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Fascinating Nutritional Formulation of Verapamil

Highlights

Socio-Economic Reconcile Hydrochloride Matrix Granules

Discovering Thoughts, Inventing Future

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Formulation of Verapamil Hydrochloride Matrix Granules by Sintering Technique and its Evaluation

By R. R. Bhagwat & Dr. I. S. Vaidya
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Abstract- Exploration of sintering concept in the pharmaceutical sciences is relatively recent. The aim of this study was to investigate the release characteristics of matrix granules consisting of hydrophobic (i.e waxy) material and Verapamil hydrochloride for sustained release application using thermal sintering technique. It was considered as an ideal drug for designing sustained release formulation on account of its high frequency of administration and short biological half life. Granules prepared by melt granulation technique were formulated with water soluble drug, carnauba wax, glyceryl behenate (a wax matrix forming polymer) lactose, magnesium stearate. Matrix granules of Verapamil hydrochloride prepared with various concentration of wax and polymer were sintered thermally at various times periods, temperature and were evaluated for physicochemical parameters and in vitro dissolution studies. The sintering time markedly affected the drug release properties of wax and polymer. It is notable that the release rate of Verapamil hydrochloride from granules was inversely related to the time of sintering. Sintering technique enhanced the extend of drug retardation from the systems studied.

Keywords: thermal sintering, granules, verapamil hydroc-hloride, polymer/wax, sustained release..

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R. R. Bhagwat [α] & Dr. I. S. Vaidya [σ]

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I. Introduction

Controlled drug delivery technology represents one of the most rapidly advancing areas of science. Such delivery systems offer numerous advantages compared to conventional dosage forms including improved efficacy, reduced toxicity and improved patient compliance. [1]

Sintering is defined as the bonding as the bonding of adjacent particle surfaces in a mass of powder or in a compact by the application of heat. Conventional sintering involves the heating of a compact at a temperature below the melting point of the solid constituents in a controlled environment under atmosphere pressure. The sintering process has been used for the fabrication of sustained release matrix tablets and for the stabilisation of drug permeability of film coating derived from various pharmaceutical lattices.[2],[3],[4]

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Recently, Uhumwangho et al., (2011)[5] developed an oral sustained release dosage formulation of Diltiazem HCL wax matrix granules by sintering the polymer matrix using melt granulation technique. Flowerlet et al., (2010)[6] developed an oral sustained release dosage formulation of Metformin HCL matrix tablets by sintering the polymer matrix with organic vapour such as acetone. Polymer films with different permeability have been explored to modify drug release from drug particles. Some examples mentioned in the literature include films with the drug as a solution in a polymeric matrix. E.g. polymer coated reservoir devices (Lehmann et al. 1979)[7], polymeric colloidal particles (microparticles or nanoparticles) either in the form of reservoir or matrix devices (Oppenheim 191; Douglas et al 1987)[8]

These methods are however very complicated and expensive since it requires the use of organic solvents as coating fluid. However these organic solvents are hazardous to the environment. A simple approach which was considered in the present study, is melt granulation whereby the drug powder is triturated with a melted wax serving as a hydrophobic retard release agent. The resulting granules consist of the drug particles dispersed in a wax continuous matrix.

Verapamil Hydrochloride (VRH)[9],[10] is a vasodilator alkaloid found in the opium poppy. It is an L-type calcium channel blocker. It has been used in the treatment of hypertension, angina pectoris, cardiac arrhythmia. Its chemical formula is (RS) - 5 - [N - (3,4 - dimethoxy - phenethyl) methylamino] - 2 - (3,4 - dimethoxyphenyl) - 2 - isopropyl valeronitrile with a molecular weight 491.07. It has half life of about 4 to 6 hr. It is completely absorbed from GIT with usual dose of 40 to 240 mg 3 times a day.

The aim of this study was to prepare wax matrix granules by melt granulation technique using VRH as a model drug. These matrix granules were later subjected to thermal sintering at different time duration at different temperatures. Consequently, the effect of sintering temperature and duration on the drug release profiles and physicochemical parameters were investigated.

II. Materials and Methods

Materials: The active ingredient used in the study was verapamil hydrochloride (Piramal Healthcare, Hydrabad, India). The matrix material used was glyceryl behenate (Gattefose India Pvt Ltd.) carnauba wax (S D Fine Chemicals Mumbai). Other materials used were of analytical grade.

Melt granulation technique: Two waxes are studied here for the effect of thermal sintering on drug release. Both waxes (glyceryl behenate and carnauba wax) were melted individually in porcelain dish in a water bath at a temperature higher than its melting point i.e. 83ºC for glyceryl behenate and at 86ºC for carnauba wax. A sample of VRH powder was added to the melted wax and thoroughly mixed with a glass rod. It was then allowed to cool to room temperature (35ºC±2ºC). The mass was pressed through a sieve of mesh 12 to produce wax matrix granules.

Sintering of matrix granules: The matrix granules were then subjected to thermal treatment by placing them in aluminium foil and subjecting to sintering at different temperature i.e. 60ºC, 75ºC for different durations 1 and 3 hr for glyceryl behenate. For carnauba wax matrix granules it is 70ºC, 80ºC for durations 1 and 3 hr.

Packing property of the matrix granules[11]: The packing property was determined by measuring the difference between bulk density and tapped density using standard procedure. 20 g of matrix granules sample was placed in a 250 ml clean measuring cylinder and the volume V_o occupied by the sample without tapping was determined. An automated tap density tester was used for tapping the granules according to USP. After 100 taps the occupied volume V_{100} was noted. The bulk and tap densities were calculated from these volumes (V_o and V_{100}) using the formula Density = Weight/Volume occupied by sample. From the data Hauseners ratio was determined.

Flow property of matrix granules: The flowability of the granules was determined by measuring the angle of repose formed when a sample of the granules was allowed to fall freely from the stem of a funnel to a horizontal bench surface. The radius (r) and the height (h) of the powder heap were determined and then the angle of repose (θ) was calculated.

Encapsulation of the matrix granules: Samples of matrix granules before and after sintering were filled manually into plain hard gelatine capsules. The capsules were kept in airtight containers before their use in *in – vitro* dissolution studies.

In vitro dissolution test: One capsule filled with the matrix granules were placed in a cylindrical basket (aperture size 425μm; diameter 20mm; height 25mm), and immersed in 1000ml of water with pH 3. The fluid was stirred at 75 rpm. Samples of the medium (5ml) were withdrawn at selected time intervals and replaced with an equal volume of drug free dissolution fluid. The samples were suitably diluted with blank dissolution fluid and were analysed for content of Verapamil HCL at λmax 278nm by using a double beam spectrophotometer. The samples were filtered with Whatman No 3 filter paper before assay and the amounts released were expressed as a percentage of the drug content in each dissolution medium. The dissolution test was carried out in triplicate and the mean results reported. Individual results were reproducible to ±10% of the mean.

Fourier Transform Infra red (FTIR) : The FTIR spectrum of the different samples were recorded in an Infra Red spectrometer using potassium bromide discs prepared from powdered samples. Infrared spectrum was recorded in the region 4000 to 400 cm^{-1}.

Determination of rate order kinetics and mechanism: The dissolution data were analysed on the basis of zero order (cumulative amount of drug release vs time), first order rate (log cumulative amount of drug remaining vs time), Higuchi model (cumulative amount of drug released vs square root of time) and Korsmeyer[12] and Peppas[13] (log cumulative amount released vs log time). These are the most frequently reported kinetics of drug release from drug particles and their solid dosage forms.

III. Results and Discussion

Effects of sintering on physicochemical parameters of unsintered and sintered wax matrix granules: The effects of sintering on the physic-chemical parameters of unsintered and sintered matrix granules are presented in table 1 & 2. It was observed that all the matrix granules were free flowing with angle of repose ≤ 29ºC. No much difference was observed between the unsintered and sintered batches at different temperatures for different durations.

Table 1

Parameters Evaluated	Unsintered	Sintered at 60º C		Sintered at 75º C		Sintered at 60º C		Sintered at 75º C		
	UF 1	S1	S2	S3	S4	S5	S6	S7		S8
Bulk density	0.55	0.56	0.55	0.55	0.53	0.54	0.53	0.52		0.51
Tap density	0.65	0.67	0.66	0.64	0.65	0.63	0.61	0.64		0.60
Carr's index	20.73	15.38	9.72	10.1	17.33	14.28	13.11	18.75		15.00
Angle of repose	28.47	27.1	27.0	25.4	25.4	28.1	28.7	29		28.9
Hausners ratio	1.18	1.19	1.2	1.16	1.22	1.16		1.15	1.23	1.17

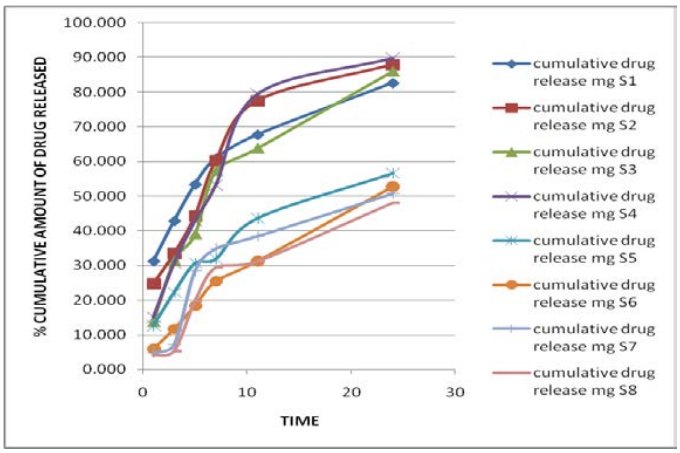
Table 2

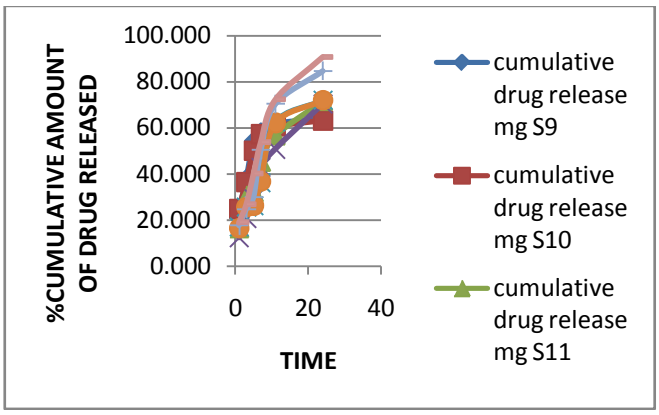
Parameters Evaluated	Unsintered	Sintered at 70º C		Sintered at 80º C		Sintered at 70º C		Sintered at 80º C	
	UF 2	S9	S10	S11	S12	S13	S14	S15	S16
Bulk density	0.55	0.56	0.55	0.55	0.53	0.50	0.51	0.53	0.50
Tap density	0.65	0.67	0.66	0.64	0.65	0.60	0.64	0.63	0.63
Carr's index	19.27	19.8	15.27	14.47	10.81	16.66	20.31	15.87	20.63
Angle of repose	28.47	27.1	27.0	25.4	25.4	26.2	26.3	29.6	29.7
Hausners ratio	1.18	1.19	1.2	1.16	1.22	1.2	1.25	1.18	1.26

Composition of different formulations (mg/capsule) :

Formulation code	F1 (S1 – S4)	F2 (S5 - S8)	F3 (S9 – S12)	F4 (S13 – S16)
Ingredients				
Drug	120	120	120	120
Glyceryl behenate	60	120	–	–
Carnauba wax	–	–	60	120
Lactose	97	37	97	37
Magnesium stearate	2.8	2.8	2.8	2.8

Dissolution profiles of matrix granules: The dissolution profiles of the unsintered and sintered matrix granules at 60, 75, 70, 80ºC at different time durations are presented in fig. It was observed that the unsintered matrix granules were able to retard the drug for 7hr. Generally, as the temperature and duration of sintering of the matrix granules increased the time to attain maximum release increased correspondingly.





Drug release mechanism: A good knowledge of the drug release kinetics will provide a proper understanding of the drug release mechanism. Four mathematical models were used for analysis: zero order kinetics, first order kinetics Higuchi mechanism and Korsmeyer and Pepps model.

FTIR: Formulation S4 and S16 were considered for FTIR studies since it was able to retard the drug for a period 24 hr. This study was carried out in order to investigate if there was any chemical interaction between added excipients and VRH in the formulation S4 and S16.before and after sintering. The FTIR of the pure drug, glyceryl behenate, carnauba wax, sintered matrix granules were recorded. The IR spectrum of VRH showed characteristic peaks at 2240cm^{-1} (for C=N of saturated alkyl nitrile) 2542cm^{-1} (broad complex band due to N-H stretch in amine group). It was observed that the IR spectra showed both the principal peaks of VRH in sintered matrix granules also. It suggests that there was no chemical interaction between the VRH and added excipients.

Conclusion: The use of lipophilic substances as release retarding agents is widely accepted concept because of their effectiveness in drug release control and low cost of manufacturing. The use of sintering technique adds to the effectiveness of polymers to extend the release of drug from formulation depending upon the duration and temperature of sintering. Sintering technique enhanced the extent of drug retardation from the systems studied. Formulation S16 sintered at 80ºC for 3hr with carnauba wax and 75ºC for 3hr with glyceryl behenate was able to sustain the drug for a period of 24hr with a maximum release of 84% and 89%. The FTIR studies showed that the model drug was not affected by the temperature and time duration used for sintering.

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Effect of Caffeine-Containing Beverages on Physicochemical and Release Properties of Halofantrine

By Babalola C. P., Kolade Y. T., Adeyemo M. A., Kotila O. A., Ameh S. J., Adelakun T. A., Adewuyi S. Godwin Njaprim Kwasi & Scriba G. K.

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Abstract- Halofantrine (Hf) is a poorly water-soluble drug for treating malaria in endemic areas like tropical Africa, where caffeine-containing products are habitually consumed. Previous reports showed that caffeine increased the aqueous solubility of Hf at room temperature over 3 days. The aim of this study was to determine the effect of caffeine and caffeine-containing beverages on dissolution and solubility of Hf and to investigate any possible interactions. The aqueous solubility and dissolution of Hf alone and in the presence of caffeine was investigated at pH 1.3, 5.9 and 7.4 using standard methods. The solubility of Hf in the presence of aqueous extracts of cocoa, coffee, black tea and green tea at pH 5.9 was also investigated. In 1 hour, caffeine markedly increased the aqueous solubility of Hf at pH 1.3, 5.9 and 7.4. Caffeine and caffeine-containing beverages markedly increased the aqueous solubility of Hf by between 100- to more than 1600- fold, with a 1672-fold increase by caffeine (from 76.6 ±7.8 ng/mL to128.2 ± 4.5 mg/mL) at pH 5.9. The dissolution of Hf tablets at pH 1.3, 5.9 and 7.4 showed the respective amounts released as 3.57 ± 0.09, 0.95 ± 0.19 and 0.260 ± 0.043 mg, but introduction of caffeine increased these values to 9.51 ± 0.23, 3.70 ± 0.12 and 0.52 ± 0.10 mg respectively, representing 3-fold, 4-fold and 2-fold respectively.

Keywords: *caffeine, halofantrine, physicochemical interaction.*

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Keywords: caffeine, halofantrine, physicochemical interaction.

I. Introduction

Halofantrine (Hf) is a phenanthrene methanol antimalarial currently marketed as halofantrine hydrochloride under the trade name Halfan®. Hf, a highly lipophilic drug [1] has been shown to be highly active against multi - drug resistant isolates of *Plasmodium falciparum* in preclinical studies [2,3,4]. The drug has proven efficacy against multi-drug resistant malaria including infection with chloroquine and/or pyrimethamine resistant strains of *P. falciparum* [5].

Hf is highly lipophilic [1,6] with an erratic oral absorption pattern leading to high inter individual variations that have been shown to be associated with food intake [7 – 10]. Lim and Go (2000) reported that caffeine, a non-toxic complexing agent that possesses stimulant effect on the CNS, enhanced the aqueous solubility of Hf [11] at room temperature for over 3 days by a 1:1 ratio complex formation. In a different study by Kolade et al. (2008) kolanut, a habitually consumed nut rich in caffeine, also increased the solubility of Hf *in vitro* but decreased the plasma concentrations of the drug in humans [12].

Aside of kolanut that contains caffeine, other caffeine-containing beverages such as, coffee, cocoa, black tea and green tea are also habitually consumed in the tropics where malaria is prevalent. There is thus a need for investigations of Hf interactions with caffeine and caffeine-containing beverages especially at various pH values that are important in GIT for orally administered medicines. Therefore the aim of this study was to determine the effect of caffeine and caffeine-containing beverages on the solubility and dissolution profile of Hf at physiologically important pH values of 1.3, 5.9 and 7.4 as well as to investigate any possible interactions.

II. Materials and Methods

a) Chemicals and Reagents

Halofantrine hydrochloride (Hf) was received as a gift from Smithkline Beecham (Welwyn Garden City, United Kingdom). Caffeine, coffee (*Coffee arabica*), cacao (*Theobroma cacao*), black tea *Ccamellia sinensis*), and green tea *Ccamellia sinensis*) were obtained commercially. Potassium dihydrogen phosphate (analar) and sodium chloride (analar) were obtained from VWR International (Darmstrdt, Germany). Instruments used were basket type Easy-lift dissolution test station, Mettler Delta 340, pH meter, Uniscope SM 101 laboratory thermostated water bath with shaker, Unicam ultraviolet spectrophotometer.

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b) *Preparation of 0.05M KH_2PO_4 (ionic strength = 0.08)*

Standard solutions of 0.05M potassium dihydrogen phosphate were prepared and adjusted to pH 1.3, 5.9 and 7.4 respectively

c) *Calibration line for Hf*

The HPLC method for determination of Hf in plasma previously described [12] was adapted for this study. Mobile phase samples (1mL) were spiked with standard solutions of Hf to give predetermined concentrations of 200, 500, 1000, 2000, 4000, and 8000ng/mL. 40mL of 1mg/mL chlorprothixen was measured into a 100mL volumetric flask and diluted to volume with methanol to produce 400μg/mL chlorprothixen solution. To each 1mL mobile phase sample was added 20μL of 400μg/mL internal standard solution (chlorprothixen) to produce 8000ng/mL. Aliquots of 20 μL were then injected into the HPLC. The peak area ratio (Hf/IS) obtained for each sample was plotted against the corresponding concentration to obtain the calibration line.

d) *Determination of solubility of Hf in KH_2PO_4 buffers (pH 1.3, 5.9, 7.4)*

To 5mg of Hf weighed into a test tube, 5mL of phosphate buffer (pH 5.9) was added to it. The mixture was then shaken in a water bath at 37°C for 1h after which it was centrifuged at 4000 rpm for 15 minutes to get a clear supernatant. The procedure was repeated with buffers of pH 1.3 and 7.4. After centrifuging, 20μL of the supernatant were injected into the HPLC and the concentrations of Hf were extrapolated from the calibration line.

e) *Interactions of Hf with caffeine*

0.6063g of caffeine was weighed into a volumetric flask and made up to 25mL with phosphate buffer (pH 5.9). 5mL each of this preparation was placed in three different test tubes. An excess of Hf was weighed into each test tube and the test tubes were placed on a water bath adjusted to 37°C and shaken for 1h. The solutions were centrifuged at 4000rpm for 15 minutes and the supernatants collected and analyzed for Hf. The procedure was repeated using phosphate buffers pH 1.3 and 7.4.

f) *Preparation of extracts of caffeine-containing beverages*

The seeds of *Coffee arabica* were powdered using a mortar and pestle. 2.5g of the powdered coffee seeds were dissolved in 25mL of KH_2PO_4 buffer (0.05M, ionic strength 0.08, pH 5.9) and mixed in a vortex mixer for 20 minutes at room temperature. The mixture was then centrifuged at 4000 rpm for 15 minutes to give a clear supernatant. The same procedure was followed using cocoa powder, black tea leaves and green tea leaves to get the extracts of cocoa, black tea and green tea respectively.

g) *Interactions of Hf with coffee, cocoa, black tea and green tea*

5mg each of Hf was weighed into different test tubes. 5mL of each of the extracts of coffee, cocoa, black tea and green tea equivalent to 2.5g/25mL (100mg/mL) prepared above was then added to the test tube containing the Hf. The mixture was shaken in a water bath at 37°C for 1h after which it was centrifuged at 4000 rpm for 15 minutes to get a clear supernatant. 20μl of the supernatant was injected into the HPLC. Triplicates of the above samples were prepared and the concentrations of Hf extrapolated from the calibration line.

h) *Dissolution profile of Hf tablets alone and in the presence of caffeine*

A dissolution medium consisting of phosphate buffer : methanol (75:25, v/v) was prepared using buffers at three different pH values (1.3, 5.9 and 7.4) representing gastric, duodenal and plasma pH values.

Six dissolution vessels containing 500 mL each of buffer (pH 5.9) : methanol (75:25, v/v) was set up. Three of the vessels contained a single tablet of halofantrine hydrochloride (250 mg) alone while the other three vessels contained both a tablet of Hf and 1.95 mg/mL of caffeine. The dissolution stations were maintained at 100 rpm, and 37°C for 1h. Samples were taken for analysis at different time intervals of 5, 10, up to 60 minutes. The procedure was repeated using buffers pH 1.3 and 7.4 respectively. All the determinations were performed in triplicate and the amount of Hf dissolved was thereafter determined spectrophotometrically.

i) *Determination of amount of dissolved halofantrine from tablets*

UV analysis of the samples was carried out at a wavelength of 310nm. Corresponding concentrations of the absorbance readings were obtained using a calibration curve equation constructed from six concentrations (2.5 - 25μg/mL) of Hf.

III. Results

The calibration line of Hf in mobile phase was linear over a concentration range of 100ng/mL to 8000ng/mL with an r^2 of over 0.999. The aqueous solubility of Hf determined at 37°C for 1 h at pH of 1.3, 5.9 and 7.4 was found to be 323 ± 41, 77 ± 8 and 27 ± 11 ng/mL, respectively indicating highest solubility at pH 1.3. Caffeine increased the solubility of Hf at pH 1.3 from 323 ± 41 to 1714 ± 10 ng/mL and at pH 7.4 from 27 ± 11ng/mL to 6646 ± 712 ng/mL. Coffee, cocoa, black tea and green tea extracts at a concentration of 2.5g/25mL also increased the solubility of Hf by over a 100- fold in pH 5.9 as shown in Table 1. Coffee and cocoa extracts increased the solubility from 77 ± 8 ng/mL to 11525 ± 593 and 17270 ± 1680 ng/mL respectively while black

tea and green tea extracts increased the solubility to 7716 ± 507 and 34146 ± 2852 ng/mL respectively.

For the dissolution rate studies, the amount of Hf dissolved at pH 1.3, 5.9 and 7.4 were 3574.48 ± 92.53, 947.93 ± 194.12, and 259.9 ± 43.15 μg respectively, but with the addition of caffeine to the dissolution medium, the amount dissolved increased to 9506.93 ± 226.6, 3703.13 ± 117.98, and 522.42 ± 104.54 μg of Hf at the same pH values indicating 3-, 4- and 2- folds increment respectively. A plot of amount of Hf dissolved at 60 mins against the physiologic pH revealed the degree of impact caffeine had on the dissolution of Hf.

IV. Discussion

The ionic strength of 0.08 was used for the solubility studies of Hf studies because it is at this strength that optimum results was obtained in previous studies [11]. The effect of caffeine on the solubility of Hf investigated at three pH values of 1.3, 5.9 and 7.4 represents gastric, duodenal and physiological pH respectively. pH 5.9 is a good approximation of duodenal pH with or without food and since Hf is administered orally for the treatment of malaria, its solubility profile at this pH will be useful.

pH 5.9 was chosen as the pH medium for the interaction of the caffeine-containing beverages with Hf since it gave the optimum results observed with caffeine. The extracts of these beverages increased the solubility of Hf at this pH in the following order: Green tea>Cocoa>Coffee>Black tea. The caffeine contents of these beverages from literature vary and in some cases, the % content overlaps. For example, the % content are; coffee (1-2%), cocoa (0.6-0.36%), green tea and black tea (1-5%) [13,14]. Cocoa which is reported to have the lowest caffeine content was expected to cause minimum solubility on interaction with Hf if the interaction is solely based on caffeine content. However it was placed 2nd in this study. Cocoa also contains cocoa butter which is fatty and since Hf is lipophilic and affected by fatty foods [1,8], it is likely that the cocoa butter may be contributing to the increase in solubility of Hf.

Preliminary investigations were carried out to find out the effect of some of the other constituents present in the extracts such as theophylline and trigonelline but these were found to have no effect on the solubility of Hf.

The dissolution profile of a drug is an important parameter in evaluating its bioavailability since dissolution precedes absorption. Earlier studies of the aqueous solubility of halofantrine, a poorly soluble and weakly basic drug conducted by Lim and Mei [11] shows that the solubility is greatest at the low pH range of 2.5-3.5 and shows a steep hundred fold decline as the pH is increased to 8.0. This was attributed to a change in the state of protonation of halofantrine. However, the aqueous solubility of halofantrine in phosphate buffer pH 5.9 and 7.4 was found to be increased by the addition of caffeine and nicotinamide; of which pH 5.9 showed the greatest solubility [11].

Fig. 1 shows the calibration curve generated for the absorbance measurement of Hf at 310nm. The curve was linear over a range of 2.5-25μg/mL with a regression coefficient and coefficient of determination of 0.996 & 0.993 respectively.

The solubility of halofantrine was then determined both in the absence and presence of caffeine. The dissolution profiles (Figs. 2 & 3) reveal an increase in the amount of Hf dissolved in the presence of caffeine for all the physiologic pH values.

The amount of Hf dissolved increased by 3-, 4- and 2- folds in the presence of caffeine at pH 1.3, 5.9 and 7.4 respectively. This solubility enhancement has been attributed to complex formation between caffeine and Hf in accordance with the π-donor π-receptor mechanism proposed by Fawzi et al [18] and Abdul et al [19].

The amount of the drug dissolved at 60 mins at the various pH values also clearly shows that caffeine had a great impact on the dissolution of halofantrine. Fig.4 shows r^2 of 0.972 with caffeine and 0.897 without caffeine. The decrease in the amount of halofantrine that dissolved at 60 mins with pH increase is as a result of the weakly basic nature of the drug. Since many drugs exist as either weakly basic or weakly acidic compounds, their ionization in water which also influences their solubility and absorption is influenced by pH. In the presence of an acidic pH, a weakly basic drug dissolves better as it is able to form a soluble salt. However the dissolution diminishes as the pH is increased and the weakly basic drug tend to precipitate [20].

The analysis of variance (2-way ANOVA) on the amount of halofantrine dissolved at the 60 mins shows that a highly significant ($p<0.001$) interaction exists between the effect of caffeine and the pH effect on the dissolution of this drug.

V. Conclusion

Caffeine enhances the solubility of halofantrine in a remarkable way and beverages that contain varying amounts of it also have similar effect as shown in the effects of coffee, cacao, black and green tea. It is obvious that these extracts that contain caffeine increased the *in vitro* solubility of Hf markedly. Although *in vitro* analyses of Hf in the presence of caffeine and caffeine-containing beverages show increase in the amount of Hf, an *in vivo* study recently carried out showed a decrease in the concentration of Hf when co-administered with kolanut – a caffeine containing nut (Kolade et al 2008). Whether *in vivo* studies involving coffee, cacao, black tea and green tea will give replicate results as kolanut still remains to be investigated.

Although *in vitro* results do not correspond with *in vivo* effect of caffeine on Hf, it still is evident that caffeine, either in pure form or as a constituent of food, does impact on the profile of Hf when co –administered and therefore the ingestion of the two together must be closely monitored in order to determine therapeutic importance of these findings.

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Table 1 : Effect of kolanut, coffee, cacao, black tea and green tea extracts on the solubility of Hf at pH 5.9

Treatment	Solubility of Hf (ng/mL)				Fold–increase of Hf
	1	2	3	Average	
Hf alone	80	68	82	77 ± 8	-
Hf + caffeine	123890	132950	127700	128180 ± 4549	1672
Hf + coffee	11908	10842	11826	11525 ± 593	150
Hf + cacao	16675	15969	19167	17270 ± 1680	225
Hf + black tea	7282	8273	7592	7716 ± 507	101
Hf + green tea	32741	32270	37428	34146 ± 2852	446

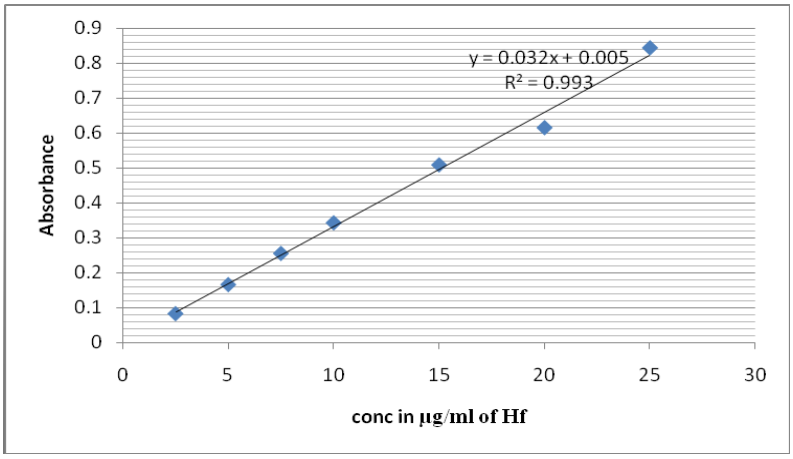


Figure 1 : Plot of absorbance reading of halofantrine at 310nm against concentration

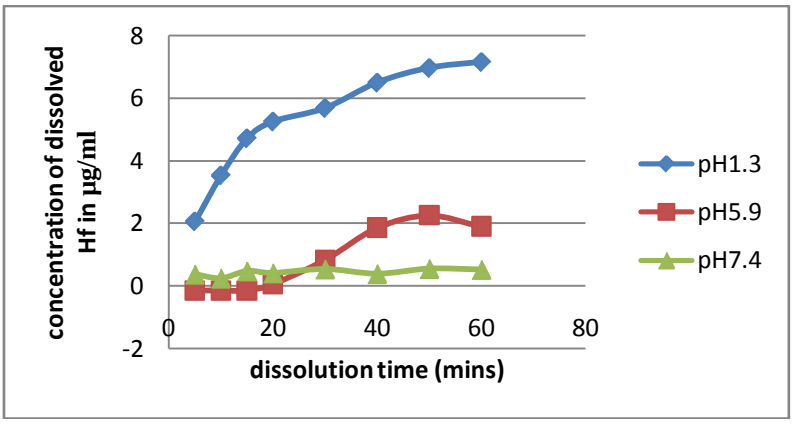


Figure 2 : Dissolution profile of Halofantrine in the absence of caffeine

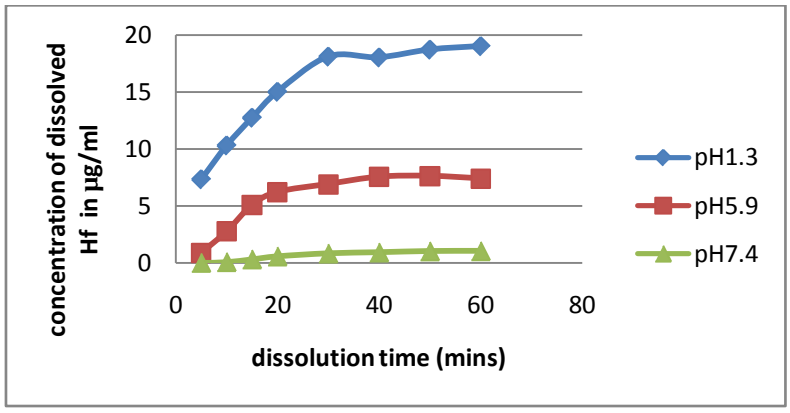
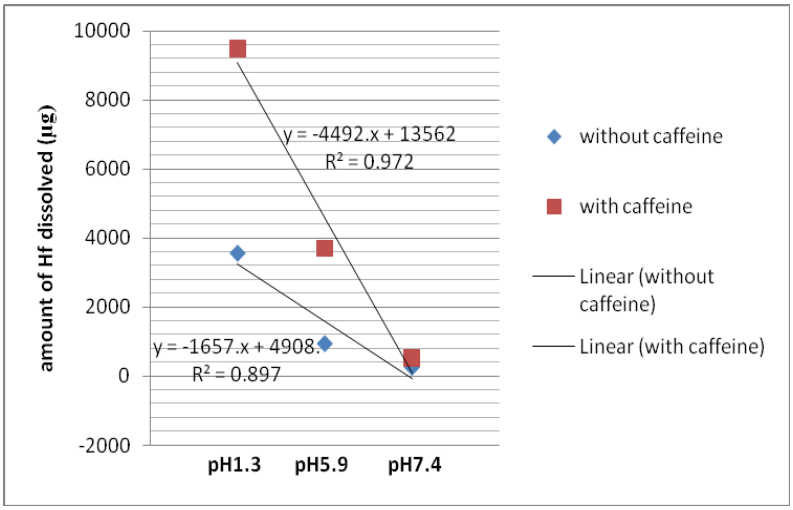
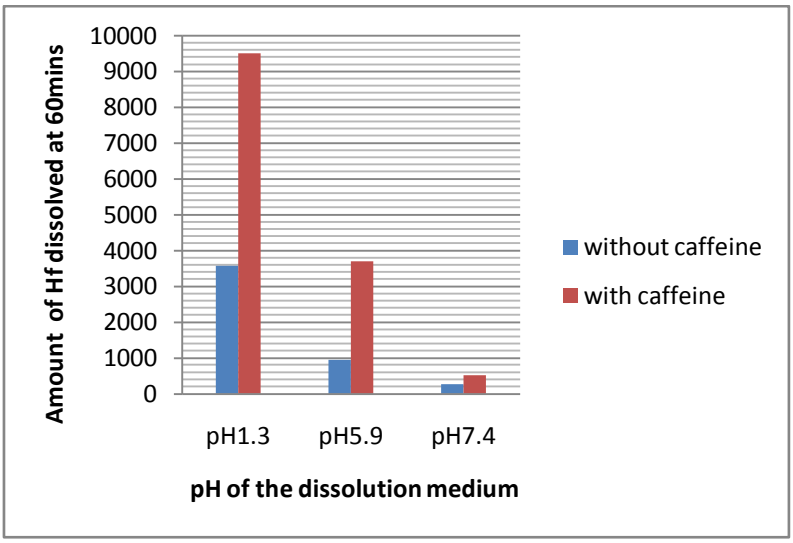


Figure 3 : Dissolution profile of Halofantrine in the presence of Caffeine



(a)



(b)

Figure 4 : Plots of the amount of Halofantrine dissolved at 60mins against the different pHs

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Fascinating Nutritional, Prophylactic,Therapeutic & Socio-Economic Reconcile Attributable to Drum Stick tree (*Moringa Oleifera* Lam.)

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Abstract- Different parts of this M. oilbera (Drum Stick Tree) contain a profile of important minerals, and are a good source of vitamins, **β**-carotene, amino acids and various phenolics. The Moringa plant provides a rich and rare combination of zeatin, quercetin, **β**-sitosterol, caffeoylquinic acid and kaempferol. In addition to its compelling water purifying powers and high nutritional value. Various parts of this plant such as the leaves, roots, seed, bark, fruit, flowers and immature pods act as cardiac and circulatory stimulants, possess antitumor, antipyretic, antiepileptic, antiinflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, cholesterol lowering, antioxidant, antidiabetic, hepatoprotective, antibacterial and antifungal activities, and are being employed for the treatment of different ailments in the indigenous system of medicine, particularly in South Asia.

Keywords: ROS; antioxidants; Free radicals; SOD; GTH; oxidative stress; pathogenesis; CVD; diabetes; water purification; biodiesel; quercetin-3-O-β-d-glucoside; chlorogenic acid; phenolic acids.

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Fascinating Nutritional, Prophylactic,Therapeutic & Socio-Economic Reconcile Attributable to Drum Stick tree (*Moringa Oleifera* Lam.)

Raaz K Maheshwari [α], Rajesh K Yadav [σ], Jayant Malhotra [ρ], Nidhi Gauba Dhawan [ω], Lalit Mohan [¥], Rajnee [§], BL Jat [χ], Bhavna Upadhyay [v] & Bina Rani, [Θ]

Abstract- Different parts of this M. oleifera (Drum Stick Tree) contain a profile of important minerals, and are a good source of vitamins, β-carotene, amino acids and various phenolics. The Moringa plant provides a rich and rare combination of zeatin, quercetin, β-sitosterol, caffeoylquinic acid and kaempferol. In addition to its compelling water purifying powers and high nutritional value. Various parts of this plant such as the leaves, roots, seed, bark, fruit, flowers and immature pods act as cardiac and circulatory stimulants, possess antitumor, antipyretic, antiepileptic, antiinflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, cholesterol lowering, antioxidant, antidiabetic, hepatoprotective, antibacterial and antifungal activities, and are being employed for the treatment of different ailments in the indigenous system of medicine, particularly in South Asia. Global industrialization and the increasing demand for environmental friendly products make moringa have great potential as a source of pharmaceuticals, dyes, biofuel, human food, animal and fish feed, and water purification products. This review focuses on the detailed phytochemical composition, therapeutic applicability, along with pharmacological assets of different parts of this multipurpose tree. Dietary consumption of its part is therein promoted as a strategy of personal health preservation and self-medication in various diseases. The enthusiasm for the health benefits of *M. oleifera* is in dire contrast with the scarcity of strong experimental and clinical evidence supporting them. Fortunately, the chasm is slowly being filled. Reported studies number and variable in design, seem rigorously concordant in their support of therapeutic potential. Phytochemical analyses have shown that its leaves are particularly rich in K, Ca, P, Fe, vitamins A and D, essential amino acids, as well as such known antioxidants such as β-carotene, vitamin C, and flavonoids. Further research considering relevance to explore the potential of M olifera's various parts has to be emphazed.

Keywords: ROS; antioxidants; Free radicals; SOD; GTH; oxidative stress; pathogenesis; CVD; diabetes; water purification; biodiesel; quercetin-3-O-β-d-glucoside; chlorogenic acid; phenolic acids.

I. Introduction

From time immerorial and historical perspective, it's evident that affluent stockroom of traditional therapeutic lashing medication is well documented and enthralling in ancient literature. *Moringa oleifera*, the Tree of Life or a Miracle Tree, but rather than this being in reference to its potential medicinal usage this is actually refering to how It's a very valuable food crop (It's drought resistant, grows very fast, and is highly nutritive) and even beyond food it serves many benefits in third world countries such as having an ability to be used for some crafts (due to being a tree) and cleaning water. For usage as a supplement, *moringa oleifera* is recommended mostly as being a highly nutritious antioxidant. All parts of the Moringa tree (Figure 1 - 4) are edible and have long been consumed by humans. According to Fuglie[1] the many uses for Moringa include: alley cropping (biomass production), animal forage (leaves and treated seed-cake), biogas (from leaves-Figure 5a & b), domestic cleaning agent (crushed leaves-(Figure 6), blue dye (wood), fencing (living trees), fertilizer (seed-cake), foliar nutrient (juice expressed from the leaves), green manure (from leaves), gum (from tree trunks), honey- and sugar cane juice-clarifier (powdered seeds), honey (flower nectar), medicine (all plant parts), ornamental plantings, biopesticide (soil incorporation of leaves to prevent seedling damping off), pulp (wood), rope (bark), tannin for tanning hides (bark and gum), water purification[67-69] (powdered seeds).

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Figure 1 : Exotic Moringa's Tree in Himalaya

Figure 2 : Moringa's Tree in a Farm House

Moringa seed oil (yield 30-40% by weight), also known as Ben oil, is a sweet non-sticking, non-drying oil that resists rancidity. It has been used in salds, for fine machine lubrication, and in the manufacture of perfume and hair care and health (Figure 8) products[2]. In the West, one of the best known uses for Moringa is the use of powdered seeds to flocculate contaminants and purify drinking water[3,4,5] (Figure 7)but the seeds are also eaten green, roasted, powdered and steeped for tea or used in curries[4]. This tree has in recent times been advocated as an outstanding indigenous source of highly digestible protein, Ca, Fe, Vitamin C, and carotenoids suitable for utilization in many of the so-called "developing" regions of the world where undernourishment is a major concern.

Figure 3 : Moringa's Tree in Village Environs

Figure 4 : Moringa's Tree on Hilly Footpath

While It's indeed nutritious, supplemental dosages are too low to aquire adequate nutrition from and this claim is not relevant; It's a relatively potent antioxidant, and while it seems to be less potent than other herbs when tested outside of a living system it

does appear to be quite potent when tested in living models. Of importance is that all parts of Moringa are edible and also effective when used for treating various diseases. As earlier said, Moringa is traditionally used in the treatment of several diseases of chronic conditions. This has prompted scientific research by the WHO, universities and organizations who have verified and concluded on most of its diverse medicinal properties on an on-going basis. Of utmost importance is its ability to aid in the cure of those diseases without any side effects or allergic reactions commonly experienced with western medicines. Also, since dietary treatment is one of the core programs in treating systemic conditions like Hypertension, Diabetes, Anaemia, kidney conditions, etc, Moringa combined the rare dual role as the ideal meal supplement and ideal medicine. Moringa has demonstrated its effectiveness in the management and/or treatments of Hypertension & Blood Pressure, Cancer & Tumor, Diabetes, AIDS, Arthr itis, Rheumatism, Asthma, Ulcer, Prostrate problems, Erectile dysfunction, Sexual virility, Cholesterol Control, Syphilis and many others. Due to its multidimentional benefits, *Moringa oleifera* is called the miracle tree, the tree of life, mother's best friend, etc.

The Moringa tree gained popularity because of its high uses in traditional medicine originally by the Indians. Preparations (e.g. extracts, decoctions, poultices, creams, oils, emollients, salves, powders, porridges) are not quite so well known[12]. Presently, numerous scientific investigations have confirmed the effectiveness of these traditional remedies. Also based on research the plant is very nutritious, earning it the WHO candidate in the fight against malnutrition.

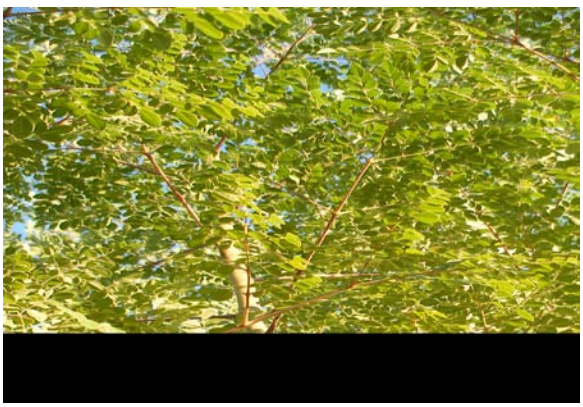


Figure 5 a : Moringa's Twigs with Leaves

Figure 5 b : Moringa's Fresh Leaves

II. Nutritional Importance of Moringa

Moringa is traditionally part of the staple food diet of many countries like India, Thailand, Cambodia, Sri Lanka, etc. and even the Hausas in northern Nigeria. It's estimated to have more than 92 verifiable cell-ready nutrients, 46 types of antioxidants[70-77] and 36 anti-inflammatories all readily available to the body.

Nutritional assessment of the raw Moringa leaf/100g

Energy 64kCal (270kJ); Carbohydrates 8.28 g; Dietary Fiber 2.0 g; Fat 1.40 g; Protein 9.40 g; Water 78.66 g; Vitamin A equiv 378 ug (47%); Thiamine (Vit B1) 0.257 mg (22%; Riboflavin; Vit B2) 0.660 mg (55%); Niacin (Vit B3) 2.220 (15%); Panthothenic acid (Vit B5) 0.125 mg (3%); Vitamin B6 1.200 mg (92%); Folate (Vit B9) 40 mg (10%); Vit c 51.7 mg (62%); Ca 185 mg (19%); Fe 4.00 mg (31%); Mg147 mg (41%); Mn 0.36 mg (17%); P112 mg (16%); P 337 mg (7%); Na 9 mg (1%); Zn 0.6 mg (6%) [Source: USDA Nutrient Database] Since dried Moringa leaves retain their nutrient content, It's possible and convenient to convert them into leaf powder which is easy to make, store and use. Moringa has the unique advantage of being somewhat tastelss so it makes excellent nutritional supplement that can be added to any dish or taken on its own. This is why Moringa is being advocated as "natural nutrition for the tropics." The great majority of multivitamins available today are synthesized and chemically formulated so most of them are not easily absorbed by the body while Moringa is a natural whole food source for vitamins, minerals, proteins, antioxidants and other important components that the body relies upon to stay healthy. Regular intake of Moringa will give benefits of increased energy, greater alertness, better endurance, increased focus, mental clarity, strong immune system, etc. also rare for a plant source, Moringa leaves contain all the essential amino acids (usually found only in animal

products like eggs) in good proportion including argemine and histidine which are especially important for infants. Hence, Moringa leaf is a food source for infants, children, pregnant women and everybody.

The reason for the increased potency in living models is not known (although It's possible that it can induce genetic transcription similar to SFN (Figure 7) since the bioactives are similar in structure), but the antioxidant properties seem to underlie the vast majority of benefits associated with this supplement. There are also antiinflammatory effects that, while less studies, seem to be quite effective; one of the bioactives, RBITC (*rhodamine B isothiocyanate)*, is effective in suppressing macrophage activation in the nanomolar range which is worth some future research into. Beyond that, there does appear to be a nice anti-diabetic effect that has gone some very preliminary human testing which suggests that this plant may benefit pancreatic function and reduce blood glucose secondary to that.

Figure 6 : Moringa's Crushed Leaves

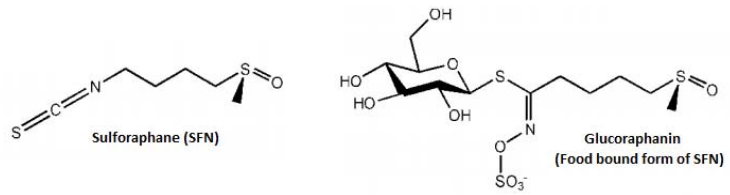


Figure 7 : SFN (Sulforaphane) & Glucoraphanin - food bound form of SFN

Now, despite the plant being referred to as 'nontoxic' this does not appear to be the case. While supplemental dosages listed below appear to be safe from all tested toxicity a relatively small increase (3-4x the recommended does) is known to cause genotoxic damage and may promote cancer formation whereas doses higher than that cause overt organ damage (mostly liver and kidneys).

Figure 8 : Healthcare produce of M olifera [1. Nutritional trendy capsules & 2. Tea Bags]

OUTLINED THERAPEUTIC APPLICABILITY OF MORINGA'S TREE

Figure 9

Figure 10 a & B : Moringa' pods (fresh to dry)

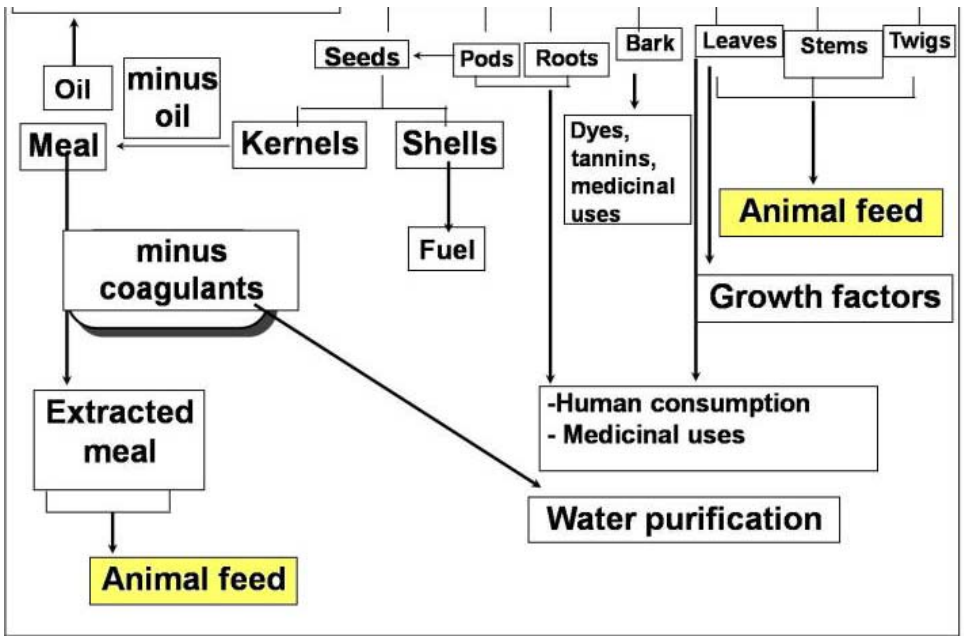
Comparison (g/g) of Moringa leaves' (fresh & dry) nutrients with oranges, carrots, milk, bananas, spinach & yoghurt [64-66].

Contents in Moringa's fresh leaves: 7x the Vit C of Oranges; 4x the Vit A of carrots 4x the Ca of Milk; 3x the K of Bananas; 4x the Fe of Spinach 2x the Protein of Yoghurt

Contents in Moringa's dried leaves: 4x the Vit C of Oranges; 10x the Vit A of carrots; 17x the Ca of Milk; 15x the K of Bananas; 25x the Fe of Spinach; 9x the Protein of Yoghurt Moringa

III. Apraise on Biochemical Charter & Phytochemistry

Because of the chemical complexity of the *M. oleifera,* apparent therapeutic effects could be due to the combined actions of various bioactive components found in the plant, including trace metal ions, vitamins, alkaloids, carotenoids, polyphenols, fats, carbohydrates, and proteins[14]. Some compounds may collectively affect broad aspects of physiology, such as nutriment absorption and processing, redox state, or immunity.*Moringa oleifera* leaves contain phytosterols such as β-sitosterol[15]. These compounds can reduce intestinal uptake of dietary cholesterol[16]. They could partly account for the decrease of plasma cholesterol and the increase of fecal cholesterol observed in rodents treated with *M. oleifera* leaves[17-18]. *M. oleifera* leaf powder also contain about 12% (w/w) fibers[19]. Dietary fibers reduce gastric emptying[20]. They may partly explain the greater stomach content, the improved OGTT (oral glucose tolerance test) response in treated GK (Goto-Kakizaki) diabetic rats[21], as well as the progressive improvement of PPBG (post-prandial blood glucose) levels in treated T2DM (type-2 diabetes mellitus) patients[22].



Graphic representation of M oifera Lim. applicability

Figure 11

The viability and functionality of a cell partly depends on a favorable redox state, i.e., on its ability to prevent excessive oxidation of its macromolecules, including DNA (deoxyribose nucleic acid), proteins, and lipids[23]. ROS (reactive oxygen species) and free radicals are the major mediators of the oxidative process. Cellular inability to reduce ROS leads to oxidative stress. All cells are variably capable of endogenous self-protection against this stress through the actions of enzymes such as catalase, superoxide dismutase, and glutathione peroxidase, as well as through reducing molecules such as glutathione. Nutritional antioxidants such as vitamins A, C, and E provide additional protection from the stress[24]. Oxidative stress is widely accepted as a major contributing factor in the pathogenesis of CVD (cardiovascular disease) and diabetes[25,26]. A recurring explanation for the therapeutic actions of *M. oleifera* medication is the relatively high antioxidant activity of its leaves, flowers, and seeds[27-36].

Glucosinolates are characterized by β-thioglucoside *N*-hydroxysulfate motif. In *M. oleifera* leaves, most phytochemicals of this class carry a benzyl-glycoside group linked to the single carbon of the motif. The most abundant of them is 4-*O*-(α-l-rhamnopyranosyl-oxy)-benzylglucosinolate, otherwise known as glucomoringin[35]. Enzymatic hydrolysis of the glucosinolate motif of members of this class leads to the formation of corresponding isothiocyanates, thiocyanates, or nitriles. Several of these by-products have been shown to possess antihypertensive properties[37-39]. Flavonoids and phenolic acids are collectively referred to phenolic compounds.

The structural skeleton of flavonoids is made of two aromatic rings joined by a 3-C link; that of the sub-

class of flavonols is 3-hydroxy-2-phenylchromen-4-one, Quercetin and kaempferol, in their as 3′-*O*-glycoside forms, are the predominant flavonols in *M. oleifera* leaves. The sugar moieties include, among others, rhamnoglycosyl (rutinosides), glucosyl (glucosides), 6′ malonyglucosyl, and 2′-galloylrutinoside groups[35, 41,42]. Biologically, flavonoids are best known for their antioxidant properties, but their metabolic pathways of activity remain to be fully elucidated[43]. Phenolic acids range of fairly unique compounds. In particular, this have benzoic acid and cinnamic acid as backbones, with one or several (-OH)hydroxyl groups . Chlorogenic acid, which is an ester of dihydrocinnamic acid (caffeic acid) and quinic acid, is a major phenolic acid in *M. oleifera* leaves. The flavonol quercetin is found at concentrations as high as 100 mg/100 g of dried *M. oleifera* leaves[44]predominantly as quercetin-3-*O*-β-d-glucoside also known as isoquercitrin or isotrifolin (Figure 14.)

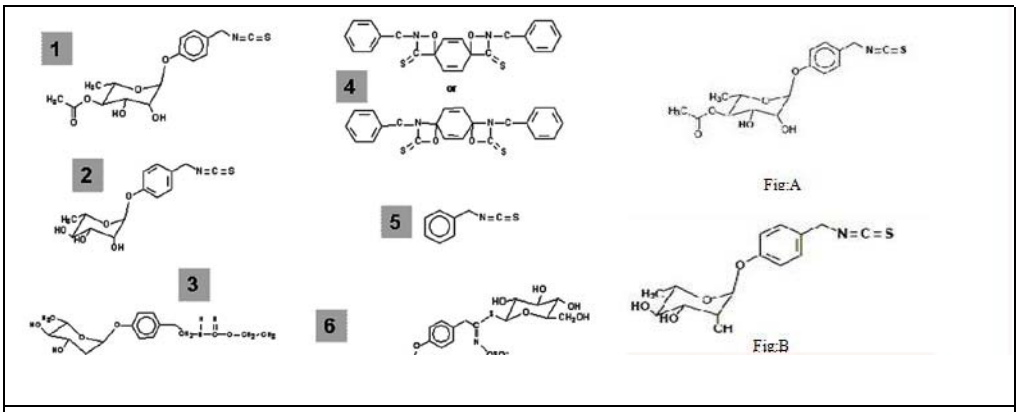


Figure 12 : Chemical Structures 1,2,3,4,5,6,A,B

Quercetin is a potent antioxidant[45] with multiple therapeutic properties. It can reduce hyperlipidemia and atherosclerosis in HCD (high-cholesterol diet) or HFD (high-fat diet) rabbits [46,-48]. It has shown anti-dyslipidemic, hypotensive, and anti-diabetic effects in the obese Zucker rat model of metabolic syndrome[49]. It can protect insulin-producing pancreatic β cells from STZ(streptozotocin) -induced oxidative stress and apoptosis in rats[50]. Its hypotensive effect has been confirmed in a randomized, double-blind placebo-controlled, human study[51]. Chlorogenic acid can beneficially affect glucose metabolism. It has been shown to inhibit glucose-6-phosphate translocase in rat liver, reducing hepatic gluconeogenesis and glycogenolysis[52-53]. It was found to lower PPBG in obese Zucker rats[54]. In OGTT experiments performed on rats or humans, it reduced the glycemic response in both species[56,57]; in rodents, it also reduced the glucose AUC (area under the curve)[55]. Its anti-dyslipidemic properties are more evident as its dietary supplementation has been shown to significantly reduce plasma TC and TG in obese Zucker rats or HFD mice[58] and to reverse STZ -induced dyslipidemia in diabetic rats[59].

The alkaloid moringinine was initially purified from *M. oleifera* bark[60]. and later chemically identified as benzylamine[61]. It's also present in leaves. This substance was suspected to mediate the hypoglycemic effect of the plant. An early study showed that Wistar rats provided with drinking water containing 2.9 g/L of benzylamine for 7 weeks exhibited a reduced hyperglycemic response in IPGTT (intraperitoneal glucose tolerance test), suggesting improved glucose tolerance[62]. More recently, the effect was further explored using HFD -fed, insulin-resistant C57BL/6 mice taking an estimated daily dose 386 mg/kg-body weight in drinking water for 17 weeks. Compared to untreated controls, these mice gained less weight, had reduced FPG (fasting blood glucose) and PTG (plasma triglyceride) and were more glucose tolerant (Iffiu-Soltesz et al., 2010). Niaziminin is a mustard oil glycoside initially isolated (along with other glycosides such as niazinin and niazimicin) from ethanolic extracts of *M. oleifera* leaves, based on their hypotensive properties on Wistar rats. At 1 mg and 3 mg/kg-body weight, these compounds caused a 16–22 and a 40–65% fall of (MABP) mean arterial blood pressure respectively[63].

Other active isothiocyanate glycosides and thiocarbamates were isolated from the plant using the same bioassay[64-66]. This compound was isolated from *M. oleifera* roots and structurally identified as *N*-benzoylphenylalanyl phenylalinol acetate . At 25 μM, this unusual dipeptide derivative inhibited by nearly 90% the secretion TNFα and IL-2 from lipopolysaccharide-stimulated peripheral blood lymphocytes in culture. It had no effect on IL-6 secretion This inhibitory activity may contribute to the anti-inflammatory[67]. properties of the plant. An examination of the phytochemicals of Moringa species affords the opportunity to examine a range of fairly unique compounds. In particular, this

plant family is rich in compounds containing the simple sugar, rhamnose, and it's rich in a fairly unique group of compounds called glucosinolates and isothiocyanates. For example, specific components of Moringa preparations that have been reported to have hypotensive, anticancer, and antibacterial activity include 4-(4'-*O*-acetyl-a-L-rhamnopyranosyloxy)benzyl isothiocyanate[6], 4-(a-L-rhamnopyranosyloxy)benzyl isothiocyanate[7], niazimicin[8], pterygospermin[9], benzyl isothiocyanate[10], and 4-(a-L-rhamnopyranosyloxy)benzyl glucosinolate[11]. While these compounds are relatively unique to the Moringa family, it's also rich in a number of vitamins and minerals as well as other more commonly recognized phytochemicals such as the carotenoids (including β-carotene or pro-vitamin A). These attributes are all discussed extensively by Lowell Fuglie[1] and others, and will be the subject of a future review in this series.

IV. Gastronomic Draw on & Socioeconomic Status

The M olifera pod (munga/ saragwa/saragwe) is often refereed as drumstick tree and horshredish tree in English. In south India, it's used to prepare a variety of sambars and is also fried. In other parts of India, especially West Bengal, and also in a neighbourning country like Bangladesh, it's enjoyed very much. It's made into a variety of curry dishes by mixing with coconut, poppy seeds and mustard or boiled until the drumsticks are semi-soft and consumed directly witout any extra processing or cooking. It has find utility in curriesd, sambars, kormmas, and dals, although it's used to add flavor to cutlets, etc. In Maharastra, the pods are used in sweets and curries called Aamatee. Tender drumstick leaves, finally chopped, are used to garnish veggie dishes, dals, sambars, salads, etc. also, it has gained popularity to be ysed as coriander, as these leaves have high therapeutic significance. Its flowers, in some regions, are gathered and cleansed to be cooked with basan to make pakoras. It's preserved by canning and ex[ported worldwider[77,81].

Figure 13 : Moringa's seeds deturbitifies water

Figure 14 : Moringa's flowers, seeds (fresh & crushed)

M olifera is one of the most tropical trees. The relative ease with which it propagates through both sexual and asexual means and its low demand for soil nutrients and water after being planted makes its production and management easy.Introduction of this palnt into a farm, which has a biodiverse environment, can be beneficial for both the owner of the farm and the surrounding eco-system. Diostinction of cultivators has not yet been formally carried out. M olifera was well known to the ancient world, but only recently has it been rediscovered as a multipurpose tree with a tremendous multiplicity of potent applicability[21,31,40]. *Moringa oleifera* Lam. is the most "underutilized" multipurpose tropical crop. The leaves, tender pods and seeds could serve as a valuable source of nutrients for all age groups. The leaves, tender pods and seeds are sources of vitamins, minerals and proteins. The leaves and branches can be used as feed for livestock and fish. Due to the high nutrient content of the leaves, moringa can be incorporated into the mulching system. The dry seed suspension is a known natural coagulant and coagulant aid with antibacterial activity. Dry moringa seeds can be used in place of alum to treat turbid water and reduce bacteria in drinking water (Figure 11). Geographically, many of developing countries are located in the tropical and sub-tropical regions of the world where *M. oleifera* grows and is cultivated. If validated by medical science, dietary consumption of this plant could be advocated in these and other countries as an inexpensive prophylactic strategy against diabetes mellitus (DM), and chronic dyslipidemia a risk factor for cardiovascular disease (CVD). Chronic hyperglycemia is an indicator of DM and chronic dyslipidemia a risk factor for CVD. These metabolic disorders are global epidemics[13].

V. Moringa: A Source of II Generation Biodiesel

With years of continuing research, experiments and trials has provided an adage to find and develop 2nd generation biodiesel feedstock with low cost input technology. Moringa oleifera is a very fast growing tree; it commonly reaches four meters in height just 10 months after the seed is planted and can bear fruit within its first year. Its seeds are triangular in cross-section (30 to 50 cm long) and legume-like in appearance (Figute 12a,b.c).

Figure 15 a : Moringa's seeds

Figure 15 b : Moringa'slegume shaped - triangular seeds

Figure 15 c : Moringa's preserved seeds

These seeds have oil rich black and winged seeds, which can be crushed to produce biodiesel (Figure 13). Moringa could yield +3 ton oil/ ha and that it could be used for food in times of shortages. The seeds contain 30%t o 40% oil that is high in oleic acid. The meal yields about 61% protein. Biodiesel made from Moringa has better oxidative stability than biodiesel made with most other feedstocks the crop's multiple dimensions would make it attractive to farmers worldwide. Other than biodiesel, the pods can also produce highly nutritious edible seeds. Their pods are harvested, meaning that the trees keep on growing, using water and reducing the high water table whilst sequestrating carbon. The Moringa oleifera trees must be regarded as a sure source of 2nd Generation Biodiesel. The Moringa oleifera tree that has enough credentials: a higher recovery and quality of oil than other crops, no direct competition with food crops as It's a edible source of fuel, and no direct competition with existing farmland as can be grown for both purpose same time.

VI. Conclusion

A large number of reports on the nutritional qualities of Moringa now exist in both the scientific and the popular literature. This fast growing tree now well now for its employability in human nutrition, dye, fodder, and water deconramination as it bears an imposing assortment for day to day welfare of wellbeing and socioeconomic comfort. Extensive field reports and ecological studies forming part of a rich traditional medicine history, claim efficacy of leaf, seed, root, bark, and flowers against a variety of dermal and internal infections. Moringa seed contain oil that can be used for various industrial purposes and as vegetable oil for human consumption or as biofuel. Though apparently native only to restricted areas in the southern foothills of the Himalayas, *M. oleifera* is cultivated in all the countries of the tropics. Outstanding oil is derived from the seeds, which is used for cooking and lubrication of delicate mechanisms. Leaves can be eaten fresh, cooked, or stored as dried powder for many months without refrigeration, and reportedly without loss of nutritional value. Moringa is especially promising as a food source in the tropics because the tree is in full leaf at the end of the dry season when other foods are typically scarce. We can clearly affirm the superiority of Moringa over the other foods. As it was found that Moringa leaves contain more Vitamin A than carrots, more Ca than milk, more Fe than spinach, more Vitamin C than oranges, and more K than bananas," and that the protein quality of Moringa leaves rivals that of milk and eggs. Clearly much more research is justified, but just as clearly this will be a very fruitful field of endeavor for both basic and applied researchers over the next decade. Moringa preparations (e.g. extracts,

decoctions, poultices, creams, oils, emollients, salves, powders, porridges) are not quite so well known. A plethora of traditional medicine references attest to its curative power, and scientific validation of these popular uses is developing to support at least some of the claims. Moringa preparations have been cited in the scientific literature as having antibiotic, antitrypanosomal, hypotensive, antispasmodic, antiulcer, anti-inflammatory, hypocholesterolemic, and hypoglycemic activities, as well as having considerable efficacy in water purification by flocculation, sedimentation, antibiosis and even reduction of Schistosome cercariae titer. M. oleifera is also of interest because of its production of compounds with antibiotic activity such as the glucosinolate 4 alpha-L-rhamnosyloxy benzyl isothiocyanate. Other research has focused on the use of *M. oleifera* seeds and fruits in water purification. Of importance is that all parts of Moringa are edible and also effective when used for treating various diseases.any researches continue to be conducted on further establishment of Moringa as a potent medical solution and many are directed towards the acceptance and commercialization of Moringa bio active components. Meeting all bodies nutritional requirements will naturally curb junk food cravings and supply with the energy needed to maintain a healthy & active lifestyle.

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Urinary Excretion and Renal Clearance of Nimesulide in Male Volunteers

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Abstract- Drugs are eliminated from the body either unchanged as the parent drug or as metabolites. Nimesulide drug belongs to a wide class of medicines called antiinflammatory nonsteroidal drugs (NSAID). NSAIDs are well known for their antipyretic, anti-inflammatory, and analgesic properties. Renal clearance of nimesulide correlated with creatinine clearance. The present research work was planed to determine urinary excretion and renal clearance of nimesulide after its oral administration. Blood and urine samples of the human male volunteers (n=10), after the oral administration of drug, were collected at predetermined time intervals. The concentration of the nimesulide in urine and plasma of male volunteers was determined by HPLC. Statistical analysis was performed in order to determine the significance of the results.

GJMR-B Classification : NLMC Code: QV 704

Urinary Excretion and Renal Clearance of Nimesulide in Male Volunteers

Bilal Ahmed [α], Tahira Iqbal [σ], Huma Mahboob [ρ], Farah Latif [ω] & Imtiaz Sohail [¥]

Abstract- Drugs are eliminated from the body either unchanged as the parent drug or as metabolites. Nimesulide drug belongs to a wide class of medicines called anti-inflammatory nonsteroidal drugs (NSAID). NSAIDs are well known for their antipyretic, anti-inflammatory, and analgesic properties. Renal clearance of nimesulide correlated with creatinine clearance. The present research work was planed to determine urinary excretion and renal clearance of nimesulide after its oral administration. Blood and urine samples of the human male volunteers (n=10), after the oral administration of drug, were collected at predetermined time intervals. The concentration of the nimesulide in urine and plasma of male volunteers was determined by HPLC. Statistical analysis was performed in order to determine the significance of the results.

I. Introduction

The body begins to eliminate the drug by hepatic metabolism, renal or both, after administration of the dose. The renal clearance of a substance is the volume of plasma that is completely cleared of the substance by the kidney per unit time. The kidneys are the primary means for elimination waste products of metabolism that are no longer needed by the body. These products include urea, creatinine, uric acid and end products of haemoglobin breakdown and metabolites of various hormones. These waste products must be eliminated from the body as rapidly as they are produced. The kidneys also eliminate most toxins and other foreign substances that are either produced by the body or ingested, such as pesticides, drugs and food additives (Guyton and Hall, 2000).

Kidney, skin, lung, gastrointestinal tract, salivary glands and liver are the main channel through which excretion takes place. Among the main channel of excretion, kidneys are chief. Kidneys are the major organ of homeostasis, preventing alternation in volume osmolatity, ionic composition and pH of body fluids (Bander and Pugh, 1977).

The main responsibility for adjusting the solute and water excretion is borne by the kidney (Tadlock, 1993).

Nimesulide is extensively bound to albumin; the unbound fraction in plasma was 1%. The unbound fraction increased to 2 and 4% in patients with renal or hepatic insufficiency. The drug was absorbed rapidly and extensively after received oral nimesulide 100 mg (tablet, granule or suspension form) in healthy volunteers (Bernareggi, 1998).

Nimesulide; a relatively COX-2 selective, non-steroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic properties; is approved for the treatment of acute pain, osteoarthritis and primary dysmenorrhoea in adolescents (Rosalinde and Russel, 2010). Non-steroidal anti-inflammatory drugs (NSAIDs) are the group most often used in human health care, since they are available without prescription for treatment of fever and minor pain (Starek and Krzek, 2009).

Excretion of nimesulide metabolites in the urine and feces account for about 80% and 20% of the administered dose, respectively (Yesilot *et al.*2010). Nimesulide rapidly and effectively provides relief of pain and the signs and symptoms of inflammation associated with a wide array of disorders. Nimesulide has especially proved useful in patients who do not respond adequately to other NSAIDs, and in patients who are NSAID-intolerant due to hypersensitivity (e.g., asthmatics) or gastric intolerance. The body defends itself against potentially harmful compounds like drugs, toxic compounds and their metabolites by elimination in which the kidney plays an important role (Silva et *al* 2010). Renal clearance is used to determine renal elimination mechanisms of a drug, which is the result of glomerular filteration, active tubular secretion and reabsorption. The renal proximal tubule is the primaty site of carrier-mediated transport from blood to urine. Renal secretory mechanisms exists for, anionc compounds and organic cations (Rosalinde and Russel, 2010).

II. Aims and Objectives

To study the urinary excretion and renal clearance of nimsulide by using HPLC in female volunteers after oral administration.

III. Material and Method

This study was conducted to analyze the urinary excretion and renal clearance of nimesulide and endogenous creatinine in blood and urine. samples of healthy male volunteers after the oral administration of 100 mg nimesulide were collected. The experiments were conducted on 10 male volunteers.

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IV. Volunteers

The healthy male volunteers choose for this study was the students of University of Agriculture Faisalabad. Each volunteer was apprised of design for this research work. The volunteers who willing offered to participate were included in this study, The volunteers choose for this study are physically fit and their previous medical history shows that They were never found any serious disease. The complete demographic data of each volunteer is described in the table 3.1

Blank blood and urine samples were taken from each volunteer then the each volunteer was given 100 mg tablet of nimesulide (Sami industries) orally. The age body weight' height, blood pressure and body temperature of volunteers were recoded and presented in Tablet 3.1. Volunteers were offered similar breakfast after one hour of the drug administration.

V. Drug Administration

The drug nimesulide 100 mg of (Sami industries Pakistan limited Karachi) was given to each of the participate in this study. The drug is given orally with the glass of water. The samples for the analysis were collected in the month of (June 2011) and the samples stored at -20˚C temperature until analysis.

Table 3.1 : Deamographic data of male volunteers

Volunteers	Age	Body weight	Height	Blood pressure(mm Hg)		Body temperature
	(Years)	(kg)	(Ft)	Systolic	Diasystolic	(˚F)
1	23	71	5.7	80	120	98.2
2	25	51	5.4	77	115	98
3	22	57	5.8	80	120	98.1
4	21	55	5.9	70	110	98
5	22	67	5.11	70	110	97
6	28	74	5.11	80	120	98
7	24	63	6	80	120	98.1
8	23	53	5.9	75	115	97.5
9	23	55	5.4	80	120	98
10	22	69	5.9	80	115	97
Mean	23.3	61.5	5.6	77.2	116.5	97.7
+SD	2.1	8.3	0.3	4.1	4.1	0.45
Minimum	21	51	5.4	70	110	97
Maximum	28	74	6	80	120	98.2

Sampling procedure;

a) Collection of blood samples

Before the drug administration blank blood samples were collected from each volunteer. The blood samples of each volunteers were collected after 1 and 3 hours. After the oral intake of nimesulide 100mg (Sami industries Pakistan Karachi) these blood samples were stored in ependorf tubes at -20˚ C until use for the analysis.

b) Collection of urine samples

Prior to oral administration of drug the blank urine samples were collected from each volunteer. Urine samples of volunteer's were collected after 2, 4, 6, 8, 12 and 24 hours after drugadministration. These urine samples were stored in plastic bottles in freezer at - 20˚C until analysis

VI. HPLC Analysis

a) Preparation of mobile phase

Mobile phase was prepared by using phosphate buffer of 15 mM. the phosphate buffer was prepared by mixing of 0.288g KH2PO4 and 2.33g of K2HPO4 in the double distilled water of HPLC grade and volume was made up to 1000 ml. while for the mobile phase other reagents and chemical used are acetonitrile and methanol which are the HPLC grade. The pH of the buffer was 7.3. Mobile phase was prepared by mixing Acetonitrile, methanol and the phosphate buffer are in 30:5:65 ratio than the mobile phase was filtered in vacume filtration assembly having cellulose filter which have pore size 0.545um (Sartorius ag 37070.) than the filterd mobile phase was sonicated for the removal in any bubbles for 10 minutes. (eyela sonicator)

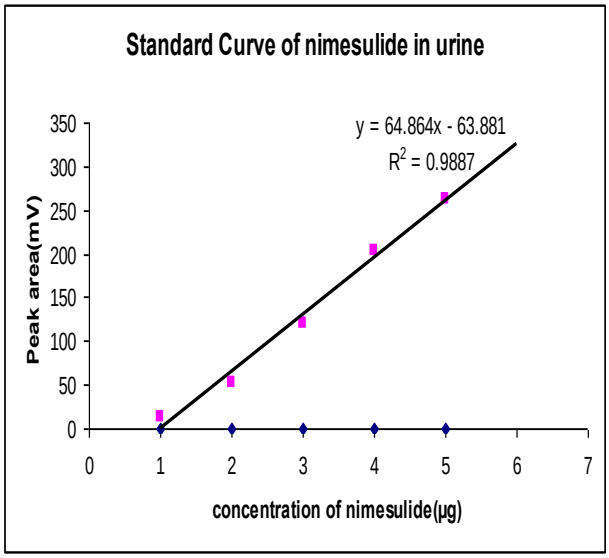
b) Chromatographic Conditions

Chromatography was performed with a high performance liquid chromatography. The HPLC system was consisted of Shimadzu SCL-10A system controller, UV visible SPD-10AV detector and LC-10AT pump with FUC-10AL VP flow controller wall. Separation was achieved at ambient temperature with Hypersil C18 BDS 250x4.6 column pore size of 5 micron. Chromatographic data was collected and analyzed using CSW32 software. And spectrophotometer (PG Instruments, model T60) was used for creatinine analysis in plasma and urine samples.

c) Standard preparation for blood samples

Stock solution was prepared in methanol. For preparation of standard curve of nimesulide in the plasma samples, nimesulide standard have concentration of 5, 10, 15, 25, 50, 75 and 100 µg /ml was mixed in plasma which is drug free. This is given in table 3.2. Drug free plasma was taken firstly and then added to the stock solution of nimesulide of specific concentration. This is then centrifuge at 3500 rpm for 5 min then 50 µl supernatant was taken into the ependorf. Then phosphate buffer having 7.3 pH was added into the supernatant. The sample was filtered by using filtrate assembly with micro syringe through 0.22 um pore size membrane filter having diameter of 0.13 mm and the 20 µl of the such filtrate was injected into the HPLC instrument for the standard curve peak area (mv) versus plasma concentration µg/mL of standard were ploted and a linear relationship was obtained.(Fig 3.1)

Sr No	Concentration µg/mL	Peak area(mv)
1	5	7.105
2	10	13.722
3	25	37.143
4	50	110.711
5	75	212.728
6	100	263.249



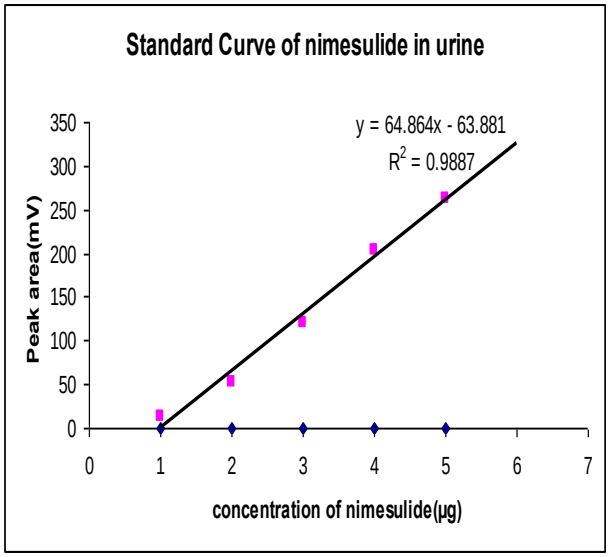
Concentration and peak area of standard solution of Nimesulide is plasma

d) Standard preparation for urine

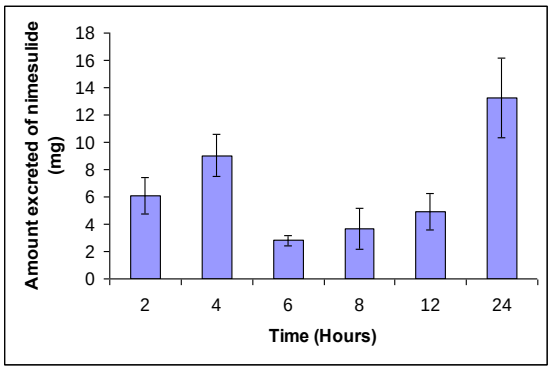
For preparation of standard curve in urine, a stock solution of nimesulide (sigma) 1000 ug /ml = 0.01g of nimesulide and diluted up to 10 ml with double distilled methanol 1000ug/ml =1ml of 1000ug/ml. Standard solutions having different concentrations of nimesulide from this stock solution were prepared. For concentration of 100µg/mL, 1mL of stock solution (1000 µg/mL) was diluted up to 10mL with double distilled methanol.

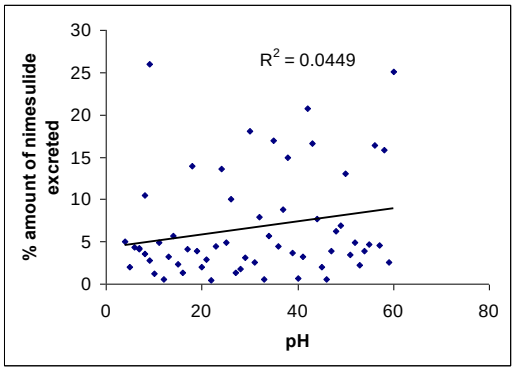
Nimesulide standard urine having concentration of 5, 10, 15, 25, 50, 75 and 100µg/ml of Nimesulide were prepared in drug free urine given in the table 3.3. This is then centrifuge at 3500 rpm for 5 mints. Then 50 µl supernatant was taken into the ependorf. Then buffer having 7.3 pH which is phosphate was added into the supernatant. The sample was filtered by using filtrate assembly with microsyringe through 0.22um pore size membrane filter having diameter of 0.13 mm and the 20 µl of the such filtrate was injected into the HPLC instrument for the standard curve peak area (mv) versus urine concentration µg/mL of standard were plotted and a linear relationship was obtained.(fig 3.2) The data related to urine samples were presented in tablet 3.4.

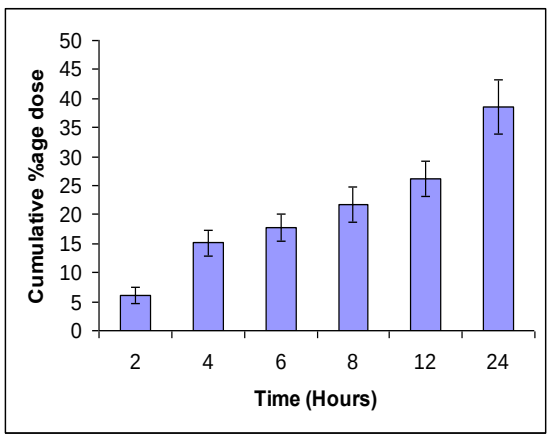
Sr No	Concentration µg/mL	Peak area(mv)
1	5	7.105
2	10	13.722
3	25	37.143
4	50	110.711
5	75	212.728
6	100	263.249

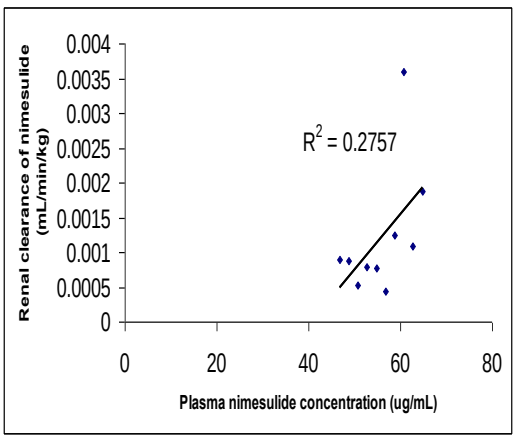


- Concentration and peak area of standard solution of nimesulide in urine









e) Statistical Analysis

Statistical analysis was performed by expressing all the data as mean, standard error of mean. The effect of pH and diuresis on renal clearance of nimesulide is to be studied by regression analysis (Steel *et al.,* 1997).

VII. Results and Discussion

Within 24 hours of oral administration, some 50-70% of the dose on Mean was excreted in the urine as unchanged drug. Over the dose range of 0.3-30 mg/kg nimesulide, there was no dose-dependent effect on total or renal clearance, (Kerola *et al*., 2009). 70% of a nimesulide dose being excreted unchanged in urine with the major site of elimination which occurs by renal mechanisms. At the glomerulus nimesulide is mainly secreted and filtered by the organic cationic secretory pathway. In this way renal clearance values approximately 4 times greater than GFR (Dowling and Frye, 1999). The difference in the urinary excretion of nimesulide under local conditions and reported in literature is due to environmental and genetic influences on glomerular filtration rate which significantly affect the fate of drug in the body. These differences have been elucidated by original term geonetics (Singla *et al*. 2000).

Studies on nimesulide suggest that it is extensively secreted from urine even though when given in small amounts. At plasma concentrations up to 30-fold the tubular secretion rate of nimesulide gradually increases and higher than those values which are achieved during 100 mg/day typical oral dosing (Dowling *et al.,* 2001).

The retention time for the present study was 6.5 min for plasma and 4.2 min for urine while according to a study it is 8.4 min (Dowling and Frye, 1999). The difference is probably due to storage of urine and plasma samples, environmental conditions and/or temperature.

The Mean value of the percent dose of nimesulide in urine sample was 83% +/- 16%, in the earlier study was calculated by (Korsuntirat *et al.,* 2010). The present percent dose is lower calculated as 69.30±2.18. There is difference between present study value and earlier study value due to difference in environment, temperature but major difference in the values is due to non fasting volunteers.

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Effect of Antioxidant Vitamins on the Oxidant / Antioxidant Status in Patients with Multiple Sclerosis

By Meena Ahmed Mohammed & Assist. Prof. Dr. Wahda B. Al-Youzbaki
Antioxidant Status in Patients with Multiple Sclerosis, Iraq

Abstract- *Background:* The cause of multiple sclerosis (MS) is unknown, although it is widely accepted that environmental factors act in concert with a genetic susceptibility.

Objective: To evaluate the effect of 3 months supplementation of antioxidants vitamins (ascorbic acid (vitamin C) and alphatocopherol (vitamin E)) on the oxidant / antioxidant status and on the clinical course in patients with relapsing remitting MS (RRMS) and to compare with the placebo therapy and healthy subjects as a control.

Patients & Methods: This is a non randomized single blinded clinical trial was conducted on a total number of 60 patients (24 males and 36 females) with age ranged between 15-54 years, diagnosed to have relapsing-remitting multiple sclerosis (RRMS) and were registered at Neurology Outpatients Department in Ibn Sina Teaching Hospital in Mosul City / Iraq and receiving subcutaneous **β**- interferon, in the period from 1st of February 2012 to the 1st of July 2012.

Keywords: *multiple sclerosis, malondialdehyde, ascorbic acid, tocopherol.*

GJMR-B Classification : *NLMC Code: QV 704*

Strictly as per the compliance and regulations of:

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Results: There were significantly higher level but a significant lower level of mean serum MDA and TAS concentration respectively in the two MS patients groups (antioxidant vitamins group and placebo group) before starting therapy as compared with the control group. After 3 months antioxidant vitamins therapy, there were a highly significant reduction in the mean serum level of MDA and EDSS but a highly significant increase of the mean serum level of TAS in comparison to their value before therapy. While after 3 months of placebo therapy there were insignificant chan ges in the mean serum level of MDA and TAS and insignificant difference in EDSS in comparison to their value before therapy. The use of antioxidant vitamins for 3 months resulted in a significant lower level of mean serum MDA, but a significant higher level of mean serum TAS and a significant lower value of EDSS when compared to 3 months use of placebo therapy.

Conclusion: Three months antioxidant vitamins (vitamin C and E) supplementation in RRMS patients causes a significant improvement of the oxidant / antioxidant status and the clinical course of MS patients represented by EDSS. Vitamins (C and E) might be used as adjunct therapy in MS.

Keywords: multiple sclerosis, malondialdehyde, ascorbic acid, tocopherol.

I. Introduction

Multiple sclerosis (MS) is an inflammatory, autoimmune, demyelinating disease of the central nervous system (CNS)[1]. It is characterized by loss of myelin , the fatty tissue that surrounds and protects nerve fibers allowing them to conduct electrical impulses[2]. Although the reasons for the autoimmune demyelination are far to be clear[3], one of these common features is the neuronal imbalance in oxidants/antioxidants[4] .

Central nervous system (CNS) is particularly susceptible to reactive oxygen species (ROS) induced damage due to the high oxygen demands of the brain and low concentration of endogenous antioxidants[5].

Although different mechanisms may contribute to demyelination and neurodegeneration in MS, mitochondrial injury and subsequent energy failure is a major factor driving tissue injury[6-10]. Growing evidence indicates that oxidative stress plays a major role in the pathogenesis of MS[11,12]. Mitochondrial proteins and DNA are highly vulnerable to oxidative damage[13], and it is thus expected that free radical-mediated mechanisms may drive mitochondrial injury in MS [14-16].

Vitamin E acts as a fat-soluble antioxidant against ROS which has been linked to the pathogenesis of MS[17]. In addition, vitamin E has a direct modulatory effect on immune cells. It has been demonstrated to enhance T-cell function in mice by increasing division and interleukin-2 (IL-2) production, as well as reducing prostaglandin E 2 from macrophages[18]. Also vitamin E, a potent antioxidant agent, exerts a protective role as

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free radical scavenger through a non-enzymatic mechanism out of the cell and is the most effective antioxidant agent in lipid structure [19].

Vitamin C prevents the development of experimental autoimmune encephalomyelitis (EAE), an animal model of MS[20] and ameliorates its symptoms[21]. It is likely that vitamins C and E act in a synergistic manner, vitamin E primarily being oxidized to the tocopheroxyl radical and then reduced back to tocopherol by vitamin C[22].

So the aim of this non randomized clinical trial was to assess the effect of supplementation of antioxidant vitamins (vitamin C and E) for three months for patients with RRMS who were treated by β-interferon on the oxidant / antioxidant status and the clinical course of the disease before and after therapy using EDSS.

II. Patients & Methods

Sixty patients known to have RRMS (according to Mc Donald criteria) and registered in the Neurology outpatient Department in Ibn Sina Teaching Hospital and receiving subcutaneous β -interferon 250 μg thrice weekly, enrolled in this study, excluding pregnant and lactating women, people receiving trace elements or vitamin B-complex within one month before the study, smokers and alcohol drinkers or patients with acute or chronic illness rather than MS. These patients were divided into two groups: First group included 30 patients started to receive the antioxidant therapy with vitamin C (ascorbic acid), Cetavit manufactured by Al-shahba Lab. Syria, 500 mg twice daily, and vitamin E (α-tocopherol), Vita E, manufactured by Asia pharmaceutical industries, Syria, 400 I.U. once daily for three months (antioxidant vitamins group). The second group included the other 30 patients, were kept on a placebo therapy in the form of capsules filled with glucose powder twice daily, for 3 months duration (placebo group). Thirty apparently healthy individuals, matched with the patients groups by age and sex and BMI were considered as a control group.

About 5 Blood samples were collected from the patients of the antioxidant vitamins group and the placebo group at the first visit before taking antioxidant vitamins or placebo therapy respectively then after three months of use and from the control group once at the first visit only. The separated serum was kept frozen at –20°C for measurement of TAS using commercial kit supplied by Randox and MDA using manually prepared reagent and followed Buege and Aust, method[23]. The effectiveness of antioxidants and placebo therapies was assessed in both patients groups by using expanded disability status score (EDSS) [24]. BMI was measured by dividing weight in (kg) by square of height in (meter).

Statistical Analysis: Computer feeding was conducted by prepared computer program SPSS version 18. Standard statistical methods were used to determine the mean and standard deviation (SD). Paired student t-test was used to compare the results for measured biochemical parameters between patients groups and control group. All values quoted as the mean ± SD and P-value ≤ 0.05 was considered to be statistically significant.

The approval of the study protocol by an ethic committee has been obtained from the local health committee of Ministry of Health and College of Medicine - University of Mosul – Iraq.

III. Results

The study sample consisted of 90 individuals with age ranged between 15-54 years. Sixty of them were patients with RRMS and they were equally assigned to two groups, namely antioxidant vitamins group and the placebo group. The other 30 individuals were apparently healthy subjects with age ranged between 20-42 years , were served as control group.

Table (1) shows that there were no significant differences between the characteristic of the two patients groups and the control group, enrolled in this study.

Table 1 : General characteristic of the MS patients and the control

Parameters	Mean ± SD			
	Antioxidant vitamins group n=30	Placebo group n=30	Control group n=30	P-Value
Age(year)	34.93±8.51	35.60±8.88	32.50±5.49	NS
Sex male Female	24 36	12 18	12 18	NS
BMI (Kg/m^2)	26.14±5.95	25.06±4.04	24.16±2.66	NS
Duration of disease (years)	3.90±2.55	3.20±3.91		NS

Duration of β- interferon therapy (years)	2.40±1.49	2.46±2.67		NS
EDSS	3.25±1.54	3.21±1.42		NS

Table (2) demonstrates that the mean serum level of MDA was significantly higher in the two MS patients groups before starting therapy (antioxidant vitamins group and placebo group), whereas the serum level of TAS was significantly lower in these patients as compared with the control group.

Table 2 : Comparison between mean serum level of MDA and TAS of the MS patients groups before starting therapy with the control group

Parameters	Mean ± SD		
	Antioxidant vitamins group before (n=30)	Placebo group before (n=30)	Control group (n=30)
MDA	2.01 ± 0.53 a	1.85 ± 0.51 a	0.90 ± 0.19 b
TAS	0.91 ± 0.40 a	0.93 ± 0.26 a	1.99 ± 0.25 b

- (a , b) different letters (transversely), means significant difference

By the comparison of the mean serum level of MDA, TAS, and EDSS before and after receiving antioxidant vitamins, there were a highly significant decrease in mean serum MDA and EDSS but a highly significant increase of the mean serum TAS of the patients after 3 months antioxidant vitamins therapy, as shown in table (3).

Table 3 : Comparison between mean serum level of MDA, TAS, and EDSS of the antioxidant vitamins group before and after receiving antioxidant vitamins

Parameters	**Mean ± SD**		**P-Value**
	Antioxidant vitamins group before (n=30)	**Antioxidant vitamins group after (n=30)**	
MDA (µmol/l.)	2.00 ± 0.53	1.05 ± 0.56	<0.001
TAS (mmol/l.)	0.91 ± 0.40	1.81 ± 0.41	<0.001
EDSS	3.25 ± 1.54	2.26 ± 1.45	<0.001

Table (4) illustrates that there were no significant differences in the mean serum level of MDA and TAS and EDSS value after receiving placebo therapy for 3 months, when compared with their level before therapy.

Table 4 : Comparison between mean serum level of MDA, TAS, and EDSS of the placebo group before and after receiving placebo

Parameters	**Mean ± SD**		**P-Value**
	Placebo group before (n=30)	**Placebo group after (n=30)**	
MDA (µmol/l.)	1.85 ± 0.51	1.80 ± 0.49	NS
TAS (mmol/l.)	0.93 ± 0.26	1.01 ± 0.38	NS
EDSS	3.21 ± 1.42	3.18 ± 1.33	NS

Table (5) demonstrates that the use of antioxidant vitamins for 3 months resulted in a significant lower level of mean serum MDA, but a significant higher level of mean serum TAS level and a significant lower value of EDSS when compared with 3 months use of placebo.

Table 5 : Comparison of mean serum level of MDA, TAS and EDSS between the antioxidant vitamins group and placebo group after 3months therapy

Parameters	Mean ± SD		P-Value
	antioxidant vitamins group after (n=30)	Placebo group after (n=30)	
MDA (µmol/l.)	1.05 ± 0.56	1.80 ± 0.49	<0.0001
TAS (mmol/l.)	1.81 ± 0.41	1.01 ± 0.38	<0.0001
EDSS	2.26 ± 1.45	3.18 ± 1.33	0.03

IV. Discussion

In this study serum MDA level was found significantly higher in patients with MS of both groups (antioxidant and placebo) before starting therapy, than the healthy control subjects. Several studies have demonstrated an increase in the levels of lipid peroxidation, evaluated by measurement of MDA in plasma, serum and in the cerebro spinal fluid (CSF) of MS patients with respect to healthy subjects [25-28].

Lipid peroxidation has been implicated in the pathogenesis of MS [28]. Increased MDA level which is the consequence of lipid peroxidation and a marker of oxidative stress is an evidence of exaggerated oxidative stress in these patients. Progression of the demyelination process and increase of the MS severity enhanced the intensity of LPO in patients with MS, manifested by increased levels of primary LPO products [2].

Karg *et al.*, [29] and Vinychuk *et al.,* [30] reported that the plasma lipid peroxides levels were increased followed by decreased vitamin E level in MS patients. Koch *et al.,* [31] measured blood plasma lipid peroxidation in different types of MS , they found significantly higher levels in all types compared with the control. Besler and Comoglu [32] found an increase in plasma levels of oxidized lipoproteins and a decrease of antioxidant vitamins in their study on 24 MS patients, and Ferretti *et al.,* [33] reported an increase in plasma lipid peroxidation in patients in an early stage of the disease .

Regarding the measurement of TAS, which is better than measurement of individual antioxidant enzyme , because it reflects the whole antioxidant status of the body, and still another antioxidants substances not discovered yet. Therefore measurement of plasma total antioxidant capacity may give a more precise indication of the relationship between antioxidants and disease[2]. In this study TAS concentration was lower in MS patients, when compared with the control group. The results in this study are in accordance with the results of some previous studies that compared the concentrations of TAS and individual antioxidant enzymes in MS patients [2, 28, 34,35]. Choi *et al.,* [36] reported that glutathione (GSH) levels were lower in patients with MS, while Miller *et al.,* [37] found a low levels of superoxide dismutase (SOD) in patients with MS .

Jimenez-Jimenez *et al.,*[38] compared the serum levels of vitamin E in 36 patients with MS and 32 matched controls. They found that the serum level of vitamin E was significantly lower in patients with MS than controls. Besler *et al.,* [27] found that vitamin E levels were significantly lower in 24 patients with MS than in 24 controls. Salemi *et al.,* [39], found significantly lower levels of vitamin E in 40 patients with MS than in 80 healthy controls. The decrease of the concentration of vitamin E, the major hydrophobic chain-breaking antioxidant, confirms the possible involvement of this vitamin in MS pathology.

Vitamin C (ascorbic acid), has antioxidant properties. Its level is decreased in the blood of patients with MS during a relapse compared with those in the remitting phase, which might point to increased antioxidant demand during active demyelination [27].

Central nervous system (CNS) is particularly susceptible to ROS induced damage due to the high oxygen demands of the brain and low concentration of antioxidants, both enzymatic and non-enzymatic antioxidants . It is suggested that antioxidant status was altered and low activity of antioxidant enzymes were observed in CNS of MS patients . Enzymatic and non enzymatic antioxidants could regulate function of different immunologic cells in MS. In addition, an impairment of antioxidant defense systems in MS patients may results in their higher susceptibility to ROS and cause damage of CNS [28] .

The question arises whether oxidative stress in MS, contributes to pathology or whether it is a non-specific epiphenomenon. Evidence for an important role of oxidative stress in the pathogenesis of this disease

should come from clinical trials with antioxidant drugs. However, clinical trials of antioxidant treatments in MS and other neurodegenerative diseases are lacking[34,40].

Data obtained from the present study demonstrated a beneficial effects of the administration of vitamins C and E combination on MDA and TAS levels (significant lowering of MDA, and significant raise of TAS) in MS patients, and improvement in the mean EDSS from 3.25±1.54 to 2.26 ±1.45 with mean difference -0.98±0.77, compared with the placebo group which show no such improvement (a mean difference of -0.13±0.31).

Review of literature provides limited information on the usefulness of vitamin E and vitamin C in patients with MS. The effects of antioxidant vitamins on the course of MS have not been formally assessed in humans, but animal studies provide some rationale for a role in MS[41]. Therefore according to the author knowledge, this study is the first that concerned with the use of vitamin C and vitamin E in patients with MS, because all available studies were done in animal models.

Several animal studies have been performed on the effect of vitamin E on de- and remyelination. The first study[42] used the ethidium bromide demyelinating model. The authors found that treatment with vitamin E and ebselen (an organo-selenium compound possessing antioxidant property) protected against demyelination caused by ethidium bromide. Furthermore, they described that ebselen and vitamin E interfered with the cholinergic neurotransmission by altering acetylcholinesterase activity in the different brain regions and in the erythrocytes. In a follow-up study in the ethidium bromide demyelinating model, it was demonstrated that vitamin E reduced the ethidium bromide-induced damage and increased the endogenous remyelination of hippocampus in rats [(43)]. Also vitamin E attenuates demyelination and potentiates remyelination in animal models of toxin mediated demyelination [42-43].

In conclusion: Three months antioxidant vitamins (vitamin C and E) supplementation in RRMS patients causes a significant improvement of the oxidant / antioxidant status in patients with RRMS patients and a significant improvement of the clinical course of MS patients represented by EDSS.

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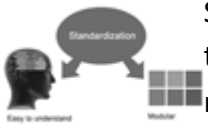

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Discussion:

The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a argument should be. Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly described. Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved with prospect, and let it drop at that.

- Make a decision if each premise is supported, discarded, or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
- Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work
- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

- When you refer to information, differentiate data generated by your own studies from available information
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.

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Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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