications such as birth defects and neurological disorders [8][4][7]. More severe deficiencies of this vitamin are associated with haematological disorders such as anaemia, leukopaenia and thrombocytopaenia, promoting haematological shortages, resulting in increased mean corpuscular red cell volume (MCV) and anaemia through the alteration of erythropoiesis [9]. When haemoglobin levels falls below a certain range, an individual may develop symptoms of anaemia [6]. A deficiency in vitamin B_{12} can affect the production of red blood cells, decreasing the number of red blood cells circulating in the blood, and therefore decreasing the amount of haemoglobin available for oxygen transport [7]. Low vitamin B_{12} levels in community-dwelling adults are usually corrected with supplements [8] among which include De-Deon of Haemoglobin Syrup.

Folate is also known as folic acid, folacin, vitamin B_9, Vitamin M, Folvite, Acifolic, Folcidin, and scientifically as Pteroylglutamic acid, was first found by Lucy Wills, a consultant pathologist at the Royal Free Hospital in London through her work, which resulted in correcting macrocytic anaemia of pregnancy in female textile workers in Bombay [10][11]. Folic acid was first isolated in 1941 from spinach hence its name folium (leaf) [12]. Folate is naturally present in a wide variety of foods, including vegetables (especially dark green leafy vegetables), fruits and fruit juices, nuts, beans, peas, seafood, eggs, dairy products, meat, poultry, and grains [13].

De-Deons of Haemoglobin Vitamin B_{12} Syrup, finds its usefulness in the treatment or management of conditions that affect red cell productions and result to anaemia. It is a very popular drug used in Nigeria and prescribed by many clinicians to their patients. Based on the wide usage of this drug on folate, it becomes imperative to determine the effects of this drug on blood production, hence the study.

MATERIALS AND METHODS

Study Design

An experimental and comparative study aimed at evaluating the effects of De-Deon of Haemoglobin syrup on vitamin B_{12}, folate and some haematological parameters among selected female subjects. The study was carried out from November, 2020 to December 2020. A total of ten (10) adult female subjects between the ages of 20 to 30 years were recruited for the study and blood samples for analysis were collected before (control), between and after taking De-Deon of haemoglobin syrup on each interval days of collection and analyzed respectively. Samples were collected before administration of the drug, after the third (3rd) day of administration of the drug and after the sixth (6th) day of administration of the drug.

Study Area

The study was conducted in Port Harcourt metropolis, Rivers State, Nigeria. Port Harcourt, the capital of Rivers State, Nigeria.

Study Population

The subjects in this study comprised of apparently healthy adult females aged between 20-30 years. Blood samples were drawn from the ten (10) healthy volunteer subjects into tube containing 0.5ml of 1.2mg/ml dipotassium ethylene diamine tetra acetic acid (K_2 EDTA) aniticoagulated-preservative container.

Inclusion Criteria

Only apparently healthy adult females were recruited for this study. Participants were eligible for inclusion in the study if they were females between the ages of 20 to 30 years, not on drugs, menstruation, and not alcoholics.

Exclusion Criteria

Participants were excluded if they had severe anaemia (Haemoglobin <8g/dL), were suffering from acute or chronic infections that could affect their haemoglobin and ferritin levels at the time of the blood sampling, were on medication or were

found to be drug addicts, were diagnosed with thalassemia trait.

Informed Consent

Informed consent was obtained from all subjects who willingly participated in the study and gave their blood samples for analysis.

Sample Collection, Transportation, Processing and Preservation

Venous blood sample was collected with the use of vacutainer needle from each subject, of which 4.0 ml of blood was collected and added into individualized vacutainer tube containing 0.5ml of 1.2mg/ml dipotassium ethylene tetra-acetic acid (K_2EDTA). The samples were preserved using ice pack in a thermo cool container and then transported to the haematology laboratory where they were analysed. Full blood count was carried out, and then the blood samples were centrifuged to obtain plasma. The plasma obtained was used for vitamin B_{12} and folate analysis using an ELISA reader capable of reading absorbance at 450nm wavelength.

Sample Analysis/Methodologies

All samples for full blood count were analysed using the automated machine (SYSMEX, manufactured by KOBE, Japan, Model No: KX-21N). All samples for vitamin B_{12} were analysed using Human Vitamin B_{12} ELISA Kit, CALBIOTECH, Inc., El Cajon, U.S.A. Lot No VBE5948: Expiry Date: 2021/02; while samples for folate were analyzed using using Human Folate ELISA Kit, CALBIOTECH, In, EI, Cajon, U. S.A. Lot number VBE598: Expiry date: 2021/01.

Determination of Full Blood Count Using Sysmex KX-21N Auto-analyser, Kobe, Japan.

Procedure of Full Blood Count Using Sysmex

The procedure is such that the sample for analysis is mixed using a vortex mixer and the lid of the sample container is opened and the sample fed into the Sysmex auto-analyser via the probe. The analysis was done by the machine and the results of the analysis displayed at the read-out screen which can be printed out.

Determination of Vitamin B12 Using Human Vitamin B12 ELISA Kit, CALBIOTECH, Inc., El Cajon, U.S.A. Lot No VBE5948: Expiry Date: 2021/02.

Procedure of Vitamin B_{12} Estimation Using Human Vitamin B_{12} ELISA Kit

The EDTA blood samples were centrifuged to obtain plasma. All reagents and specimens were allowed to come to room temperature before use. Desired numbers of coated strips were placed into the holder. 50ul of extracted Vitamin B_{12} standards, controls and samples were dispersed into appropriate wells. 50ul of biotinylated intrinsic factor reagent was dispersed into each well. The microplate was shaken gently for 20-30 seconds to mix and was incubated for 45 minutes at room temperature (25^{0}C). 50ul of enzyme conjugate was added into all the wells, the microplate was gently shaken for 20-30 seconds to mix and was incubated for 30 minutes at room temperature (20-25^{0}C). The contents were briskly shaken out of the wells, the wells were rinsed 3 times with 1X wash buffer, and the wells were stroked sharply on absorbent paper to remove residual water droplets. Using a multi-channel pipette, 100 ul of TMB Substrate was dispensed into each well which resulted in the development of a blue-coloured solution. The absorbance of the colour was read spectrophotometrically at 450nm wavelength.

Determination of Folate using Human ELISA Kit, CALBIOTECH, In, EI, Cajon, U. S.A. Lot number VBE598: Expiry date: 2021/01

Procedure for Folate Estimates Using Human ELISA Kit

The EDTA blood sample were centrifuged to obtain the plasma. All reagent and sample were allowed to come to room temperature for use. Desired numbers of coated strips were placed into the holders. 5µl of extracted Folate standard, control

and sample were dispensed into the appropriate wells. 50µl of biotinylated intrinsic factor reagent was dispensed into each well. the microplate was shaken gently for 20 to 30 seconds to mix and was incubated for 30 minutes under a temperature of (25°C). 50µl of enzyme conjugate was added into all the wells, the microplate was gently shaken for 20-30 seconds to mix and was incubated for 60 minutes at room temperature (20-25°C). The content was briskly shaken out of the wells, the wheels were rinsed 3 times with wash buffer and the wells were stroked sharply on absorbent paper to remove residual water droplets. Using a multi- channel pipette, 10ul of TMB substrate was dispensed into each well which result in development of blue colour solution. The absorbance of the colour was read spectophotometrically at 450nm wavelength.

Data Analysis

Data generated from this study was statistically analyzed using Statistical Package for Social Sciences computer database (Version 10.0; SPSS Inc., Chicago, IL, USA) to obtain mean, standard deviations, Pearson's correlation, p-value and t-value using analysis of variance (ANOVA). Tukey's multiple comparison tests was done to check for significance between groups. A p-value of <0.05 was considered statistically significant in all statistical comparison.

RESULTS

Demographic Characteristics of the Studied Subjects

A total of ten (10) apparently healthy female participants within the ages of 20 to 30 years were recruited for the study. Samples were collected from all ten (10) participants before administration of De-deon syrup which served as control, between administration and after administration of De-deon syrup. Samples were collected before administration of the drug, after the third (3rd) day of administration of the drug (4^{th} day) and after the sixth (6th) day after administration of the drug (7^{th} day). Details of the demographic characteristics of the study population are shown in Table 3.1.

Analysis of Variance of the Studied Parameters Before Day 1, After Day 3 of Treatment and After Day 6 of Treatment

Analysis of variance of treatment before (day 1), during (day 4) and after treatment (day 7) of full blood counts from the study as presented in Table 3.2, shows no significant change ($p>0.05$) in haematological parameters of red blood cells (RBC), white blood cells (WBC), haemoglobin (Hb), haematocrit (HCT), platelet count (PLT), mean cell volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), RDW-SD and RCW-CD before treatment (Day 1), during treatment (day 4) and after treatment (day 7) of De-Deon syrup administration.

Correlation Analysis of Vitamin B_{12} Against Full Blood Count for Day 1

Correlation Analysis of Vitamin B_{12} against FBC and Red Cell Indices before day 1 as reported in Table 3.3 shows a significant positive correlation in Vitamin B_{12}, and Platelet ($r= 0.6731$ respectively) and a reverse significant correlation in HGB level ($r= -0.5868$) and non-significant negative and positive in WBC, RBC, HCT, MCV, MCH, MCHC.

Correlation Analysis of Vitamin B_{12} Against Full Blood Count after Day 3

Correlation Analysis of Vitamin B_{12} against FBC and Red Cell Indices after day 3 as reported in Table 3.4 shows a significant positive correlation in Vitamin B_{12}, and Platelet ($r = 0.6795$respectively) and non-significant positive and negative correlation in the other parameters.

Correlation Analysis of Vitamin B_{12} Against Full Blood Count after Day 6

Correlation Analysis of Vitamin B_{12}against FBC and Red Cell Indices after day 6 as reported in Table 3.5 showed non-significant positive and negative correlation was observed in levels of all parameters

Correlation Analysis of Folate with Red Cell Indices

Table 3.6a-c shows the correlation between folate and the red cell indices (RBC, HBG, HCT, MCV, MCH, MCHC) before administration of drug, after day 3 and after day 6. No statistical significance was observed using Pearson correlation.

Table 3.1 Demographic Characteristics of the Studied Subjects

Parameters	Frequency
Total human subjects	10
Control (Before administration)	10
Day 4 (During administration)	10
Day 7 (After administration)	10
Number of females	10

Table 3.2: ANOVA Of Treatment Before (Day 1), After (Day 3) and After Treatment (Day 6) of the Studied Parameters

Parameters	Before Day 1	After Day 3	After Day 6	p-value	I value	Remark
WBC (10^3/µL)	6.13±1.51	5.66±0.88	5.42±1.10	0.4145	0.9100	NS
RBC (10^6/µL)	4.47±0.67	4.38±0.63	4.49±0.68	0.5299	0.5022	NS
HGB (g/dL)	12.19±0.69	12.00±0.65	11.96±0.81	0.4480	0.7668	NS
HCT (%)	37.12±2.57	35.97±2.50	36.69±2.83	0.3319	1.134	NS
PLT (10^3/µL)	213.7±61.30	222.6±57.27	206.8±50.73	0.4804	0.6678	NS
MCV (fl)	83.84±7.34	83.25±7.35	82.88±7.45	0.3051	1.230	NS
MCH (pg)	27.59±2.85	27.80±2.94	27.06±2.98	0.1982	1.875	NS
MCHC (g/dL)	32.89±1.14	33.37±0.90	32.62±1.13	0.0848	2.983	NS
RDW-SD (fl)	42.44±3.68	41.68±2.97	41.80±3.30	0.395	0.9938	NS
RCWCD (%)	13.37±1.07	13.30±1.16	13.08±1.28	0.4554	0.6945	NS
$VitB^{12}$(pg/mol)	1785±1500	1863±1632	2027±1729	0.5004	0.5672	NS
Folate	10.77±4.228	11.31±3.513	10.82±5.138	0.04718	0.9540	NS

KEY: NS= Not-significant; S=Significant; applicable to all Tables.

Table 3.3: Correlation Analysis of Vitamin B12 Against Haematological Parameters before Day 1

Vitamin B_{12}	WBC	RBC	HB	PCV
	r = -0.2491	r = -0.1496	r = 0.6731	r =0.4676
	p = 0.2438	p = 0.3400	p = 0.0373	p = 0.0865
	Platelets	MCV	MCH	MCHC
	r = 0.6731	r = -0.2371	r = -0.2141	r = 0.0122
	p = 0.0165	p = 0.2548	p = 0.2763	p = 0.4866

Table 3.4: Correlation Analysis of Vitamin B12 against Haematological Parameters after day 3

Vitamin B_{12}	**WBC**	**RBC**	**HB**	**PCV**
	r = -0.2915	**r = -0.1168**	**r = -0.3824**	**r = -0.3777**
	p = 0.2069	**p = 0.3740**	**p = 0.1378**	**p = 0.1409**
	Platelets	MCV	MCH	MCHC
	r = 0.6795	r = -0.1995	r = -0.1132	r = 0.2480
	p = 0.0153	p = 0.2903	p = 0.3777	p = 0.2448

Table 3.5: Correlation Analysis of Vitamin B12 Against Haematological Parameters after Day 6

Vitamin B_{12}	**WBC**	**RBC**	**HB**	**PCV**
	r = -0.1936	**r = -0.1623**	**r = -0.4641**	**r = -0.3617**
	p = 0.2961	**p = 0.3271**	**p = 0.0883**	**p = 0.1522**
	Platelets	MCV	MCH	MCHC
	r = 0.4531	r = -0.1219	r = -0.1483	r = -0.0969
	p = 0.0942	p = 0.3686	p = 0.3413	p = 0.3950

Table 3.6a: Correlation Analysis of Folate against Red Cell Indices for Day 0

'arameters	Folate	RBC (10^6/µL)	HGB (g/dL)	HCT (%)	MCV (fl)	MCH (pg)	MCHC (g/dL)
value	1.0000	0.3910	0.0137	0.0731	-0.3020	-0.2906	-0.0864
value	0.0000	0.2639	0.9699	0.8408	0.3964	0.4154	0.8123

Table 3.6b: Correlation Analysis of Folate against Red Cell Indices after Day 3

'arameters	Folate	RBC (10^6/µL)	HGB (g/dL)	HCT (%)	MCV (fl)	MCH (pg)	MCHC (g/dL)
value	1.0000	0.09608	-0.4762	-0.3429	-0.0654	0.3064	-0.0792
value	0.0000	0.7918	0.1641	0.3321	0.8574	0.3893	0.8278

Table 3.6c: Correlation Analysis of Folate against Red Cell Indices after Day 6

Parameters	Folate	RBC (10^6/µL)	HGB (g/dl)	HCT (%)	MCV (fl)	MCH (pg)	MCHC (g/dL)
value	1.0000	0.4594	0.4056	0.3683	-0.3449	-0.4733	-0.0048
p value	0.0000	0.1817	0.2449	0.2950	0.3290	0.1671	0.9894

DISCUSSION

The present study investigated the effects of De-Deon Syrup of Haemoglobin Vitamin B_{12} on vitamin B_{12}, folate and some haematological parameters among selected female subjects. From the study, there was no significant increase ($p>0.05$) in all analyzed haematological parameters before treatment (Day 1), after treatment (day 3) and after treatment (day 6) with De-Deon syrup administration. This non-significance in the result is in agreement with results obtained by Smelt *et al.* [14] where they reported a non-significant measurable change in routine haematological parameters after treatment with vitamin B_{12} in older persons with either normal or vitamin B_{12} below the reference range 200-900pg/mL. The results are also in line with previous analyses in the Leiden 85-plus study, where no relationship was found between vitamin B_{12} and changes in MCV over time [15]. The results of the study are not in agreement with results obtained by Abed *et al.* [16] where they observed significant increase in haematological parameters of Hb, MCH and MCHC and no significant increase in MCV among children suffering from anaemia after receiving syrup containing vitamin B_{12} for 3 months [16], though the duration of administration in this study was 6 days. Also, Bolaman, [17] reported increased haematologic responses in both oral and intramuscular vitamin B_{12} supplemented for 1 month and observed improvements of cognitive function, sensory neuropathy and vibration sense but the difference between both groups were not statistically significant [17].

There are several possible explanations for the lack of effect. First, the study consisted of participants without any apparent vitamin B_{12} deficiency. As these participants may not have had a true tissue deficiency of vitamin B_{12}, this may have diluted the effect of supplementation. Secondly, participants in this study were without clinical features of anaemia. Perhaps haemoglobin levels in non-anaemic patients are less likely to increase in response to vitamin B_{12} treatment than haemoglobin levels in anaemic patients [18]. Thirdly, a low vitamin B_{12} concentration alone may not be the only reason to develop anaemia, and treatment of these low levels may not be sufficient to raise haemoglobin levels especially when the drug was not taken for a long period of time as in the present study; samples were collected after 3 and 6 days of administration. The 3 and 6 days might not have been enough to elicit an effect which may cause a significant change in the studied parameters. However, vitamin B_{12} levels in the subject were not within the levels or cut off for deficiency as the reference range for vitamin B_{12} is 200-900pg/ml [14] although it differs according to population.

In this study, no significant change was observed in the levels of Vitamin B_{12} after day 3 and day 6 of treatment. Although there was a slight increase in the levels of vitamin B_{12}, the increase was

not enough to cause a significant change. This non significance in result is in agreement with results obtained by Smelt *et al.* [14].

De deon syrup contains vitamin B_{12} [1], and thus, it's supplementation should cause a rise in its value as reported by Smelt *et al.* [14] who recorded an increase in vitamin B_{12} after supplementation without statistical significance. The non-significance in the result may be as a result of the short duration of supplement administration.

In this study, analysis was done before day 1, after day 3 and after day 6 of administration as opposed to higher duration of administration (more than 1 month) in other studies. This duration may not have been enough to elicit an effect which might be significant when statistically analyzed. This can also be seen in the correlation analysis of vitamin B_{12} before day 1 as presented on Table 4.3 where there was a positive correlation in haemoglobin and platelet concentration; after day 3 as presented on Table 4.4, there was a positive correlation in platelet concentration and on day 6 as presented on Table 4.5, showing that De-Deon of haemoglobin syrup might have some effect on haematological parameters with increased duration. The levels of vitamin B_{12} as reported on table 4.2 were also increasing from 1785±1500 before day 1, to 1863±1632 after 2 and to 2027±1729 after day 6. These observed increase and significant positive correlation may be as a result of the supplement administration where the vitamin B_{12} content of De-Deon syrup [1], having an additive effect on vitamin B_{12} level in the body resulting in the observed difference, but the increase was not enough to cause a significant difference when statistically analysed. Secondly, the study consisted of participants without any apparent vitamin B_{12} deficiency or just below the cutoff values. As these participants may not have had a true tissue deficiency of vitamin B_{12}, this may have diluted the effect of supplementation.
In this study there was no significant on effects of the De-Deon hemoglobin syrup on folate. The results obtained shows no significant increase in folate after administration of the drug. Further analysis was carried out, correlating folate and red cell indices (Table 4.4a-c) but no significance was observed in the result. There are several possible explanations for the lack of effect, first, some studies consisted mostly of participants without folate deficiency or just below the cut-off values. As these participants may not have had a true tissue deficiency of folate, this may have diluted the effect of supplementation. Secondly, many of the included studies mainly consisted of participants without anaemia. Perhaps haemoglobin levels in non-anaemic patients are less likely to increase in response to De Deon Haemoglobin treatment than haemoglobin levels in anaemic patients [18]. Unfortunately, the low numbers of people with anaemia refrained us from drawing definite conclusions on the effects of supplementation in an anaemic population with low folate concentrations. Third, a low folate concentration alone may not be the only reason to develop anaemia, and treatment of these low levels may not be sufficient to raise haemoglobin levels. Other genetic or environmental factors may be involved in the onset of anaemia [19]. Also, other causes such as chronic inflammation may play a role in the development of anaemia [20].

White blood cells (WBCs) are a part of the immune system that helps fight infection and defend the body against other foreign materials. The most important function of vitamin B_{12} is DNA synthesis, necessary for cell division, whereby it could modulate human immunity [21]. Addition of vitamin B_{12} in B_{12}-deficient patients facilitates the production of T lymphocytes recruited in cellular immunity, restores an abnormally increased CD4/CD8 ratio and maintains the count of lymphocyte subgroups in the normal range [22]. Erkurt *et al.* [23] demonstrated that the intake of vitamin B_{12} in patients with anaemia causes an increase in the absolute number of CD8+ as well as a slight increase in CD4+ lymphocytes [23]. In this present study, B_{12} supplementation did not affect white blood cells as no significant increase was seen in white blood cell concentration. This is in agreement with results obtained by Lewicki *et al.* [24] in their study on the effect of vitamin B_{12} supplementation on white blood cells. The result of the study is not in agreement with results obtained by Erkurt *et al.* [23] and Tamura *et al.* [25]. The

reason for this difference may be as a result of participants in our study consisting of apparently healthy subjects without any clinical features of pernicious anaemia as addition of vitamin B_{12} in B_{12}-deficient patients facilitates the production of T lymphocytes recruited in cellular immunity, restores an abnormally increased CD4/CD8 ratio and maintains the count of lymphocyte subgroups in the normal range [25] and participants in their study consisted of subjects with anaemia.

Red blood cells contain a protein called haemoglobin, which carries oxygen from the lungs to all parts of the body. De-Deons of Haemoglobin syrup contains vitamin B_{12} which is a required nutrient that helps allow proper red blood cell production and function in the body. Thus, its supplementation tends to increase the vitamin B_{12} concentration which was increased although not significant. Vitamin B_{12} has been seen to have a well-defined role in haematopoiesis as it is essential for normal maturation and development of blood cells [26]. Thus, an increase in Vitamin B_{12} should increases hematopoiesis which also increases the concentration of red blood cells (RBC), haemogblobin and also haematocrit values.

In this study, no significant increase was observed in red blood cell concentration, haematocrit and also levels of haemoglobin after day 3 and day 6 of administration, the result of this study is in agreement with results obtained by Smelt *et al.* [14]. The result is not in agreement with results obtained by Abed *et al.* [16]. The reason for this discrepancy may be as a result of the participants in the study without clinical features of anaemia. Perhaps red blood cell, haematocrit and haemoglobin levels in non-anaemic patients are less likely to increase in response to vitamin B_{12} treatment than the levels in anaemic patients [18]. A low vitamin B_{12} concentration alone may not be the only reason to develop anaemia, and treatment of these low levels may not be sufficient to raise red blood cell, haematocrit and haemoglobin levels [14] especially when the drug was not taken for a long period of time in the present study.

No significant difference was found in the levels of haematological indices of MCV, MCH and MCHC after day 3 and day 6 of treatment. Haematological indices also known as red blood cell indices provide information about the haemoglobin content and size of red blood cells. Abnormal values indicate the presence of anaemia and which type of anaemia it is. Deficiency of vitamin B_{12} has been known to cause a type of anaemia known as pernicious anaemia [27]. Pernicious anaemia, a type of anaemia caused by vitamin B_{12} insufficiency is thought mainly to be caused by an autoimmune process which inhibits the production of a substance in the stomach called intrinsic factor. This vitamin is needed to make red blood cells, which carry oxygen to all parts of the body. The non-significant increase in the results of this study is in agreement with results obtained by Smelt *et al.* [14] and Weck *et al.* [27]. The non-significant increase in the results of haematological indices of MCV, MCH and MCHC in this study is not in agreement with results obtained by Abed *et al.* [16].

Platelets, also called thrombocytes are a component of blood whose function (along with the coagulation factors) is to react to bleeding from blood vessel injury by clumping, thereby initiating a blood clot [5] (Waugh and Grant, 2007). In this study, no significant difference was observed in platelet concentration after day 3 and day 6 of administration of De-Deon of Haemoglobin syrup. However, a significant positive correlation between platelet volumes was reported after day 3 of administration. The result of this study is in agreement with results obtained by Smelt *et al.* [14]. The body needs vitamin B_{12} for tetrafolate production and methylation reactions that are required for DNA synthesis. In addition, B_{12} is required for the formation from homocysteine to methionine and the formation of shaped elements in the bone marrow. Therefore, it was reported that thrombocytopenia might occur as well as anaemia and leukopenia as a result of the damage of DNA synthesis due to B_{12} and/or folate deficiency which may lead to observed difference in platelet count upon treatment in vitamin B_{12} deficiency. In this study, participants consisted of only apparently healthy subjects without any clinical features of vitamin B_{12} deficiency thus resulting in the non-

significant difference in the study.

The red cell distribution width (RDW-SD) (fl) reflects erythrocyte anisocytosis and the extent variability of erythrocytes [28] and also related to inflammation. Activated red blood cells may have a crucial role in inflammation and it has been known that vitamin B_{12} deficiency which is a leading cause of pernicious anaemia increases the red cell distribution width [29] and it also potentiate inflammation via the red cell distribution width platelet ratio (RPR). In our study, no significant increase was seen in the levels of red cell distribution width after day 1, day 3 and day 6 of treatment with De deon of Haemoglobin syrup. RDW indicates heterogeneity and equivalent of anisocytosis of red blood cell. It was documented that high levels of RDW predicts elevated red blood cell destruction in iron, folate, and vitamin B_{12} deficiency [30][31]. Eventually, different-sized erythrocytes may be expressed and elevated RDW may result in increased RPR [31]. The result in our study is not in agreement with results obtained by Yin *et al.* [32]. The reason for the discrepancy is as a result of the participants in our study were apparently healthy subjects without any clinical features of pernicious anaemia as opposed to that in their study whose participants consisted of those with known vitamin B_{12} deficiency.

Red cell width (RCW-CD%) is a measure of the range of variation of red blood cell (RBC) volume that is reported as part of a standard complete blood count. Usually, red blood cells are a standard size of about 6–8 μm in diameter. Certain disorders, however, cause a significant variation in cell size. Higher RCW values indicate greater variation in size. Normal reference range of RCW-CD in human red blood cells is 11.5–15.4% [28]. Red cell width is often used together with mean corpuscular volume (MCV) to determine the possible causes of anaemia. It is mainly used to differentiate anaemia of mixed causes from anaemia of a single cause.

CONCLUSION

De-Deon syrup when taken within a short period of time (acute administration), there is no positive effect on haematological parameters rather vitamin B12 was increased albeit without any significance. Although no significance was observed in the study, the significant positive correlation between Vitamin B_{12} and platelet count points out the relevance of Vitamin B_{12} supplementation as it is required in the metabolism of every cell of the human body and also a cofactor in DNA synthesis in both fatty acid and amino acid metabolism, particularly important in the normal functioning of the nervous system via its role in the synthesis of myelin and in the maturation of developing red blood cells in the bone marrow.

Recommendations

Further studies are recommended which will include a large sample size and analysis of other variables such as methylmalonic acid and total homocysteine as these variables reflects a true tissue deficiency of vitamin B_{12}. Such studies could reveal the impact of vitamin B_{12} supplementation on the concentrations of the mentioned biomarkers. Also, it is recommended that the duration of drug administration be increased as the limitations of the present study are the small sample size, the lack of analysis of other variables that reflect the tissue deficiency of vitamin B_{12}, such as methylmalonic acid (MMA) and total homocysteine and low duration of drug administration. The marked areas in this study such as duration of administration and sample size should be looked into as it will help provide more robust bases to establish the effect of this drug in particular on its effect on vitamin B_{12} and haematological parameters.

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