# Influence of Occupational Noise on Insulin, Blood Glucose, Homocysteine, Blood Pressure and Heart Rate

Mohammed Fezil, Narmadha*. M. P, Babu Benson

Department of Pharmacy practice, Nehru college of Pharmacy, Pampady, Thiruvilwamala, Thrissur district

E.Mail narmadhasam@yahoo.co.in

## ABSTRACT

**Background:** It has been suggested that noise exposure increases the risk of hypertension. Occupational noise is the dominant source of community noise exposure.

**Objective:** To study the influence of Occupational Noise on Insulin, Blood Glucose, Homocysteine, Blood Pressure and Heart Rate

**Methods:** The study group included 100 workers employed in different firms exposed to occupational noise exceeding 80 dB and 50 workers exposed to occupational noise less than 80 dB. The noise levels were measured using standard noise level meter. Selected subjects were screened for the above mentioned parameters.

**Results:** Values of the above said parameters were significantly higher in the groups exposed to noise above 80 dB. For the high noise exposed group all the parameters, except homocysteine were significantly higher than the normal values.

**Conclusion:** The results indicate that industrial occupational noise could be a possible contributing factor for the development of hypertension and diabetes.

## INTRODUCTION

The term noise is commonly used to describe all sounds that are disagreeable or unpleasant and are produced by acoustic waves of random intensities and frequencies[1]. Some authors define noise as any audible acoustic energy that adversely affects the physiological or psychological well being of the people[2].

Noise is insidious of all industrial pollutants, involving every industry and causing severe hearing loss across every country in the world. Exposure to excessive noise is the major avoidable cause of permanent hearing impairment[3]. In 1971 OSHA (Occupational Safety and Health Administration) of U.S.A was the first organization to set standards in noisy work places. Later several organizations and nations including India (The Noise Pollution Rules, 2000) brought their own rules to set the noise limit in work places. But the noise standard to which it was amended has actually been around for a quite while.

Excessive noise pollution has been blamed not only for hearing damage and community annoyance but also for hypertension, cardiovascular diseases, lack of concentration, fatigue, heart trouble, plasma viscosity, high glucose, and reduced motor efficiency. Exposure to noise causes physiological activation including increase in heart rate and blood pressure, peripheral vasoconstriction and thus increased peripheral vascular resistance[3].

In India, occupational permissible exposure limit for 8 h time weighted average is 90 dBA[4]. The major industries responsible for excessive noise and exposing workers to hazardous levels of noise are textile, printing, saw mills, power houses, mining, etc. Studies carried out by the National Institute of Occupational Health, India, showed that the sound pressure levels are very high in various industries of India. Hearing protectors should be used when engineering controls and work practices are

not feasible for reducing noise exposure to safer levels, which is unfortunately still not brought into practice in India.

Noise Induced Hearing Loss (NIHL) has been a compensable disease since 1948 under the Employees State Insurance Act (1948) and the Workmen's Compensation Act (1923) [3]. But still there is very little awareness regarding this fact. Awareness should be created among the workers about the harmful effects of noise on hearing and other body systems by implementing compulsory education and training programs.

Studies have been conducted to analyze the impact of noise on several parameters. It has been found that noise exposure has a relation with blood pressure, heart rate, Catecholamine and Cortical secretion 5-10. The results from the previous studies are heterogeneous and inconsistent.

The other chronic risk factors like raised Blood Pressure, Homocysteine and Blood Glucose level which can lead to Hypertension and Diabetes are still not taken into account. The chances of such disease occurrence should be seriously studied and taken sufficient and suitable action to reduce the severity and probability.

Many studies have been conducted in western countries to study the impact of road traffic noise, aircraft noise and occupational noise on health. Most of them states that a negative impact exists. Moreover, the occupational environments in the developed nations are strictly controlled and standardized. In India, the regulatory standards are not strictly brought into practice. Hence chances for unsafe occupational environment exist and the workers might be exposed to high levels of noise.

There are very few published studies of occupational noise induced health effects in India. More extensive studies are needed to know the exact prevalence of occupational noise induced health effects in India.

## Methods

### Subjects and experimental protocol

The study subjects were workers operating in high noise atmospheres like Spinning mills, Hydroelectric power house, Traffic police, and International Airport and for distinguishing the parameters, subjects were also selected from less noisy atmosphere like Textile shops and Higher Secondary Schools. 25 individuals were selected from each working environment after preliminary interview; their details were recorded and were asked to answer a questionnaire. The selected patients had a minimum of 5 years experience in the specific working class and were of normal BMI. Subjects with a history of smoking, inner ear diseases, cardiovascular diseases, renal dysfunction, diabetes, hypertension and use of ototoxic drugs were excluded.

### Measurement of sound level

Existing noise levels were measured using sound level meter (MEXTECH industries, Chennai, India) during the first visit. The readings were taken in 'A' weighted decibels (dBA). From each study centre, Lmax (maximum sound level) and Leq (equivalent continuous A sound level) for one hour was measured. In case of traffic police and school teachers, the sound prevalence near each subjects were measured and average value was recorded.

### Blood pressure and haematological assessment

At the day of blood collection patients were asked to take food early in the morning and to assemble in the study centre (minimum three hours after food). 8ml of blood was drawn from the forearm site of each subject and placed equally into two separate tubes. Blood in the first tube was centrifuged and plasma sample was kept in a mobile freezer (-20 C) for further analysis of homocysteine and glucose levels. In the second tube, serum was prepared and was saved in the mobile freezer for further analysis of insulin. Care was taken to clearly label each tube properly.

Blood pressure and heart rate of each patient were measured after keeping them at rest for 5 minutes before they started work and after their work schedule in the noisy atmosphere.

### Statistical analysis

Statistical analysis was done, by using Instat Graphpad3, version 3.01. Results were expressed as Mean ± Standard Deviation (SD). Multiple ANOVA followed by Tukey-

Kramer Multiple Comparisons Test was performed for comparing 3 or more groups. Students't' test was used to analyse the significance in two groups. Values of 'P' less than or equal to 0.05 were considered statistically significant

## Results

All the subjects selected in this study were in the age group of 30-45 years. Table 1 shows the noise level at different sites. According to the level of noise recorded from the study sites, the subjects were broadly classified into high noise exposed, moderate noise exposed, and low noise exposed

**Table 1: Noise level of the study sites**

| Study centres | Type of noise | Lmax (dbA) | Leq (dbA) |
|---|---|---|---|
| *Highly noise exposed* | | | |
| Spinning mill | Continuous | 109 | 101 |
| Hydroelectric powerhouse | Continuous | 116 | 109 |
| *Moderately noise exposed* | | | |
| Traffic police station | Intermittent | 106 | 89 |
| Airport | Intermittent | 111 | 91 |
| *Low noise exposed* | | | |
| Textile shop | Intermittent | 63 | 56 |
| Higher secondary school | Intermittent | 65 | 58 |

Comparison of Homocysteine, Blood glucose and Serum insulin levels (Fig 1, 2 and 3) among the groups showed a significant difference among the groups (P value <0.0001, =0.0008 and <0.0001 respectively).

**Figure:1** Comparison of homocysteine level

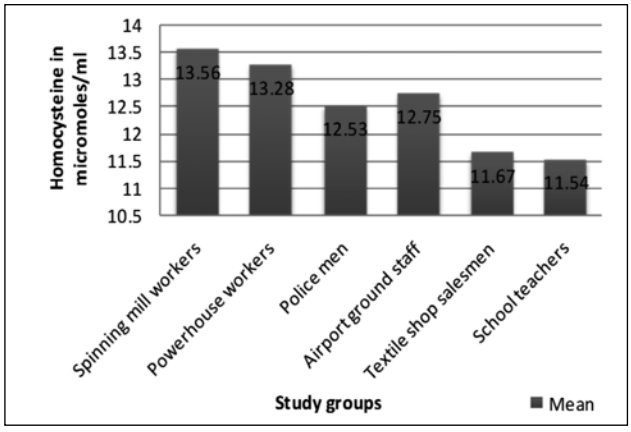



**Figure: 2** Comparison of glucose level

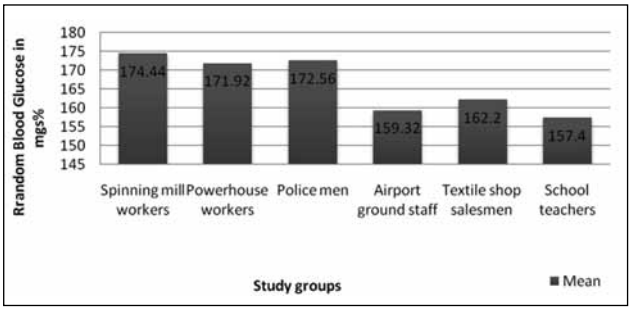
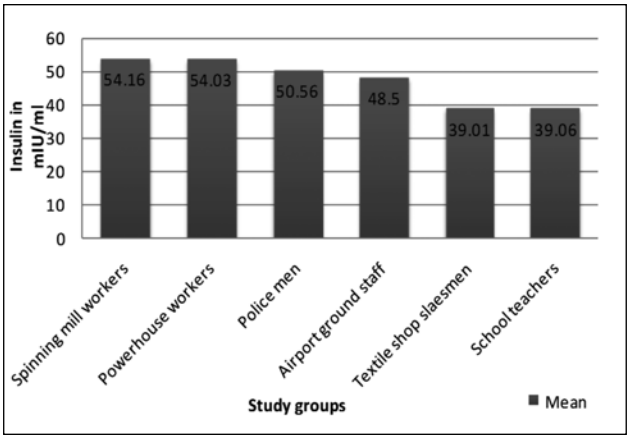


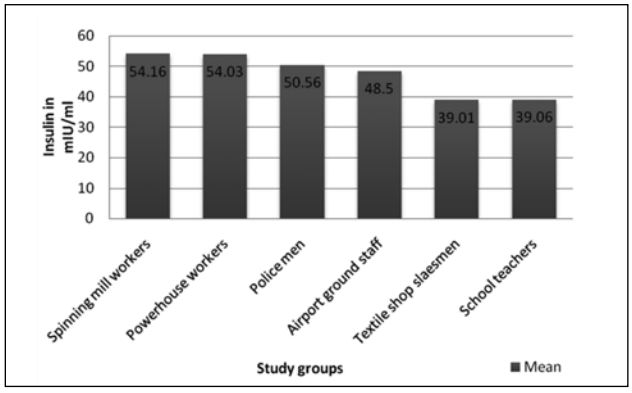

**Figure: 3** Comparison of insulin level




The order of level of the parameters are spinning mill workers > Powerhouse workers > Police men > Airport ground staff > Textile shop salesmen > School teachers.

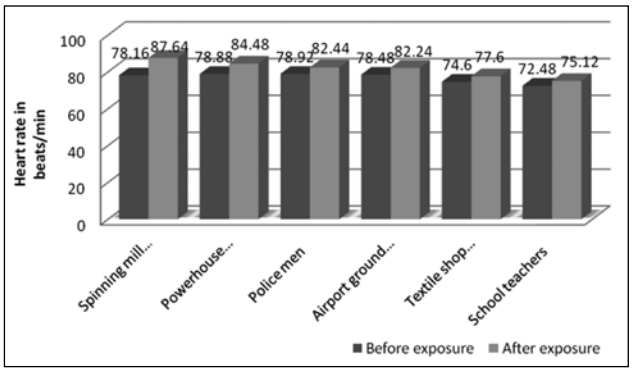
When before exposed and after exposed blood pressure readings were compared, the highly noise exposed groups showed a high increment in the blood pressure upon exposure to noise. Fig 4 shows the increment in the blood pressure after being exposed to noise (after exposed blood pressure minus (-) before exposed blood pressure).

**Figure 4:** Increase in blood pressure after exposed to occupational noise.



From the comparison of heart rate (Fig 5) we found that highest heart rate was seen in highly noise exposed groups. The highest increase in heart rate was also seen in highly noise exposed groups.

**Figure 5:** Comparison of heart rate of study population




The order of increase in heart rate is, spinning mill workers > Powerhouse workers > Police men > Airport ground staff > Textile shop salesmen > School teachers.

In order to correlate the findings with the duration of exposure, subjects of each group were subdivided according to the duration of exposure. The years of working experience in the field was taken as duration of exposure [Present age minus (-) age of initializing to the job]. The mean values of homocysteine, glucose and insulin was calculated for each sub group and correlated with the duration of exposure (Table 3). The correlation results show that the high noise exposed groups showed a significant increase with time in the glucose and insulin values. There was no significant increase in homocysteine levels with respect to time.

**Discussion**

Authorities have set the limit of permissible noise to 55 – 45 dbA at residential zones and 80 – 75 dbA at industrial zones. 80 dbA is the maximum sound limit at which hormonal homeostasis is maintained. Above 80 dbA 11, the sympathetic nervous system gains dominance. Occupational noise is one of the main annoying stressor to which a large population is being exposed for a long duration daily. Most blue collar workers in the industry have no control over their jobs. With the rising unemployment, workers in India, have no control over the type of work environment they can choose for themselves. This creates a sense of helplessness.

Loud occupational noise may serve as a potent external stressor, similar to sudden emotional stress and physical exertion, to activate the sympathetic nervous system and endocrine system. Nocturnal sleep architecture is also disturbed in healthy subjects who are exposed to loud occupational noise 12.

Previous studies have proven the association between noise and blood pressure, heart rate, cardiovascular diseases, cortisol imbalance, etc; but the results are inconsistent. The reason for discrepancies may be related to difference in workplace noise levels, duration of exposure, exposure assessment methods, study designs, and sample size across different studies.

In order to rule out the influence of other occupations (both previous and side), we have selected individuals who have worked only in the present job. We have selected only male subjects due to the difficulty in availability of female patients in all working classes. We have also taken care to eliminate all the confounding parameters like smoking, diabetes, ear diseases, ototoxic drug use, hypertensive patients, and those with history of cardiovascular diseases 13.

We have set an age limit (30-45 years) because the age itself can affect the measured parameters. Subjects with a minimum of 5 year experience in the specific working class were only selected. A minimum time of exposure will be necessary for a chronic change in biochemical parameters.

This study did not take into account other environmental factors that are associated with cardiovascular diseases such as noise exposure from road traffic noise, air pollution in residence area, etc. These relevant factors may be non-differentially distributed in the highly noise exposed group and low noise exposed group, and thus may not substantially affect the observed associations.

Previous studies have already proved that exposure to noise will increase Cortisol section.5, 14-16. The aim of measuring insulin and glucose level in our study was to rule out the role of Cortisol in inhibiting insulin action as proven in the previous studies.17, 18

The results of this study, impact of occupational noise on the measured biochemical parameters gave marginal support to the hypothesis that, noise has a negative impact on the health.

As far as glucose levels are concerned, there is a statistically significant difference in glucose levels between the groups (P=0.0008). The highest level was found in the spinning mill workers (174.44 ± 15.24) and the lowest level was found in school teachers (157.4 ± 9.13). The glucose levels are gradually decreasing while travelling from highly noise exposed groups to low noise exposed.

Comparison of insulin levels also shows that the levels are high in highly noise exposed groups. The mean insulin levels of spinning mill workers and generator circle technical staff are 54.16 ± 7.67 and 54.03 ± 7.46 respectively. This levels itself comes in the abnormal range of insulin (Normal Insulin level [Random] = <50 mIU/ml). The lowest levels are seen in textile shop salesmen (39.01± 6.5).

Taking glucose and insulin levels into consideration, we can assume that the subjects are gradually proceeding to insulin resistance, which may in future make them diabetic. The previous studies have proved that exposure to loud noise will increase the Cortisol secretion and Cortisol levels in body. By opposing the actions of insulin, glucocorticoids could contribute to insulin resistance and its association with other cardiovascular risk factors 19. Chronic increase in insulin levels can gradually progress into Diabetes mellitus. Correlating insulin and glucose levels with the duration of exposure, a significant increase with time was shown by highly noise exposed groups ($r = 0.7221$ for glucose and $r = 0.4241$ for insulin, $P < 0.0001$), lesser by moderately exposed and a non significant increase by low exposed groups. Comparing the homocysteine levels between the groups showed a statistically significant difference ($P< 0.0001$), with the highest level in spinning mill workers (13.56 ± 1.73) and lowest in school teachers (11.54 ± 1.04). But the increase in homocysteine was not biologically significant (Normal homocysteine level = <13 micromoles/ml). Increasing the level from 13-30 micromoles/ml is considered as moderate hyperhomocysteinaemia. In our study population, the maximum mean level of homocysteine seen is 13.56 ± 1.73, which is the lower limit of moderate hyperhomocysteinaemia. This by itself may not produce any major cardiovascular complications.

We also found an elevated risk of hypertension for subjects exposed to high levels of noise. When the blood pressure after exposure and before exposure was compared, an approximate increment of 8.96 and 6.88 mmHg was seen in systolic and diastolic blood pressure respectively in the highly exposed groups. The increment in the blood pressure on exposure to noise was comparatively less in moderately exposed (5.84 and 2.6 mmHg) and less exposed working groups (2.44 and 1.2 mmHg).

The morning blood pressure before exposure to noise was also seen raised in spinning mill workers (132.68 ± 10.52 systolic and 81.2 ± 9.8 diastolic) and generator circle technical staff (133.48 ± 11.68 and 82.28 ± 7.2 diastolic). The blood pressure before exposure in school teachers are 120.04 ± 5.4 systolic and 79.04 ± 5.07 diastolic. The details point out that an elevated risk of hypertension exist in highly noise exposed groups. We were also able to see an increase in heart rate in highly noise exposed groups.

Our findings of increased risk of hypertension in highly noise exposed groups are in agreement with several recent studies. The association with hypertension is relatively well established for occupational noise exposure as pointed out in recent reviews and research articles 20-24.

The subjective responses to the questionnaire were as expected. Subject from the highly exposed groups scored more than the other groups. Subjects from highly exposed groups reported to have frequent headaches, problems in sleeping and impaired concentration.

From our study; apart from homocysteine, all other measured parameters showed a significant and proportional relation to the amount of noise. The overall view was that all the parameter showed an increase: highest in highly exposed groups, moderate in moderately exposed groups, and least in low noise exposed groups.

Exposure to high noise levels makes chronic changes in insulin, glucose, and blood pressure levels in the subjects. Increased levels of insulin for a long time can cause insulin resistance, which can progress into diabetes mellitus. Our study proves that individuals exposed to high occupational noise are also under the

risk of hypertension. Increased blood pressure along with increased glucose level may alleviate the chances for cardiovascular diseases. Previous studies suggest that the subjects exposed to high occupational noise can also have a sudden sensorineural hearing loss, most probably after their retirement 25, 26.

Even though the subjects of high noise environments are progressing into diseases like diabetes and hypertension due to their occupational atmosphere, these are not covered under their insurance and they will have to carry the burden themselves. Hearing loss is mainly acquired after the retirement, which also falls after their insurance coverage period. Increasing unemployment, lack of awareness, and lack of alternatives are the factors inhibiting the workers from the change of job.

The subjects participating in the study were counselled about the undesired effects of occupational noise. They were also educated about the methods of reducing the harmful effects like, usage of ear plugs (commercially available or simple cotton plug), avoiding practice of hearing music or watching TV in high volume, etc.

During this study, we too have experienced the irritability of high noise. We have also experienced a confused state when exposed to the noise in our study site. Very few studies have been conducted regarding the impact of noise on the central nervous system and the cognitive functions. In a previous study it was found that traffic noise has a linear association with impaired reading comprehension in students 27. Another study conducted shows that noise significantly elevates stress among children at ambient levels far below those necessary to produce hearing damage 28. We suggest that studies may be conducted to explore the effect of noise on the mental and cognitive functions.

**Conclusion**

The study revealed that continuous exposure to high occupational noise increases glucose and insulin levels in the body. The study observed that subjects working in noisy atmospheres are at high risk of developing hypertension as they showed elevated blood pressure and heart rate levels. Stress questionnaire response revealed that the subjects working in noisy atmosphere are also under mental stress.

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# The Impact of Lifestyle Modification on Metabolic Syndrome; An Evidence Based Approach Through Systematic Review and Meta-Analysis

Vijayakumar Arumugam*, Geethu George, Jyothi Susan George, Merin Joseph, Sreedevi Paramboorillam Vasudevasharma and Manisenthilkumar KT

Regional Pharmacovigilance Center (PvPI), Department of Pharmacy Practice, KMCH College of Pharmacy, Coimbatore - 641 048

*Corresponding Author: vijayspharm@gmail.com

## ABSTRACT

Lifestyle modification intervention is a known non-pharmacological management for Metabolic Syndrome. The previous results of impact of LMI on patients with metabolic syndrome were controversial. The present study was aimed to provide Evidence Based Information on the impact of lifestyle modification intervention on metabolic syndrome by comparing subjects who treated with usual care and those with usual care and lifestyle modification intervention, by assessing the metabolic components such as waist circumference, diastolic blood pressure, systolic blood pressure, fasting blood sugar, triglycerides and high density lipoprotein. A systematic review was conducted in Pubmed, Science Direct, and Cochrane databases and seven randomized control trials were included based on inclusion and exclusion criteria. The comparison of metabolic syndrome components before and after lifestyle intervention was performed and the results of meta-analysis revealed that lifestyle modification was effective in resolving waist circumference [CI -0.829 to -0.055; $p<0.05$], diastolic blood pressure [-0.752 to -0.254; $p<0.001$], systolic blood pressure [-0.463 to -0.190; $p<0.001$], and triglycerides [-0.379 to -0.107; $p<0.001$]. Lifestyle modification intervention has not shown significant impact on fasting blood sugar [0.030 to 0.975; $p>0.001$] and high density lipoprotein [0.010 to 0.075; $p>0.05$]. Publication bias was observed by funnel plot. Egger's regression test and heterogeneity test were also performed. These findings are being reported with respect to metabolic syndrome and lifestyle modification intervention and our observation suggest that lifestyle modification intervention have greater impact in the management of waist circumference, diastolic blood pressure, diastolic blood pressure, triglycerides but not in case of high density lipoprotien and fasting blood glucose.

**Key words:** lifestyle modification intervention, metabolic syndrome, dietary intervention and metabolic syndrome, physical activity & exercise and metabolic syndrome,

**Abbreviations:** MetS: Metabolic syndrome; **LMI:** Life style modification intervention; **WC:** Waist circumference; **DBP:** Diastolic blood pressure; **FBS:** Fasting blood sugar; **HDL:** High density lipoprotein, **SBP:** Systolic blood pressure; **TG:** Triglycerides; **RCT:** Randomized Control Trial; **CMA:** Comprehensive Meta-Analysis; **CI:** Confidence Interval

## INTRODUCTION

Metabolic syndrome is a constellation of inter - related risk factors (elevated blood pressure, elevated plasma glucose, atherogenic dyslipidaemia) that are due to mainly abdominal obesity and insulin resistance, but also due to physical inactivity, aging ,and hormonal imbalance. These inter-related risk factors appear to directly increase the risk for developing atherosclerotic cardiovascular disease, and type 2 diabetes mellitus1-4.

There are several definitions for metabolic syndrome which are given by IDF (International Diabetes Federation), WHO (World Health Organization), NCEP (National Cholesterol Education Program Adult

Treatment Panel III), EGIR (European Group for the Study of Insulin Resistance), AHA/NHLBI (American Heart Association/Updated NCEP).5

The International Diabetes Federation defines metabolic syndrome as the presence of central obesity (defined as waist circumference with ethnicity-specific values) and any two of the following:

- Raised triglycerides: >150mg/dL (1.7mmol/L), or specific treatment for this lipid abnormality
- Reduced HDL cholesterol: <40mg/dL (1.03mmol/L) in males, <50mg/dL (1.29mmol/L) in females, or specific treatment for this lipid abnormality
- Raised blood pressure (BP): systolic BP>130 or diastolic BP>85mmHg, or treatment of previously diagnosed hypertension
- Raised fasting plasma glucose (FPG): >100 mg/dL (5.6mmol/L), or previously diagnosed type 2 diabetes.6-8

The US National Cholesterol Education Program Adult Treatment Panel III (2001) requires at least three of the following:

- Central obesity: waist circumference ≥102 cm or 40 inches (male), ≥88 cm or 35 inches(female)
- Dyslipidemia: TG ≥ 1.7mmol/L (150 mg/dl)
- Dyslipidemia: HDL-C <40 mg/dL (male), <50 mg/dL (female)
- Blood pressure: ≥130/85mmHg (or treated for hypertension)
- Fasting plasma glucose ≥ 6.1mmol/L (110 mg/dl) 9-10

The prevalence of metabolic syndrome is increasing day by day. MetS is more prevalent with increasing age, affecting half of adults aged 60 years and over. It is more common in men when WHO or IDF criteria are used, but there is little difference between the sexes when the NCEP definition is used. The National Health and Nutrition Examination Survey 1999–2002 estimated the age-adjusted prevalence of Mets in US adults aged 20 years and over to be between 34.6% (NCEP) and 39.1% (IDF). The metabolic alterations and several factors occur simultaneously, which thereby increases cardiovascular risk over and above the risk associated with the individual factors alone. The risk increases with the number of MetS components present. Ethnicity also influences Mets prevalence. The prevalence of MetS is increasing in parallel with population ageing and "epidemic" obesity. The rise in childhood obesity presents a challenging problem for the future.11-15

Lifestyle modification is the first-line therapy to prevent and treat MetS. The main lifestyle intervention includes nutritional intervention, promotion of physical activity, psychosocial care, and education. The most important therapeutic intervention effective in subjects with MetS should focus on modest weight reduction and regular leisure-time physical activities.16-19

Even though many randomized controlled trials were conducted on "Impact of Life Style Modification on Metabolic Syndrome", it is very difficult to compare the results of each article because of the controversies existing in types of life style interventions done and the results after the interventions. The extent of Impact of lifestyle modification on patients with MetS is yet to be identified. So this study was an attempt to assess the effects of lifestyle modification (Diet + Exercise) on the reduction of MetS and the extent of improvement in values of MetS components in subjects with this syndrome. We conducted a systematic review and meta analysis of seven randomized controlled trials (RCTs) that assessed the impact of lifestyle interventions on MetS using the software CMA(Comprehensive Meta Analysis) .We also performed further statistical analysis which are relevant to the topic.

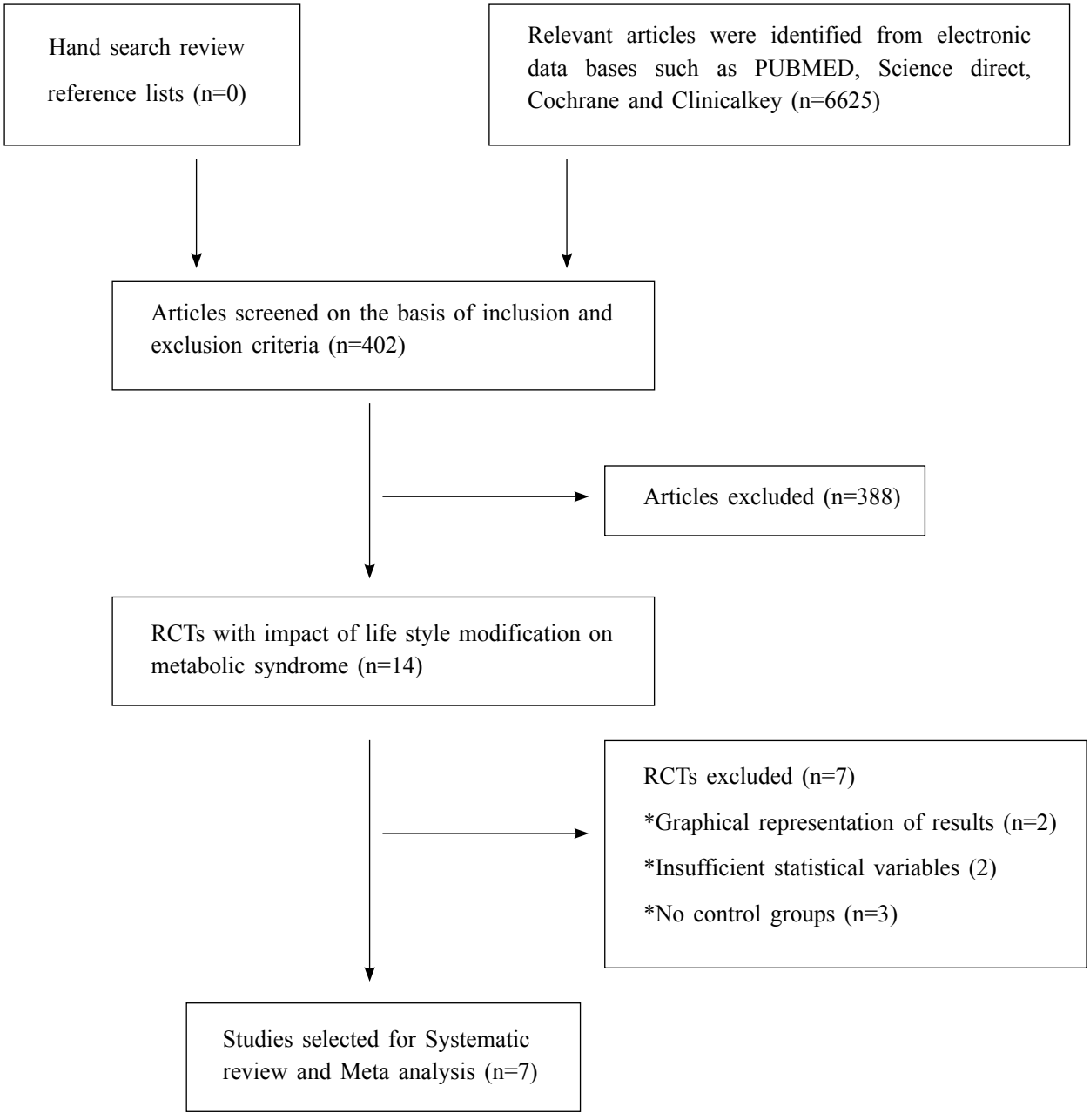
## MATERIALS AND METHODS

The objective of this meta-analysis was to determine the effect of lifestyle modification on metabolic syndrome by assessing the impact on waist circumference, systolic blood pressure, diastolic blood pressure, fasting blood sugar, triglycerides, and high density lipoprotein. Relevant studies were selected by means of hand search and electronic databases such as PUB MED, Science direct, Clinicalkey and Cochrane in a time period from 2000 January to 2013 December. We hand-searched the content pages of Journal of Australian Prescriber, British Medical Journal, Journal of Clinical Science, and Journal of Diabetic Care. The search was performed from 4 January 2014 – 22 February 2014.

The inclusion criteria were: randomised control trials published in English language, ttudies that were conducted from 2000 to 2013, RCT's studying the impact of lifestyle modifications on metabolic syndrome, RCT's that includes the metabolic parameters such as WC, DBP, SBP, HDL, TG, FBS, studies that considered both intervention and control group, Adult participants with metabolic syndrome were considered. Search terms used on the electronic databases were:-Lifestyle modification AND metabolic syndrome, Impact of lifestyle modification on metabolic syndrome, Dietary intervention AND metabolic syndrome, Physical activity AND metabolic syndrome, Exercise AND metabolic syndrome.

6625 articles were identified in the initial search by means of electronic databases (fig:1).

**Figure: 1** Flow diagram: Impact of Life Style Modification on Metabolic SyndromS



Eligibility assessment of these articles were performed independently in an unblended standardised manner by three reviewers. Disagreement between the reviewers was resolved by the fourth reviewer. Finally we selected the articles Boer et al20, Nanri et al21, Landaeta et al22, Marcelo et al23, Pettman et al24, Simona et al25, and Watkins et al26.All analysis were performed by using the software Comprehensive Meta-analysis (trial version).

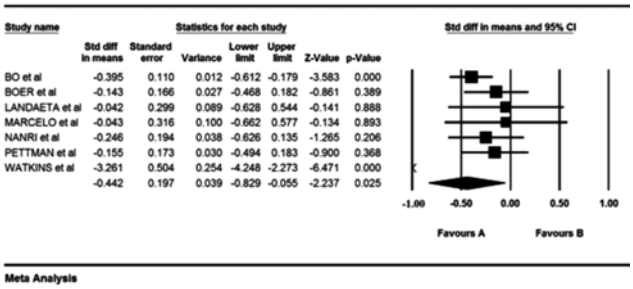
All effect measures obtained from original studies were continuous data, so standard difference in mean was used to calculate the effect size. All analysis was performed by using the Comprehensive Meta-analysis (trial version) software 27. Standard differences in mean with 95% CI for continuous variables were used to assess the effect of WC, DBP, SBP, HDL, TG, and FBS.

## RESULTS

### Waist circumference

Individuals assigned to the intervention group had decreased waist circumference than individuals randomized to the control group. There is a decrease of 4.16cm in the intervention and 1.14cm in the control group (Table I &II). From the forest plot, we observed that LMI favours intervention group ($p<0.05$) with narrow confidence interval (-0.829 to -0.055) (Figure:2).

**Figure 2: Forest plot that shows the impact on waist circumference before and after LMI using random effect model. The pooled SMD is represented by a diamond plot of standard height, with the width indicating 95% CI.**




The effect size was similar in all the studies and p-values differ only because of sample size which is larger in Bo et al., and Watkins et al. The funnel plot was computed and we obtained an asymmetrical plot which implicated chances of bias. The eggers' test was performed (p=0.186) which indicated absence of publication bias (Table: I).

**Table I: Results of publication bias study (Egger's Test)**

| S.no | Study | Egger's test p-value (one-tailed) |
|---|---|---|
| 1 | Waist circumference | 0.186 |
| 2 | Diastolic blood pressure | 0.050 |
| 3 | Systolic blood pressure | 0.455 |
| 4 | High density lipoprotein | 0.223 |
| 5 | Triglycerides | 0.152 |

The imputed funnel plot showed asymmetry and filled circles which may be due to heterogeneity. It was further confirmed using the heterogeneity test which gave an I2 value of 84.351, p value of 0.000 and Q value of 38.34 which indicates considerable heterogeneity (Table: II). The precision plot confirms accuracy of the forest plot. From the plot, it was observed that all the seven studies were within the confidence limits. The filled diamond from the imputed precision plot denotes the adjusted standard mean difference to get the précised results.

Table II: Results of heterogeneity study

| S.no | Study | Heterogeneity | | |
|---|---|---|---|---|
| | | I-squared | p-value | Q-value |
| 1 | Waist circumference | 84.351 | 0.000 | 38.340 |
| 2 | Diastolic blood pressure | 61.763 | 0.016 | 15.692 |
| 3 | Systolic blood pressure | 0.000 | 0.762 | 3.361 |
| 4 | High density lipoprotein | 40.631 | 0.120 | 10.106 |
| 5 | Triglycerides | 0.000 | 0.446 | 5.803 |
| 6 | Fasting blood sugar | 89.693 | 0.000 | 58.212 |

**Diastolic blood pressure**

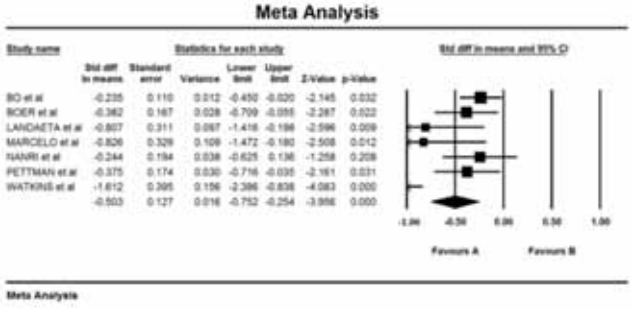
**Table III: Results of heterogeneity study**

| S.no | Study | Heterogeneity | | |
|---|---|---|---|---|
| | | I-squared | p-value | Q-value |
| 1 | Waist circumference | 84.351 | 0.000 | 38.340 |
| 2 | Diastolic blood pressure | 61.763 | 0.016 | 15.692 |
| 3 | Systolic blood pressure | 0.000 | 0.762 | 3.361 |
| 4 | High density lipoprotein | 40.631 | 0.120 | 10.106 |
| 5 | Triglycerides | 0.000 | 0.446 | 5.803 |
| 6 | Fasting blood sugar | 89.693 | 0.000 | 58.212 |

**Diastolic blood pressure**

LMI produced significantly reduced mean values in a population of 912 patients with metabolic syndrome compared with the control and intervention group when determined by the random effect model (fig 3). Seven trials reported an average decrease of 5.9mmhg in the intervention group. The effect size was similar in most of the studies and more significant p-value ($p<0.001$) reflected a larger effect size on intervention group. The funnel plot was computed and an asymmetrical plot was observed which indicated chances of bias. Egger's regression analysis was performed ($p=0.003$) and it indicated the presence of publication bias (Table I). The imputed funnel plot showed asymmetry which could be due to the presence of bias. The heterogeneity test gave an I2 value of 61.763, p value of 0.016 and Q value 15.692 which indicated substantial heterogeneity (Table II). From the precision plot, it was observed that studies were not within the confidence limits.

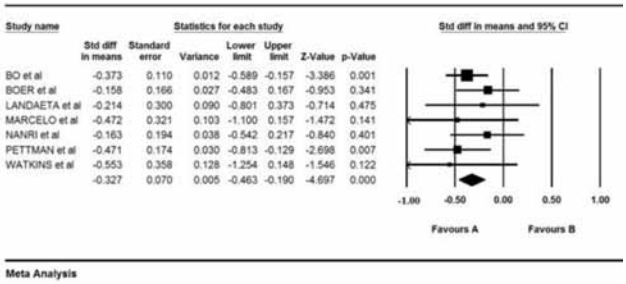
**Figure 3: Forest plot that shows the impact on diastolic blood pressure before and after LMI using random effect model. The pooled SMD is represented by a diamond plot of standard height, with the width indicating 95% CI.**




**Systolic blood pressure**

Physical activity and dietary interventions significantly decreased systolic blood pressure. There was an average decrease of 8.2mmhg in the intervention group and 3.4mmhg in the control group. Among the studies, only Bo et al., and Pettman et al., gives significant values, all others are insignificant. BO et al., gives the most significant p-value of 0.001 (95%CI 0.589 to -0.157) with more weightage to overall summary effect with relative weightage of 39.78. From the overall summary effect it was observed that LMI favours intervention group ($p<0.001$) with narrow confidence interval of -0.463 to -0.190 (fig 4).

**Figure 4: Forest plot that shows the impact on systolic blood pressure before and after LMI using random effect model. The pooled SMD is represented by a diamond plot of standard height, with the width indicating 95% CI.**

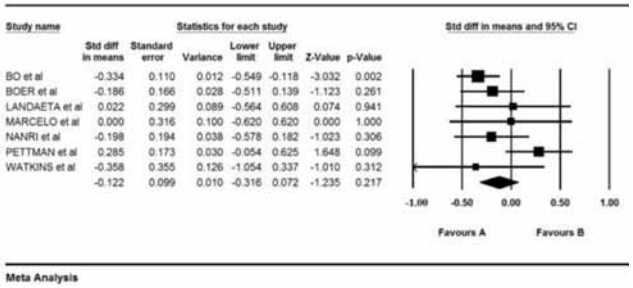



Symmetrical funnel plot indicating absence of bias was obtained. Egger's regression analysis ($p=0.455$) indicated absence of publication bias (Table I). The heterogeneity test gave an I2 value of 0.000, p value of 0.762 and Q value of 3.361 which denoted absence of heterogeneity (Table:II). From the precision plot it was observed that all the seven studies were within the confidence limits.

**High density lipoprotein**

All the seven studies (Bo et al., Boer et al., Landaeta et al., Marcelo et al., Nanri et al., Pettman et al., and Waktins et al) measured the effect of HDL. The level of HDL increased in both intervention and control group. In intervention group there is an increase of 1.2 and 0.2mg/dl in control group. The overall summary effect shows there is no significant effect of LMI on intervention group ($p> 0.05$; CI: -0.316 to 0.072) (fig 5).

**Figure 5: Forest plot that shows the impact impact on high density lipoprotein before and after LMI using random effect model. The pooled SMD is represented by a diamond plot of standard height, with the width indicating 95% CI.**

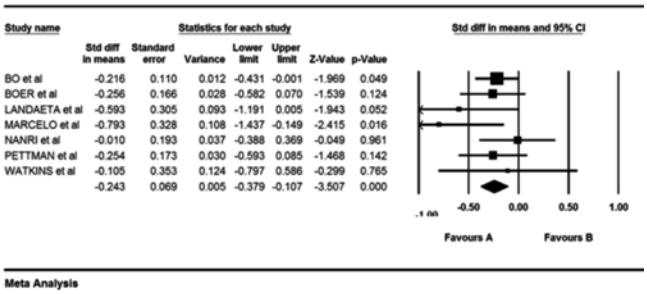


Asymmetrical funnel plot indicating presence of bias was obtained. Egger's regression analysis (p=0.223) indicated absence of publication bias (Table I). The heterogeneity test gave an I2 value of 40.631, p value of 0.120 and Q value of 10.106 which denoted moderate heterogeneity (Table III). From the precision plot, it was observed that all the seven studies were within the confidence limits.

**Triglycerides**

Intervention group had a greater decrease than control group in triglycerides, an average decrease of 38.3mg/dl and 15.05mg/dl in intervention and control group respectively. Among the studies, only Bo et al., and Marcelo et al., gives significant values and all others gives insignificant values. From the forest plot it was observed that LMI has larger effect on intervention group (p<0.001) with narrow confidence interval -0.379 to -0.107 (Fig 6).

**Figure 6: Forest plot that shows the impact on triglycerides before and after LMI using random effect model. The pooled SMD is represented by a diamond plot of standard height, with the width indicating 95% CI.**

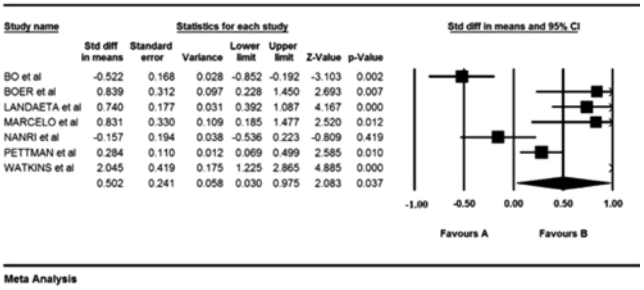



The effect was similar in all studies in which Bo et al trial contributed more with relative weightage of 40.01%. The funnel plot was plotted and obtained a symmetrical plot which indicated absence of bias. Egger's regression analysis (p=0.152) indicated the absence of publication bias (Table I). The heterogeneity test gave an I2 value of 0.000, p value of 0.4446 and Q value of 5.803, which denoted absence of heterogeneity (Table II). In the precision plot, all the studies were within the confidence limit which indicated the accuracy of forest plot.

**Fasting blood sugar**

Fasting blood sugar was measured in 912 patients with Mets. Bo et al., and Nanri et al., trial favours intervention. The level of FBS was found to be increased by 1.5mg/dl in the intervention group and reduced by 0.75mg/dl in the control group. From the forest plot it was observed that LMI had no effect on intervention group (fig 7).

**Figure 7: Forest plot that shows the impact on fasting blood sugar before and after LMI using random effect model. The pooled SMD is represented by a diamond plot of standard height, with the width indicating 95% CI.**




The overall summary effect gives a significant p-value (p<0.05) with confidence interval of 0.030 to 0.975. The funnel plot was computed and obtained an asymmetrical plot, which indicated possibilities of bi Egger's regression analysis (p=0.128) and indicated the absence of publication bias (Table I). The imputed funnel plot showed asymmetry and filled circles, which may be due to heterogeneity. The heterogeneity test gave an I2 value of 89.69, p-value of 0.00 and Q value of 58.212 which indicated considerable heterogeneity (Table II). From the precision plot, it was observed that studies were not within the confidence limits. The filled diamond from the imputed precision plot denotes the adjusted standard mean difference to get the précised result

There was only one meta-analysis work published on the same topic. "Effects of lifestyle modification on metabolic syndrome: a systematic review and Meta analysis"28 conducted by Kazue Yamaoka and Toshiro Tango and which was published in the journal of BMC in 2012. They evaluated the effect of lifestyle modifications on Mets. A cumulative meta-analysis by the random-effects model was performed to determine the point at which sufficient evidence was available

to show a beneficial effect of LMI. In our study we performed meta analysis individually for all the Mets components using Comprehensive meta analysis software and subgroup analysis was also conducted using the variables age an funnel plot in the previous study, where as, we did eggers test to soundly tell our publication bias results. The study concluded that values for five (fasting blood glucose, waist circumference, systolic blood pressure and diastolic blood pressure, triglycerides) of the six components of Mets (excluding HDL) were significantly reduced in the LMI groups compared with their control groups. These results are comparable with our results. The study found a weak tendency toward reduction in fasting blood glucose, and they mentioned the need for further evidence in order to confirm whether fasting blood glucose level is a good index as the component for glucose intolerance.. The study included both 'diet only' and 'diet plus exercise' intervention for the review but we included only those trials providing both diet and exercise intervention. The present study found that LMI has more impact on the Mets components except HDL, FBS, and hba1c values.

**DISCUSSION**

In this systematic review and meta-analysis, 402 relevant articles were identified through electronic databases and are screened for the inclusion exclusion criteria. Finally, seven articles were selected for conducting meta-analysis. The study was conducted in order to analyze the extent of impact of life style modification on Mets components. The pooled standard difference in mean value at 95% confidence interval and the results from the forest plot suggested that LMI contributes varied effect on each component. Our result shows that the components such as WC, SBP, DBP, and TG were significantly influenced by LMI. At the same time, the variables such as HDL and FBS were not influenced by the LMI.

The present study has several limitations. Dietary intervention in each trial was varied and the physical activity duration recommended in each trial was different. Most of the trials recommend 30 minutes exercise. Gender wise assessment is also not done as the articles doesn't mention it separately. Even though we have taken seven trials for the review we got good population size of 912 subjects with Mets. Future studies involving different type of combination therapies along with LMI are further needed to be identified.29-32

In conclusion, this meta-analysis provides strong evidence that a lifestyle modification reduces the metabolic syndrome abnormalities. Metabolic components such as waist circumference, diastolic blood pressure, systolic blood pressure, triglycerides, and fasting blood sugar were assessed and all components except fasting blood sugar and high density lipoprotein were found to be reduced after LMI.

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# Opimization of Microwave Assisted Extraction of *Pseudarthria viscida* timLeaves

Sheeba Jasmin.T S[1]*, N A Aleykutty[2], Jyoti Harindran[3], Aravind A[1], Neethu V[4]

[1]College of Pharmaceutical Sciences, Government Medical College, Thiruvananthapuram - 695011, Kerala, India.

[2]Caritas College of Pharmacy, Kottayam.

[3]University College of Pharmacy, M G University, Cheruvandoor, Kottayam

[4]Al Shifa College of Pharmacy, Perinthalmanna, Malappuram

*Corresponding author : sheebajasmints2003@yahoo.co.in

## ABSTRACT

Extraction of plant materials by hot percolation using soxhlet is time consuming and expensive. Microwave assisted extraction of plant material is getting more popular these days. Microwave assisted extraction is utilizing microwave energy. It helps for the partition of analytes from the matrix to the solvent. This work is focused on the optimization of various parameters for microwave assisted extraction of *Pseudarthria viscida* leaves in ethanol. The processing parameters are the proportion of the solvent, extraction time, and microwave power. The optimum extraction conditions were 30 ml of ethanol for 1g of the sample and extraction time 5 minutes at 850 W. Under these conditions maximum percentage yield was obtained by microwave assisted extraction. Major advantages are the reduced extraction time and solvent volume. In the present study sample to solvent ratio, total extraction time, microwave power etc. were optimized. But beyond a certain limit, a decrease in the extraction efficiency was noted.

**Key words:** *Pseudarthria viscida*, microwave assisted extrac

## INTRODUCTION

Phytoconstituents from the natural source can be separated by the process of extraction. Extraction is the method of release and solubilisation of secondary metabolites from the matrix. By the use of solvents, soluble target compounds can be separated. There are number of procedures adopted for the extraction of medicinal plants. Maceration, percolation, soxhlet extraction, infusion, decoction, supercritical fluid extraction etc are few among them. Microwave assisted extraction; molecular distillation, microdistillation etc are some of the modern methods of extraction.

Microwave assisted extraction is the method to extract active components by the application of microwave energy. It was first used in 1975 for acid digestion1. Ionic conduction and dipole rotations are the two processes under microwave assisted extraction. Moisture is present within the plant cell. Due to the microwave energy, the moisture gets heated and evaporated. On the cell wall it generates tremendous pressure. The cell wall gets ruptured and the active constituents are coming out to the surrounding solvent. Solubility, dielectric constant, extraction time, power of microwave energy etc may affect the microwave assisted extraction2. In case of microwave assisted extraction, extraction time is shorter and the solvent consumption is also lesser3. Heat labile components can also be extracted by microwave assisted extraction1. Flavanoids4 and plant phenolics also can be extracted by the application of microwave assisted method2. To achieve maximum extraction efficiency, the extraction methodology is to be optimized.

Pseudarthria viscida is known as Salaparni5 and more than 70 ayurvedic formulations contain

Pseudarthria viscida as one of the ingredient. The plant is a perennial shrub growing up to 50 cm to 1m in height. The branches are slender, contain 3 foliolate leaves. The leaflets are 7-10 x 5-7 cm and they are broadly ovate in shape. The apex of the leaflet is acute and the base is round. The petiole is 7cm long. The stipule is lanceolate. The flowers are small, papilionaceous, and the calyx is 2 tipped. The lobes are lanceolate. The petals are red or pink in colour. It is orbicular, retuse and wings are 3mm long. It is oblong and stamens are 9+1. Ovary is sessile and style inflexed. Pods are 2-3 x 0.4-5 cm oblong, hairy, and compressed. It contains 3-5 seeds, compressed, subreniform and brownish black in colour 5,6,7.

Habitat: Moist deciduous forest, forest plantation and plains, scrub jungles.

Flowering and fruiting: It flowers in May and fruits in June.

Distribution in Kerala: Throughout the state.

Part used: Leaf

The study focused to optimize the extraction time, microwave power ,and sample to solvent ratio for microwave assisted extraction of Pseudarthria viscida leaves to extract many compounds as possible.

## MATERIALS AND METHODS

### Plant material

The plant Pseudarthria viscida Linn. was collected from Kizhattoor, Malappuram district, Kerala. The plant material was taxonomically identified by A K Pradeep, Department of Botany, Calicut University, Kerala. The leaves of Pseudarthria viscida Linn were dried under shade and then powdered with a mechanical grinder. The powder was passed through sieve No. 42 and stored in an airtight container for further use.

### Microwave assisted extraction

Many parameters like particle size, extraction time, amount of solvent, microwave power etc affects the microwave assisted extraction. Based on a previous study, appropriate ranges of various parameters were selected. The experiment was done with a scientific microwave oven with reflux condenser assembly8. About 1g of the powdered material was treated with different volumes of ethanol (20-50 ml), stirred well and was irradiated for different time periods (2-7 minutes) and at different microwave power units. Filtration sample and evaporation of the solvent was done by rotary evaporation. The extraction efficiency was calculated.

### Optimization of sample-solvent ratio

The sample-solvent ratio was optimized by using different volumes of ethanol (20, 30, 40 and 50 ml). Quantity of material used was constant (1g), the time set was also constant (5 minutes) and the microwave power was set at 850W. Results are depicted in table 1.

Optimization of total extraction time

**Table 1: Microwave assisted extraction of *Pseudarthria viscida* leaves in different volumes of ethanol at constant time and power and the percentage yield obtained**

| S.NO. | Volume of solvent (ml) | Percentage yield obtained (%) |
|---|---|---|
| 1 | 20 | 6 |
| 2 | 30 | 10 |
| 3 | 40 | 10 |
| 4 | 50 | 10 |
| 5 | 60 | 10 |

### Optimization of total extraction time

For identifying the accurate extraction time for maximum efficiency the extraction was carried out at different time periods 2 to 7 minutes by using ethanol as the solvent. The power was kept constant at 850W. The observations are given in table 2.

table 2.

**Table 2: Microwave assisted extraction of *Pseudarthria viscida* at different time periods at constant power and the percentage yield obtained**

| S.NO. | Time (minutes) | Percentage yield obtained (%) |
|---|---|---|
| 1 | 2 | 4 |
| 2 | 3 | 5 |
| 3 | 4 | 8 |
| 4 | 5 | 10 |
| 5 | 6 | 10 |
| 6 | 7 | 10 |

### Optimization of power

For identifying the accurate power for maximum efficiency the extraction was carried out at different microwave power (800W, 850W, 900W, and 950W).

The quantity of material used was 1g and the solvent used was 30 ml. The time was kept constant at 5 minutes. The results are given in table 3.

**Table 3: Microwave assisted extraction of Pseudarthria viscida leaves at different microwave power at constant time.**

| S.NO. | Power (W) | Percentage yield obtained (%) |
|---|---|---|
| 1 | 800 | 6 |
| 2 | 850 | 10 |
| 3 | 900 | 10 |
| 4 | 950 | 10 |

## RESULTS AND DISCUSSION

*Pseudarthria viscida* extraction by using soxhlet method in ethanol is already reported9. But the method has so many disadvantages as the consumption of solvent and also the reaction time is more. Present study is focused to carry out the microwave assisted method of extraction to extract out the active constituents from the leaves of *Pseudarthria viscida*

### Optimization of sample to solvent ratio

From the table 1 it is clear that the highest efficiency of the extraction was obtained by keeping sample: solvent ratio at 1:30 (g/ml). So from the result it is clear that volume of solvent used for extraction is an important parameter to be considered.

### Optimization of total extraction time

The extraction was done at different time intervals with the power (850 W), material weight (1g) etc kept constant. Maximum percentage yield was obtained for an extraction time of 5 mins.

### Optimization of power

At constant time (5 minutes), using 1g of powdered plant material in 30ml of solvent, the extraction was carried out at different microwave power settings. The extraction efficiency was found to be highest at 850W.

## CONCLUSION

The Pseudarthria viscida leaf extraction by microwave assisted method was carried out at different time periods. For maximum yield, the time required was 5 minutes. Sample to solvent ratio 1:30 g/ml was the optimum one for highest extraction efficiency. But when the extraction time and volume of solvent was increased, there is no further increase in the efficiency. This study reveals that if we do the extraction of plant materials by using the microwave assisted method, better yields could be obtained and time can also be saved while comparing with the soxhlet extraction method. This study is to be continued to find out the various biological activities of microwave assisted ethanol extract of *Pseudarthria viscida* leaf.

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# Simvastatin Loaded Nanostructured Lipid Carrier Based Hydrogel Expedites Diabetic Wound Healing

Abdul Hasan Sathali A[1]*, Sudhakar S[1], Chidambaranathan N[2], Vijaya C[3]

[1]Department of Pharmaceutics, College of Pharmacy, Madurai Medical College, Madurai- 625 020, Tamil Nadu,

[2]Department of Pharmacology, K.M College of Pharmacy, Madurai, Tamil Nadu, India.

[3]Department of Pharmaceutics, Ultra College of Pharmacy, Madurai, Tamil Nadu, India.

*Corresponding author: Email: drabdulhasan@rediffmail.com

## ABSTRACT

A loaded nanostructured lipid carrier (SNLC) based hydrogel (HG) was developed as a potential Simvastatin dermatological formulation for diabetic wound healing. Hot homogenization followed by ultrasonication was employed to produce NLCs using lipids: stearic acid and oleic acid, surfactants: phospolipon 90G and Tween 80. The mean particle size of SNLC was 140.6 nm and the nanodispersion showed 40.6% drug encapsulation efficiency and 70.9% in vitro drug release at 12 hrs. Ex vivo drug release and drug deposition studies on the SNLC based chitosan hydrogel showed prolonged drug release (61.1% at 12 h) and better penetration capacity (87.4% at 24 h) respectively as compared to drug loaded HG (ex vivo drug release 46.7% in 12 h & 59% in 24 h). Kinetic analysis reveals Fickian diffusion as the release mechanism with SNLC dispersion and anomalous non-Fickian diffusion with both SNLC based HG and simvastatin loaded HG. SNLC based hydrogel possesses desirable mechanical properties such as firmness, adhesiveness, and viscosity as revealed by the texture analysis. The safety of the formulations was confirmed by the absence of skin irritation in rats. The results of the in vivo diabetic wound healing study indicate that SNLC based HG promotes wound healing in diabetic rats significantly at a faster and greater rate to 100% within 12 days when compared to chitosan HG without simvastatin ($p<0.0001$) as well as drug loaded chitosan HG ($p<0.001$).

**Key words:** Chitosan Hydrogel, Diabetic wound healing, Nanostructured Lipid Carriers, Simvastatin, Texture Analysis.

## INTRODUCTION

Diabetes mellitus, a metabolic disorder, impairs the sequences of normal wound healing. Wound healing in diabetics gets prolonged due to accelerated apoptosis and weakened angiogenesis & lymphangiogenesis [Asai et al., 2012]. Moreover, it is assumed that diabetes mellitus is accompanied by a decrease of the immunologic defense mechanism leading to a defect in bactericidal activity. Thicker and weaker blood vessel walls and poor phagocytosis by neutrophils and macrophages are some of the other reasons related to complex wounds in Diabetes [McLennan et al., 2006; Boulton et al., 1999].

Simvastatin, a member of the most effective antihyperlipidaemic drug category, inhibits the HMG-CoA reductase essential for the sterol synthesis thereby lowering the plasma cholesterol levels. Clinical evidences reveal that statins exhibit diabetic wound healing activity via enhanced angiogenesis and lymphangiogenesis. Simvastatin inhibits NADPH oxidase leading to suppression of superoxide formation and oxygen free radical scavenging which could help in increasing the activity of eNOS, angiogenesis, and reducing oxidative damage. The other mechanisms involved in the pro-healing activity of statins include proper stimulation of the endothelial cell migration, proliferation, and differentiation [4,5]. Angiogenesis forms the basis of wound repair since it is required for restoration of blood flow for growing tissue which is essential for the supply of oxygen & other nutrients required in the cellular & biochemical process of the repair. Moreover, statins are known for their excellent safety and even their major side effects are rare and reversible [6, 7].

The present study was aimed at developing a more potent topical delivery system for simvastatin for the treatment of chronic wounds associated with diabetes mellitus.

NLCs are one of the promising formulations that can be used for topical delivery of drug and used as an alternative to liposomes and emulsions. Owing to the unstructured matrix composed of a solid fat and an oily lipid, nanostructured lipid carriers (NLC) developed next to solid lipid nanoparticles in lipid based formulation systems, possess increased drug loading capacity, show reduced drug expulsion from the nanoparticulate matrix at storage, and are amenable to modulations in drug release [8, 9]. NLC, with lipid particle size less than 400 nm, causes greater skin hydration due to more skin occlusion leading to improved percutaneous absorption of drugs [10]. The other desirable features of NLC compared to the available topical drug products include low level of toxicity and improved stability of the entrapped drug in lipid matrix [11, 12].

Hydrogels find an increasing use in pharmaceuticals as dressing for healing of wounds and as controlled drug delivery vehicles. The degree of flexibility of hydrogels imparted by the network of natural or synthetic hydrophilic polymers is more akin to natural tissues [13]. Hydrogels can provide a moist environment for the wound while delivering the incorporated drug to the wound in a sustained manner which distinguishes it from other topical vehicles. Hydrogels of natural origin are known to have several advantages over synthetic origin hydrogels such as high biodegradability, non-toxicity, and anti-microbial potency [14-16].

NLC based hydrogels fall in the preferred topical drug product owing to the facilitated drug delivery to the skin tissues and convenient application feasibility with the product [12].

Chitosan hydrogel was selected as the external matrix for NLC as delivery system for wound treatment in the present study. By reducing the loss of water through epidermis, chitosan produces sustained moisturizing effect on the skin and thus improves the dermatological compatibility of formulations. With respect to sustained hydrating effects, chitosan is more promising than hyaluronic acid. Chitosan through its film-forming nature imparts a soothing effect to the skin besides protecting it from adverse environmental conditions. In addition, chitosan has been used as an antibacterial and antifungal agent [17 - 19].

The objective of the present study was to prepare formulations of simvastatin loaded NLC based chitosan hydrogel, characterize them for various physico-mechanical properties, and evaluate them for safety, ex vivo drug permeation, and wound healing potential in alloxan-induced diabetic male albino rats.

## MATERIALS AND METHODS

### Materials:

Simvastatin was a gift sample from Bafna Pharmaceuticals, Chennai. Phospolipon 90G (phospholipid GmbH, Germany), stearic acid, oleic acid, and acetonitrile ( CDH Pvt Ltd, New Delhi), chitosan (Himedia Lab, Mumbai), sodium Dodecyl Sulphate (SDS) (Rankem Laboratory Reagent) were used in the study. All other chemicals were of pharmaceutical and analytical grade.

### Preparation of Simvastatin loaded Nanostructured Lipid Carriers (SNLC):

Lipids used for NLCs of simvastatin were stearic acid and oleic acid in the ratio of 70:30 at 5% concentration. Lipophilic surfactant (Phospolipon 90G) at 3% level and hydrophilic surfactant Tween 80 at 2% level were employed. Simvastatin nanostructured lipid carriers were prepared by a modified method of high speed hot homogenization followed by ultrasonication [11]. The modifications followed were addition of both lipophilic and hydrophilic surfactant to the lipid phase and incorporation of aqueous phase in two stages as reported by Hung et al earlier [20]. Briefly, Simvastatin, lipids, and surfactants were dissolved in 10 ml of 1:1 mixture of Chloroform:Methanol. Complete removal of organic solvents was effected after which the drug loaded lipid matrix was melted at 5-10°C above the melting point of the lipid. The volume of water was divided into two portions such that the volume of the first was the same as that of the lipid phase. The first portion of the water was heated to the same temperature of lipid mixture (65°C) and mixed with oil phase and homogenized (at 2000 rpm) in a mechanical stirrer for 1 hour period. During the homogenization process,

the remaining water (25° C) was added to the mixture slowly by stirring at 400 rpm for 30 min to form the pre-emulsion. This was then ultrasonicated using a probe sonicator (Vibronics ultrasonicator processor p2) for 10 min. Finally, the NLC dispersion of simvastatin was rapidly cooled to room temperature in an ice bath.

**Characterization of simvastatin loaded nanostructured lipid carriers (SNLC):**

**Particle size and zeta potential:**

Particle size (z-average diameter), polydispersity index and zeta potential of Simvastatin loaded NLC dispersion were determined by dynamic light scattering also known as photon correlation spectroscopy(PCS) using a Malvern Zetasizer 3000 Nano ZS (Malvern instruments, UK) at 25°C. The diluted SNLC dispersion was taken in the disposable sizing cuvette, which was then placed in the cuvette holder of the instrument and analysed. Before measurement, air bubbles were removed from the capillary. All measurements were done in triplicate.

**Scanning electron microscopy (SEM):**

Scanning electron microscopy is an excellent tool for observing the physical features of the formulated NLC such as particle shape and surface characteristics of the nanoparticles. The formulation was incorporated in circular aluminium stubs using double adhesive tape and coated with gold in HUS – 5GB vacuum evaporator and then observed in Hitachi S – 3000N SEM at an acceleration voltage of 10 KV and a magnification of 5000X.

**Entrapment Efficiency:**

The entrapment efficiency (EE) of the formulations was the ratio of the content of drug entrapped in NLC to the total drug content. The estimation of non-entrapped drug in SNLC was done with the separation of the aqueous phase by centrifugation (Remi Centrifuge, India) followed by membrane filtration using 0.45µ membrane filter and further spectrophotometric measurement of the drug present in the filtrate (Shimadzu UV-1700 Pharma Spec, Japan) at 239 nm.

The entrapment efficiency of simvastatin loaded NLC was determined after centrifugation using the formula,

$$\%EE = \frac{\text{Total amount of drug taken - Non-entrapped drug}}{\text{Total amount of drug taken}} \times 100.$$

**In vitro drug release studies:**

In vitro release of simvastatin from SNLC formulation was determined by using dialysis bag diffusion technique [21]. The bags were soaked in distilled water for one day previous to use. SNLC equivalent to 10 mg of Simvastatin was placed in dialysis bag and both ends of the bag were sealed. The dialysis bag was immersed in receptor medium of 100 ml of pH 7.4 saline phosphate buffer containing 0.15% SDS maintained at 37°C ± 2°C and stirred using a magnetic stirrer. Aliquots were taken at half an hour time interval for first 2hrs and then for every 1 h for 10 hrs. Equal volume of fresh phosphate buffer solution was replaced after each time of sampling in order to maintain sink condition. The samples were analysed for drug content by UV spectrophotometer (Shimadzu UV-1700 Pharma Spec, Japan) at 239 nm. Drug release studies were conducted in triplicate and the average values were taken.

**Formulation of Simvastatin loaded Hydrogel:**

For the preparation of blank HG, Chitosan was dispersed in 1 % acetic acid solution and subsequently, glycerine 1.5 ml was added to the aqueous solution. The mixture was stirred manually for 10 minutes and sonicated to remove air bubbles. The formed hydrogel was left equilibrating for 48 hours in a sealed container at room temperature. Simvastatin and SNLC were added to the blank HGs, and mixed for 1 min to yield drug loaded plain HG and SNLC based HG (1% drug content) respectively [22,23].

**Evaluation of the SNLC based Hydrogel:**
**Measurement of pH:**

The pH of the simvastatin hydrogel formulations was determined by dissolving 1g of gel in 10 ml of distilled water using a pH meter thrice in a month post to preparation i.e 1st day, 15th day and on 30th day to identify pH changes [24].

**Drug content:**

One gram of gel formulation, which contains approximately 5mg of drug, was dispersed in 10 ml of methanol. The dispersion was filtered through 0.45µ pore size membrane filter and was analysed for drug content spectrophotometrically after suitable dilution with pH 7.4 saline phosphate buffer containing 0.15% SDS [25]. The solution prepared from NLC based hydrogel without simvastatin served as the blank.

**Texture Analysis:**

Rheological characteristics of semisolids play a significant role in topical application by fixing the feasibility of application and residence time of the formulations on the skin. The consistency and viscosity of the formulations were determined using Texture Anayser TA- XT Plus (Stable Microsystems, UK) with the accessory Back extrusion cell with a 35mm disc and extension bar using 50 kg load cell [26].

Tests were performed in a standard size back extrusion container (50mm diameter), approximately 75% full, immediately after removal from storage at a specific temperature e.g. 25°C. The extrusion disc (35 mm) was positioned centrally over the sample container. For comparing cohesiveness and 'work of cohesion' the probe was returned to the start position above the samples at the end of the test. The averages of three readings are used to calculate the viscosities of formulations. When the surface trigger is 10g i.e. the point at which the disc's lower surface and the formulation were in complete contact, the disc will proceed further 20 mm down to penetrate into the formulation, after which point (the maximum force), the probe will return to its initial position.

**Ex vivo skin permeation studies:**

Ex vivo skin permeation of SNLC based HG, drug loaded plain HG, and NLC dispersion were studied using Franz diffusion cell indigenously fabricated (effective permeation area 2.54cm2 and cell volume 15ml). Rats (male albino) 6 to 8 weeks old, of body weight 150 to 200g were sacrificed for abdominal skin. After removing the hair, the abdominal skin was excised and cleaned from the connective tissue. Then the adhered fat( if any) and subdermal tissue remnants on the dermal side of the skin were gently removed. Care was taken to ensure that the skin samples for transdermal permeation studies were free from fine holes or cervices. The ex vivo study was approved by the Institutional Animal Ethical Committee.

The skin was mounted in the diffusion cell with the stratum corneum side facing upside into the donor compartment. Phosphate buffer saline pH 7.4 containing 0.15% SDS was filled in the receptor compartment. The SNLC based gel formulation was applied on the skin and covered with aluminium foil to prevent any evaporation. Samples were removed through the sampling port of diffusion cell at time intervals for 12 hours, diluted to 10µg/ml concentration with phosphate buffer saline pH 7.4 containing 0.15% SDS and analyzed for drug content by UV-Visible spectrophotometer at 239 nm. Equal volume of fresh medium was added in to the receptor compartment for sink conditions to be maintained. Experiments were conducted in triplicate. Graphical plots were made between cumulative percentage of drug release and the time to obtain the drug release profile [27].

To reveal the release pattern of simvastatin, the ex vivo drug release data were fit into equations viz. cumulative % release vs. Time (zero order), log % drug remaining vs. time (first order), cumulative % drug release vs. square root time (Higuchi's plot), cubic root of % drug remaining vs time (Hixson Crowell) and log cumulative % drug release vs log time (Korsmeyer -peppas model). Values of R2 and k were estimated for these plots by regression analysis.

**Drug deposition study:**

After 24 h of ex vivo release study, drug remaining on skin surface was determined by scraping and then washing the skin 3-4 times with diffusion medium. These washings were filtered through 0.45µm membrane filter. This sample was analyzed by diluting with medium to make 10 µg/ ml and absorbance was measured at 239 nm using UV-VIS spectrophotometer (Shimadzu UV-1700 pharma spec, Japan). [27].

**Skin Sensitivity Test:**

Rats (200 -250 g) of either sex were used for testing of skin irritation as per the animal experimentation protocol approved by IAEC, K.M. College of Pharmacy, Madurai (IAEC/125/KMCP/261220708/2013-2014). The animals were maintained at standard laboratory conditions. Hair was removed on the dorsal side and an area of 4 cm2 was marked on both the lateral sides; one side as control whereas the other side was test. Gel application (500 mg/animal) was done two times a day for a one week period and the area was noticed for any signs of sensitivity, the reaction being graded as 0 for no reaction, 1 for slight patchy erythema, 2 for moderate patchy erythema, and 3 for severe erythema with or without edema, respectively [27].

**Wound healing activity in diabetic Wistar rats:**

The study was carried out after the approval by the Institutional Animal Ethics Committee of KM College of Pharmacy, Madurai, registered with CPCSEA (proposal number of IAEC/125/KMCP/261220708/2013-2014). The studies were conducted on Wistar albino rats of either sex, weighing 200 ± 20 g. Rats fasted overnight were made diabetic by injecting alloxan i.p (120mg/kg). After 72 hours, animals with blood glucose levels in the range of 300-400 mg/dl were divided into 3 groups of 6 animals each. All the groups were treated with required dose of insulin for diabetes in order to fulfil the ethical consideration. The first group served as the control group of diabetic rats bearing wounds treated with chitosan hydrogel without simvastatin, second group was the diabetic rats treated for wounds with simvastatin loaded plain gels for wound healing and the third group animals were treated with SNLC based gels.

The hairs from the dorsal part of the rats were removed and then surgical spirit was applied. When the animals were under light anaesthesia, wounds of approximately 2cm2 in surface area and 2mm in depth were created with sterile scalpel on the prepared area of the back skin below the shoulder blades. The animals cannot reach for this wound area so that there will not be self-licking. The wounding day was taken to be day zero. The wound areas were treated with topical application of prepared gel (100mg once daily) until the wounds were healed completely [24]. The healed as well as the unhealed wound area were traced on butter paper and the areas were found out by square counting procedure after replication on a graph paper [28]. The number of complete squares ((Nc = 0.01cm2) and partial squares (Np) in the tracings were counted and area was calculated using the following formula:

Total area of the wound = Area of complete and partial squares = (Nc + 0.4xNp) x 0.01.

The area of wound was monitored from the wounding day followed by 4,7,10 & 12th day. The percentage of wound contraction was computed as per the following formula [24, 28]

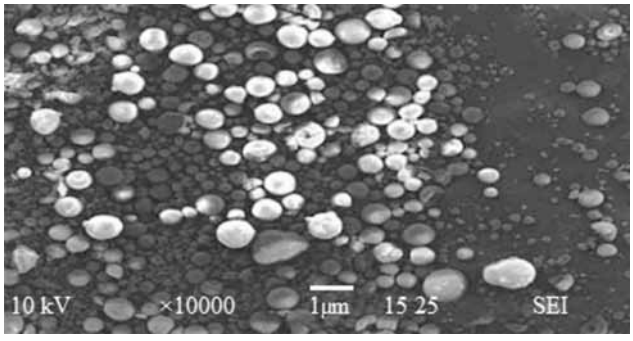
$$\% \text{ wound contraction} = \frac{\text{Wound area on day (zero)} - \text{Wound area on day (n)}}{\text{Wound area on day (zero)}} \times 100$$

**RESULTS AND DISCUSSION**

The method followed for preparing SNLC, ultrasonication subsequent to hot homogenization is reliable and simple as has been reported [29]. The appearance of the prepared NLC was uniform and homogenous.

**Characterization of simvastatin loaded Nanostructured Lipid Carriers (NLCs):**

The mean size of simvastatin loaded NLC was found to be 140.6 ± 7.2 nm with a poydispersity Index (PDI), of 0.347. If PDI, a parameter describing the variation of particle size is closer to 0, it indicates a narrow size range of the nanodispersion [30]. Zeta potential or electrophoretic mobility indicates the electric charge on the surface particle forming an electrical barrier responsible for the repulsion between the dispersed particles. Zeta potential of a particulate formulation helps in predicting the physical stability of Nanodispersion [31]. The prepared NLC has a zeta potential of -13.6 mV, which may be sufficient to inhibit aggregation of particles. The scanning electron microscopy (SEM) photograph of the formulation (Fig. (1)) reveals that the nanoparticles are spherical in shape with smooth surface.

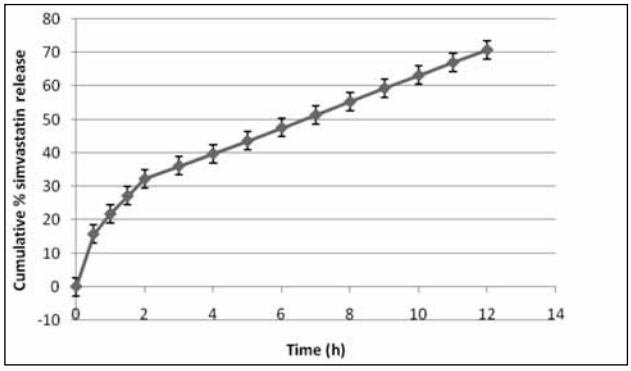



The drug entrapment efficiency of the NLC formulation was found to be 40.64 ± 1.36 % (mean ± SD of triplicate determinations).

**In vitro release studies:**

The drug release pattern from SNLC was biphasic with an initial burst release in the first 2 hours followed by a slower sustained release as depicted in Fig.(2). The initial burst effect might have been caused by the sudden release of the non-entrapped drug in SNLC. The same may be reasoned out in another way that the liquid lipid rich soft outer shell of the nanoparticle might have dissolved the lipophilic drug leading to greater drug

load in the outer region and consequent increase in the drug release from the surface of the nanoparticles in the initial stages [32]. After the 2 hours of burst release, NLC formulations showed sustained release until 12 hours. The controlled release of simvastatin could have resulted out of the presence of low melting lipid stearic acid as well as slow diffusion of the drug from the solid lipid matrix [33]. Both phases of drug release are useful in topical application; burst release takes care of improving the drug permeation for rapid onset of action, while a sustained release supplies the drug over a prolonged period of time.




**Formulation and Evaluation of Simvastatin loaded Hydrogel:**

NLC gel formulation was yellow in colour, translucent, smooth, and homogeneous in texture.

**pH measurement and Drug content:**

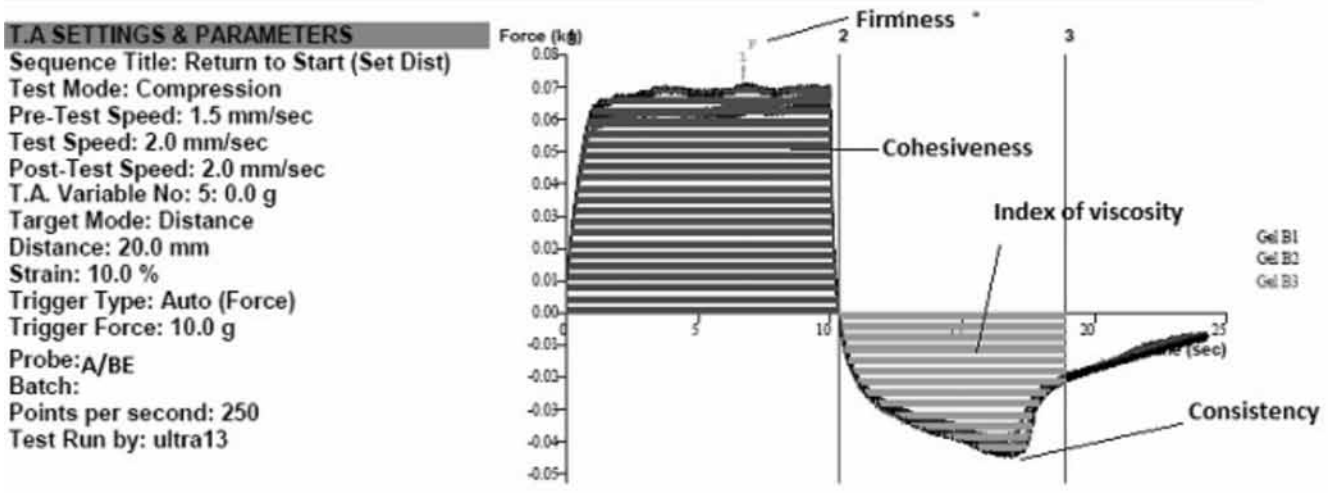
The pH of the gel was 5.3, which is compatible with normal skin pH in healthy people and did not change significantly at various times in a month period when tested on the 0th, 15th, and 30th day. This may render the topical application safe without any irritation [34]. Drug content was found to be 96.51 % and 97.71% for NLC based gel and drug loaded HG gel respectively, which indicates a uniform distribution of drug in prepared gel formulations.

**Texture analysis:**

The texture of the gels and NLC formulation were evaluated using back extrusion cell in a Texture analyser in triplicate and the data are given in Table 1.

The results of that of SNLC based hydrogels are graphically depicted in Fig.(3). Firmness of the semisolid gel formulations was measured by the maximum force (peak); higher force value indicating firmer sample. Consistency of the sample was determined by the area under the curve up to this peak point; greater value designating thicker consistency of the formulation.

| S.No | Formulation tested | Firmness (Kg) | Cohesiveness (Kg.sec) | Consistency (Kg) | Index of viscosity (Kg.sec) |
|---|---|---|---|---|---|
| 1 | Drug loaded HG | 0.166±0.009 | 1.246±0.035 | 0.077± 0.006 | 0.404±0.050 |
| 2 | SNLC Based HG | 0.069±0.003 | 0.629±0.031 | 0.042±0.003 | 0.268±0.014 |
| 3 | SNLC | 0.010±0.001 | 0.108±0.015 | 0.005±0.000 | 0.002±0.000 |



The curve on the negative side of the axis formed due to the return of the probe to the original position results because of the weight of sample lifted mainly on the upper surface of the disc while returning, i.e. back extrusion and therefore indicates again the consistency/ resistance to flow off the disc. The maximum negative force is the cohesiveness of the sample; more cohesive samples will have more negative value. The area of the negative side of the curve is taken as the 'work of cohesion'; higher values considered to be 'more resistant' to withdrawal of the sample, i.e, cohesiveness and also viscosity of the sample. High drug release facilitates the topical application. Both the parameters depend on the rheological behaviour, i.e, the viscosity of the formulations. Adhesiveness and optimal firmness are the two desirable characteristics of a semi-solid formulation for topical delivery, the former increases the contact time of the applied formulation with the skin allowing for the complete release.

The obtained data indicate that the viscosity of the SNLC was very low because of larger water content, suggesting the incorporation of SNLC into a hydrogel in order to obtain a convenient topical drug product with an appropriate semisolid consistency [11, 35, 36]. Moreover, NLC based hydrogels have been noted to enhance the viscoelastic behaviour i.e. thixotropic nature of topical formulations [37]. The cohesiveness of SNLC based HG formulation was higher than SNLC far enough to adhere on skin surface, which enhances the residence time and penetration across the skin, thereby increasing drug availability at site of action in a controlled manner and also the subsequent skin absorption. The drug loaded plain HG is firmer, more cohesive, and of a thicker consistency than SNLC based HG. Though the presence of SNLC within HG reduces the firmness and cohesiveness, it assists spreadability .

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**Ex vivo Skin permeation study:**

Ex vivo skin permeation studies were done to compare the transport of the drug from the optimized SNLC, SNLC Based HG, and drug loaded plain HG preparation containing 5mg of simvastatin through excised rat skin. The permeation values for 12 hours are given in **Fig.(4).**

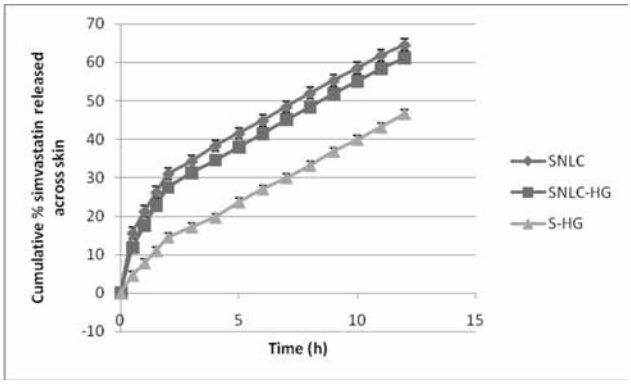


Fig.(4) shows the burst drug release profiles of 31.01% and 27.44% for SNLC and SNLC based HG, respectively, in the first 2 hours, whereas, it was only 14.53% for drug loaded plain HG. The cumulative % drug release at 12 hours was 64.54%, 61.08% and 46.66% for SNLC, SNLC based HG and drug loaded plain HG respectively. These release changes can be attributed to the higher viscosity of drug loaded plain HG when compared to that of SNLC based HG, as the rheologic behaviour of semi-solid formulations greatly influences the drug release profile [36].

Furthermore, SNLC and SNLC based HG formulations showed a prolonged drug release over a period of 12 h, but a more pronounced sustaining was observed with SNLC based HG formulation when compared to SNLC, which could have been attributed to the resistance to drug release by the gel matrix. The controlled release of simvastatin from SNLC and SNLC based gels resulted in maintenance of the drug concentration over a longer

period of time. These results are in accordance with the report on voricanazole loaded NLC based hydrogels, where effective regulated drug release was observed [12]. Also, the results of rat skin permeation indicate the possible permeation of simvastatin through human skin also [11].

**Drug deposition study:**

The percentage of drug remained on the skin from SNLC dispersion, SNLC based HG and drug loaded HG at 24 hours in ex vivo studies was found to be 15.01% ± 0.75%, 12.56% ± 0.59%, and 41.11% ± 1.34% respectively. It implies that the percentage of simvastatin permeated through the skin from SNLC dispersion, SNLC based HG, and drug loaded HG at 24 hours were 84.99%, 87.44% and 58.89% respectively. It shows the better penetration ability of NLC based HG and it was more than that of drug loaded HG and SNLC dispersions. Thus, drug-retaining effect in the skin is shown to be possible with SNLC, which being a lipid based nanocarrier, accelerates the drug permeation into the skin facilitated by the submicron size and assists in skin targeting of the drug through drug localization due to its lipoidal nature [38,39].

**Ex vivo release kinetics:**

To analyze the drug release mechanism, ex vivo release data was fit into various release equations and kinetic models (Zero order, First order, Hixson – Crowell, Higuchi and Korsemeyer – Peppas models). The release kinetic data for all the formulations are shown in Table 2.

| FORMULATION CODE | FIRST ORDER | | HIGUCHI MODEL | | KORSMEYER-PEPPAS | |
|---|---|---|---|---|---|---|
| | $R^2$ | $K_1^{(h-1)}$ | $R^2$ | $KH^{h(-1/2)}$ | $R^2$ | n value |
| SNLC | 0.993 | -0.030 | 0.995 | 17.03 | 0.995 | 0.432 |
| SNLC Based HG | 0.992 | -0.028 | 0.994 | 17.05 | 0.993 | 0.491 |
| Drug loaded HG | 0.995 | -0.020 | 0.986 | 14.85 | 0.995 | 0.700 |

Among the models tested, the drug release profile of all formulations were best fit with first order with R2 values ranging from 0.992-0.995 and Higuchi model with R2 values ranging from 0.986 - 0.995. It can be concluded that, all formulations fit very well to the Korsmeyer-Peppas model with different R2 and n values, which means that the drug release processes were different. Classical Fickian diffusion was the release mechanism for NLC dispersion with n value of 0.432 and anomalous non-Fickian diffusion (combination of diffusion and erosion mechanisms) for both NLC based HG and drug loaded HG with n value of 0.49 and 0.7 respectively.

**Skin irritation study:**

The results of the skin irritation study revealed that SNLC based gel exhibited considerably no signs of irritation or erythemas and all the animals tolerated the applied gels during the whole period of study. Thus the gel formulation is suitable for topical application with a high degree of safety.

**Diabetic wound healing activity:**

Results of diabetic wound healing studies are presented in Table 3. SNLC based HG treated groups showed an increase in % wound contraction with time, which is significantly greater than other groups.

| S. No | Day of Observation | Wound Contraction (%) | | |
|---|---|---|---|---|
| | | Control (Chitosan HG without simvastatin) | Drug loaded HG | SNLC Based HG |
| 2 | 4 | 26.80±0.60 | 41.34±1.13* | 48.29±0.69*[a] |
| 3 | 7 | 48.54±1.59 | 73.49±1.16* | 81.94±1.16*[a] |
| 4 | 10 | 62.74±1.37 | 84.71±0.62* | 94.96±0.35*[a] |
| 5 | 12 | 78.69±1.60 | 96.42±1.39* | 100± 0.0*[b] |

By day 4, SNLC based HG treated wounds were 48.29% healed ($p<0.0001$) compared with 41.34% (p) and 26.80% in the drug loaded HG treated and control group (treated with chitosan hydrogel without simvastatin) respectively. In diabetic animals treated with SNLC based HG on wounds, about 80% wound closure was observed in a week's time. In control group animals, wounds healed more slowly and only after 12 days wound closure of about 80% was achieved. Also, there was a significant difference ($p<0.001$) between SNLC based HG (100% groups (96.42% wound closure by day 12). It was also revealed that none of the rats in

simvastatin treated groups showed scab formation but rats in control group showed formation of scab with delayed wound healing. The lipids present in the SNLC based hydrogels must have added to the soothing effect along with wound healing [11].

The findings indicate that the wound healing in both SNLC based HG and drug loaded HG treated groups was faster than the control group, while complete wound closure and covering of wounds area with hair was observed on 12thday of wound induction in SNLC based HG treated groups only. This reveals the superiority of SNLC based HG in faster wound healing due to both the drug and the vehicle NLC.

The results of the present study indicate that simvastatin promotes the healing in diabetic wounds. The faster and higher wound contraction rate should have been contributed by both the drug and the carrier, NLC based chitosan HG.

**CONCLUSION**

Simvastatin loaded nanostructured lipid carrier was successfully prepared by hot homogenization followed by sonication. SNLC based chitosan hydrogel showed sustained release of simvastatin and better skin permeation in ex vivo drug release studies using rat skin. The mechanical properties of the SNLC based hydrogels indicate the incorporation of SNLC in hydrogels has transformed SNLC into a suitable formulation amenable to topical administration. The wound healing in diabetic rats was more rapid and superior with SNLC based hydrogel when compared to that with drug loaded hydrogel and plain hydrogel, confirming the enhanced drug permeation at the wound site contributed by the improved skin-uptake of nanosized lipid particles from the chitosan gel matrix. Thus the NLC based HG can be used as novel drug delivery carrier for improved skin permeation of simvastatin for its diabetic wound healing action.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest

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# Development and Characterization of Valsartan Loaded Eudragit Nanoparticles

A. Abirami, S. Mohamed Halith*, K.KulathuranPillai, R.Umaprabha, S.Venkataraman and M.Nagarajan

Department of Pharmaceutics, K.M College of pharmacy, Madurai - 625 107, India.

*Corresponding author : halithsm@rediffmail.com

## ABSTRACT

Valsartan loaded Eudragit RL 100 and RS 100 nanoparticles were developed for the hypertension therapy and prepared by nanoprecipitation method. Nanoparticles of different core: coat ratio were formulated and evaluated for process yield, loading efficiency, particle size, zeta potential, invitro drug release, kinetic studies, and stability studies. There was a steady increase in the entrapment efficiency on increasing the polymer concentration in the formulations. The in vitro release behaviour from all the drug loaded batches were found to follow zero order kinetics and provided sustained release over a period of 24 h. No appreciable difference was observed in the drug content of product for 3 months during which the nanoparticles were stored at 4°C, room temperature and 45°C/70% RH. According to the data obtained, Eudragit RL 100 based delivery system opens new and interesting perspectives as drug carriers.

**Key words:** Valsartan, Eudragit RL 100, Nanoprecipitation, cooling centrifuge technique.

## INTRODUCTION

Blood transports oxygen and nutrients to the organs. The heart pumps the blood through the blood vessels. This action causes blood pressure[1]. The normal level for blood pressure is below 120/80. The top number, the systolic blood pressure, corresponds to the pressure in the arteries as the heart contracts and pumps blood forward into the arteries. The bottom number, the diastolic pressure, represents the pressure in the arteries as the heart relaxes after the contraction [2].

Cardiovascular disease caused 2.3 million deaths in India in the year 1990, and this is projected to double by the year 2020. Hypertension is the most common cardiovascular disease. It is responsible for 57% of all stroke deaths and 24% of all coronary heart disease deaths in India. The prevalence of hypertension in Indian population is about 30-40%[3,4]. Hypertension is persistent elevation of systolic BP to 140 mmHg or greater and/or diastolic BP of 90 mmHg or greater.[5] Essential hypertension is a far more common condition and accounts for 95% of hypertension. Essential hypertension develops only in groups or societies that have a fairly high intake of salt, exceeding 5.8 grams daily. Approximately 30% of cases of essential hypertension are attributable to genetic factors [6,7]. The term "secondary hypertension" implies that a patient's blood pressure elevation is the result of an underlying discoverable disease process.

Selective drug delivery systems or targeted drug delivery systems are designed to improve the benefit/ risk ratio of any drug and make the drug available more in the required tissue by decreasing its distribution in unwanted tissue.

Valsartan belongs to a class of antihypertensive agents called angiotensin II receptor blockers (ARBs). Valsartan is a specific and selective type-1 angiotensin II receptor (AT1) antagonist which blocks the blood pressure increasing effects angiotensin II via the renin-angiotensin-aldosterone system (RAAS). RAAS is a homeostatic mechanism for regulating hemodynamics, water and electrolyte balance. It is used as a first line

agent to treat uncomplicated hypertension, isolated systolic hypertension, and left ventricular hypertrophy. This can be also used as a first line agent to delay progression of diabetic nephropathy. Losartan may be also used as a second line agent in the treatment of congestive heart failure, systolic dysfunction, myocardial infarction, and coronary artery disease in those intolerant of ACE inhibitors [8, 9, 10].

Valsartan has a short biological half life of 6hrs and requires frequent administration for a prolonged period of time. The bioavailability of the valsartan after oral administration is low (10-30%) with higher variability. To reduce the dose frequency the study was aimed at preparing nanoparticles of valsartan. The present study was aimed at developing nanoparticles of valsartan in order to improve the bioavailability and efficacy in treatment of hypertension.

## MATERIALS AND METHODS

Valsartan was obtained as a gift sample from Hetero Laboratory Ltd (Chennai, India). Eudragit RL 100 was furnished from Micro labs, Hosur (Karnataka,India). Ethanol was purchased from SD Fine chemicals,(Mumbai,India).

## METHOD OF PREPARATION OF VALSARTAN NANOPARTICLE

### NANOPRECIPITATION METHOD

All batches of nanoparticles were prepared by nanoprecipitation method. The required quantity of polymer was dissolved in 3ml ethanol and drug was dissolved in 3ml of ethanol and mixed. To this mixture, 2% of DMSO was added and mixed together. The mixe was homogenized in a vortex mixture for 1 min and then the final volume of the preparation was made up to 10ml. Then this preparation was centrifuged at 15,000rpm at 40c for half an hour using ultra cooling centrifuge. The supernatant was discarded and precipitate was washed 3times with distilled water. The nanoparticles thus obtained were dried at 550C and stored in desiccators [11, 12].

The prepared formulation were characterized for loading efficiency, entrapment efficiency, particle size, particle size distribution , zeta potential, and drug polymer compatibility studies

**Table1: Various Composition of Valsartan Nanoparticles Formulation**

| Formulation code | Drug | Eudragit RLl00 | Eudragit RS100 | DMSO |
|---|---|---|---|---|
| F 1 | 40 | 20 | - | 2% |
| F2 | 40 | 40 | - | 2% |
| F3 | 40 | 60 | - | 2% |
| F4 | 40 | 80 | - | 2% |
| F5 | 40 | - | 20 | 2% |
| F6 | 40 | - | 40 | 2% |
| F7 | 40 | - | 60 | 2% |
| F8 | 40 | - | 80 | 2% |
| F9 | 40 | - | 100 | 2% |
| F10 | 40 | - | 120 | 2% |

**Note: Drug and polymers value represented by mg, DMSO - dimethyl sulfoxide**

### Characterisation of Valsartan Nanoparticles

### FT-IR spectroscopy

Infrared spectroscopy by potassium bromide pellet method was carried out on pure substance (such as Valsartan, Eudragit RL 100 and Eudragit RS 100 separately and their physical mixtures) compressed under 15 tonnes pressure in a hydraulic press to form a transparent pellet. The pellet was scanned from 4000 to 500 cm-1 in FTIR 8400S model spectrophotometer [13, 14].

### Drug Entrapment Study

The entrapment efficiency study was determined by free drug content in the supernatant, which was obtained after centrifuging the suspension at ( 15,000rpm for 20 min at zero using ultra centrifuge ) The absorbance was measured at 248 nm by UV spectrophotometrically.

The entrapment efficiency was calculated using following equation.

$$\text{Percentage drug loading (PDL)} = \frac{\text{Entrapped drug (mg)}}{\text{Total Drug added (mg)}} \times 100$$

The % Entrapment Efficiency of the optimized formulation (F8) was determined in the supernatant remains after centrifugation and it was found to be 97.42 %, which is higher than other formulations [15, 16].

**INVITRO DRUG RELEASE STUDIES**

**UV Spectrophotometric Method:**

The invitro drug release study was carried out by using the diffusion membrane technique.The nanoparticles were placed in a dialysis membrane and dropped in a beaker containing 200ml of diffusion medium (phosphate buffer saline pH 7.4). The medium was maintained at 37o C under magnetic stirrer at constant speed. At fixed time intervals, 1ml sample was taken from the diffusion medium for every 1 hr and it was replaced by 1 ml fresh medium. This process was carried out for 24 hrs. The sample was measured UV spectrometrically at 248nm. The percentage of drug released at various time intervals was calculated from the calibration graph [17, 18].

**Scanning Electron Microscopy**

The optimized formulation was morphologically characterized by scanning electron microscopy( SEM). The sample for SEM analysis was mounted in the specimen by using a scotch double adhesive tape. The sample was analyzed in a scanning electron microscope operated at 15 kv and image was taken [19, 20].

**Surface Charge (Zeta Potential Determination)**

Zeta potential is an important parameter to evaluate and establish an optimum condition for stability of colloidal or dispersed systems. The prepared nanoparticle suspension were characterized with respect to zeta potential by using zeta potential analyser (Malvern Zeta seizer). The effect of Eudragit RL 100(polymer) on the surface characteristics of the nanoparticle was studied[21, 22, 23]

**Stability Studies of Nanoparticles**

The stability studies of nanoparticles involved observing the formulation at 45ºC /70% RH, which constitutes accelerated condition and at 4oC and 30 oC. The formulations were kept in the above mentioned temperatures for 3 months and sufficient amount of sample were taken at periodic intervals, for performing the following tests [24].

a. Physical appearance

b. pH of the solution

c. In vitro drug release (Dissolution)

d. Percentage of drug entrapment

**Drug Release Kinetic Model**

The optimized formulation was subjected to graphical treatment to assess the kinetics of drug release.

**Zero Order Plot**

The zero order plot was obtained by plotting cumulative % drug release versus time.

**Higuchi Plot**

The Higuchi plot was obtained by plotting cumulative percentage (%) drug release versus square root of time.

**Koresmeyer Plot**

The graph was obtained by log cumulative percentage (%) drug release versus log time [25].

**Results and Discussion**

**FT-IR Study**

FT I.R studies ruled out the possibility of interaction between the selected polymer and drug. The spectrum obtained from I.R studies at wavelength from 4000cm-1 to 400cm-1 showed that there are neither major shifts nor any loss of functional peaks between the spectra of drug. The drug was entrapped into the polymer matrix without any chemical interaction. FT-IR of pure drug and nanoparticles suggest that no significant interaction was seen when formulating nanoparticles with eudragit RL100 polymer.

**Fig-1: FT-IR Graph of Pure Drug (Valsartan)**

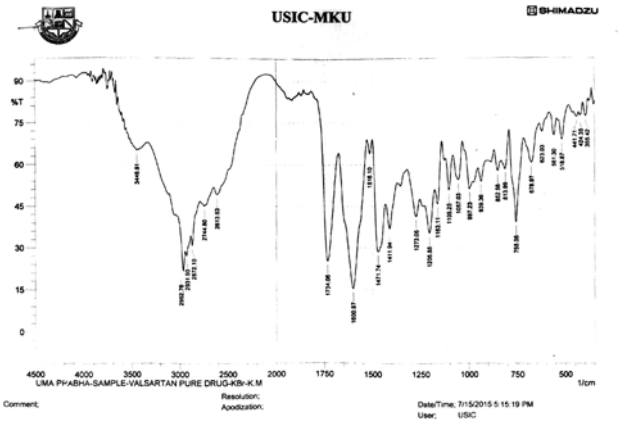
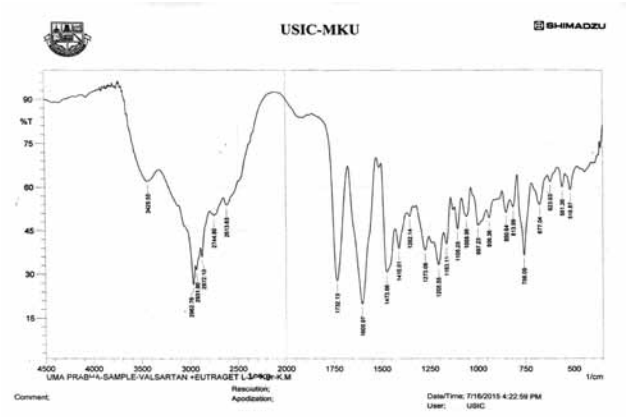


**Fig 2:Ft-Ir Graph of Optimized Formulation (F8) Drug – Eudragit Rl 100**




**Encapsulation efficiency**

Sufficient loading capacity is obtained by nanoprecipitation method. The amount of drug to be incorporated into the delivery system is dependent on the physicochemical property of drug and preparation process. Polymer concentration was more for F8 formulation, resulting in more entrapment efficiency than other formulations. When polymer concentration increases, surface tension reduces and it also promotes wettability and amount of drug entrapped. Nanoparticle using Eudragit RS100 shows lower entrapment efficiency than Eudragit RL 100. This is due to the chemical structure of drug and polymer.Eudragit RL 100 polymer showed higher entrapment efficiency ( 97.42 % ), thereby making this polymer suitable for valsartan nanoparticles.

**Particle size analysis**

The zeta potential of the nanoparticle formulation with Eudragit( RL100) (formulation F8) particles, which were present in the formulation were de-aggregated was measured. The zeta potential remains same and more stable in the substance (zeta potential (mV) is 13.4and PDI 0.353). Therefore, this polymer is more suitable for nanoparticles preparation and the result shows smooth surface character and efficient repelling action and decrease in the opsoniazation.

Zeta sizer (Malvern instruments .UK) was used for analysis of particle size. All measurements and analysis setting were controlled using standard operation procedure.

**FIG 3: Size distribution report by intensity**

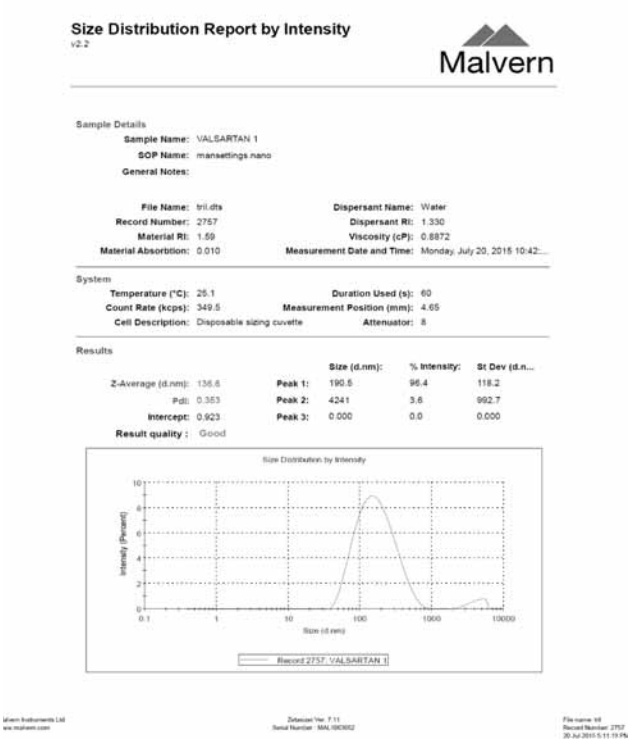
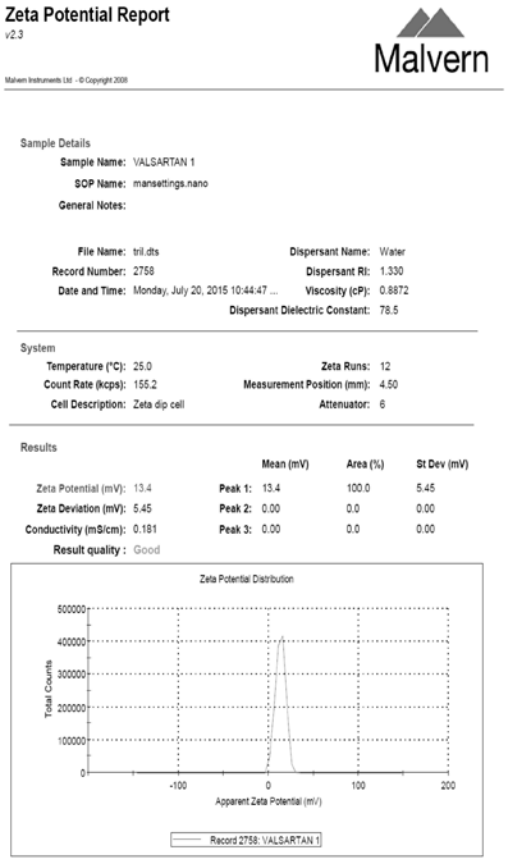


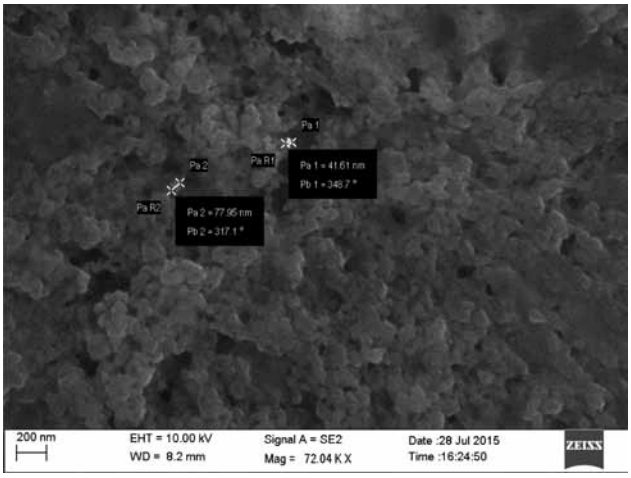

**FIG 4: Zeta potential report of valsartan nanoparticles**



**Scanning electron microscopy**

The surface characteristics of the optimized formulation (F8) particle size were studied by scanning electron microscope. The SEM image of prepared nanoparticle formulation shows the coating of polymer mixture on drug particles.The appearance of nanoparticles in scanning electron microscope is in granule form and aggregated, which indicates a thin and uniform coating over the drug. SEM image revealed that the valsartan nanoparticles were in nano size range and smooth spherical shape in this F8 Formulation with a particle size diameter ranging from 41.61nm to 77.95nm.

**FIG 5: SEM image of valsartan nanoparticle**




**Stability studies of valsartan nanoparticles**

The stability tests of optimized nanoparticle formulation F8 were carried out for a period of 3 months at various storage conditions. The results showed that the formulation remains stable throughout the period of study. Nanoparticles were stable with respect to the amount of drug retained for a period of 3 months at 4ºC and also affirm that the drug leakage increased at a higher temperature

**Table 2: Stability studies for valsartan nanoparticle**

| S.NO | Storage Condition | Test parameters | 1st month | 2th month | 3rd month |
|---|---|---|---|---|---|
| 1 | 4°C | pH<br>colour<br>% Invitro drug release | 7.5<br>Clear& colour less<br>99.17 | 7.5<br>Clear& colour less<br>98.26 | 7.5<br>Clear & colour less<br>97.12 |
| 2 | Room Temperature | pH<br>colour<br>% Invitro drug release | 7.4<br>Clear & colour less<br>98.52 | 7.4<br>Clear & colour less<br>95.42 | 7.3<br>Clear & colour less<br>92.48 |
| 3 | Acceleration condition at 45°C/70% RH | pH<br>Colour<br>% Invitro drug release | 7.4<br>Clear & coloureless<br>96.34 | 7.3<br>Clear& coloureless<br>92.26 | 7.3<br>Clear& coloureless<br>89.54 |

**In vitroDrug release and kinetic Model**

Invitro drug release studies showed drug release profile from nanoparticles, which confirmed the release is controlled and prolonged. All the characterization studies revealed that the prepared nanoparticles have good physical stability and distinct particle size and shape. The release of drug from nanoparticles optimized formulation showed regression coefficients forboth zero order and Higuchi (0.9952 & 0.879 respectively). The 'n' value for Peppa's model of 0.927 stated that the release followed a diffusion controlled mechanism. The Zero order, Higuchi, and Peppa's model are depicted in fig no: 8-10.

**Fig.No8: zero order In-vitro drug release of (F8) optimized nanoparticles**

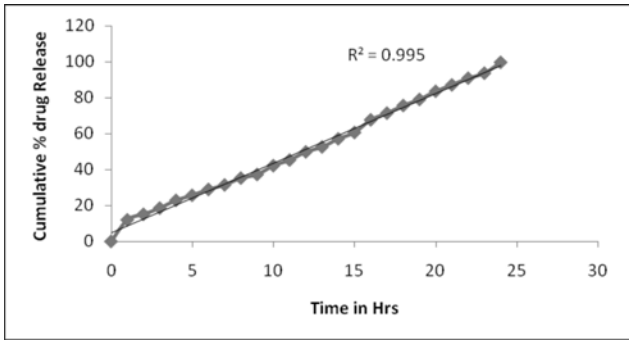



**Fig.No 9:Higuchi's plot for formulation F8**

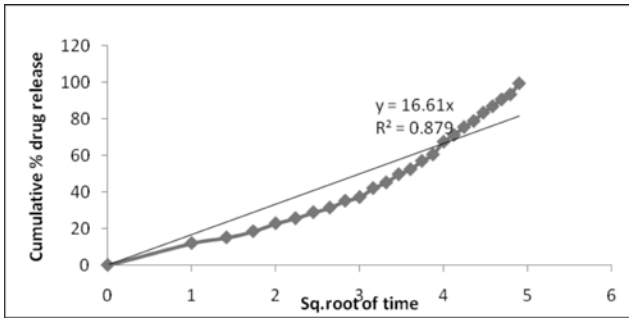
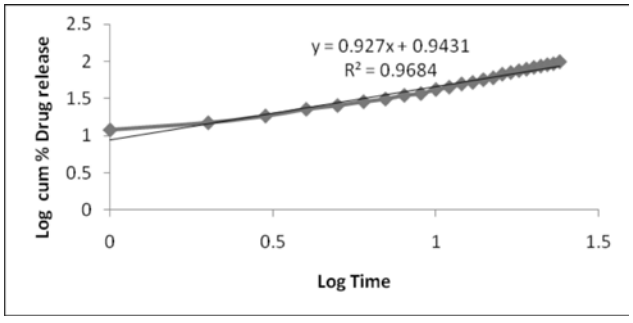



**Fig.No.10: Korsemeyer'splot forformulation F8**




**4 CONCLUSION**

The present work was an attempt to develop nanoparticles of Valsartan using Eudragit RL 100 by nanoprecipitation method. The optimized formulationF8 (drug 40mg, Eudragit RL 100 80mg) was selected based on entrapment efficiency and Invitro drug release studies. The entrapment efficiency of the optimized formulationF8 (drug 40mg, Eudragit RL 100 80mg) was 97.42 ±0.09 and the invitro drug release was 99.40% after 24 hours. It also obeys the zero order and follows the diffusion and erosion mechanism of release.

These findings enable the conclusion that nanoparticles represent a promising particulate carrier showing sustained drug release and release the drug in systemic circulation for a longer time, which is the prime requirement for the management of hypertension.

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