Online ISSN : 2249-4618
Print ISSN : 0975-5888
DOI : 10.17406/GJMRA

Global Journal

of Medical Research: B

Pharma, Drug Discovery, Toxicology & Medicine

Highlights

- Effect of Biofield Energy
- Medicated Tolnaftate Nail
- Bio-Flexy Film Approach
- Deliver Nanosized Atorvastatin

Discovering Thoughts, Inventing Future

VOLUME 18 ISSUE 5 VERSION 1.0

Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology & Medicine

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Volume 18 Issue 5 (Ver. 1.0)

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Contents of the Issue

Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology & Medicine
Volume 18 Issue 5 Version 1.0 Year 2018
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Formulation and Evaluation of Medicated Tolnaftate Nail Lacquer

By Farsana. P, Baby Shahanas, Anu Sebastian & Ashly Merin George

Abstract- The present study was aimed towards the design and formulation of medicated nail lacquer of tolnaftate to control onychomycosis condition and improve the patient compliance. The present work investigated the amount of tolnaftate released from different formulations containing different concentration of ethyl cellulose and different proportions of thioglycolic acid and dimethyl sulfoxides for treatment of onychomycosis. Then these lacquers were compared for drying time, nonvolatile content, drug content, drug diffusion and antimicrobial studies. The stability test showed that the formulation were stable at 37° ± 2°C for 1 month. The results obtained from in-vitro diffusion studies showed that formulation F3 have completed drug release of 94.48% over 24 hrs. The F3 formulation had salicylic acid as keratolytic agent and 0.5ml of 1% w/v of thioglycolic acid as penetration enhancer. From diffusion studies, it was concluded that thioglycolic acid containing formulation (F2 and F3) have better penetration enhancement as compared to DMSO containing formulation. The best formulation was evaluated for antifungal sensitivity test against the Candida albicans. From the above study, it can be concluded that medicated nail lacquers proved to be a better tool.

Keywords: *medicated nail lacquer, keratolytic agent penetration enhancer, DMSO, onychomycosis.*

GJMR-B Classification: *NLMC Code: QV 752*

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Formulation and Evaluation of Medicated Tolnaftate Nail Lacquer

Farsana. P [α], Baby Shahanas [σ], Anu Sebastian [ρ] & Ashly Merin George [ω]

Abstract- The present study was aimed towards the design and formulation of medicated nail lacquer of tolnaftate to control onychomycosis condition and improve the patient compliance. The present work investigated the amount of tolnaftate released from different formulations containing different concentration of ethyl cellulose and different proportions of thioglycolic acid and dimethyl sulfoxides for treatment of onychomycosis. Then these lacquers were compared for drying time, nonvolatile content, drug content, drug diffusion and antimicrobial studies. The stability test showed that the formulation were stable at 37° ± 2°C for 1 month. The results obtained from in-vitro diffusion studies showed that formulation F3 have completed drug release of 94.48% over 24 hrs. The F3 formulation had salicylic acid as keratolytic agent and 0.5ml of 1% w/v of thioglycolic acid as penetration enhancer. From diffusion studies, it was concluded that thioglycolic acid containing formulation (F2 and F3) have better penetration enhancement as compared to DMSO containing formulation. The best formulation was evaluated for antifungal sensitivity test against the *Candida albicans*. From the above study, it can be concluded that medicated nail lacquers proved to be a better tool .In this work, the main goal is to develop medicated nail lacquer, for maximum drug release for treating onychomycosis and achieve better patient compliance.

Keywords: medicated nail lacquer, keratolytic agent penetration enhancer, DMSO, onychomycosis.

I. Introduction

The major constrains of the preungual drug delivery (drug delivery through the nail) to nail is lack of understanding about barrier property related to the nail formulations. Topical drug delivery system owes many advantages in case of antifungal drugs such as it avoids hepatotoxicity, high tissue concentration which is required for the treatment of fungal infection of nails. Most of topical formulations in form of gels, lotions etc. pose limitations such as removal by whipping, rubbing and less adherence of formulation to the affected site of nail[1]. Medicated nail lacquer is an excellent alternative for the treatment of fungal infection of nails and high efficacy of drug can be achieved. It also provides an optimized and sustained release of drug by formation of an occlusive film which acts as "depot" after the application of lacquer on the nail.[2]

The advantages of nail lacquer include it cannot be easily removed by rubbing, washing etc the effect is long lasting, depot formation. Factors affecting drug delivery include molecular size of compound or diffusing species, degree of ionization, binding of the drug to keratin and other nail constituents, nail thickness and presence of disease. Nail lacquers containing drug are fairly new formulations and have been termed transungual delivery systems. These formulations are essentially organic solutions of a film-forming polymer and contain the drug to be delivered. When applied to the nail plate, the solvent evaporates leaving a polymer film (containing drug) onto the nail plate[3]. The drug is then slowly released from the film, penetrates into the nail plate and the nail bed. The drug concentration in the film is much higher than concentration in the original nail lacquer as the solvent evaporates and a film is formed.

Here tolnaftate is used as a drug which is a synthetic antifungal agent comes under thiocarbamate derivative with antimicrobial and antifungal activity. Salicylic acid used in the formulation as a keratolytic agent and DMSO as penetration enhancer. In this work, tolnaftate loaded nail lacquer was formulated and produce a drug release over 24 hours.

II. Materials and Methods

a) Materials

Tolnaftate was purchased from Yarrow Chem Products. Ethyl Cellulose and DMSO were purchased from Nice Chemicals, Cochin. Salicylic acid, Sodium hydroxide, Potassium Di-hydrogen Phosphate were purchased from Spectrum Reagents and Chemicals Pvt. Ltd, Cochin. Glycerin was obtained from Isochem Laboratory, Palakkad and Thioglycollic Acid from SDFCL Mumbai and Nutrient Agar Medium from Himedia Labortaries. Pvt. Ltd. Distilled Water was obtained from Grace College of Pharmacy. All other reagents used are of high purity.

b) Method

i. Preparation of Tolnaftate Nail Lacquer

Tolnaftate nail lacquer was prepared by simple mixing method. Where in the formulation, the drug concentration was kept constant. The amount of ethyl cellulose, salicylic acid, glycerine were mixed till it gives the uniform distribution of component which is used as the nail lacquer.

Tolnaftate was mixed properly and then the thioglycolic acid added in F2, F3 and DMSO in F4 and F5 and mixed the solution in the magnetic stirrer. Then the solvent mixed and volume made up to fix quantity and mixed properly.

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Table 1: Composition of Tolnaftate Nail Lacquer

Sl. No.	Ingredients	F1	F2	F3	F4	F5
1	Tolnaftate (mg)	20	20	20	20	20
2	Salicylic Acid (mg)	40	40	40	40	40
3	Ethyl Cellulose (g)	2	2	3	2	3
4	Glycerine (ml)	2	2	2	2	2
5	Thioglycolic Acid (%)	--	0.2	0.5	--	--
6	DMSO (ml)	--	--	--	0.2	0.5
7	Ethanol (ml)	20	20	20	20	20

20 mg of Tolnaftate was loaded in this formulations and various combination were tried to get effective nail lacquers. Tolnaftate was added to the ethanol which containing salicylic acid which is to improve the drug permeation and 2 g of ethyl cellulose as one of the polymer are added which is also act as film former. Glycerin was mixed till it gives the uniform distribution of the component which is used in the nail lacquer. To enhance the penetration level of the formulation, thioglycolic acid and dimethyl sulfoxide were added. Now the solution was kept on the magnetic stirrer till tolnaftate get completely mixed and volume made upto fix quantity and mixed properly. After preparation of nail lacquer, stored and further used for evaluation studies.

III. Evaluation Study

a) Drying Time

A film of sample was applied on a glass petri dish with the help of brush. The time to form a dry to touch film was noted using a stopwatch.

b) Nonvolatile Content

8 ml of sample was taken in a glass petri dish of about 8cm in diameter. Samples were spread equally. The dish was placed in the oven at 105^0C for 1 hr. The petri dish was removed, cooled, and weighed. The difference in weight of sample after drying was determined that gives the volatile content present. The difference in weights was recorded.

c) Water Resistance

This is the measure of the resistance towards water permeability of the film. This was done by applying a continuous film on a surface and drying, then immersing it in water. The weight before and after immersion was noted and increase in weight was calculated. Higher the increase in weight, lower the water resistance.

d) Stability Study[4]

Stability study was conducted by storing the optimized formulation at 40°C and 37±20°C for 1 month. The formulation was then evaluated for drying time, non-volatile content, in-vitro adhesion, water resistance and drug content.

e) Smoothness to Flow

The sample was poured on a glass slide on an area of 1.5 square inches and spread on a glass plate by making glass slide to rise vertically. And smoothness of flow was determined by comparing with standard marketed nail lacquer.

f) Drug Content Estimation[5]

Nail lacquer equivalent to 200 mg was dissolved in 50 ml phosphate buffer solution of pH 7.4. Then the solution was ultra sonicated for 15 mints. The resulting solution was filtered, made up to 100 ml with phosphate buffer solution of pH 7.4. From the above solution take 10ml and made up to100ml with PBS of pH 7.4. Then the diluted solution was estimated spectrophotometrically at wavelength of 254 nm and determined the drug content.

g) Diffusion Studies Across Artificial Membrane[6]

Diffusion studies were performed using artificial membrane (cellophane).The membrane was soaked for 1hr in solvent system (phosphate buffer, pH 7.4), and the receptor compartment was filled with solvent. Test vehicle equivalent to 4 mg was applied evenly on the surface of the membrane. The prepared membrane was mounted on the cell carefully to avoid entrapment of air bubbles under the membrane. The whole assembly was maintained at 37°C, and the speed of stirring was kept constant (600 rpm) for 24 hrs. The 2 ml aliquot of drug sample was taken after a time interval of 2 hrs and was replaced by the fresh solvent. Each experiment was replicated at least thrice. The drug analysis was done using UV spectrophotometer at 254 nm.

h) In-Vitro Transungual Permeation Studies[7]

In Hooves from freshly slaughtered cattle, free of adhering connective and cartilaginous tissue, were soaked in distilled water for 24 hrs. Membranes of about 1-mm thickness were then cut from the distal part of hooves. In-vitro permeation studies were carried out by using Franz diffusion cell (respective volume, 100 ml) the hoof membrane was placed carefully on the cell, and the surface area available for permeation was 1.4 cm^2. Then the test vehicle equivalent to 4 mg was applied evenly on the surface of the nail membrane. The receptor compartment was filled with solvent A (phosphate buffer, pH 7.4), and the whole assembly

was maintained at 37°C with constant stirring for 30 h. The 5 ml aliquot of drug sample was taken after a time interval of 2 h and was replaced by the fresh solvent A. The drug analysis was done by using double-beam UV spectrophotometer at 254 nm.

i) Determination of Zone of Inhibition[8]

Agar cup-plate method was used to determine in vitro antifungal activity against Candida albicans. Nutrient agar plates were prepared and sterilized by autoclaving at 120°C, 15 pounds pressure for 15 min. 70 ml nutrient agar media was then inoculated with fungal strain i.e. C. albicans (2 mL of inoculum to 100mL of nutrient agar media). The mixture was then poured in two sterilized petri plates and five wells of 5 mm diameters were prepared via sterile cork borer in each petri plate. 0.2 ml each of optimized formulation, control formulation were transferred to the cups aseptically and labelled accordingly as optimized and control formulation. Negative and positive controls were also prepared which consist of un-inoculated media and media seeded with test organism but deprived of antifungal agent, respectively. The prepared petri plates were maintained at room temperature for 2 h to allow the diffusion of the solutions in to the medium and then incubated at 28°C for 48 hrs. The diameter of zone of inhibition surrounding each of the well was recorded.

IV. Results

The prime objective of the work was to formulate tolnaftate nail lacquer containing two different penetration enhancers and different concentrations of polymer and to find out which polymer concentration and concentration of penetration enhancers gave better release as well as to carry out the antifungal testing on the best formulation obtained.

a) Identification by FTIR Spectroscopy

IR spectrum of tolnaftate was compared with the standard spectrum and the sample spectrum (Fig. 1) showed all the characteristic peaks in the relevant region. So IR spectra verified the authenticity of the procured sample. The IR spectrum of tolnaftate, ethyl cellulose and salicylic acid combination does not show deviation as compared to standard spectrum of tolnaftate is shown in (Fig. 2).

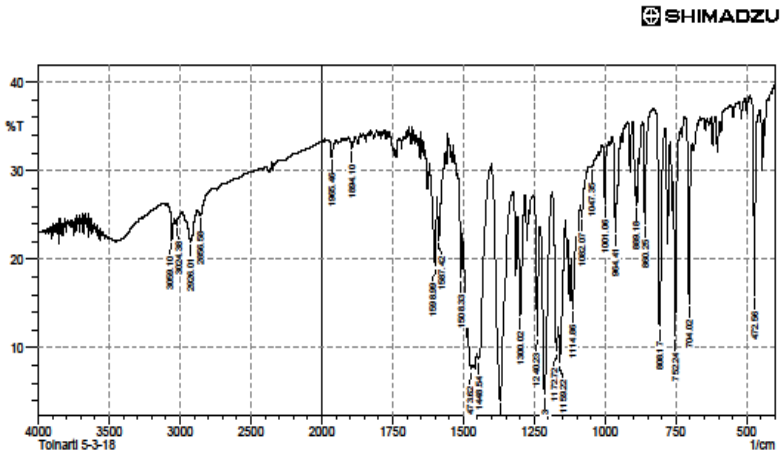

Fig. 1: IR Spectrum of Tolnaftate

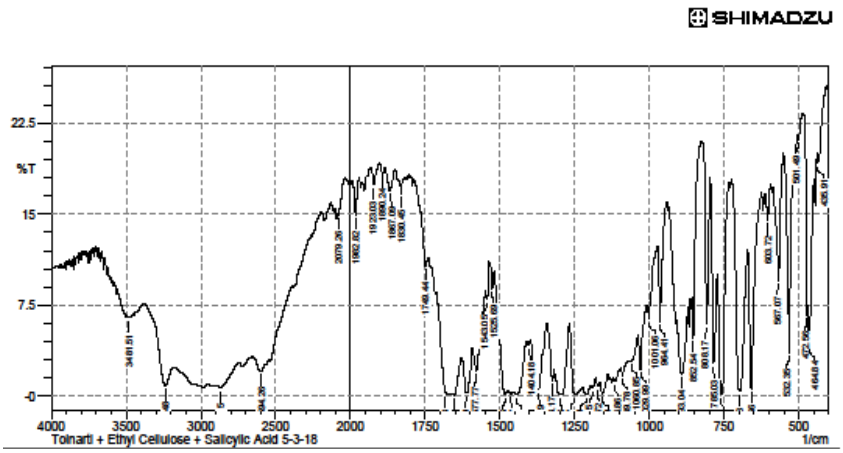

Fig. 2: IR Spectrum of Tolnaftate Ethyl Cellulose Salicylic Acid Combination

V. Formulation of Tolnaftate Nail Lacquer using Different Penetration Enhancers

Tolnaftate nail lacquers with different penetration enhancers combinations were prepared by simple mixing method. For that 20 mg of tolnaftate was loaded in various solutions, so as to get a concentration of 1mg/ml. Different penetration enhancers such as thioglycolic acid and dimethyl sulfoxides were used in the F1, F2, F3, F4, F5. Different formulations containing different concentration of ethyl cellulose and different proportions of thioglycolic acid (F2 and F3) and dimethyl sulfoxides (F4 and F5) combinations were prepared.

VI. Evaluation of Nail Lacquer

Formulated nail lacquers were subjected to preliminary evaluation tests. Nail lacquers with any imperfection in smoothness of flow, Water resistance, Drying time and in stability were excluded.

a) Drying Time

Drying time for formulations F1 to F5 was found between 61 seconds to 70 seconds. It was found that as the polymer concentration increases from 2% w/v to 3% w/v the drying time increases respectively. The time required for the solvent to evaporate from the more viscous solution is more than the less viscous solution.

b) Non Volatile Content of Tolnaftate Nail Lacquer

It was seen that as the polymer concentration increases from 1% w/v to 2% w/v the non-volatile content increases. The formulation which had higher concentration of polymer showed higher non-volatile content as the amount of polymer present in the sample for determination of nonvolatile content was more as compared to the formulation which contained lower concentrations of polymer. Non-volatile content depends and varyupon the concentration of polymer used.

c) Water Resistance Test

Table 2: Water Resistance Test

Sl. No.	Formulation Code	W1(g)	W2(g)	Difference In Weight (g)
1	F1	6.00	6.24	0.24
2	F2	6.00	6.22	0.22
3	F3	6.00	6.22	0.22
4	F4	6.00	6.51	0.51
5	F5	6.00	6.50	0.50

W1 and W2 - Weight of glass slide along with nail lacquer before and after dipping in water.

From the water resistance test, it can be seen as the polymer concentration increases the water resistance increases, as the concentration of polymer decreases the water resistance decreases. Formulations F1, F2, and F4 showed lower water resistance as compared to F3 and F5.

d) Stability Study

The stability study data indicated that the medicated nail lacquer, showed good stability for 1 month when it was stored at temperature of 37±2ºC. There is no significant change is observed in color, non volatile content, viscosity, drying time and smoothness.

e) Smoothness to Flow

Smoothness of flow for formulations F1, F2, F3, F4 and F5 showed satisfactory flow property compared to marketed product.

f) Drug Content Estimation

Table 3: Drug Content

Formulation	Drug Content (%)
F1	90.00± 0.209
F2	92.50±0.167
F3	94.28±0.006
F4	91.25±0.474
F5	92.76±0.178

Percentage drug content for all the lacquers was found to be satisfactory and in between 90% to 94% which is reported in table 3. Highest % of drug content was found to be 94.28 % (F3) and the lowest % of drug content was 90.00 % (F1). Drug content more than 90% in the formulation shows the high amount of drug present in the formulation, ensuring that the methods of formulation and the ingredients selected are not affecting the stability of drug. High drug content also gives the assurance that, a good therapeutic outcome can be expected.

g) In vitro Diffusion Studies Across Artificial Membrane

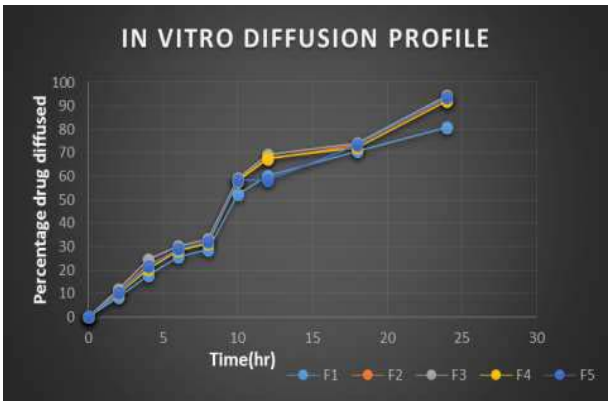

Fig. 3: In Vitro Diffusion Profile of Tolnaftate

In vitro diffusion studies were conducted using diffusing cell for 24 hours. Formulation F3, F5 containing highest concentration of penetration enhancer (thioglycolic acid and DMSO) showed the highest release of 94.48 % and 93.58 %. It was found that as the penetration enhancer concentration increases, the release of drug increases. From the data obtained by evaluation of nail lacquer, formulation F3 was found to be best formulation among all the four formulations.

h) In vitro Transungual Permeation Studies

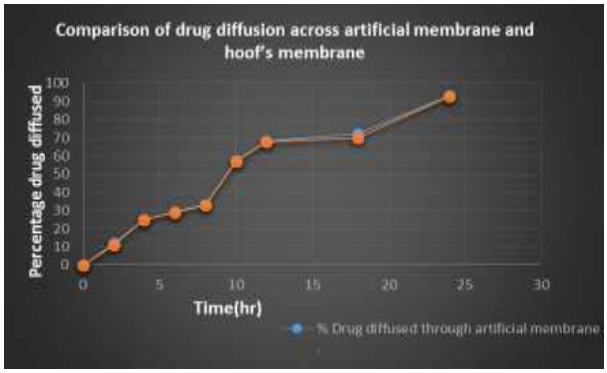

Fig. 4: In-Vitro Permeation Studies of Formulations F1 to F5

In vitro permeation studies, it was found that formulation F3 showed release of 92.78% at the end of 24 hours. From in vitro diffusion studies and in-vitro permeation studies it was found that thioglycolic acid was proved to a better penetration enhancer as compared to dimethyl sulfoxide. The effect of thioglycolic acid was attributed to its small molecular weight and damage caused on the keratin network and decrease in lipid content in the dorsal nail layer; this act which loosened the nail structure, allowing tolnaftate to penetrate easier.

i) Determination of Zone of Inhibition

Formulation prepared with ethyl cellulose, salicylic acid, and glycerine respectively having 1mg/ml dose of tolnaftate were subjected to antifungal activity. 20 mg tolnaftate was loaded in various combinations and compared the obtained zone diameter as that of zone of inhibition of tolnaftate in ethanol. The zone of inhibition obtained were determined in Candida albican organism and compared with tolnaftate standard. From the analysis, formulation showed comparable zone of inhibition with that of tolnaftate standard solution.

Fig. 5: Antifungal Activity of Various Tolnaftate Loaded Formulations

F1-Tolnaftate nail lacquer without penetration enhancer.
F2-Tolnaftate nail lacquer with 0.3ml of thioglycolic acid.
F3-Tolnaftate nail lacquer with 0.5ml of thioglycolic acid.
F4-Tolnaftate nail lacquer with 0.3ml of DMSO.
F5-Tolnaftate nail lacquer with 0.5ml of DMSO.

Comparative antifungal activity of tolnaftate loaded thioglycolic acid formulation with marketed suspension.

The antifungal activity of tolnaftate loaded with various combinations were studied using nutrient agar medium. Here ketoconozole in DMSO taken as standard and compared the zone diameter obtained by tolnaftate with that of various penetration enhancers. It was found that thioglycolic acid tolnaftate loaded lacquer have similar zone diameter as those of ketoconozole drug standard. Moreover presence of thioglycolic acid in all formulations can also contribute to prevent the development of onychomycosis because it inhibits Candida albicanto nail plate.

VII. Summary and Conclusion

The present study aimed to produce a formulation for treating onychomycosis. This formulation includes antifungal agents, penetration enhancers (DMSO and thioglycolic acid) and keratolytic agent salicylic acid for additional benefits.

The nail formulations excluding polymer with omitted as the formulation showed tackiness, dullness etc. Out of 36 formulations, best 5 were chosen for further formulation and evaluations was done. FTIR studies revealed that no chemical interaction between drug and polymer. Then these lacquers were compared for drying time, non volatile content, drug content, drug diffusion and antimicrobial studies. All formulations showed good film formation and other parameters. The stability test showed that the formulation were stable at 37° ± 2° C for 1 month.

The results obtained from in-vitro diffusion studies showed that formulation F3 have completed drug release of 94.48% over 24 hrs. The F3 formulation had salicylic acid as keratolytic agent and 0.5 ml of 1 % w/v of thioglycolic acid as penetration enhancer. This indicates the combination of permeation enhancer and keratolytic agent resulted in an improved permeation and sustained drug release. The nonvolatile content of F3 was found to be 1.04. F3 formulation showed rapid drying rate. From diffusion studies, it was concluded that thioglycolic acid containing formulation (F2 and F3) have better penetration enhancement as compared to DMSO containing formulation. From the above study, it can be concluded that medicated nail lacquers proved to be a better tool as a drug delivery system for ungual drug delivery of an antifungal in the treatment of onychomycosis.

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Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology & Medicine
Volume 18 Issue 5 Version 1.0 Year 2018
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Cytoprotective Effect of Biofield Energy Treated Test Item against *Tert*-Butyl Hydroperoxide (*T*-BHP) - Induced Cell Damage in Hepg2 Cell-Line

By Alice Branton & Snehasis Jana

Abstract- Emerging data indicate that the mortality rate is rising due to liver disorders day-by-day in the developed countries. The present study was conducted to evaluate the potential of the Biofield Energy (The Trivedi Effect®) Treated test item (DMEM) in HepG2 cell-line. The test item was divided into two parts. One part of the test item received Consciousness Energy Healing Treatment by a renowned Biofield Energy Healer, Alice Branton and was labeled as the Biofield Energy Treated DMEM and the other part defined as untreated DMEM, where no Biofield Treatment was provided. Cell viability of the test items using MTT assay showed 113% and 129.9% viable cells in the untreated DMEM and Biofield Energy Treated DMEM groups, respectively suggested that the test items were nontoxic and safe in nature. The Biofield Energy Treated DMEM showed significant ($p \leq 0.001$) protection of cells by 15% against oxidative stress induced by *t*-BHP, while untreated DMEM group showed 0.4% protection.

Keywords: the trivedi effect®, hepg2, liver health, interleukin-8, alt, cholesterol, albumin.

GJMR-B Classification: NLMC Code: QV 4

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Cytoprotective Effect of Biofield Energy Treated Test Item against *Tert*-Butyl Hydroperoxide (*T*-BHP) - Induced Cell Damage in HepG2 Cell-Line

Alice Branton [α] & Snehasis Jana [σ]

Abstract- Emerging data indicate that the mortality rate is rising due to liver disorders day-by-day in the developed countries. The present study was conducted to evaluate the potential of the Biofield Energy (The Trivedi Effect®) Treated test item (DMEM) in HepG2 cell-line. The test item was divided into two parts. One part of the test item received Consciousness Energy Healing Treatment by a renowned Biofield Energy Healer, Alice Branton and was labeled as the Biofield Energy Treated DMEM and the other part defined as untreated DMEM, where no Biofield Treatment was provided. Cell viability of the test items using MTT assay showed 113% and 129.9% viable cells in the untreated DMEM and Biofield Energy Treated DMEM groups, respectively suggested that the test items were nontoxic and safe in nature. The Biofield Energy Treated DMEM showed significant ($p \leq 0.001$) protection of cells by 15% against oxidative stress induced by *t*-BHP, while untreated DMEM group showed 0.4% protection. The level of IL-8 was significantly ($p \leq 0.01$) reduced by 32.15% in the Biofield Energy Treated DMEM group compared to the untreated DMEM group. Moreover, the level of ALT enzyme activity was significantly ($p \leq 0.01$) reduced by 53.2% in the Biofield Energy Treated DMEM group compared to the untreated DMEM group. Cholesterol level was significantly ($p \leq 0.001$) reduced by 37.35% in the Biofield Energy Treated DMEM group compared to the untreated DMEM group. Besides, the Biofield Energy Treated DMEM group showed 43.13% increased the level of albumin compared to the untreated DMEM group. Altogether, results suggested that Biofield Treatment significantly improved liver function. Thus, Consciousness Energy Healing (The Trivedi Effect®) Treatment could be utilized as a hepatoprotectant against several hepatic disorders such as Gilbert's disease, cirrhosis, steatosis, alcohol abuse, hemochromatosis, Budd-Chiari syndrome, Wilson's disease, cholangiocarcinoma, etc.

Keywords: the trivedi effect®, HepG2, liver health, interleukin-8, ALT, cholesterol, albumin.

I. Introduction

Hepatocellular carcinoma (HCC) is the fifth most common malignancy in the world. As per global statistics it has been reported that the incidence of chronic liver cirrhosis is increasing worldwide ranging from 3% to 9% per year[1]. Cancer, aging, coronary heart disease, neurodegenerative disorders (*i.e.,* Alzheimer's disease), diabetes, and liver damage are all associated with an increased level of reactive oxygen species (ROS) formation. More selectively the mitochondrial electron transport chain is another main source of cellular ROS generator[2,3]. For the assessment of hepatoprotective activity *in vitro* model is more advantageous than *in vivo*[4]. Human hepatoma cell line (HepG2) has been widely used as an alternative model to human hepatocytes *in vitro* for the assessment of hepatoprotectant activity of a test substances[5]. HepG2 cell line has many advantages compared to others cells lines as it is an immortalized cell line, easily available and cryopreserved, and even after cultivation the metabolizing ability not reduced[6]. Numerous experimental data reported the useful effects of Biofield Energy Healing Treatment in cases of cancer patients *via* therapeutic touch[7], massage therapy[8], etc. Biofield Therapy is one of the Complementary and Alternative Medicine (CAM) therapies to enhance physical, mental, and emotional human wellness. The National Center of Complementary and Integrative Health (NCCIH) has recognized Biofield Therapy as a CAM health care approach including other therapies, medicines and practices such as natural products, chiropractic/ osteopathic manipulation, deep breathing, Tai Chi, yoga, meditation, relaxation techniques, Qi Gong, special diets, progressive relaxation, massage, healing touch, homeopathy, guided imagery, rolfing structural integration, acupuncture, movement therapy, hypnotherapy, pilates, mindfulness, acupressure, traditional Chinese herbs and medicines, Ayurvedic medicine, Reiki, aromatherapy, naturopathy, essential oils, and cranial sacral therapy. The Biofield Energy can be harnessed and transmitted by the Healers into living and non-living things *via* the process of Biofield Energy Healing Treatment. The outcomes of The Trivedi Effect® -Consciousness Energy Healing Treatment has been reported with a significant revolution in a wide spectrum of areas including materials science[9-11], agriculture [12,13], microbiology [14-16], biotechnology [17,18], nutraceuticals [19,20], cancer research [21,22]. Apart from this, The Trivedi Effect® also tremendously

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improved bioavailability of various low bio available compounds [23-25], an improved overall skin health [26,27], bone health [28-30], human health and wellness. Based on the excellent outcome of The Trivedi Effect® and importance of liver health authors intend to develop a new treatment modality to study the impact of the Biofield Energy Healing Treated (The Trivedi Effect®) test item (DMEM) on liver hepatocyte cells.

II. Materials and Methods

a) Chemicals and Reagents

Antibiotics solution (penicillin-streptomycin) was purchased from HiMedia. Dulbecco's Modified Eagle Medium (DMEM) and fetal bovine serum (FBS) were obtained from Gibco, India. Alanine aminotransferase (ALT) 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-Diphenyltetrazolium Bromide (MTT) and ethylenediaminetetraacetic acid (EDTA) were obtained from Sigma Chemical Co. (St. Louis, MO). The positive controls silymarin and mevinolin were procured from Sanat products ltd., India and Zliesher Nobel, respectively. All the other chemicals used in this experiment were analytical grade procured from India.

b) Biofield Energy Healing Strategy

The test item (DMEM) was used in this experiment and one portion was considered as the untreated DMEM group, where no Biofield Treatment was provided. Further, the untreated group was treated with "sham" healer for comparison purpose. The sham healer did not have any knowledge about the Biofield Energy Healing Treatment. The other portion of the test item was received Biofield Energy Treatment and defined as the Biofield Energy Treated DMEM group. Biofield Energy Healing Treatment (known as The Trivedi Effect®) was received under laboratory conditions for ~5 minutes through Alice Branton's unique Biofield Energy Transmission process. Biofield Energy Healer was located in the USA; however the test items were located in the research laboratory of Dabur Research Foundation, New Delhi, India. Biofield Energy Healer in this experiment did not visit the laboratory, nor had any contact with the test samples. After that, the Biofield Energy Treated and untreated test items were kept in similar sealed conditions and used for the study as per the study plan.

c) Assessment of Cell Viability using MTT Assay

The cell viability was performed by MTT assay in HepG2 cell line. The cells were counted and plated in a 96-well plate at the density corresponding to 10 X 10^3 cells / well / 180 µL in DMEM + 10% FBS. The cells in the above plate(s) were incubated for 24 hours in a CO_2 incubator at 37°C, 5% CO_2, and 95% humidity. Following incubation, the medium was removed and the following treatments were given. In the Biofield Treated test item (DMEM) group, 200 µL of the Biofield Energy Treated test item (DMEM) was added to wells, and in the untreated DMEM group, added 200 µL of untreated DMEM. Besides, in the positive control groups, added 180 µL of DMEM with 20 µL of positive controls were added from the respective 10X stock solutions. After incubation for 48 hours, the effect of test items on cell viability was assessed by MTT assay. 20 µL of 5 mg/mL of MTT was added to all the wells and incubated at 37°C for 3 hours. The supernatant was aspirated and 150 µL of DMSO was added to all wells to dissolve formazan crystals. The optical density (OD) of each well was read at 540 nm using Biotek Reader.

Effect of the test items on viability of HepG2 cells was determined using Equation (1):

$$\%\ Cell\ viability = (100 - \%\ Cytotoxicity) \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where, % Cytotoxicity = {(O.D. of cells of untreated DMEM - O.D. of cells Biofield Treated DMEM / positive controls) / O.D. of cells of untreated DMEM}*100

For test items and positive controls, concentrations resulting≥70% cell viability were taken as safe / non-cytotoxic concentration.

d) Evaluation of Cytoprotective Effect of the Test Item

Cells were trypsinized and a single cell suspension of HepG2 was prepared. Cells were counted on an hemocytometer and seeded at a density of 10 X 10^3 cells / well / 180 µL in DMEM + 10% FBS in a 96-well plate. Cells were incubated in a CO_2 incubator for 24 hours at 37°C, 5% CO_2 and 95% humidity. After 24 hours, the medium was removed and the following treatments were given. In the test item groups, 180 µL of the test items were added to wells. In the positive control group, 160 µL of serum free medium and 20 µL of positive control from the respective 10X stock solution was added to wells. After 24 hours of treatment, cells were treated with *t*-BHP at 250 µM (20 µL from the respective 10X stock) for 4 hours. After 4 hours, the protective effect of the test items on cell viability was assessed by MTT assay as per study protocol.

e) Estimation of Interleukin-8 (IL-8)

HepG2 cell suspension in DMEM containing 10% FBS was plated at a density of 0.3 X 10^6 cells /well / 1 mL in a 12-well plate. Cells were incubated in a CO_2 incubator for 24 hours at 37°C, 5% CO_2, and 95% humidity. Cells were sera starved by replacing the medium with DMEM + 10% FBS for 24 hours. After 24 hours of sera starvation, medium was removed and pre-treatment were provided to the different treatment groups. After 24 hours of treatment, cells were stimulated with inflammatory stimulus TNF-α at a final concentration of 10 ng/mL. After treatment, cells were incubated in a 5% CO_2 incubator for 24 hours.

After 24 hours of incubation, culture supernatants were collected from each well and stored at -20°C until analysis. The level of cytokine (IL-8) in culture supernatants of HepG2 cells was determined using ELISA as per manufacturer's instructions.

f) Estimation of ALT

Cells were trypsinized and a single cell suspension of HepG2 was prepared and counted on an hemocytometer. Cells were seeded at a density of 10 X 10^3 cells / well / 180 µL in DMEM + 10% FBS in a 96-well plate. Cells were incubated in a CO_2 incubator for 24 hours at 37°C, 5% CO_2, and 95% humidity. After 24 hours, medium was removed and different treatments were given as per study plan. After incubation for 24 hours, cells were treated with 250 µM of *t*-BHP. After 4 hours of incubation, culture supernatants were collected from each well and stored at -20°C until analysis. The level of ALT in culture supernatants of HepG2 cells was determined using commercial kit as per manufacturer's instructions.

g) Estimation of Cholesterol

Cells were trypsinized and a single cell suspension of HepG2 was prepared. Cells were counted using an hemocytometer and seeded at a density of 1 million cells / well / mL in DMEM + 10% FBS in a 6-well plate. Cells were incubated in a CO_2 incubator for 24 hours at 37°C, 5% CO_2 and 95% humidity. After 24 hours, medium was removed and treated with different treatment groups. After 24 hours of incubation, cell lysates were prepared in the following manner. Lysis buffer containing chloroform: isopropanol: IGEPAL CA630 in the ratio of 7:11:0.1 was prepared. Medium was removed from each well and 400µL of the above buffer was added to each well, which led to detachment of cells and formation of white layer. Cells were scrapped off and transferred into a labeled centrifuge tubes. The cells were homogenized in ice using a tissue homogenizer for 4-5 minutes until the solution was turned turbid in appearance. After homogenizing, the cells were centrifuged at 13000*g* for 10 minutes. The supernatant was collected in a prelabeled centrifuge tube and the pellet was discarded. The tube containing the supernatant was kept at 37°C for 24 hours for evaporation of buffer. After 24 hours, the tube was removed from 37°C and the dried lipids (small yellow colored pellet) were obtained, which was stored at -20 °C until analysis. The level of cholesterol in cell lysates of HepG2 cells was determined using a commercial kit as per manufacturer's instructions.

h) Estimation of Albumin

Cells were trypsinized and a single cell suspension of HepG2 was prepared. Cells were counted using an hemocytometer and seeded at a density of 0.25 million cells / well / 1 mL in DMEM+10 % FBS in a 24-well plate. Then, the cells were incubated in a CO_2 incubator for 24 hours at 37°C, 5% CO_2, and 95% humidity. Further, the cells were sera starved by replacing the medium with DMEM + 10% FBS for 24 hours. After 24 hours, medium was removed and various treatments were given. After 48 hours of incubation, culture supernatants were collected from each well and stored at -20°C until analysis. The level of albumin in culture supernatants of HepG2 cells were determined using a commercial kit as per manufacturer's instructions.

i) Statistical Analysis

All the values were represented as Mean ± SEM (standard error of mean) of three independent experiments. For two groups comparison student's *t*-test was used. For multiple group comparison, one-way analysis of variance (ANOVA) was used followed by post-hoc analysis by Dunnett's test. Statistically significant values were set at the level of $p \leq 0.05$.

III. Results and Discussion

a) Cell Viability Assay (MTT)

The results of the cytotoxicity using MTT cell viability assay after treatment with the positive controls (silymarin and mevinolin), untreated DMEM, and the Biofield Energy Treated DMEM in HepG2 cells are shown in Figure 1. Silymarin showed more than 136% cell viability upto 25 µg/mL and mevinolin showed greater than 97% cell viability upto 20 µg/mL. Further, the untreated and Biofield Energy Treated DMEM groups showed 113% and 129.9% cell viability, respectively (Figure 1). Therefore, the positive controls and the test items were found more than 97% cell viability, which indicated a safe and nontoxic profile in the tested concentrations.

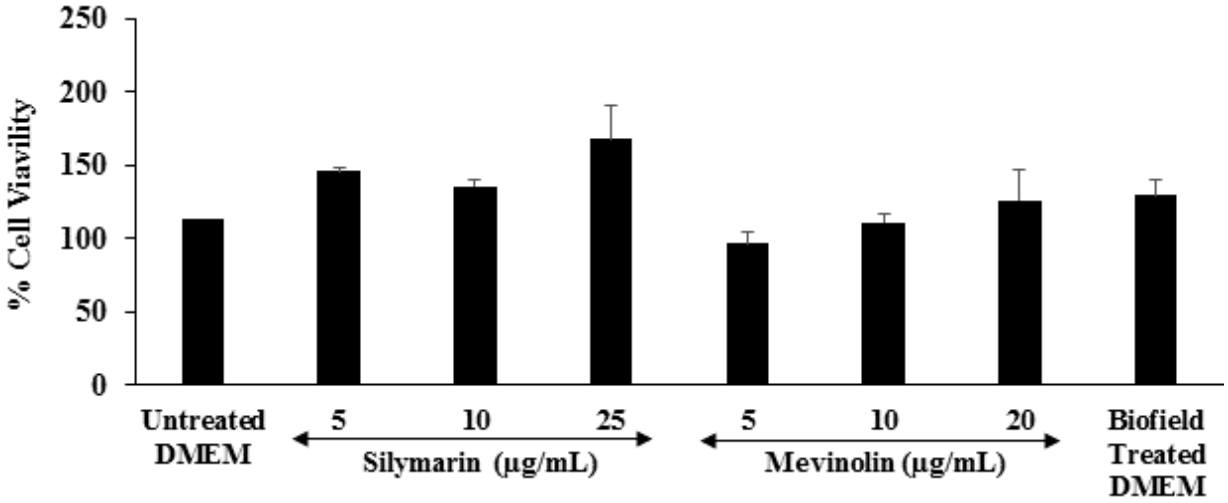

Figure 1: Effect of the test items (DMEM) and positive controls on cell viability in HepG2 cells after 48 hours of treatment. All the values are represented as mean ± SEM of three independent experiments.

b) Cytoprotective Activity

The cytoprotective activity of the Biofield Energy Treated test items on the protection of cell viability in HepG2 cells was determined against *t*-BHP induced cell damage after 4 hours of treatment is presented in Figure 2. Silymarin showed 4.9%, 38.4% ($p \leq 0.001$), and 66.1% ($p \leq 0.001$) cellular protection at 1, 5, and 25 µg/mL, respectively compared to the *t*-BHP induced group. Besides, the Biofield Energy Treated test item (DMEM) showed significant ($p \leq 0.001$) restoration of cell viability by 15%, while untreated DMEM group showed 0.4% protection under the *t*-BHP induction (Figure 2). *t*-BHP is known to generate ROS and induce lipid peroxidation in cells and simultaneously reduced the primary antioxidant of cells *i.e.,* glutathione (GSH)[31,32]. In this experiment from Figure 2, it was observed that Biofield Energy Treated Test item effectively restored cellular function by 15%. The findings showed that Biofield Energy Treatment has the significant cytoprotective and antioxidant activities, which could be due to the effect of The Trivedi Effect®- Energy of Consciousness. Thus, The Trivedi Effect® Treated test item (DMEM) could be utilized against liver disorders.

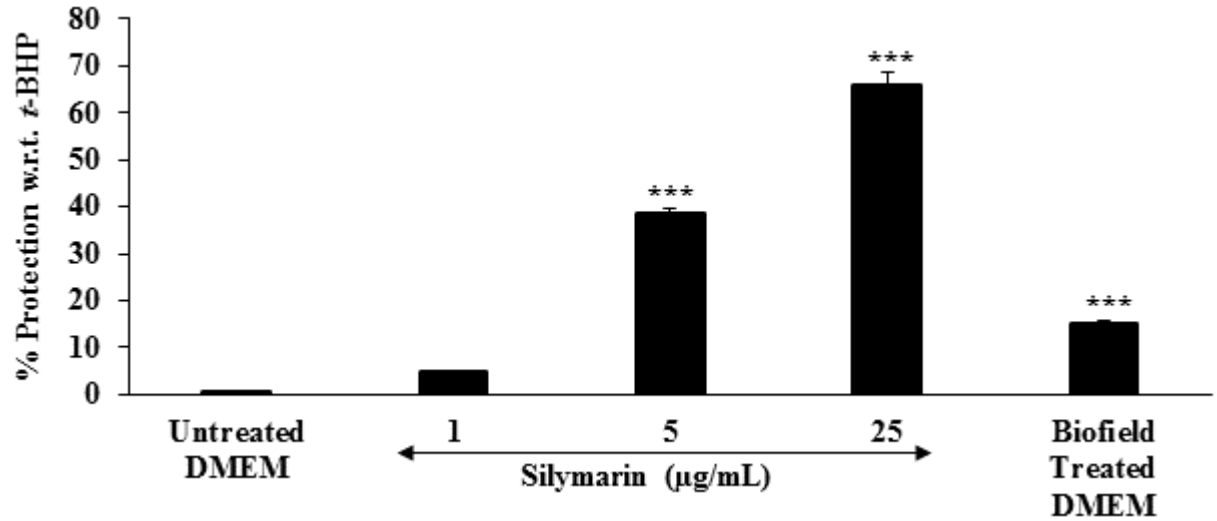

Figure 2: Assessment of cytoprotective effect of the test items (DMEM) in HepG2 cells against *tert*-butyl hydroperoxide (*t*-BHP) induced damage after 4 hours of treatment. All the values are represented as mean ± SEM of three independent experiments. ****p*≤*0.001* vs. untreated DMEM group.

c) Estimation of Interleukin-8 (IL-8)

Interleukin-8 (IL-8) is a potent chemoattractant for neutrophils and causes acute liver inflammation[33,34]. The effect of the test items on IL-8 is shown in Figure 3. Increase level of oxidative stress causes increase secretion of IL-8, and ultimately recruit the inflammatory cells causes' localized inflammation[35]. In this experiment, after treatment with TNF-α at 10 ng/mL can

significantly induced oxidative stress and the proinflammatory cytokines IL-8, because oxidative stress and TNF-alpha are the mediators in IL-8 response[36]. The level of IL-8 in the untreated DMEM group was 964.4 ± 40.65 pg/mL. On the other side, the Biofield Energy Treated DMEM group showed significant ($p \leq 0.01$) reduction of IL-8 by 32.15% compared to the untreated DMEM group under the stimulation of TNF-α at 10 ng/mL (Figure 3).

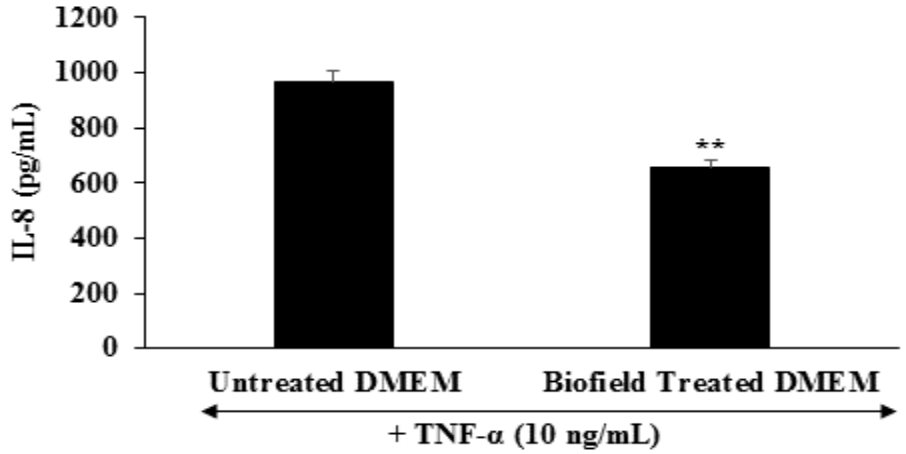

Figure 3: The effect of the test items on proinflammatory cytokine - interleukin-8 (IL-8) against TNF-α stimulation after 24 hours of treatment. TNF-α: Tumor necrosis factor alpha. All the values are represented as mean ± SEM of three independent experiments. ***p≤0.01* vs. untreated DMEM group.

d) Estimation of Alanine Aminotransferase (ALT)

The effect of the test items on alanine aminotransferase (ALT) is shown in Figure 4. The positive control, silymarin showed 8.4%, 25.6%, and 79.2% ($p \leq 0.01$) reduction of ALT level at 1, 5, and 25 µg/mL, respectively with respect to the untreated DMEM group. Besides, the Biofield Energy Treated DMEM group showed a significant ($p \leq 0.01$) reduction of ALT by 53.2% compared to the untreated DMEM group (Figure 4). The aminotransferase enzymes catalyze the reversible transformation of α-ketoacids into amino acids. Increased serum level of ALT is directly proportional to the severity of the diseases like hepatocellular injury and death[37]. Thus, the elevation of serum ALT enzyme chances of liver disorders[38]. Here, the Biofield Energy Treated test item (DMEM) has significantly protect liver hepatocytes in terms of reducing the level of transaminase enzyme, ALT compared to the untreated DMEM group.

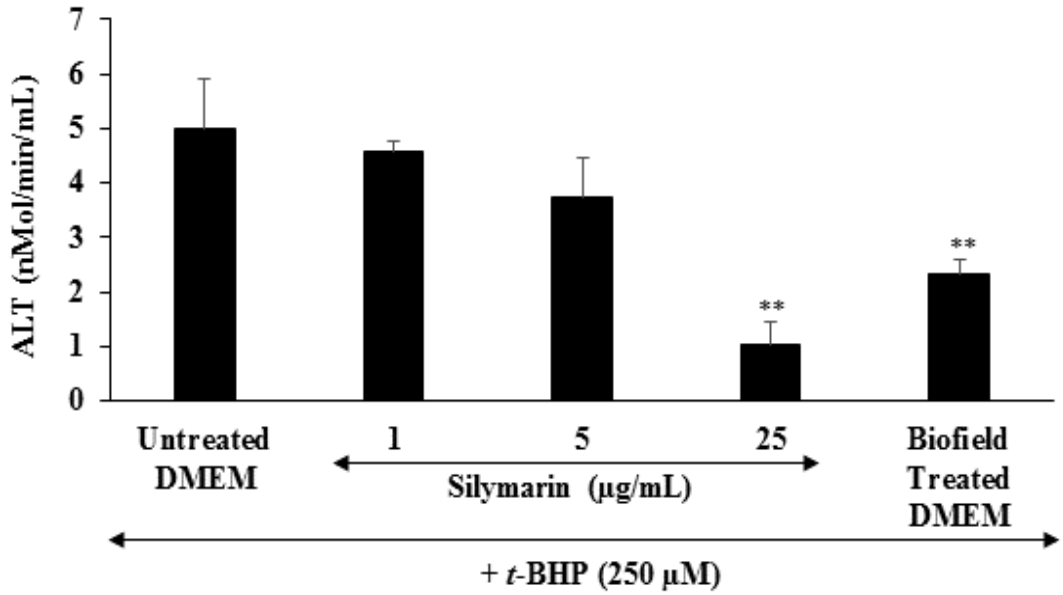

Figure 4: The effect of the test items on alanine amino-transaminase (ALT) activity against *tert*-butyl hydroperoxide (*t*-BHP) induced damage after 4 hours of treatment. All the values are represented as mean ± SEM of three independent experiments. ***p≤0.01* vs. untreated DMEM group.

e) Estimation of Cholesterol

The effect of the test items on cholesterol in shown in Figure 5. Mevinolin (positive control) showed 17.45%, 25%, and 80.19% ($p \leq 0.001$) reduction of cholesterol at 5, 10, and 20 μM, respectively compared to the untreated DMEM group. On the other side, cholesterol level was significantly ($p \leq 0.001$) reduced by 37.35% in the Biofield Energy Treated DMEM group compared to the untreated DMEM group (Figure 5). Cholesterol, its metabolites, and immediate biosynthetic precursors of cholesterol plays a vital role in salt and water balance, calcium metabolism, and stress responses[39]. Over accumulation of cholesterol leads to nonalcoholic fatty liver disease (NAFLD)[40].

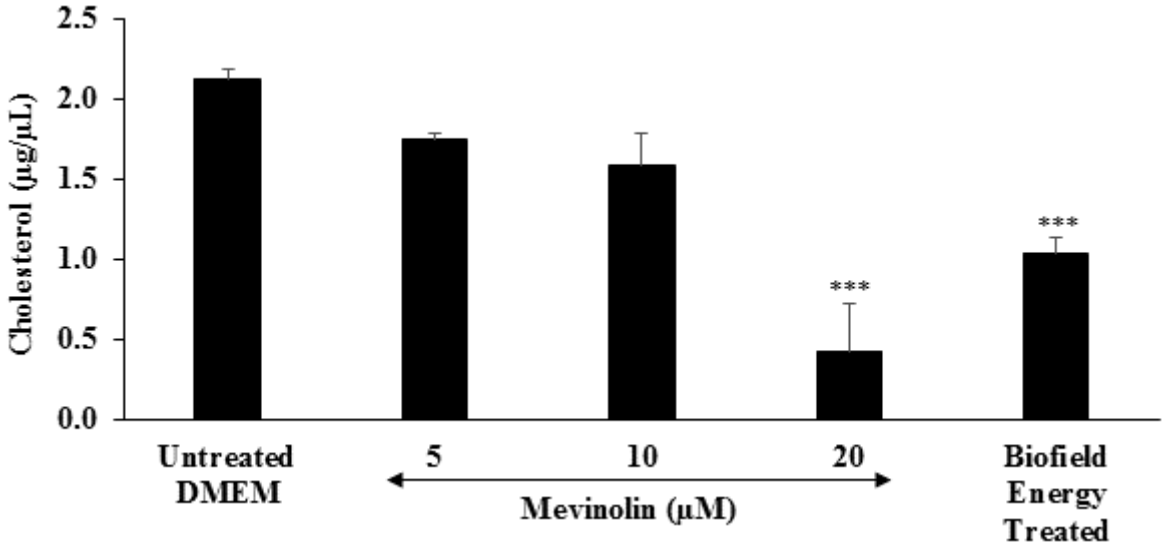

Figure 5: The effect of the test items on the level of cholesterol in HepG2 cells after 24 hours of treatment. All the values are represented as mean ± SEM of three independent experiments. ****p≤0.001* vs. Untreated DMEM group.

f) Estimation of Albumin

The effect of the test items on albumin concentration is shown in Figure 6. The level of albumin was significantly increased by 29.65%, 69.51%, 100.21% ($p \leq 0.001$), and 142.78% ($p \leq 0.001$) at 0.5, 1, 5, and 20 μM, respectively in the positive control (silymarin) group compared to the untreated DMEM group. Besides, the Biofield Energy Treated DMEM group showed 43.13% increase the level of albumin compared to the untreated DMEM group (Figure 6). From literature it has been reported that albumin plays a multiple physiological effects like volume expansion, anti-oxidation[41,42], and endothelial protection[43], hence was recommended for the management of liver cirrhosis patients and in acute/chronic liver failure[44,45]. In this experiment, the Biofield Treated DMEM significantly increased the level of albumin, which could be due to The Trivedi Effect® - Energy of Consciousness Healing Treatment.

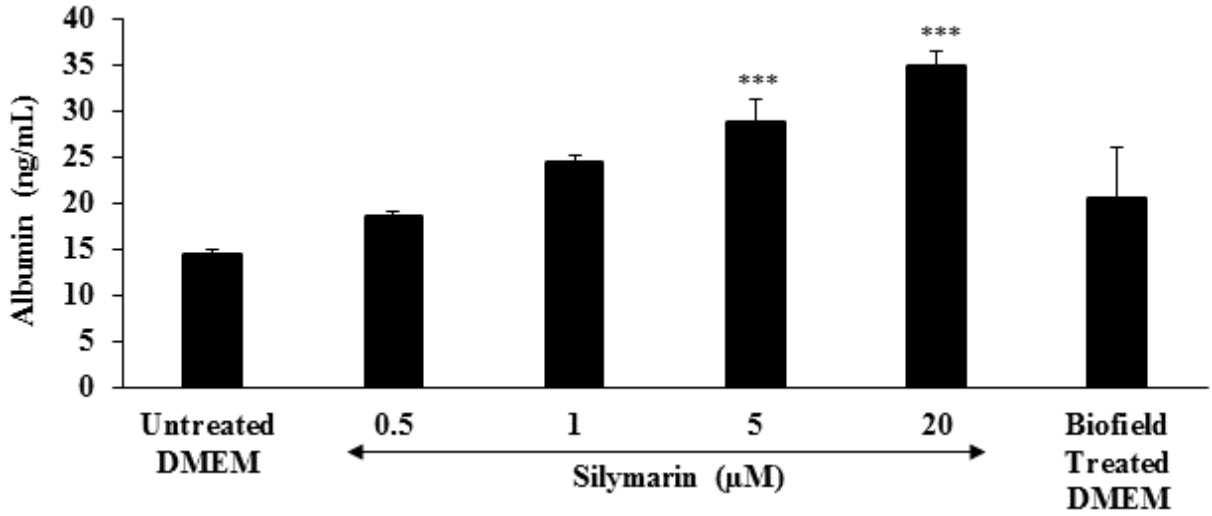

Figure 6: Effect of the test items on the level of albumin in HepG2 cells after 48 hours of treatment. All the values are represented as mean ± SEM of three independent experiments. ****p≤0.001* vs. untreated DMEM group.

IV. Conclusions

The study results showed that the test items were safe and non-toxic based on MTT cell viability assay. The Biofield Energy Treated test item (DMEM) showed significant ($p \leq 0.001$) protection of cells by 15% from the oxidative damage induced by *t*-BHP, while untreated DMEM group showed 0.4% protection. The proinflammatory cytokine, IL-8 was significantly ($p \leq 0.01$) reduced by 32.15% in the Biofield Energy Treated DMEM group compared to the untreated DMEM group. Moreover, ALT enzyme activity was significantly ($p \leq 0.01$) reduced by 53.2% in the Biofield Energy Treated DMEM group compared to the untreated DMEM group. Cholesterol level was significantly ($p \leq 0.001$) reduced by 37.35% in the Biofield Energy Treated DMEM group compared to the untreated DMEM group. Further, Biofield Energy Treated DMEM group showed 43.13% increased the level of albumin compared to the untreated DMEM group. In conclusion, The Trivedi Effect®-Consciousness Energy Healing Treatment significantly protect hepatocytes cells oxidative stress and it can be used as a complementary and alternative treatment for the prevention of various types of hepatobiliary disorders *viz.* acute hepatitis A, B, C, D, and E, chronic viral hepatitis, portal hypertension in schistosomiasis, toxoplasmosis, hepatosplenic schistosomiasis, liver abscess, autoimmune hepatitis, primary biliary cholangitis (primary biliary cirrhosis), phlebitis of the portal vein, granulomatous hepatitis, cholestasis, necrosis, cirrhosis, etc. Further, it could be useful to improve cell-to-cell messaging, normal cell growth and differentiation, cell cycling and proliferation, neurotransmission, skin health, hormonal balance, immune and cardiovascular functions. Moreover, it can also be utilized in organ transplants (*i.e.,* kidney, liver, and heart transplants), hormonal imbalance, aging, and various inflammatory and immune-related disease conditions like Alzheimer's Disease (AD), Ulcerative Colitis (UC), Dermatitis, Asthma, Irritable Bowel Syndrome (IBS), Hashimoto Thyroiditis, Pernicious Anemia, Sjogren Syndrome, Multiple Sclerosis, Aplastic Anemia, Hepatitis, Graves' Disease, Dermatomyositis, Diabetes, Parkinson's Disease, Myasthenia Gravis, Atherosclerosis, Systemic Lupus Erythematosus (SLE), stress, etc. with a safe therapeutic index to improve overall health and Quality of Life.

Acknowledgements

Authors gratefully acknowledged to Trivedi Global, Inc., Trivedi Science, Trivedi testimonials and Trivedi master wellness for their support. In addition, authors are thankful for the support of Dabur Research Foundation for conducting this study.

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Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology & Medicine
Volume 18 Issue 5 Version 1.0 Year 2018
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

A Review on Microvascular Complications in Diabetes

By Priya Sharma, Neeraj Kumar & Amit Singh
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Abstract- Diabetes may be a chronic global health issue, that affects children's and adult both, when insulin level or resistance to insulin action becomes insufficient to control systemic glucose levels. The number of available agents to manage diabetes continues to expand rapidly; the maintenance of euglycemia by individuals with diabetes remains a substantial challenge. Many patients with type 1 (it mostly affects children's because it is a genetic disease) and type 2 (it is mostly affects adults) diabetes will ultimately experience diabetes complications. Diabetes can lead to many serious microvascular degenerative complications (e.g., retinopathy, nephropathy, and neuropathy) resulting in an increased risk of morbidity and mortality and with this significant health care system costs.

Diabetic retinopathy will have an effect on the peripheral retina, that macular or each both and leading cause of visual disability and blindness in individuals with diabetic retinopathy. Diabetic neuropathy is a varity of microvascular complication that affects the nerves of individuals. Diabetic kidney disease is a very serious microvascular complication that affects the kidney. Diabetes affects many organs of the body like muscles, skin, heart, brain, and kidney. A very common risk issue for diabetes is hyperglycemia, insulin resistance, dyslipidemia, cardiovascular disease, and fleshiness.

Keywords: complications, diabetic retinopathy, diabetic kidney disease, diabetic neuropathy.

GJMR-B Classification: NLMC Code: WD 200

AREVIEWONMICROVASCULARCOMPLICATIONSINDIABETES

Strictly as per the compliance and regulations of:

A Review on Microvascular Complications in Diabetes

Priya Sharma [α], Neeraj Kumar [σ] & Amit Singh [ρ]

Abstract- Diabetes may be a chronic global health issue, that affects children's and adult both, when insulin level or resistance to insulin action becomes insufficient to control systemic glucose levels. The number of available agents to manage diabetes continues to expand rapidly; the maintenance of euglycemia by individuals with diabetes remains a substantial challenge. Many patients with type 1 (it mostly affects children's because it is a genetic disease) and type 2 (it is mostly affects adults) diabetes will ultimately experience diabetes complications. Diabetes can lead to many serious microvascular degenerative complications (e.g., retinopathy, nephropathy, and neuropathy) resulting in an increased risk of morbidity and mortality and with this significant health care system costs.

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I. Introduction

Diabetes describes a collection of chronic disorders within which insulin production is insufficient to maintain normal glucose homeostasis. Whether insulin insufficiency is due to loss of pancreatic islet beta cells or resistance to insulin action, the result the chronic elevation of systemic glucose levels, trials such as the Diabetes Control and Complications trial (for type 1 diabetes) [1]. The United Kingdom Prospective Diabetes Study (for type 2 diabetes) has demonstrated the benefits of intensive management on long-term disease complications. However, the implementation of intensive management strategies has remained a challenge particularly with the increasing number of patients with diabetes worldwide, and many patients struggle to maintain euglycemia. Also, emerging evidence suggests that in some circumstances, intensive glucose control alone may be insufficient to completely prevent the complications associated with diabetes [2].

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Diabetes can lead to many sever microvascular degenerative complications (e.g., retinopathy, nephropathy, and neuropathy) resulting in an increased risk of morbidity and mortality and with this significant health care system costs. Many prospective experimental studies have made public the role of intensive glucose control in reducing the risk of microvascular complications in diabetes. A number of the necessary medication that square measure wide utilized in the treatment of T2DM square measure antidiabetic drug, sulfonylureas, and thiazolidinediones class of molecules [3,4,5]. Dipeptidyl peptidase-4 (DPP-4) inhibitors were introduced within the treatment of T2DM in 2006 [6].

UK Prospective Diabetes Study (UKPDS) reported that compared with the conventional group, the intensive group showed a significant risk reduction by 12% in any diabetes-related aggregate endpoint, which was mainly due to a 25% risk reduction in microvascular finish points [7]. Moreover, this intensive glycemic management crystal rectifier to the lower rates of cardiovascular events and diabetes-related mortality ten years later [8]. However, in the Korean diabetic population, the prevalence of diabetic complications remains high; the prevalence of diabetic nephropathy (DN) was 30.3% in 2016, and that of diabetic retinopathy (DR) was 15.9% in 2015 [9].

USA; it absolutely was calculable that nearly 21 million Americans (or approximately 7% of the US population) consummated the diagnostic criteria for diabetes mellitus. Diabetic retinopathy at the time of the diagnosis of diabetes is lower with type I being 0.4% in kind I while 7.6% in kind II [10].

II. Microvascular Complications

Diabetes will cause several severe microvascular degenerative complications (e.g., retinopathy, nephropathy, and neuropathy) resulting in an increased risk of morbidity and mortality and with this significant health care system costs. Hence, while, ideally, the treatment of diabetes demands a holistic approach that may address varied complications related with diabetes, the first target of achieving an adequate blood glucose level as measured by hemoglobin A1c (HbA1c) level appears still essential. In fact, in previous studies in patients with T2DM, associate between the degree of hyperglycemia and a high risk of microvascular complications have been shown [11, 12].

Microvascular complications divided into three totally different parts:

- Diabetic retinopathy
- Diabetic nephropathy
- Diabetic neuropathy

III. Diabetic Retinopathy

DR may be a major diabetic microvascular complication that may cause minimized visual acuity and sightlessness[13]. Increased vascular permeability, edema, recruitment of inflammatory cells, elevated cytokine levels, tissue damage, and revascularization have been observed in DR, implicating oxidative stress and inflammation as the key mechanisms [14].

Diabetic retinopathy is a vision-threatening process that leads to almost 10,000 new cases of sightlessness in the US each year. It is the leading cause of sightlessness between the ages of 25 to 74 years, and is responsible for about 12% of sightlessness in the US. It's reported within the T1DM population that children have a negligible risk of developing retinopathy during the first decade of life, even when diagnosed before age two years. In adults after seven years of T1DM, about 50% of patients have some degree of retinopathy; while after twenty years, approximately 90% demonstrate retinopathy [15,16].

DR affects the peripheral retina, the macula, or both and is a leading cause of visual disability and blindness in people with diabetes [17]. The severity of DR ranges from non-proliferative and pre-proliferative to more severely proliferative DR, in which the abnormal growth of new vessels occurs [18]. Total or partial vision loss will occur through a vitreous hemorrhage or retinal, and vision loss will occur through retinal vessel leakage and ensuant macular lump [19].

IV. Stages Of Diabetic Retinopathy

- Mild non-proliferative retinopathy (Figure 1)
- Moderate non-proliferative retinopathy (Figure 2)
- Severe non-proliferative retinopathy (Figure 3)
- Proliferative retinopathy (Figure 4)

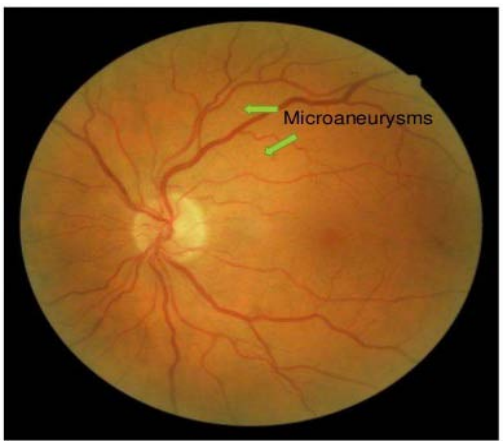


Figure 1: Mild non-proliferative retinopathy

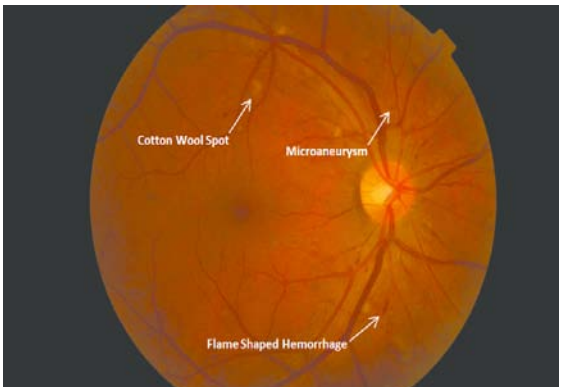


Figure 2: Moderate non-proliferative

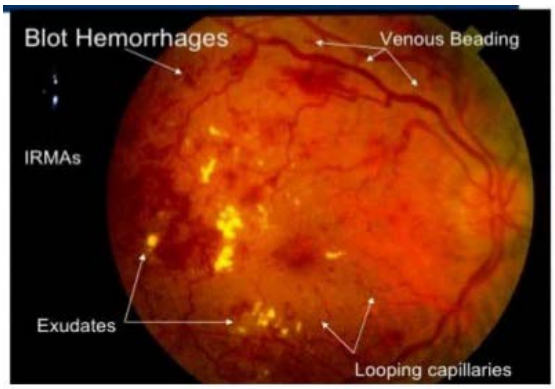


Figure 3: Several non-proliferative retinopathy

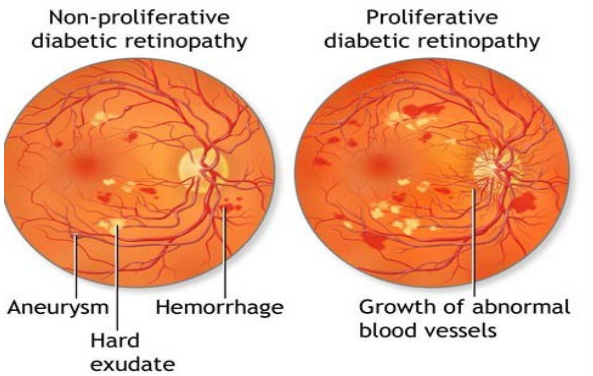


Figure 4: Several Proliferative retinopathies

V. Diabetic Nephropathy

Diabetic nephropathy (DN) may be a thoughtful and progressive complication of each kind 1 DM and kind 2 DM. Diabetic nephropathy is a condition that may cause end-stage renal disease requiring dialysis and eventual transplant. Patients may initially increase microalbuminuria that can develop into gross proteinuria. Gross proteinuria is an indication of widespread microvascular disease. These patients also develop elevated blood pressures and decreased glomerular filtration, eventually leading to renal failure. In the past, diabetic nephropathy has been reported to develop in about 40% of patients with T1DM and about 20% of patients with T2DM [20].

Table 1: Different stage of kidney disease in nephropathy.

Stages	Chronic kidney disease	GFR	% Kidney function
Stage 1	Kidney damage with normal kidney function	90 or higher	90-100
Stage 2	Kidney damage with mild loss of kidney function	89-60	89-60
Stage 3a	Mild to moderate loss of kidney function	59-40	59-45
Stage 3b	Moderate to severe kidney function	44-30	44-30
Stage 4	Sever loss of kidney function	29-15	29-15
Stage 5	Kidney failure	<15	<15

Diabetic neuropathy is the result of a slowed motor and sensory nerve conduction that most commonly develops between 5 and ten years after the onset of disease. Neuropathy can present as peripheral sensorimotor, cranial, peripheral motor, and autonomic neuropathy. The peripheral sensorimotor neuropathy is symmetric and mostly affects the feet, leading to diminished sensation and paresthesia. The diminished sensation can cause an altered perception of foot pressures and altered foot architecture. This change can result in injury, non-healing wounds, and eventual amputations. Alternatively, diabetic neuropathy can lead to painful and debilitating hyper sensation and burning dysesthesias, which makes ambulation difficult [21]. The prevalence of peripheral neuropathy in the pediatric population has been reported to range between 7% to 57% depending on the diagnostic criteria used, with subclinical neuropathy reported to occur in 57% of children and adolescents with T1DM [22].

People with diabetes also frequently have autonomic neuropathy, involuntary cardiovascular autonomic dysfunction that is manifested as abnormal vital (HR) and vascular control [23].

Physical therapists unremarkably encounter diabetes- associated PN within the analysis and treatment of balance and movement disorders as a result of these disorders frequently have an effect on lower-extremity sensation and may cause lower-extremity pain in individuals with diabetes. Loss of lower-extremity sensation let alone impaired peripheral vascular function can contribute to lower-extremity (commonly foot) ulceration [24].

a) Symmetrical polyneuropathies

i. *Relatively stable conditions*

- Symmetrical distal sensory polyneuropathy (SDSP) Variants: acute, severe SDSP in the beginning of diabetes, pseudosyringomyelia neuropathy, pseudodiabetic neuropathy, autonomic neuropathies.
- Episodic (transient) symptoms: Diabetic cachexia neuropathy Hyperglycemic neuropathy Treatment-induced diabetic neuropathy or insulin neuritis chronic inflammatory demyelinating polyneuropathy (CIDP-plus) hypoglycemic neuropathy.

b) Asymmetrical/focal and multifocal neuropathies

Diabetic lumbosacral radiculoplexus neuropathy (DLSRN; Bruns-Garland syndrome, diabetic amyotrophy, proximal diabetic neuropathy). Cervicobrachial radiculoplexus neuropathy Trunk neuropathie(thoracic/abdominal radiculopathy) cranial neuropathies Mononeuropathies (median, ulna, fibular).

i. *Risk factors*

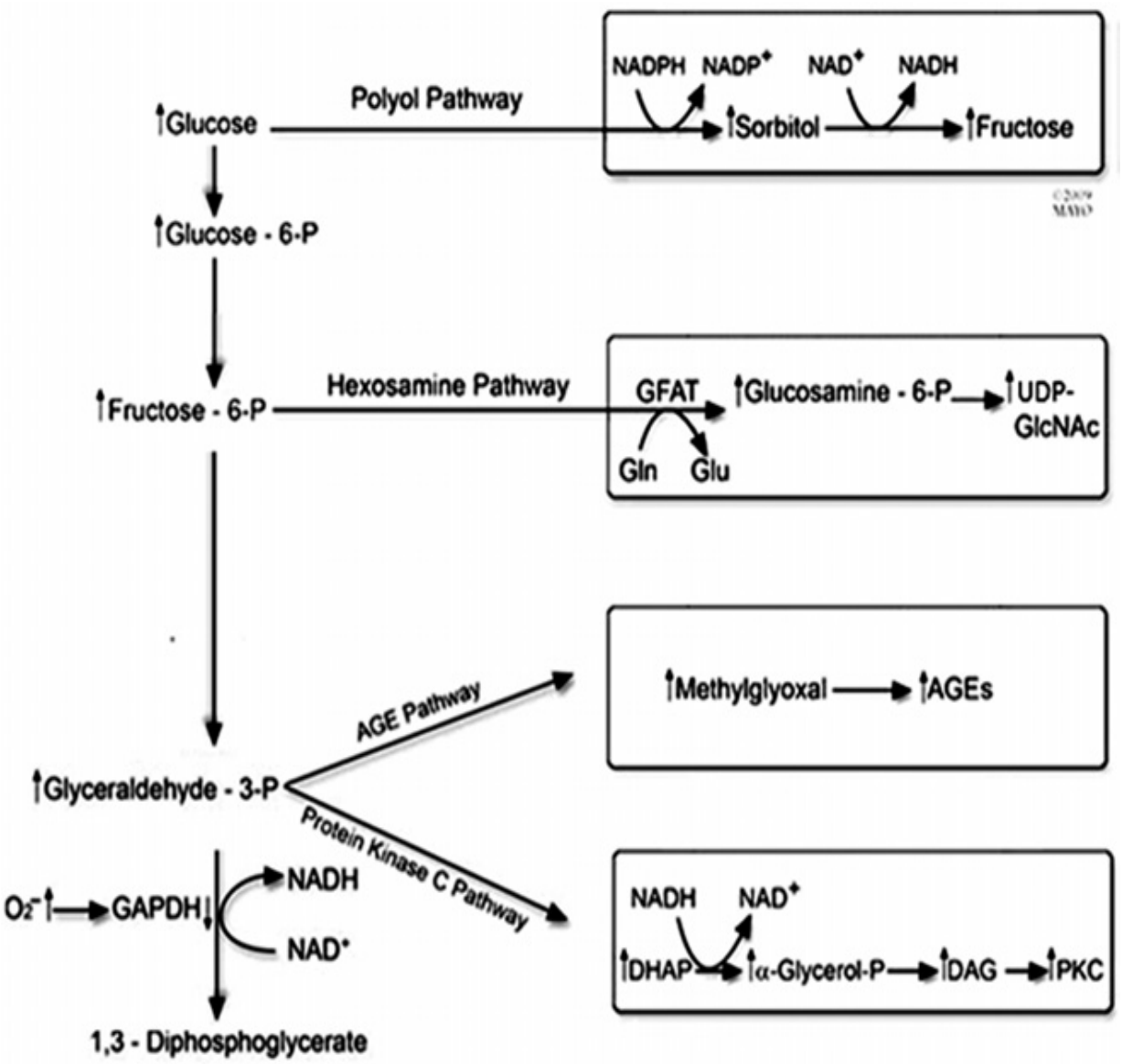
There are plenty of risk factors connected microvascular complications. Retinopathy, neuropathy and nephropathy diabetes have many risk factors like hyperglycemia, hyperinsulinemia, age, tobacco use, insulin treatment, etc.

There is a table which explains different factors for different types of diabetic complications (retinopathy diabetes, neuropathy diabetes, nephropathy diabetes).

Table 2: The risk factors of microvascular diabetes

Risk Factor	Retinopathy	Neuropathy	Nephropathy
Hyperglycemia	Yes	Yes	Yes
Hyperinsulinemia			
Age	Yes	Yes	Yes
Tobacco use	Yes	Yes	Yes
Insulin treatment	Yes		
Dyslipidemia	Yes	Yes	Yes
Pregnancy	Yes		
Renal disease	Yes		
Elevated homocysteine level	Yes		
High-fat diet	Yes		
Chronic diabetes mellitus		Yes	
Hypertension		Yes	
Obesity			
Atrial fibrillation			
Heart failure			
Proteinuria			Yes
Microalbuminuria		Yes	Yes
Hyperuricemia			
Blood inflammatory molecules			
Elevated blood fibrinogen level			
Physical inactivity			
Elevated height		Yes	
Ketoacidosis		Yes	
Carotid artery stenosis			

VI. Pathogenesis



This schematic shows the four biochemical pathways that lead to diabetic retinopathy. DHAP, dihydroxyacetone phosphate; DAG, diacylglycerol; PKC, protein kinase C; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; AGEs, advanced glycation end products, UDP-GlcNAC, N-acetylglucosamine.

VII. Conclusion

Studies were performed of the effect of treating streptozotocin type 1 diabetic rats with vildagliptin, a Dipeptidyl peptidase IV inhibitor, on retinal, vascular and nerve dysfunction. We found that treatment with vildagliptin improved some neural, vascular and retinal complications. It is becoming clear that dipeptidyl peptidase IV inhibitors have multiple affects and may improve outcome by mechanisms unrelated to the preservation of GLP-1 or GIP [25].

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Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology & Medicine
Volume 18 Issue 5 Version 1.0 Year 2018
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Liver Cirrhosis: Common Clinical Problem

By Ruchi Singh, Dr. Afroze Alam, Vinod Kumar, B.D. Tripathi & Amrendra Yadav
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Abstract- The liver is the multi-functional and vital organ of the body. It is found in the upper abdomen region of the vertebrates. Due to long-term damage, liver stops functioning properly which may lead to cirrhosis. This long-term damage occurred when scar tissue replaces the normal tissue of the liver. This disease develops slowly and has no early symptoms, but when it develops and become worse, then it leads to tiredness, itchiness, weakness, yellow skin, swelling in the lower legs, spider-like blood vessels and an easy bruise on the skin with fluid in the abdomen. The severe complications like bleeding dilated veins in esophagus or stomach, hepatic encephalopathy leading to confusion and unconsciousness and liver cancer may occur in the body. This review article is focusing on the effect of liver damage in the human body.

Keywords: *cirrhosis, splenomegaly, autoimmune hepatitis.*

GJMR-B Classification: *NLMC Code: WI 725*

Strictly as per the compliance and regulations of:

Liver Cirrhosis: Common Clinical Problem

Ruchi Singh [α], Dr. Afroze Alam [σ], Vinod Kumar [ρ], B.D. Tripathi [ω] & Amrendra Yadav [¥]

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I. Introduction

Cirrhosis is the most common condition occurred due to hepatitis B & C, alcohol abuse and several other factors [1]. There are basically two types of liver cirrhosis, i.e. Alcoholic and nonalcoholic. Alcoholic cirrhosis may occur to those people who are having over a no. of years 2-3 alcoholic drinks per day [2]. Non- alcoholic fatty liver disease may occur due to following causes like-high blood pressure, high blood fats, diabetes, and overweight. Several medications, autoimmune hepatitis, hemochromatosis, gallstones, etc. are the less common causes of cirrhosis [2, 3].

Liver biopsy, blood testing, and medical imaging are the basis for diagnosing liver cirrhosis. Hepatitis B may be prevented by vaccination [4]. Antiviral medications may treat Hepatitis B as well as C [5]. Steroid medications may treat Autoimmune hepatitis. If disease occurred due to a blocking of the bile ducts, then Ursodiol may be used for the treatment of this disease [4, 5].

In hepatic encephalopathy, dilated esophageal or stomach veins, leg or abdominal swelling several medications may be used, and in severe cirrhosis only the option left is liver transplantation [5, 6]. Many researchers have shown that mostly men die due to cirrhosis in comparison to women. Several studies have shown that in comparison to women mostly men die in the world [7]. Every year nearly 8000 people die because of liver damage and over 800 people have liver transplantation [8]. The last stage of chronic liver disease may cause intense scarring of the liver is known as cirrhosis. It may occur due to toxins like alcohol and viral infections [9]. The liver is the large organ situated in the upper right side of the abdomen below the diaphragm. There are mainly two types of cirrhosis- compensated and decompensated [10]. The compensated cirrhosis has no signs or symptoms but have evidence of portal hypertension [11]. The decompensated cirrhosis has several complications like jaundice and also related to portal hypertension [10, 11]. The function of the liver is to remove the waste product from the body but the damaged liver could not able to eliminate the waste product from the body, and thus the waste product may enter in the brain causing several problems like loss of consciousness, confusion, sleepy, tremors, etc. [12]

Functions of the vital organ liver [13]:

- It produces bile juice which helps in absorbing cholesterol, dietary fats, vitamins A, D, E, K.
- It preserves sugar and vitamins for later purposes.
- It also creates proteins for blood clotting.
- It purifies blood and removes toxins like alcohol and bacteria from the body [13, 14].

Cirrhosis is a word derived from a Greek word: 'kirrhos' meaning 'yellowish' and 'osis' meaning 'condition'. It may affect approx 3 million people and due to this approx 1.5 million deaths have been occurred in the world [13, 15].

II. Causes

There are various types of diseases and conditions which can cause cirrhosis. These may damage the entire liver. The most possible causes are [12, 14, 15]:

- Alcohol abuse.
- Chronic hepatitis B & C.
- Deposition of fat in the liver.
- Accumulation of copper in the liver.
- Galactosemia or glycogen storage disease.
- Deposition of iron in the body (hemochromatosis).
- Genetic digestive disorder.
- Autoimmune hepatitis.
- Poor formation and destruction of bile ducts.
- Syphilis.
- Medications.

III. Symptoms

The signs and symptoms may not appear until a large area of the liver is damaged, and then the signs & symptoms may be:

- Loss of appetite.
- Nausea.

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- Fatigue.
- Weight loss.
- Swelling in legs.
- Itching.
- Bleeding.
- Bruising [16, 17].

IV. Diagnosis

The detailed history, as well as physical examination can help in diagnosing liver cirrhosis [17]. The long exposure to alcohol abuse and hepatitis C, family background of autoimmune diseases and several other risk factors may be diagnosed by the patient's history [13, 18]. There are following signs which can be diagnosed by physical examination:

- Yellow eyes (jaundice).
- Hand tremors.
- Pale skin.
- Red palms.
- Reduced alertness.
- Increased breast tissue in men.
- Enlarged spleen and/or liver [17, 18].

V. Prevention

The increased risk of cirrhosis may be reduced by taking care of the liver [11, 16, 17].

Do's and don'ts if you have cirrhosis:

- Don't be alcohol abused.
- Eat healthy food.
- Don't be obese or have weight-loss, have a healthy weight.
- Decrease the risk of hepatitis B & C [15, 18].

VI. Complications

1. Portal hypertension (High blood pressure in the veins that transports blood to the liver).
2. Fluid accumulation in legs (edema) and the abdomen (ascites) may cause swelling in the abdomen and legs.
3. Bleeding due to fewer proteins for clotting.
4. Spleen enlargement (splenomegaly).
5. Medications sensitivity as liver functions for processing medications in the body.
6. Serious infections, weakness, weight loss, and malnutrition.
7. Bruising due to decreased clotting and low platelet count.
8. Toxins accumulating in the brain.
9. Jaundice and bone disease.
10. Liver cancer and liver failure.
11. Kidney failure [19, 20, 21].

VII. Treatment

- Nitrates or beta-blockers [22].
- Intravenous antibiotics for treating peritonitis.
- Hemodialysis.
- Low protein diet.
- Stop drinking alcohol.
- Stop medications, even over-the-counter ones without consulting the doctor.
- When all the treatments fail then the last option is liver transplantation [21, 23].

VIII. Conclusion

Cirrhosis is a dynamic process, and clinicians should treat all the complications related to cirrhosis. The disease progression and the requirement of liver transplantation should be avoided by early intervention. Now in the 21st century it is the biggest challenge to stop liver transplantation. Liver cirrhosis may be treated or prevented as it may lead to morbidity and it is the major cause of mortality.

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Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology & Medicine
Volume 18 Issue 5 Version 1.0 Year 2018
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

An Innovative Gateway to Deliver Nanosized Atorvastatin by Bio-Flexy Film Approach

By Kirti Singh & N. V. Satheesh Madhav
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Abstract- Atorvastatin is an antihyperlipidemic drug which is widely used to treat hyperlipidemia and lower the cholesterol level in the body, but atorvastatin has low bioavailability due to high intestinal clearance and first-pass metabolism. The main objective of our research work was to develop a formulation to increase the therapeutic efficacy of the drug. A bio-polymer was isolated from a natural edible source *Coriandrum sativum* and was subjected for screening its filmability and adhesivity. Atorvastatin was nanosized using a novel method and using the bio-polymer and other co-processing agents five bio-flexy films of different ratios (i.e. 1:1, 1:2, 1:3, 1:4, 1:5) were formulated. The isolated bio-polymer was subjected to various analytical parameters. The drug-excipient compatibility study was performed using UV and TLC method. The formulated bio-flexy films were evaluated for various parameters like weight, thickness, content uniformity, surface pH, folding endurance, and in-vitro drug permeation. The formulation AC2 (containing 1:2 bio-polymer) was found to be the best formulation having R^2 value 0.9989 with zero order as best fit model.

Keywords: *bio-polymer, bio-flexy films, coriandrum sativum, atorvastatin.*

GJMR-B Classification: *NLMC Code: QV 785*

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Kirti Singh [α] & N. V. Satheesh Madhav [σ]

Abstract- Atorvastatin is an antihyperlipidemic drug which is widely used to treat hyperlipidemia and lower the cholesterol level in the body, but atorvastatin has low bioavailability due to high intestinal clearance and first-pass metabolism. The main objective of our research work was to develop a formulation to increase the therapeutic efficacy of the drug. A bio-polymer was isolated from a natural edible source *Coriandrum sativum* and was subjected for screening its filmability and adhesivity. Atorvastatin was nanosized using a novel method and using the bio-polymer and other co-processing agents five bio-flexy films of different ratios (i.e. 1:1, 1:2, 1:3, 1:4, 1:5) were formulated. The isolated bio-polymer was subjected to various analytical parameters. The drug-excipient compatibility study was performed using UV and TLC method. The formulated bio-flexy films were evaluated for various parameters like weight, thickness, content uniformity, surface pH, folding endurance, and in-vitro drug permeation. The formulation AC2 (containing 1:2 bio-polymer) was found to be the best formulation having R^2 value 0.9989 with zero order as best fit model. The results obtained concluded that the efficacy of atorvastatin can be effectively increased by delivering it as a trandermal formulation.

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I. Introduction

Coriander commonly known as 'dhaniya' in hindi is obtained from the seeds of *Coriandrum sativum* belonging to family Apiaceae. It is a herbal spice commonly used in culinary purposes and it possess various properties. It contains various essential oil, tannins, terpenoids, reducing sugars, alkaloids, phenolics, flavonoids, fatty acids, sterols and glycosides. It is highly rich in proteins, oils, carbohydrates, fibers, minerals, trace elements and vitamins. It has various pharmacological effects like anxiolytic, antidepressant, sedative-hypnotic, anticonvulsant, memory enhancement, improvement of orofacial dyskinesia, neuroprotective, antibacterial, antifungal, anthelmintic, insecticidal, antioxidant, cardiovascular, hypolipidemic, anti-inflammatory, analgesic, antidiabetic, mutagenic, antimutagenic, anticancer, gastrointestinal, deodorizing, dermatological, diuretic, reproductive, hepatoprotective, etc. [1,2]

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Hyperlipidemia is the presence of raised or abnormal levels of lipids and/or lipoproteins in the blood. Lipid and lipoprotein abnormalities are regarded as a highly modifiable risk factor for cardiovascular disease due to the influence of cholesterol on atherosclerosis. Hyperlipidemia includes several conditions, but it usually means that you have high cholesterol and high triglyceride levels. [3]

Atorvastatin is an antihyperlipidemic drug. It belongs to the category of statins, which acts by competitively inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase. This enzyme is involved in the conversion of HMG CoA to mevalonate which is the rate limiting step in the cholesterol synthesis. The drug has low oral bioavailability due to low aqueous solubility (belongs to BCS class II) and rapid intestinal clearance and hepatic first-pass metabolism. This can be avoided by formulating a formulation for transdermal delivery. [4]

The aim of our research work was to improve therapeutic efficacy of Atorvastatin by delivering nanosized atorvastatin through trans-nabhi route. This can be attained by formulating bio-flexy film.

II. Materials and Methods

Atorvastatin was obtained as a gift from Mylan laboratories Ltd. *Coriandrum sativum* was procured from local market. All other reagents used were of analytical grade.

a) Extraction of biopolymer from Coriandrum sativum

500 gm *Coriandrum sativum* was taken and powdered. The powder was soaked in 1000 ml of distilled water and kept in refrigerator for overnight. It was centrifuged at 3000 rpm and supernatant was collected which was treated with equal amount of propanone. It was kept in refrigerator for 24 hrs. The supernatant was centrifuged at 3000 rpm. The bio-polymer was collected and dried. The dried bio-polymer was purified by hot dialysis method. The process was repeated 6 times and the percentage yield was calculated. The purified bio-polymer was passed through 120# sieve and stored for further use. [5]

b) Characterization of the isolated bio-polymer

The isolated bio-polymer was subjected to various physicochemical analysis like color, texture, solubility, presence of carbohydrates, proteins and starch; IR, SEM, DSC, NMR spectroscopy studies.

c) *Drug-excipient interaction study*

Drug interaction study with other excipients of the formulation was performed by dry and wet method. The drug was mixed with excipients in the ratios of 1:1, 1:3, and 3:1. The mixtures were stored at room temperature for a period of 3 days. The dilutions of the mixtures were prepared with methanol and the samples were analyzed by ultraviolet spectrophotometric method (Shimadzu 1800). [5]

d) *Preparation of nanosized atorvastatin loaded bio-flexy films*

Atorvastatin was nanosized by using a novel method. Atorvastatin was triturated with dextrose in a pestle mortar. Double distilled water was added to the solution drop by drop and triturated continuously. The solution was transferred to a beaker and was sonicated for six cycles of 3 min each. After each sonication cycle, percentage absorbance and transmittance was observed at wavelength 200-800nm. The solution was microcentrifuged. Nanosized atorvastatin was obtained and dried. It was kept in dessicator for 24 hrs. Nanosized drug was collected and stored in cool and dry place.

Bio-flexy films were prepared by solvent casting method. Bio-polymer isolated was accurately weighed in different ratios and dissolved in 10 ml of distilled water at room temperature. Dextrose was added to this solution. Nanosized atorvastatin used as a model drug was dissolved in little amount of ethanol. The nanosized drug solution was added to the polymeric solution. It was poured in a petri-dish for natural drying. The dried bio-flexy films were obtained and packed in tightly closed container. [6,7]

Table 1: Formula for Bio-flexy films

Ingredients	AC1	AC2	AC3	AC4	AC5
Nanosized Atorvastatin (mg)	10	10	10	10	10
Coriandrum sativum Bio-polymer (mg)	100 (1%)	200 (2%)	300 (3%)	400 (4%)	500 (5%)
Dextrose (mg)	100	100	100	100	100
Distilled water (mL)	10	10	10	10	10

e) *Evaluation of Bio-Flexy Films*

i. *Physical appearance*

The formulations were visually inspected for various factors like color, clarity, and smoothness in order to ensure the uniformity in physical appearance of the bio-flexy films.

ii. *Weight*

Three patches (1 cm^2) of each formulation were taken, weighed and average weight was calculated. [8, 9]

iii. *Thickness*

The thickness of the films for every formulation was measured using a micrometer screw gauge at three different places and the mean value was calculated. [8, 9]

iv. *Folding endurance*

Folding endurance was determined by repeatedly folding the film at the same place till it broke. The number of times the film could be folded at the same place without breaking was recorded which is known as the folding endurance. [8,9]

v. *Surface pH*

The individual film was placed in a petridish and moistened with 0.5 ml of distilled water and kept for 30 min. The surface pH was measured by using pH meter. [5]

vi. *Drug content uniformity*

The bio-flexy film was dissolved in methanol and volume was made up to 100 ml. It was sonicated and kept for 24 hours. 0.1 mL was withdrawn from this and diluted to 10 ml. The drug content was measured by using UV Spectroscopy. This was repeated for all the formulations. From the drug content, % drug content was calculated. [8, 9]

vii. *In-vitro drug release study*

The *in-vitro* drug release was carried out by using MS diffusion apparatus. This is the static method which utilizes complete replacement of the sample thus provides 100% sink condition. Egg membrane was attached on the donor compartment. A piece of formulated bio-flexy film was adhered onto the egg membrane in the donor compartment. The receptor compartment was filled with 13 ml of pH 7.4 buffer solution. Samples were withdrawn completely at regular intervals for 48 hrs and replaced completely by fresh buffer each time. The samples were analyzed by UV spectroscopy (Shimadzu 1800) at 241 nm to estimate the amount of the drug. Similarly drug diffusion study was carried out for each nanosized atorvastatin loaded bio-flexy films. [5, 10]

viii. *Stability studies*

The formulated bio-flexy films were subjected to accelerated stability studies according to the ICH guidelines for six months. [11]

III. Results And Discussion

a) *Characterization of the isolated bio-polymer*

The bio-polymer isolated from *Coriandrum sativum* was found to be smooth, amorphous, odourless, and buff in color. It was slightly soluble in water. The yield was found to be 12.40±2.13 % w/w. The bio-polymer was found positive for carbohydrates and protein content. The test was negative for starch content. The color changing point was found to be 264±5°C. The IR spectra (Fig. 1) revealed the presence of aromatic phenols (3290.26 cm^{-1}), alkanes

(2924.59 cm^{-1}), alkenes with stretching (1651.46 cm^{-1}), nitro compound (1543.89 cm^{-1}), aromatics with stretching (1455.35 cm^{-1}), sulfone (1239.50 cm^{-1}), thiocarbonyl (1151.04 cm^{-1}). These groups are responsible for bioadhesivity of the biopolymer. SEM analysis of the bio-polymer (Fig. 2) showed that the bio-polymer has smooth surface and is amorphuys in nature. It showed the morphological structure similar to the polymers which confirms that the bio-polymer is polymeric in nature.

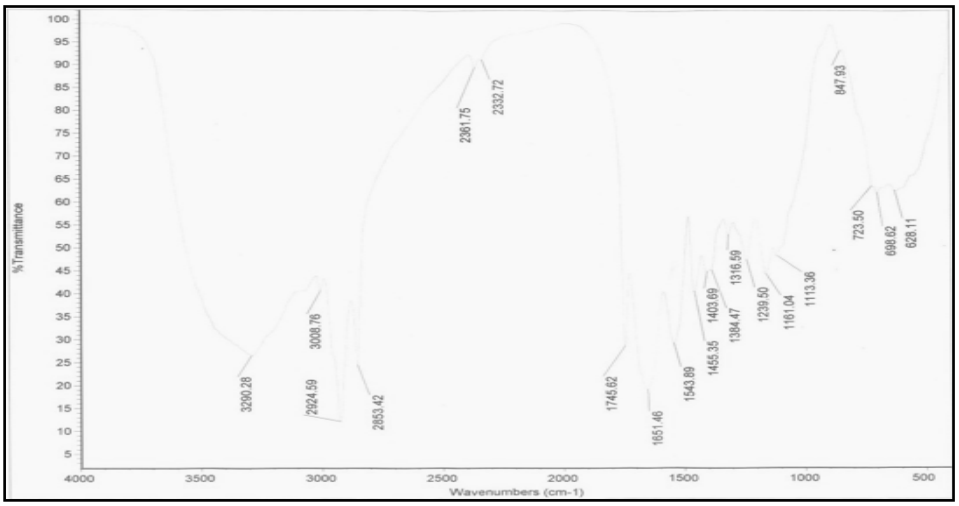

Fig. 1: IR spectrum of *Coriandrum sativum* bio-polymer

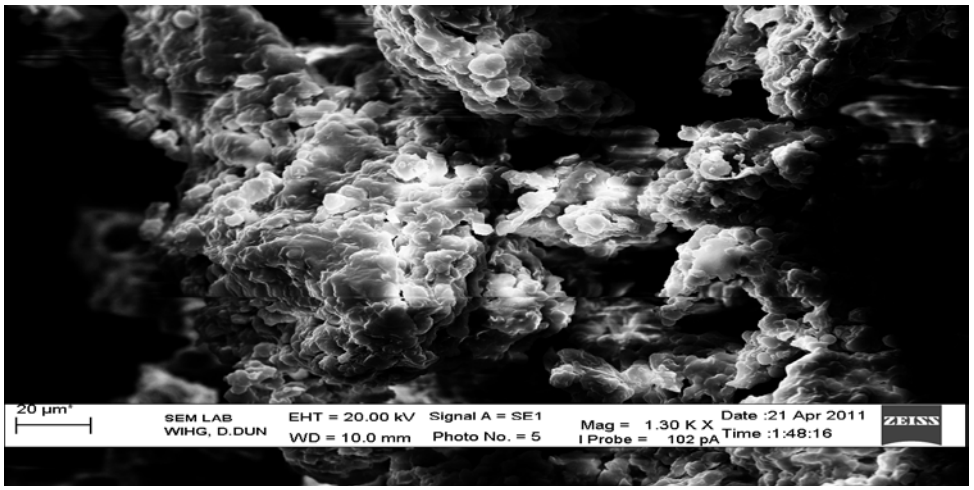

Fig. 2: SEM of *Coriandrum sativum* bio-polymer

b) Drug-excipient interaction study

The drug-excipient interaction studies revealed that there was no interaction between the drug and the exciepients as there was no change in the wavelength of the drug.

c) Nanosizing of Atorvastatin

The percentage of transmittance at different wavelength represents that the light is passed through the particles which means the particle size is below that wavelength. The percentage of the particles which are present in the mixture below 400 nm. Whereas the % blockade indicates the % particle which are above 400 nm and the data was correlated with the SEM analysis. The percentage of transmittance was measured by UV spectrophotometer and after each cycle increase in the percentage transmittance was observed which indicated that the particles may have been reduced to nano range. The effect of sonication on percentage of transmittance after each cycle is shown in fig. 2.

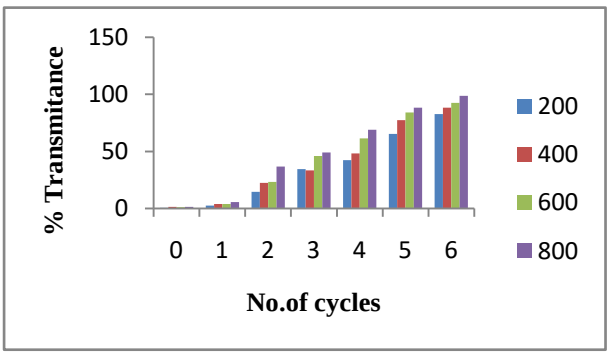

Fig. 3: Nanosizing characterisation by UV spectroscopy.

d) Thickness, weight, folding endurance and surface pH

Thickness of the bio-flexy films AC1 to AC5 containing *Coriandrum sativum* bio-penetrant ranged from 0.35 ± 0.15 to 0.39 ± 0.11 mm.

The weight of the bio-flexy films AC1 to AC5 containing *Coriandrum sativum* bio-penetrant ranged from 20.62 ± 0.12 to 38.43 ± 0.25 mg.

The micro environmental pH of the bio-flexy films ranged from 6.53 to 7.05. The pH of the bio-flexy films was found to be close to the pH of the skin. It confirms that the formulations will not cause any irritation effect.

Folding endurance of the bio-flexy films ranged from 71 to 118 (times) which indicates reasonable flexibility of the bio-flexy films.

e) Drug content uniformity

The range of drug content uniformity for the prepared bio-flexy films was found to be 86.51±0.23 to 94.47±0.45 %. No significant difference was observed in the drug content of the prepared bio-flexy films which indicated that the drug is uniformly dispersed throughout the bio-flexy films.

f) In-vitro drug release study

The drug release of bio-flexy films were analysed by using BIT-SOFT. The drug release profile was found to be in the order AC2> AC3> AC1> AC4> AC5. AC2 (1:2) was found to be the best formulation having t_{50} 3.6 hrs, t_{80} 26.8 hrs, R^2 value 0.9989, zero order as best fit model and anomalous transport as mechanism for drug transport analyzed by BIT-SOFT 1.12.

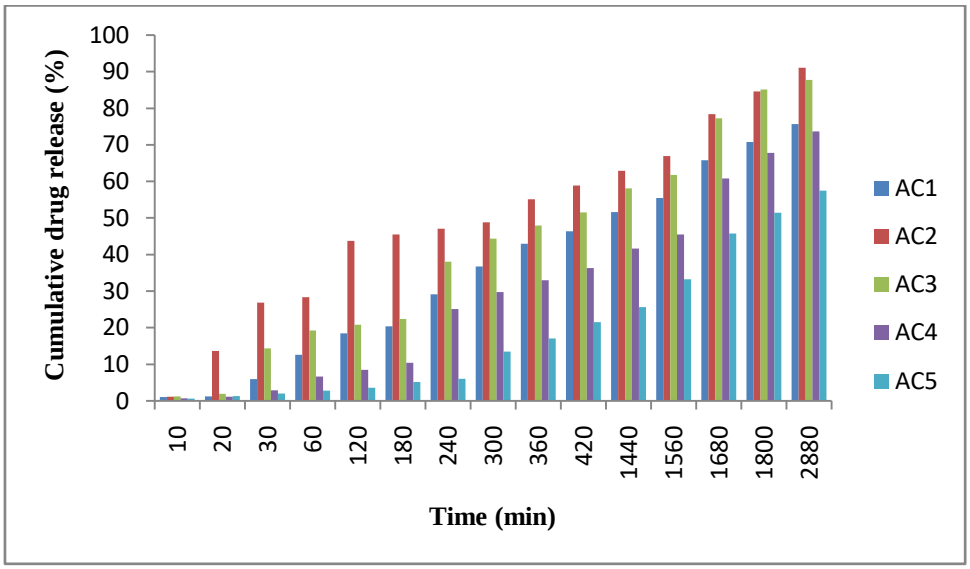

Fig. 4: In-vitro drug release of formulations.

g) Stability studies

During and at the end of stability studies, the formulations showed no change in physical appearance and flexibility. They showed insignificant difference for *in-vitro* drug release. This showed that the formulations were physically and chemically stable during the study.

IV. Conclusion

Atorvastatin is the most selling drug used for lowering the cholesterol level in the body. The problem with the drug is low bioavailability and higher risk for side effects. In this research work, an attempt has been made for formulating bio-flexy films. Bio-flexy films can act as a promising formulation for drug delivery. By nanosizing the drug, amount of the drug administered is reduced thus minimising the dose related side effects of the drug. This route by passes the first pass metabolism and thus increases the bioavailability of the drug. The bio-polymer isolated from natural edible source, *Coriandrum sativum* was found to be biodegradable, non-toxic, and non-reactive and can be effectively isolated in large quantity. Bio-flexy films were prepared using the isolated biopolymer, nanosized atorvastatin and other co-processing agents. The isolated bio-polymer can further be used as a promising excipient for formulating various pharmaceutical formulations.

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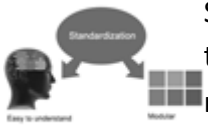

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Once you are designated as MARSM, you may send us a scanned copy of all of your credentials. OARS will verify, grade and certify them. This will be based on your academic records, quality of research papers published by you, and some more criteria.

It is mandatory to read all terms and conditions carefully.

AUXILIARY MEMBERSHIPS

Institutional Fellow of Open Association of Research Society (USA) - OARS (USA)

Global Journals Incorporation (USA) is accredited by Open Association of Research Society, U.S.A (OARS) and in turn, affiliates research institutions as "Institutional Fellow of Open Association of Research Society" (IFOARS).

The "FARSC" is a dignified title which is accorded to a person's name viz. Dr. John E. Hall, Ph.D., FARSC or William Walldroff, M.S., FARSC.

The IFOARS institution is entitled to form a Board comprised of one Chairperson and three to five board members preferably from different streams. The Board will be recognized as "Institutional Board of Open Association of Research Society"-(IBOARS).

The Institute will be entitled to following benefits:

The IBOARS can initially review research papers of their institute and recommend them to publish with respective journal of Global Journals. It can also review the papers of other institutions after obtaining our consent. The second review will be done by peer reviewer of Global Journals Incorporation (USA) The Board is at liberty to appoint a peer reviewer with the approval of chairperson after consulting us.

The author fees of such paper may be waived off up to 40%.

The Global Journals Incorporation (USA) at its discretion can also refer double blind peer reviewed paper at their end to the board for the verification and to get recommendation for final stage of acceptance of publication.

The IBOARS can organize symposium/seminar/conference in their country on behalf of Global Journals Incorporation (USA)-OARS (USA). The terms and conditions can be discussed separately.

The Board can also play vital role by exploring and giving valuable suggestions regarding the Standards of "Open Association of Research Society, U.S.A (OARS)" so that proper amendment can take place for the benefit of entire research community. We shall provide details of particular standard only on receipt of request from the Board.

The board members can also join us as Individual Fellow with 40% discount on total fees applicable to Individual Fellow. They will be entitled to avail all the benefits as declared. Please visit Individual Fellow-sub menu of GlobalJournals.org to have more relevant details.

We shall provide you intimation regarding launching of e-version of journal of your stream time to time. This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.

After nomination of your institution as "Institutional Fellow" and constantly functioning successfully for one year, we can consider giving recognition to your institute to function as Regional/Zonal office on our behalf.
The board can also take up the additional allied activities for betterment after our consultation.

The following entitlements are applicable to individual Fellows:

Open Association of Research Society, U.S.A (OARS) By-laws states that an individual Fellow may use the designations as applicable, or the corresponding initials. The Credentials of individual Fellow and Associate designations signify that the individual has gained knowledge of the fundamental concepts. One is magnanimous and proficient in an expertise course covering the professional code of conduct, and follows recognized standards of practice.

Open Association of Research Society (US)/ Global Journals Incorporation (USA), as described in Corporate Statements, are educational, research publishing and professional membership organizations. Achieving our individual Fellow or Associate status is based mainly on meeting stated educational research requirements.

Disbursement of 40% Royalty earned through Global Journals : Researcher = 50%, Peer Reviewer = 37.50%, Institution = 12.50% E.g. Out of 40%, the 20% benefit should be passed on to researcher, 15 % benefit towards remuneration should be given to a reviewer and remaining 5% is to be retained by the institution.

We shall provide print version of 12 issues of any three journals [as per your requirement] out of our 38 journals worth $ 2376 USD.

Other:

The individual Fellow and Associate designations accredited by Open Association of Research Society (US) credentials signify guarantees following achievements:

- The professional accredited with Fellow honor, is entitled to various benefits viz. name, fame, honor, regular flow of income, secured bright future, social status etc.

- In addition to above, if one is single author, then entitled to 40% discount on publishing research paper and can get 10%discount if one is co-author or main author among group of authors.
- The Fellow can organize symposium/seminar/conference on behalf of Global Journals Incorporation (USA) and he/she can also attend the same organized by other institutes on behalf of Global Journals.
- The Fellow can become member of Editorial Board Member after completing 3yrs.
- The Fellow can earn 60% of sales proceeds from the sale of reference/review books/literature/publishing of research paper.
- Fellow can also join as paid peer reviewer and earn 15% remuneration of author charges and can also get an opportunity to join as member of the Editorial Board of Global Journals Incorporation (USA)
- • This individual has learned the basic methods of applying those concepts and techniques to common challenging situations. This individual has further demonstrated an in–depth understanding of the application of suitable techniques to a particular area of research practice.

Note :

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- In future, if the board feels the necessity to change any board member, the same can be done with the consent of the chairperson along with anyone board member without our approval.
- In case, the chairperson needs to be replaced then consent of 2/3rd board members are required and they are also required to jointly pass the resolution copy of which should be sent to us. In such case, it will be compulsory to obtain our approval before replacement.
- In case of "Difference of Opinion [if any]" among the Board members, our decision will be final and binding to everyone.

"

Preferred Author Guidelines

We accept the manuscript submissions in any standard (generic) format.

We typeset manuscripts using advanced typesetting tools like Adobe In Design, CorelDraw, TeXnicCenter, and TeXStudio. We usually recommend authors submit their research using any standard format they are comfortable with, and let Global Journals do the rest.

Alternatively, you can download our basic template from https://globaljournals.org/Template

Authors should submit their complete paper/article, including text illustrations, graphics, conclusions, artwork, and tables. Authors who are not able to submit manuscript using the form above can email the manuscript department at submit@globaljournals.org or get in touch with chiefeditor@globaljournals.org if they wish to send the abstract before submission.

Before and during Submission

Authors must ensure the information provided during the submission of a paper is authentic. Please go through the following checklist before submitting:

1. Authors must go through the complete author guideline and understand and *agree to Global Journals' ethics and code of conduct,* along with author responsibilities.
2. Authors must accept the privacy policy, terms, and conditions of Global Journals.
3. Ensure corresponding author's email address and postal address are accurate and reachable.
4. Manuscript to be submitted must include keywords, an abstract, a paper title, co-author(s') names and details (email address, name, phone number, and institution), figures and illustrations in vector format including appropriate captions, tables, including titles and footnotes, a conclusion, results, acknowledgments and references.
5. Authors should submit paper in a ZIP archive if any supplementary files are required along with the paper.
6. Proper permissions must be acquired for the use of any copyrighted material.
7. Manuscript submitted *must not have been submitted or published elsewhere* and all authors must be aware of the submission.

Declaration of Conflicts of Interest

It is required for authors to declare all financial, institutional, and personal relationships with other individuals and organizations that could influence (bias) their research.

Policy on Plagiarism

Plagiarism is not acceptable in Global Journals submissions at all.

Plagiarized content will not be considered for publication. We reserve the right to inform authors' institutions about plagiarism detected either before or after publication. If plagiarism is identified, we will follow COPE guidelines:

Authors are solely responsible for all the plagiarism that is found. The author must not fabricate, falsify or plagiarize existing research data. The following, if copied, will be considered plagiarism:

- Words (language)
- Ideas
- Findings
- Writings
- Diagrams
- Graphs
- Illustrations
- Lectures

- Printed material
- Graphic representations
- Computer programs
- Electronic material
- Any other original work

Authorship Policies

Global Journals follows the definition of authorship set up by the Open Association of Research Society, USA. According to its guidelines, authorship criteria must be based on:

1. Substantial contributions to the conception and acquisition of data, analysis, and interpretation of findings.
2. Drafting the paper and revising it critically regarding important academic content.
3. Final approval of the version of the paper to be published.

Changes in Authorship

The corresponding author should mention the name and complete details of all co-authors during submission and in manuscript. We support addition, rearrangement, manipulation, and deletions in authors list till the early view publication of the journal. We expect that corresponding author will notify all co-authors of submission. We follow COPE guidelines for changes in authorship.

Copyright

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Appealing Decisions

Unless specified in the notification, the Editorial Board's decision on publication of the paper is final and cannot be appealed before making the major change in the manuscript.

Acknowledgments

Contributors to the research other than authors credited should be mentioned in Acknowledgments. The source of funding for the research can be included. Suppliers of resources may be mentioned along with their addresses.

Declaration of funding sources

Global Journals is in partnership with various universities, laboratories, and other institutions worldwide in the research domain. Authors are requested to disclose their source of funding during every stage of their research, such as making analysis, performing laboratory operations, computing data, and using institutional resources, from writing an article to its submission. This will also help authors to get reimbursements by requesting an open access publication letter from Global Journals and submitting to the respective funding source.

Preparing your Manuscript

Authors can submit papers and articles in an acceptable file format: MS Word (doc, docx), LaTeX (.tex, .zip or .rar including all of your files), Adobe PDF (.pdf), rich text format (.rtf), simple text document (.txt), Open Document Text (.odt), and Apple Pages (.pages). Our professional layout editors will format the entire paper according to our official guidelines. This is one of the highlights of publishing with Global Journals—authors should not be concerned about the formatting of their paper. Global Journals accepts articles and manuscripts in every major language, be it Spanish, Chinese, Japanese, Portuguese, Russian, French, German, Dutch, Italian, Greek, or any other national language, but the title, subtitle, and abstract should be in English. This will facilitate indexing and the pre-peer review process.

The following is the official style and template developed for publication of a research paper. Authors are not required to follow this style during the submission of the paper. It is just for reference purposes.

Manuscript Style Instruction (Optional)

- Microsoft Word Document Setting Instructions.
- Font type of all text should be Swis721 Lt BT.
- Page size: 8.27" x 11'", left margin: 0.65, right margin: 0.65, bottom margin: 0.75.
- Paper title should be in one column of font size 24.
- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word "Abstract" in bold italics.
- Main text: font size 10 with two justified columns.
- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
- The paragraph before spacing of 1 pt and after of 0 pt.
- Line spacing of 1 pt.
- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

Structure and Format of Manuscript

The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

a) A title which should be relevant to the theme of the paper.
b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.
c) Up to 10 keywords that precisely identify the paper's subject, purpose, and focus.
d) An introduction, giving fundamental background objectives.
e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition, sources of information must be given, and numerical methods must be specified by reference.
f) Results which should be presented concisely by well-designed tables and figures.
g) Suitable statistical data should also be given.
h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.
j) There should be brief acknowledgments.
k) There ought to be references in the conventional format. Global Journals recommends APA format.

Authors should carefully consider the preparation of papers to ensure that they communicate effectively. Papers are much more likely to be accepted if they are carefully designed and laid out, contain few or no errors, are summarizing, and follow instructions. They will also be published with much fewer delays than those that require much technical and editorial correction.

The Editorial Board reserves the right to make literary corrections and suggestions to improve brevity.

Format Structure

It is necessary that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.

All manuscripts submitted to Global Journals should include:

Title

The title page must carry an informative title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) where the work was carried out.

Author details

The full postal address of any related author(s) must be specified.

Abstract

The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Keywords

A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy: planning of a list of possible keywords and phrases to try.

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning searching. Think about the most important concepts related to research work. Ask, “What words would a source have to include to be truly valuable in a research paper?” Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

Numerical Methods

Numerical methods used should be transparent and, where appropriate, supported by references.

Abbreviations

Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

Formulas and equations

Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

Tables, Figures, and Figure Legends

Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.

Figures

Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

Preparation of Eletronic Figures for Publication

Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). Please give the data for figures in black and white or submit a Color Work Agreement form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution at final image size ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs): >350 dpi; figures containing both halftone and line images: >650 dpi.

Color charges: Authors are advised to pay the full cost for the reproduction of their color artwork. Hence, please note that if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a Color Work Agreement form before your paper can be published. Also, you can email your editor to remove the color fee after acceptance of the paper.

Tips for writing a good quality Medical Research Paper

1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

4. Use of computer is recommended: As you are doing research in the field of medical research then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

5. Use the internet for help: An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.

6. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right? It is a good habit which helps to not lose your continuity. You should always use bookmarks while searching on the internet also, which will make your search easier.

7. Revise what you wrote: When you write anything, always read it, summarize it, and then finalize it.

8. Make every effort: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

9. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

10. Use proper verb tense: Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. Arrangement of information: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. Go to seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. Adding unnecessary information: Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. Upon conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear: Adhere to recommended page limits.

Mistakes to avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

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

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

Approach:

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

Procedures (methods and materials):

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."

Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

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

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ISSN 9755896