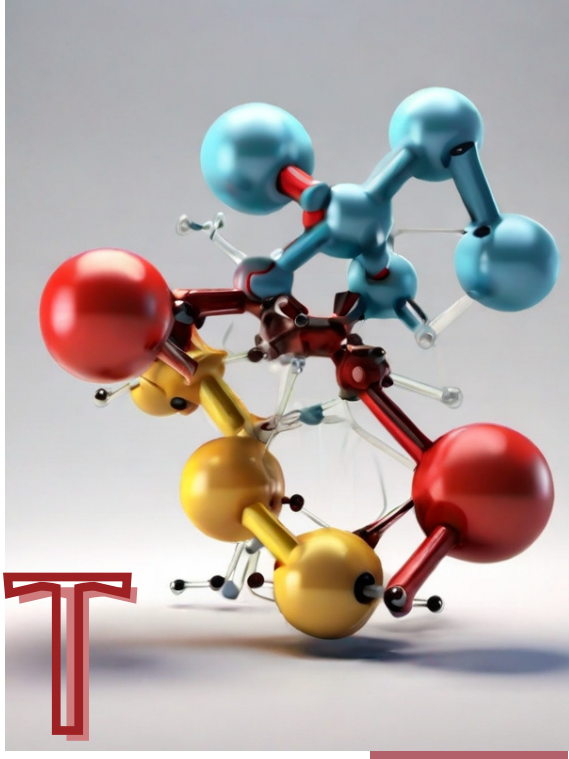
THE OFFICIAL PUBLICATION OF
DEPERTMENT OF PHARMACEUTICAL SCIENCES & TECHNOLOGY



INTERNATIONAL JOURNAL

PHARMBIT

Volume: 38 (January-December, 2022)

ISSN : 0973-6204

BIRLA INSTITUTE OF TECHNOLOGY
MESRA, RANCHI, INDIA

www.bitmesra.ac.in

FROM THE EDITOR

“

It is my privilege to publish the 38th edition of PHARMBIT, the esteemed journal of the Department of Pharmaceutical Sciences and Technology, on behalf of the Pharmaceutical Society of BIT Mesra. As an annual scholarly publication, our mission is to promote innovation, collaboration, and advancement across pharmacy and life sciences. PHARMBIT is currently abstracted in several prestigious databases including Natural Science Database, ProQuest, Index Copernicus, Google Scholar, ASCI-Database and CiteFactor.

The 38th volume provides insightful perspectives of a diverse range of topics, highlighting the expertise of our distinguished authors. The five manuscripts featured illuminate critical issues such as computer-aided diagnosis of malaria, pharmacological effects of estrogen, drug permeation principle through the Diffusion Cell Method, the role of SCUBE 1 in wound healing, and standardization of Ayurvedic formulation using ICH guidelines. I firmly believe the original research, perspectives, and reviews in this edition will furnish the readers with valuable insights and perspectives on current trends and emerging challenges in Pharmacy and life sciences. Furthermore, these contributions will inspire future investigations and collaborations to solve society’s most pressing medical needs.

I sincerely thank all authors for choosing PHARMBIT to showcase their research and perspectives. My gratitude also goes to our esteemed Editorial Board for their guidance, the HOD and faculty for their enduring support, and our Vice Chancellor whose leadership has sustained this invaluable publication legacy.

As the Chief Editor, I encourage researchers, practitioners, and students alike to engage with the content presented here, to question assumptions, challenge conventions, and contribute to the ongoing dialogue that drives progress in our field. I invite you to explore this stimulating issue and join our shared pursuit of scientific excellence and discovery. Thank you for your continued support and readership.

”

Sincerely,

Manik Ghosh

Chief Editor

TABLE OF CONTENTS

EDITORIAL ADVISORY BOARD

EDITORIAL BOARD MEMBERS

Computer-aided Diagnosis of Malaria through Transfer Learning using the ResNet50 Backbone

Sanya Sinha[1*] and Nilay Gupta[2]

[1]*Dept. of Electronics and Communications Engineering,*
Birla Institute of Technology Mesra, Patna Campus, Patna, India.
[2]*Centre for Artificial Intelligence,*
ZHAW School of Engineering, Wintherthur, Switzerland.
*Corresponding Author: ssanya0904@gmail.com

A B S T R A C T

According to the World Malaria Report of 2022, 247 million cases of malaria and 619,000 related deaths were reported in 2021. This highlights the predominance of the disease, especially in the tropical and sub-tropical regions of Africa, parts of South-east Asia, Central and Southern America. Malaria is caused due to the Plasmodium parasite which is circulated through the bites of the female Anopheles mosquito. Hence, the detection of the parasite in human blood smears could confirm malarial infestation. Since the manual identification of Plasmodium is a lengthy and time-consuming task subject to variability in accuracy, we propose an automated, computer-aided diagnostic method to classify malarial thin smear blood cell images as parasitized and uninfected by using the ResNet50 Deep Neural Network. In this paper, we have used the pre-trained ResNet50 model on the open-access dataset provided by the National Library of Medicine's Lister Hill National Center for Biomedical Communication for 150 epochs. The results obtained showed accuracy, precision, and recall values of 98.75%, 99.3% and 99.5% on the ResNet50 (proposed) model. We have compared these metrics with similar models such as VGG16, Watershed Segmentation and Random Forest, which showed better performance than traditional techniques as well.

Keywords: Artificial Intelligence, Automated Diagnosis, Computer Vision, Deep Learning, Healthcare.

1. INTRODUCTION

Malaria is a fatal, mosquito-borne disease caused mainly due to the presence of the *Plasmodium falcifarum* parasite [1]. Malaria is typically symptomized with high body temperatures, chills, and body ache like most other influenza-adjacent diseases [2]. However, if left untreated, malaria may also cause severe health complications including seizures, neuro-disability, multiple organ failure, and ultimate death [3]. Untimely malarial diagnosis unnecessarily complicates the line of treatment and causes additional adversities including drug resistance, chemo preventive abuse, and genetic mutations which are physically, mentally, and monetarily catastrophic to handle [4]. Therefore, timely, and accurate diagnosis of malaria is of critical importance.

Giemsa is a purple-colored stain that explicitly highlights the parasite in thin slides of the patient's blood [5]. Parasitologists traditionally examine the stained blood cell smears for the purple-stained parasite to confirm malarial infiltration. However, the degree of accuracy of diagnosis is largely variable and subject to the expertise of the parasitologist, the contrast, illumination and brightness of smear images obtained, as well as the quality of the testing equipment used. This may lead to discrepancies in the diagnosis pipeline, ultimately leading to misdiagnosis [6]. To

effectively shorten the detection pipeline while improving the accuracy of the diagnosis, the computer-aided diagnostic landscape was introduced. This automated the detection process and improved the speed, accuracy and cost-effectivity of the line of treatment. Several methods were introduced to develop efficient healthcare delivery systems for computer-aided malarial diagnosis.

Image Processing techniques were introduced to remove pertaining noise, correct illumination, normalize edge segmentation, and eradicate artifacts from cell images to render them useful for automated examination. Mean [7], Median [8], Geometric Mean [9], and Wiener filtering [10] are notable image denoising methods that operate in the pixel's neighborhood for image impulse noise removal and edge preservation. Savkare et al. [11] introduced a novel method to enhance malarial image resolution through Laplacian filtering and Adaptive Histogram Equalization. Razzak et al. [12] proposed top-hat transforms for correcting irregularity in image illumination. Dong et al. [13] suggested morphological operations for artifact removal and hole filling for substituting the missing frequencies in a signal with appropriate values. Once the images have been preprocessed, a segmentation algorithm is leveraged for highlighting parasitic infestation. Threshold-based segmentation algorithms such as Otsu's thresholding were amalgamated with morphological operations such as granulometry to calculate optimum threshold values for bimodal images. Watershed Segmentation for Malaria was introduced by Kim et al. [14] for extracting the boundaries of the parasite inside the cell. Marker-controlled Watershed [15] is an improvement over the traditional Watershed segmentation as it includes the segmentation of extrinsically overlapping cells as well. Bibin et al. [16] introduced Active Contour Models for malarial cell segmentation by topological levelling. Abbas et al.'s Gaussian Mixture Model [17] for malaria identification worked with the probabilistic assumption that all the defined data points arose from a mixture of Gaussian distributions. Image Processing models are simple to implement, but are only suitable for smaller-sized datasets. Learning-based methods return better results for larger-sized datasets.

Learning-based methods could be either Supervised or Unsupervised. Unsupervised learning methods such as the Naïve Bayes Tree by Das et al. [18] return substantially satisfactory results without providing training output data. However, Supervised learning methods such as the Random Forest Classifier proposed by Quinn et al. [19] return better classification accuracy. Neural Networks have proven to be remarkably efficient for modeling image data. Gopakumar et al. [20] proposed a Convolutional Neural Network (CNN) to operate on the focal parasitemia of blood smear images. However, training CNNs straight from scratch is a computationally intensive and time-consuming process.

In this paper, we propose ResNet50, a deep neural network (DNN) for classifying malarial cell images into the parasitized and uninfected classes. Since building a DNN from the ground-up is expensive, we introduce Transfer Learning (TL). TL fine-tunes pre-trained models to accommodate alien weights without having to train the model from absolute scratch.

2. TARGET IDENTIFICATION

The open-access dataset provided by the National Library of Medicine's Lister Hill National Center for Biomedical Communication [21] has been used in this paper. It was created in collaboration with the National Institutes of Health, the Centers for Disease Control and Prevention, and the Mahidol-Oxford Tropical Medicine Research Unit, and is now considered a benchmark dataset for computer vision tasks aimed at classifying malarial cell images.

The dataset contains 27,558 thin blood smear slide images clicked using light microscopy of 150 P falciparum malaria-infected and 50 healthy individuals. The dataset is equally distributed into the parasitized and uninfected classes, each containing 13,779 images, with a training, testing and validation ratio of 7:2:1.

Before implementing the classification algorithm, data augmentation is done to make the dataset more robust to image quality variations. MixUp [22] and CutMix [23] are the two algorithms used for data augmentation. MixUp involves randomly combining pairs of images and labels by taking a weighted linear combination of the two, while CutMix involves cutting and pasting random patches from one image to another with corresponding label adjustments. Both techniques aim to improve model generalization by encouraging smoother and more robust decision boundaries. **Figure 1** illustrates the output images of MixUp and CutMix augmentation. After the dataset has been augmented, it is passed through the ResNet50 classifier.

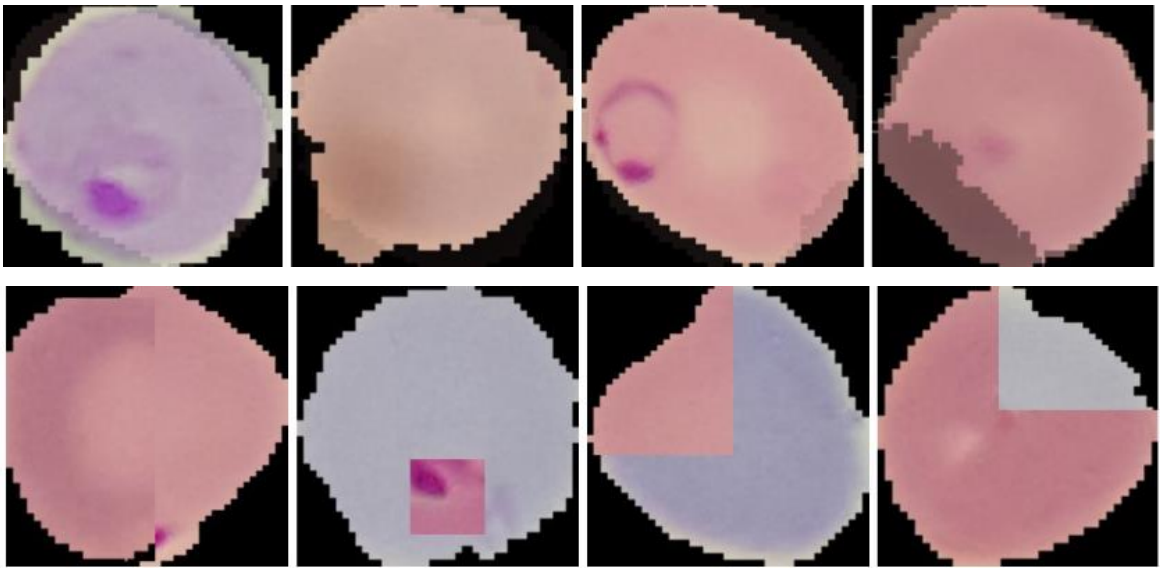


Figure 1: Results of MixUp and CutMix Augmentation

ResNet50 [24] is a member of the deep neural network family that is known as "Residual Networks". These networks have special connections called "residual connections" that permit the network to bypass certain layers and "skip connections" that enable gradients to flow more smoothly through the network. ResNets are highly effective in binary image classification tasks, especially when using the sigmoid activation function which is known to experience the vanishing gradient problem. ResNets tackle this issue by using the residual connections to create shortcuts from the input to the output, which bypasses layers and allows gradients to flow more effectively during backpropagation. ResNet50 contains 49 convolutional layers followed by a fully connected layer at the end. **Figure 2** illustrates the architecture of the ResNet50 backbone. The convolutional layers are organized into four blocks, each with a different number of layers and downsampling operations. The first block has three layers and downsamples the spatial dimensions of the input cell image by a factor of 4, while the other three blocks each have four layers and downsample the input image by a factor of 2. The final fully connected layer maps the output of the last convolutional layer to the desired output dimensionality. The model was trained for 150 epochs on the NVIDIA Tesla P100 GPU. The Adam optimizer was used with the

Binary cross entropy loss function for the training. The Sigmoid activation function is used in the classification layer.

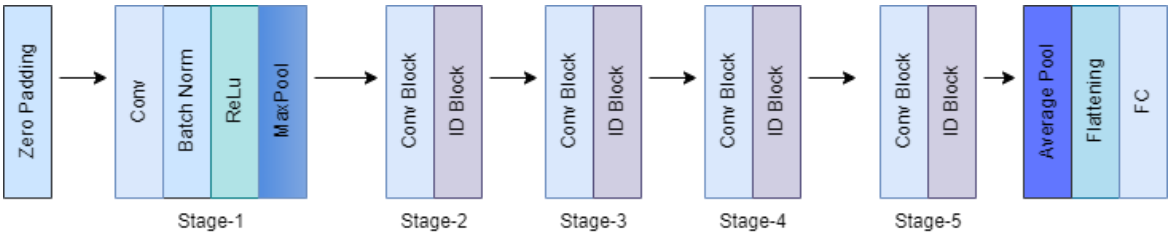


Figure 2: Architecture of ResNet50

3. RESULTS

The performance of the ResNet50 model is compared with 3 other state-of-the-art models that have been used to detect malaria in cell images. The models chosen for comparison belong to three different classes of classifiers used in medical imaging. Watershed segmentation is an example of an image processing-based thresholding technique for identifying boundaries of the parasite. Random Forest is a tree-based machine learning algorithm which culminates the outputs of multiple decision trees to reach the result. VGG16 [25] belongs to the VGG group of deep neural network models trained on the ImageNet dataset for image classification. It has 16 trainable layers.

The models have been juxtaposed based on 3 performance metrics. These metrics; accuracy, precision and recall have been used to compare the performance of the model.

Accuracy is used to show the number of correctly classified malarial cells over the total number of cells. It can be represented as:

$$Accuracy\ (\%) = \frac{TP + TN}{TP + TN + FP + FN} \times 100 \quad (1)$$

Precision is determined by dividing the genuine positives by any positive prediction. It measures how well a model can predict a particular category.

$$Precision = \frac{TP}{(TP+FP)} \quad (2)$$

Recall is the true positive rate of any model. It is computed by dividing the real positives by anything that has been predicted as positive, rightly or not.

$$Recall = \frac{TP}{(TP + FN)}$$

Table 1: Performance Metrics

Model Name	Accuracy	Precision	Recall
Random Forest	0.651	0.740	0.740
VGG16	0.937	0.529	0.744
Watershed Segmentation	0.90	0.643	0.662
Proposed Model	**0.9875**	**0.993**	**0.995**

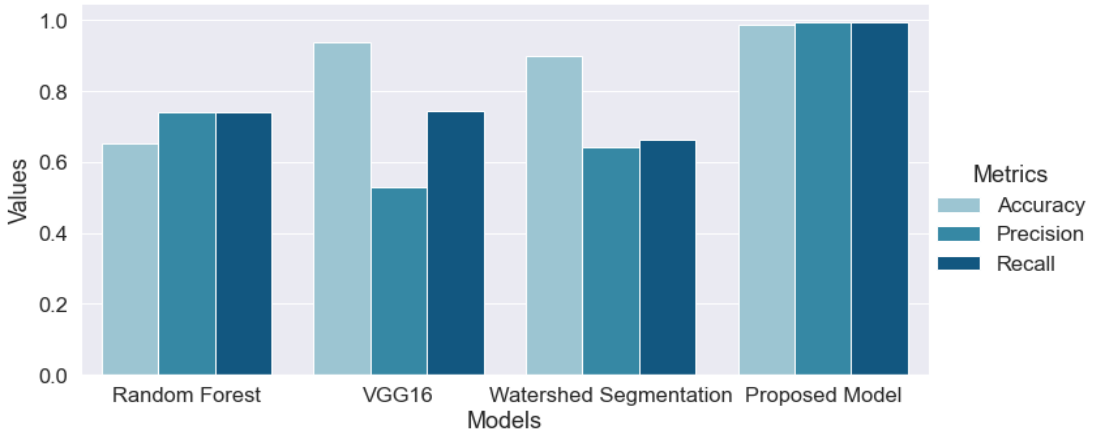


Figure 3: Performance Metrics for the four models

drug design holds tremendous opportunities. As these developments take place, in silico approaches have the potential to completely transform drug discovery and provide novel approaches to pressing global health issues [5][8].

4. DISCUSSION

It can be observed from **Table 1** and **Figure 3** that the proposed model returned the best accuracy, precision, and recall values of 98.75%, 99.3% and 99.5%. VGG16 returned comparable accuracy of 93.7%, which is acceptable but not as decent as the proposed models. It has lower precision and recall values of 52.9% and 74.4%, indicating that the model might have overfitted. This could be a result of the vanishing gradient problem associated with the Sigmoid activation function. Random Forest returned 65.1% accuracy, with 74% precision and recall values. These are extremely underperforming metrics, especially when compared to the proposed model. Watershed segmentation returned 90% segmentation accuracy, with 64.3% precision and 66.2% recall values. The poor precision and recall values are a primary by-product of the dataset's large size. **Figure 4** illustrates the true labels and the predicted labels for a set of malarial cells. It can be seen how the ResNet50 backbone returned accurate labels for each of the test samples. Hence, it can be observed that the proposed model outperforms all the 3 models used for comparison.

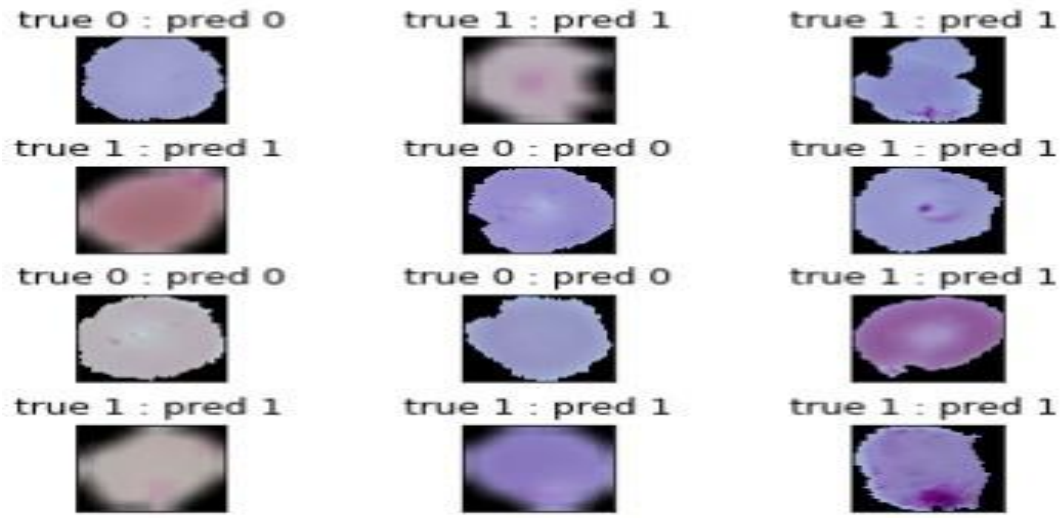


Figure 4: Classification Output of ResNet50 model

5. CONCLUSION

Therefore, it is concluded that malaria detection could be faster and more accurate due to TL-based methods. ResNet50 showed the best binary classification results when compared with other thresholding-based, learning-based, and neural network-based techniques. The only problem associated with ResNets is that a large amount of training data is required to achieve optimal performance. While the malaria dataset had an adequate number of images per class, this method could not be expanded to other diseases with smaller-sized datasets. In our future work, we aim to leverage Vision Transformers for enabling disease detection on medical datasets with a lesser number of images.

6. REFERENCES

1. *World Health Organization. (2022). World malaria report 2022. World Health Organization.*
2. *Divis, P. C., Singh, B., & Conway, D. J. (2021). Molecular epidemiology and population genomics of Plasmodium knowlesi. Advances in parasitology, 113, 191-223.*
3. *Parsel, S. M., Gustafson, S. A., Friedlander, E., Shnyra, A. A., Adegbulu, A. J., Liu, Y., ... & McArthur, C. P. (2017). Malaria over-diagnosis in Cameroon: diagnostic accuracy of Fluorescence and Staining Technologies (FAST) Malaria Stain and LED microscopy versus Giemsa and bright field microscopy validated by polymerase chain reaction. Infectious diseases of poverty, 6(1), 1-9.Karmakar, S., & Deshpande, H. (2016). Ormeloxifene. A new treatment modality in Dysfunctional Uterine Bleeding: efficacy and safety. Indian J Obstet Gynecol Res, 3(3), 225-8.*
4. *Tan, K. R., Magill, A. J., Parise, M. E., & Arguin, P. M. (2011). Doxycycline for malaria chemoprophylaxis and treatment: report from the CDC expert meeting on malaria chemoprophylaxis. The American journal of tropical medicine and hygiene, 84(4), 517.*
5. *Arco, J. E., Górriz, J. M., Ramírez, J., Álvarez, I., & Puntonet, C. G. (2015). Digital image analysis for automatic enumeration of malaria parasites using morphological operations. Expert Systems with Applications, 42(6), 3041-3047.*
6. *Yang, D., Subramanian, G., Duan, J., Gao, S., Bai, L., Chandramohanadas, R., & Ai, Y. (2017). A portable image-based cytometer for rapid malaria detection and quantification. PloS one, 12(6), e0179161.*
7. *Sinha, S., & Gupta, N. (2023). Computer-aided Diagnosis of Malaria through Transfer Learning using the ResNet50 Backbone. arXiv preprint arXiv:2304.02925.*
8. *Linder, N., Turkki, R., Walliander, M., Mårtensson, A., Diwan, V., Rahtu, E., ... & Lundin, J. (2014). A malaria diagnostic tool based on computer vision screening and visualization of Plasmodium falciparum candidate areas in digitized blood smears. PLoS One, 9(8), e104855.*
9. *Sirima, S. B., Richert, L., Chêne, A., Konate, A. T., Campion, C., Dechavanne, S., ... & Gamain, B. (2020). PRIMVAC vaccine adjuvanted with Alhydrogel or GLA-SE to prevent placental malaria: a first-in-human, randomised, double-blind, placebo-controlled study. The Lancet Infectious Diseases, 20(5), 585-597.*
10. *May, Z., & Aziz, S. S. A. M. (2013, October). Automated quantification and classification of malaria parasites in thin blood smears. In 2013 IEEE International Conference on Signal and Image Processing Applications (pp. 369-373). IEEE.*
11. *Savkare, S. S., & Narote, S. P. (2015, January). Automated system for malaria parasite identification. In 2015 international conference on communication, information & computing technology (ICCICT) (pp. 1-4). IEEE.*
12. *Razzak, M. I. (2015). Malarial parasite classification using recurrent neural*

network. Int J Image Process, 9, 69.

13. *Dong, Y., Jiang, Z., Shen, H., Pan, W. D., Williams, L. A., Reddy, V. V., ... & Bryan, A. W. (2017, February). Evaluations of deep convolutional neural networks for automatic identification of malaria infected cells. In 2017 IEEE EMBS international conference on biomedical & health informatics (BHI) (pp. 101-104). IEEE.*
14. *Kim, J. D., Nam, K. M., Park, C. Y., Kim, Y. S., & Song, H. J. (2016). Automatic detection of malaria parasite in blood images using two parameters. Technology and Health Care, 24(s1), S33-S39.*
15. *Das, D. K., Chakraborty, C., Mitra, B., Maiti, A. K., & Ray, A. K. (2013). Quantitative microscopy approach for shape-based erythrocytes characterization in anaemia. Journal of microscopy, 249(2), 136-149.*
16. *Bibin, D., Nair, M. S., & Punitha, P. (2017). Malaria parasite detection from peripheral blood smear images using deep belief networks. IEEE Access, 5, 9099-9108.*
17. *Abbas, N., Saba, T., Mohamad, D., Rehman, A., Almazyad, A. S., & Al-Ghamdi, J. S. (2018). Machine aided malaria parasitemia detection in Giemsa-stained thin blood smears. Neural Computing and Applications, 29, 803-818.*
18. *Das, D. K., Ghosh, M., Pal, M., Maiti, A. K., & Chakraborty, C. (2013). Machine learning approach for automated screening of malaria parasite using light microscopic images. Micron, 45, 97-106.*
19. *Quinn, J. A., Andama, A., Munabi, I., & Kiwanuka, F. N. (2014). Automated blood smear analysis for mobile malaria diagnosis. Mobile Point-of-Care Monitors and Diagnostic Device Design, 31, 115.*
20. *Gopakumar, G. P., Swetha, M., Sai Siva, G., & Sai Subrahmanyam, G. R. K. (2018). Convolutional neural network-based malaria diagnosis from focus stack of blood smear images acquired using custom-built slide scanner. Journal of biophotonics, 11(3), e201700003.*
21. *Rajaraman, S., Antani, S. K., Poostchi, M., Silamut, K., Hossain, M. A., Maude, R. J., ... & Thoma, G. R. (2018). Pre-trained convolutional neural networks as feature extractors toward improved malaria parasite detection in thin blood smear images. PeerJ, 6, e4568.*
22. *Zhang, H., Cisse, M., Dauphin, Y. N., & Lopez-Paz, D. (2017). mixup: Beyond empirical risk minimization. arXiv preprint arXiv:1710.09412.*
23. *Yun, S., Han, D., Oh, S. J., Chun, S., Choe, J., & Yoo, Y. (2019). Cutmix: Regularization strategy to train strong classifiers with localizable features. In Proceedings of the IEEE/CVF international conference on computer vision (pp. 6023-6032).*
24. *He, K., Zhang, X., Ren, S., & Sun, J. (2016). Deep residual learning for image recognition. In Proceedings of the IEEE conference on computer vision and pattern recognition (pp. 770-778).*
25. *Sifat, M. M. H., & Islam, M. M. (2020, June). A fully automated system to detect malaria parasites and their stages from the blood smear. In 2020 IEEE Region 10 Symposium (TENSYMP) (pp. 1351-1354). IEEE.*

Effect of Estrogen on Thermoregulation

Shivani Baskey, Monika Bhardwaj, Mansi Agrawal, and Papiya Mitra Mazumder*
Department of Pharmaceutical Sciences and Technology,
Birla Institute of Technology, Mesra, Ranchi-835215, Jharkhand, India
*Corresponding Author: pmmazumder@bitmesra.ac.in

A B S T R A C T

Estrogen is one of the major steroidal hormones responsible for the development of female sexual characteristics. Towards menopause the level of estrogen falls down drastically due to which post-menopausal symptoms occur which includes hot flushes, night sweats, mood swings, anxiety and insomnia. Fall in the level of estrogen increases the body temperature which can be detected by the hypothalamus. The hypothalamus tries to maintain the normal homeostasis either by feedback or feed forward mechanism. Additionally, post-menopausal symptoms can be cured using hormone replacement therapy. Although, hormone replacement therapy can relieve post-menopausal symptoms, it can cause serious problems like endometrial cancer. Hence, non-hormonal treatments like selective serotonin reuptake inhibitors and estrogen receptor beta agonists are used as an alternative to hormone replacement therapies. In this review the post-menopausal syndrome, its treatment using hormone replacement therapy, major side effects of hormone replacement therapy (HRT) have been discussed along with the ways to overcome HRT related side effects.

Keywords: Estrogen, Hormone replacement therapy, Hypothalamus, post-menopausal symptoms, Thermoregulation.

1. INTRODUCTION

The three interrelated systems that makes up the human body are the physiological, psychological, and pneumatological systems. Each system is made up of numerous sub systems. For instance, the physiological system, includes the skeletal, muscular, neurological, digestive, excretory, respiratory, circulatory, and metabolic systems. The endocrine system is also a very important part of the human body. The endocrine system secrets a chemical substance known as hormones that help in maintaining the homeostasis of the human body. These hormones are produced by the endocrine glands which includes the hypothalamus, pituitary gland, pineal gland, thymus gland, thyroid gland, pancreas, adrenal gland, gonads i.e. the ovaries in female and testis in male. The hormones produced by these glands are directly poured into the blood stream hence are also known as the ductless glands. Pancreas is known as the mixed gland because it has both exocrine and endocrine functions. The exocrine part of pancreas produces digestive enzymes and the endocrine part produces insulin and glucagon. There are certain special structures that produces hormones during pregnancy like the placenta. This is important for the maintainance of pregnancy. Hormones control the growth, development, and metabolism of the body, the electrolyte composition of body fluids, as well as reproduction. These hormones are of different types based on the basic building blocks like the steroidal hormones, amino acid derivatives, prostaglandins and miscellaneous

types. Depending on the receptor binding they produce different actions. During menopause the level of certain hormones especially estrogen falls down drastically due to which post-menopausal syndrome can occur. Hot flushes, irritability, mood swings, insomnia, dry vagina, difficulty concentrating, mental confusion, stress, incontinence, osteoporotic symptoms, depression, headache, vasomotor symptoms and insomnia are among the symptoms associated with postmenopausal syndrome. These symptoms can be treated by administering hormones externally in the form of hormone replacement therapy. Sometimes if these therapies are taken for long or if discontinued in the middle can cause serious side effects and can even cause cancer. In this review we have tried to discuss the post-menopausal syndrome, its treatment using hormone replacement therapy, major side effects of hormone replacement therapy (HRT) and how to overcome these HRT related side effects.

2. HISTORY

There are different forms of estrogen present in our body. Estrone was the first estrogen to be discovered in 1929 by Adolf Butandant and Edward Adelbert Doisy. In 1930 and 1933 estriol and estradiol was discovered respectively.**[3]** Later it was discovered that estrogen alone or estrogen along with progesterone can be used to treat post-menopausal symptoms like night sweat, hot flashes and mood swings. Estrogen was widely used to treat menopausal symptoms in 1950's and 1960's. **[3]** Towards 1970's it was discovered that estrogen only therapy can lead to endometrial cancer in postmenopausal women. As a result, it was advised that women with intact uterus should take estrogen along with progesterone to lower the risk of cancer. In the USA, the first HRT and chronic postmenopausal diseases clinical trials got underway in late 1990s. The Women's Health Initiative (WHI), launched one of the biggest randomized trials in 1998 to investigate the impact of HRT on the most prevalent causes of death and disability in postmenopausal women, including cardiovascular disease, cancer, and osteoporosis. **[3,4]** After these years, the use of HRT decreased in several countries. For example, the usage of HRT substantially decreased by 46% in the USA and by 28% in Canada. Such figures were also seen in European nations like Germany or the United Kingdom.**[4]**

3. CHEMICAL CLASSIFICATION OF HORMONES

Hormones are chemical messengers that are produced by the ductless endocrine glands into the blood stream from where it is taken up by the organs and tissues. Hormones can be classified into different types based upon the chemical nature, mechanism of action, nature of action and effect. **Table 1** summarizes the classification of hormones based upon the chemical nature.

3.1 Classification of hormones based upon their mechanism of action

Hormones are classified as group one and group two based upon their mechanism of action. Group one hormones act by forming hormone receptor complex (HRC). These HRC form intracellular receptor by which they exert biochemical effect. They are made up of cholesterol and are lipophilic in nature. For example, testosterone, progesterone, T4, T5 and estrogen. Group two hormones bind to cell surface receptors and produce specific molecules known as the second messengers.

3.2. Classification of hormones based upon nature

Based upon the nature hormones can be either local or general. The secretion from

local hormones has local effect for example testosterone whereas the hormones released by general hormones are transported to different target organs and tissues for example thyroid hormone and insulin.

3.3. Ring Opening Reaction: Classification of hormones based upon its effect

Hormones are classified as kinetic hormones, metabolic hormones, morphogenetic hormones based upon its effects. Kinetic hormones may cause muscle contraction, glandular secretion and colour migration. Examples of these kind of hormones are melanosite stimulating hormones (MSH) and epinephrine. Metabolic hormones function to regulate the rate of metabolism and balance the reaction in body. Examples of this type of hormones are glucagon and insulin. Morphogenetic hormones are the hormones that are responsible for growth and differentiation processes. For example, Follicle Stimulating Hormones (FSH) and thyroid hormone.

Table 1*:* Classification of hormones based upon the chemical nature

Hormone	Source	Examples
Steroid hormone	These are derived from cholesterol	Testosterone, estrogen and progesterone
Amine hormone	Hormones derived from the modification of amino acid and are referred to as amine hormones.	Melatonin, thyroid hormones and catecholamines
Peptide hormone	These hormones often take the form of a linear chain and contain just a few amino acid residues.	Oxytocin And vasopressin
Protein hormone	These hormones include a significant amount of amino acid residue.	Insulin, glucagon, somatropins
Glycoprotein hormone	These are conjugated protein bound to carbohydrates like galactose, mannose and fructose.	Luteinizing hormone, follicle stimulating hormone, thyroid stimulating hormone
Eicosanoid hormone	These are a range of arachidonic acid-containing small fatty acid derivatives	Prostaglandins

4. DISTRIBUTION OF ESTROGEN RECEPTORS

Nuclear receptors and membrane receptors are the two different types of estrogen receptors. Estrogen receptors (ER-α) and (ER-β) are examples of nuclear receptors. The G-Protein coupled receptor is a type of membrane receptor. **[6]** The digestive system and neurological system both include estrogen receptors α and β, whereas the reproductive system and bones contain estrogen receptors α, β as well as G-protein coupled receptors. These receptors are found in the bone, which makes women more vulnerable to bone fractures as they age. **[5,6]** The ER- α is present in the hypothalamus, endometrium, breast cancer cells, and ovarian stromal cells. The ER- α protein is present in the male efferent duct epithelium. It has been demonstrated that the ER-β protein is expressed by ovarian granulosa

cells, kidney, brain, bone, heart, lungs, intestinal mucosa, prostate, and endothelial cells. **[21]**

5. MECHANISM OF ACTION OF ESTROGEN

The biological effects of estrogen are mediated by nuclear receptors called estrogen receptors (ER) α and β, which are members of a large superfamily. These receptors function as transcription factors that are ligand-activated. **[22]** Through the conventional ER action mechanism, estrogen attaches to receptors in the nucleus. The receptors then dimerize and bind to particular response elements termed estrogen response elements (EREs) located in the promoters of target genes. Moreover, the ligand-binding region of the receptors undergoes a conformational shift in response to hormone binding, and this conformational change enables the recruitment of activator proteins. **[7]** Although not entirely understood, the chemical processes by which ERs control transcription at alternate response elements are becoming increasingly obvious. ERs can regulate gene expression without directly interacting with DNA by altering the function of another family of transcription factors through protein-protein interactions in the nucleus. **[8]** Such ERE-independent genomic effects are routinely observed when ERs interact with the activator protein 1 (AP-1) transcription factor complex. Moreover, certain estrogen-responsive genes that are deficient in EREs nevertheless have ERE half-sites, which act as direct ER binding sites for the orphan nuclear hormone receptor SF-1. ER α but not ER β can bind to the SFRE's.**[4]** Some of the actions of estrogens are mediated by the action of estrogen receptors (ERs) on gene expression, while some of the effects of estrogens are so quick-acting that they do not rely on the activation of RNA and protein synthesis. These processes are referred to as nongenomic processes and are thought to be mediated by membrane-associated ERs. The activation of numerous protein-kinase cascades is usually linked to the actions. However, estrogens nongenomic effects may inadvertently affect gene expression by activating signal transduction pathways that subsequently affect target transcription factors. Several transcription factors, including AP-1, have their actions controlled by protein kinase, suggesting that estrogens may operate non-genotypically on these transcription factors. This signalling route, also known as nongenomic-to-genomic signalling, offers a mechanism by which ERs can modify the activities of transcription factors and hence control the expression of genes lacking EREs that is distinct from protein-protein interactions in the nucleus. **[7,8]**

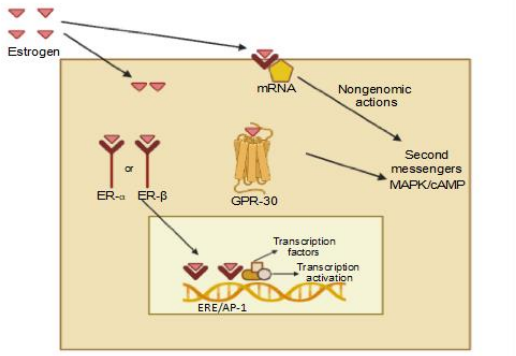


Figure 1: Mechanism of action of Estrogen through genomic and non-genomic pathway.

6. INVOLVEMENT OF HYPOTHALAMUS ON THERMOREGULATION

With the help of feed forward and feedback mechanisms, the hypothalamus serves as a key hub for regulating stable body temperature. The lateral parabrachial nucleus and spinal (or trigeminal) dorsal horn serve as relays in the feed forward pathway, which sends sensory temperature data from the periphery to the preoptic region (POA). Moreover, the POA has temperature-sensitive neurons that are

naturally able to recognise changes in the local temperature of the brain. To control various thermoregulatory responses, this information about peripheral and central temperature is included. The feed-back thermoregulatory network includes the hypothalamic nuclei such as the dorsomedial hypothalamus (DMH), ventromedial nucleus (VMH), arcuate hypothalamus (ARC), and caudal brain regions such as the raphe pallidus (RPa), where various premotor neurons independently control sympathetic activation of brown adipose tissue (BAT) thermogenesis and tail artery vasoconstriction. Each thermoregulatory effector is driven separately by a parallel but different efferent pathway, despite the thermoregulatory network's coordination of both heat production and dissipation. **[2]** Estrogens can affect body temperature by interacting with a variety of thermosensory and thermoregulatory nodes. Estrogen may specifically affect one element of thermal reactions or affect them all. For instance, warm-responsive neurons in the POA express estrogen receptors and exhibit changes in neuronal activity in response to E2. These neurons reduce heat generation and enhance heat removal. Rats' BAT thermogenesis and core body temperature are both elevated by E2 in the VMH. However, E2 can have either inhibitory or excitatory effects on kisspeptin neurons in the ARC or anteroventral periventricular nucleus, respectiely resulting in a difference in how E2 impacts neuronal activity. **[2]**

7. POST MENOPAUSAL EFFECT OF ESTROGEN

Whatever changes a woman goes through before or after her menstruation stops are often referred to as the menopause. The ovaries gradually generate less estrogen as menopause approaches, altering the menstrual cycle and leading to other bodily changes. Hot flashes, night sweats, mood changes, and vaginal changes (dryness and atrophy or thinning of the vaginal walls) are the most typical signs of menopause [10]. Hot flashes are defined as brief periods of shivering, flushing, anxiety, and transitory feelings of heat. Sweating on the face, neck, and chest as well as peripheral vasodilation are the symptoms that are typical of a heat-dissipation response. The most probable reason of hot flush is unknown, although a study has found no difference in the levels of estrogen in women who experience hot flashes and those who do not. Since the symptoms of hot flashes were lessened after estrogen administration, it was assumed that the drop in estrogen levels was the most likely cause of hot flashes. Other elements, such as a drop in serotonin and endorphin levels, as well as the kisspeptin neurokinin dynorphin (KND) signal system, are connected to the reduction in estrogen levels and may also contribute to postmenopausal symptoms. **[1,10]** When estrogen levels decline, serotonin and endorphin levels rise as a result of which there is a rise in the level of norepinephrine and upsets the hypothalamus thermostat. In women, this may cause hot flashes. These symptoms can be managed with medications called selective serotonin reuptake inhibitors, such as Fluoxetine. The selective serotonin reuptake inhibitors (SSRIs) increase the amount of serotonin in the body by inhibiting its uptake, which also aids in regulating body temperature. This can be used to treat hot flashes without using hormones. **[12,13]** The Kisspeptin Neurokinin Dynorphin (KND) signal pathway is similarly indirectly responsible for the development of heat flashes. Norepinephrine is elevated by neurokinin, which also causes the body's temperature to rise. Fezolinetant is an NK3 receptor antagonist that can be used to lower body temperature in women with hot flashes. This is another non-hormonal method of treating the symptoms of menopause**. [1]** Side effects from these non-hormonal

treatments include anxiety, dry mouth, constipation, and blurred vision. While hormone therapies like estrogen alone or in conjunction with progesterone can improve symptoms, they also carry a risk of major adverse effects like breast and endometrial cancer. Despite this, HRTs are regarded as the preferred approach for treating postmenopausal symptoms.

8. HORMONE REPLACEMENT THERAPY

For women who have not undergone hysterectomy, hormone replacement therapy (HRT) in combination of progestogen and estrogen is suggested to treat menopausal symptoms to protect the endometrium. Estrogen can be administered orally, intravaginally, topically, or transdermally as estradiol, estradiol-17, estrone, or conjugated equine estrogen. The progestogen may be administered orally, topically, or intrauterinally. In HRT regimens, progesterone is added either continuously (continuous combined regimen) or sequentially (cyclic regimen) as needed. Tibolone is a synthetic oral steroid that also functions as HRT. It has estrogenic, androgenic, and progestogenic properties. **[13]** For women who have undergone hysterectomy or who do not have an intact uterus, estrogen-only therapy is typically advised. This is true since endometrial cancer is not a possibility. However, estrogen and progesterone are advised for those with an intact uterus**. [19]** They can be given as pills, transdermal patches, sprays, or patches that adhere to the skin. HRT benefits include a reduction in the risk of osteoporosis as well as relief with hot flashes, mood swings, and vaginal dryness. The likelihood of fracture increases after menopause because the bones contain estrogen receptors, which can be reduced by hormone replacement therapy (HRT). **[13]** Increased risk of endometrial cancer, breast cancer, cardiovascular illnesses like stroke, and blood clots are some drawbacks of HRTs. All these adverse effects can occur if HRT is abruptly stopped. The risk of heart disease and stroke is increased by oral combination therapy, whereas patches or gel do not increase the risk of blood clots. **[14]**

9. RECENT ADVANCEMENT OF HORMONE REPLACEMENT THERAPY

Menopause typically starts at the age of 50, whereas the majority of disorders that affect older women start to appear 10 years later. Osteoporosis, cancer, metabolic disorders, and cognitive impairment are some of these illnesses. In the case of cardiovascular diseases, estrogen-based therapy has been found to be cost-effective and especially decrease coronary heart diseases among younger women. This is in addition to symptomatic treatment, improvements in quality of life, and a reduction in osteoporosis. The differences in the initiation age and HRT duration are important. HRT appears to reduce cardiovascular disease in younger women who are close to menopause, but it raises the chance of a coronary event in older women. Although HRT is an established method of treating and preventing osteoporosis, it is not approved as a first-line treatment for the condition. Low and ultra-low estrogen levels have been demonstrated to be more effective than regular dose therapy for the treatment of vasomotor symptoms, vaginal atrophy, and the prevention of bone loss with fewer side effects. Low and ultra-low estrogen levels have been demonstrated to be more effective than regular dose therapy for the treatment of vasomotor symptoms, vaginal atrophy, and the prevention of bone loss with fewer side effects. However, more investigation is required to assess the impact on fractures, as well as breast and cardiovascular disorders. The effects of progestins are significantly distinct from

those of other assays. The testosterone patch demonstrates how testosterone works to enhance both sexual desire and response in both surgically and naturally postmenopausal women. **[13]**

10. LIMITATIONS

In spite of the research and studies going on there are several limitations associated with Hormone Replacement Therapies. Apart from that we do not know the exact reason behind hot flashes. Thought anti-hypertensives can be used to treat flashes but the exact mechanism of action is yet not known. We know that during the menopausal period there is a decrease in the level of estrogen but if this level of estrogen causes such symptoms or there are other factors involved is not known. HRTs are used to overcome the hormonal disbalance occurring in post-menopausal women but there are several side effects associated with it. While administering these therapies care must be taken over the condition of patient. Estrogen only therapy is given to women who has undergone hysterectomy whereas estrogen progesterone combination is given to patients without an intact uterus. Side effects of HRT's appear on long term administration or on sudden discontinuation after a period of five years. There is certain non- hormonal treatment available but they are also associated with certain side effects. In the experiment carried out on long term oral administration of (EGX358) which is a ER-β agonist there was an improvement seen in the tail skin temperature as well as there was an improvement in memory of the mice but it was surprising to see that at a dose of 0.5mg/kg body weight no effect on the anxiety and depression like activity was seen. Further studies can be carried out with higher doses of ER-β agonist to see if anxiety and depression like behavior is improved on administering higher dose. **[11,12]**

11. CONCLUSION

Hypothalamus is a major gland that helps in maintaining the homeostasis of the body. There are several receptors present in different parts of our body that can sense changes in the external environment and send signals to the brain to adapt accordingly. With the help of negative and positive feedback mechanism the hypothalamus executes the commands as it receives from the brain in the form of neural circuits. Sudden decline in the level of estrogen during menopause causes certain symptoms that can be treated with estrogen replacement therapies or estrogen-progesterone replacement therapy. By influencing heat generation and dissipation, estrogen affects body temperature. Many of these actions are mediated, either specifically or concurrently, by various estrogen-sensitive areas. The type of therapy should depend on patient's condition. Long term hormonal therapies should be avoided to minimize risks of breast and endometrial cancer. Most of the risks associated with HRT are related to ER-α and not ER-β **[1]**. Hence in future ER-β should be targeted to avoid the health risks. We should work to understand the neural circuit and identify various potential targets for the development of therapeutics that could mimic the thermoregulatory effects of estrogen and prevent the negative side effects of hormone therapy because we are aware that symptoms like hot flashes can affect our day-to-day lives. **[13]**

12. FUTURE SCOPE

Hormone replacement therapy is considered to be helpful for women with post-menopausal symptoms as well as other ailments associated with HRT including cardiovascular disorders and osteoporosis. However, HRT's can cause a number of side effects if discontinued abruptly or if it is taken for a very long period of time. Numerous non hormonal

therapies like selective serotonin reuptake inhibitors (SSRI) and Estrogen receptor (ER-*β)* agonist are being studied by researchers as they are associated with lesser side effects. Additionally, the exact reason behind hot flash is yet to be explored along with the mechanism behind anti-hypertensives to treat post-menopausal symptoms. Future studies will explore additional issues, such as the potential rise in breast cancer risk over ten or more years of treatment. The creation of safe regimens that do not encourage uterine bleeding and minimize other undesirable side effects may be crucial to the broader acceptance of HRT.

13. ACKNOWLEDGEMENT

The authors are thankful to Central Instrumentation Facility and Birla Institute of Technology Mesra, Ranchi.

14. CONFLICT OF INTEREST

None.

15. REFERENCES

1. *Fleischer, A. W., Schalk, J. C., Wetzel, E. A., Hanson, A. M., Sem, D. S., Donaldson, W. A., & Frick, K. M. (2021). Long-term oral administration of a novel estrogen receptor beta agonist enhances memory and alleviates drug-induced vasodilation in young ovariectomized mice. Hormones and behavior, 126(3),130-136.*
2. *Zhang, Z., DiVittorio, J. R., Joseph, A. M., & Correa, S. M. (2021). The effects of estrogens on neural circuits that control temperature. Endocrinology, 145(2),162 -167*
3. *Van Keep, P. A. (1990). The history and rationale of hormone replacement therapy. Maturitas, 12(3), 163-170.*
4. *Inscoe, J. (2021). Sonic Waves of Grain: Paul Harvey's American Evangelism (Doctoral dissertation, University of Maryland, Baltimore County).*
5. *Shughrue, P. J., Lane, M. V., & Merchenthaler, I. (1997). Comparative distribution of estrogen receptor-α and-β mRNA in the rat central nervous system. Journal of Comparative Neurology, 388(4), 507-525.*
6. *Gg, K. (1997). Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors α and β. Endocrinology, 138, 863-870.*
7. *Turner, R. T., Backup, P., Sherman, P. J., Hill, E., Evans, G. L., & Spelsberg, T. C. (1992). Mechanism of action of estrogen on intramembranous bone formation: regulation of osteoblast differentiation and activity. Endocrinology, 131(2), 883-889.*
8. *Bentrem, D. J., Dardes, R. C., Liu, H., MacGregor-Schafer, J., Zapf, J. W., & Jordan, V. C. (2001). Molecular mechanism of action at estrogen receptor α of a new clinically relevant antiestrogen (GW7604) related to tamoxifen. Endocrinology, 142(2), 838-846.*
9. *Boulant, J. A. (2000). Role of the preoptic-anterior hypothalamus in thermoregulation and fever. Clinical infectious diseases, 31(Supplement_5), S157-S161.*
10. *Rosenberg, L., Armstrong, B., & Jick, H. (1976). Myocardial infarction and estrogen therapy in post-menopausal women. New England Journal of Medicine, 294(23), 1256-1259.*
11. *Gorodeski, G. I. (2002). Update on cardiovascular disease in post-menopausal women. Best practice & research Clinical obstetrics & gynaecology, 16(3), 329-355.*

12. *Barrett-Connor, E., & Grady, D. (1998). Hormone replacement therapy, heart disease, and other considerations. Annual review of public health, 19(1), 55-72.*
13. *Lobo, R. A. (2017). Hormone-replacement therapy: current thinking. Nature Reviews Endocrinology, 13(4), 220-231.*
14. *Barrett-Connor, E., & Grady, D. (1998). Hormone replacement therapy, heart disease, and other considerations. Annual review of public health, 19(1), 55-72.*
15. *Brincat, M. P. (2000). Hormone replacement therapy and the skin. Maturitas, 35(2), 107-117.*
16. *Lobo, R. A., Pickar, J. H., Stevenson, J. C., Mack, W. J., & Hodis, H. N. (2016). Back to the future: hormone replacement therapy as part of a prevention strategy for women at the onset of menopause. Atherosclerosis, 254, 282-290.*
17. *Nilsson, S., Makela, S., Treuter, E., Tujague, M., Thomsen, J., Andersson, G., ... & Gustafsson, J. Å. (2001). Mechanisms of estrogen action. Physiological reviews, 81(4), 1535-1565.*
18. *Nelson, H. D., Humphrey, L. L., Nygren, P., Teutsch, S. M., & Allan, J. D. (2002). Postmenopausal hormone replacement therapy: scientific review. Jama, 288(7), 872-881.*
19. *DiMicco, J. A., & Zaretsky, D. V. (2007). The dorsomedial hypothalamus: a new player in thermoregulation. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 292(1), R47-R63.*
20. *Rosenberg, L., Armstrong, B., & Jick, H. (1976). Myocardial infarction and estrogen therapy in post-menopausal women. New England Journal of Medicine, 294(23), 1256-1259.*
21. *Shughrue, P. J., Lane, M. V., & Merchenthaler, I. (1997). Comparative distribution of estrogen receptor-α and-β mRNA in the rat central nervous system. Journal of Comparative Neurology, 388(4), 507-525.*
22. *DeMAYO, F. J., Zhao, B., Takamoto, N., & Tsai, S. Y. (2002). Mechanisms of action of estrogen and progesterone. Annals of the New York Academy of Sciences, 955(1), 48-59.*

How to Evaluate the Permeation of Drugs Through Skin Using the Diffusion Cell Method

Randa S.H. Mansour*

Faculty of Pharmacy, Philadelphia University (19392), Jordan

*Corresponding Author: rmansour@philadelphia.edu.jo

ABSTRACT

The diffusion cell method stands as a cornerstone in pharmaceutical research, facilitating the investigation of drug permeation across biological membranes. This technique offers a robust platform for evaluating the behavior of formulations, and drugs by providing crucial insights into their characteristics like permeability, absorption kinetics, and bioavailability. At its core, the diffusion cell method simulates the physiological conditions encountered by drugs upon administration, typically employing synthetic or biological membranes as barriers. These barriers or diffusion medium can be extracted through various animal sources but they are not widely used due to high degree of variations. So, in practical work, the synthetic barriers are favorable. This article presents a comprehensive overview of the diffusion cell method, encompassing its principles and experimental setup.

Keywords: Diffusion cell method, Drug Permeability, Transdermal Drug Delivery System

1. INTRODUCTION

Transdermal drug delivery system enables a drug to showcase its pharmacological effects when administered over the intact skin layers [1]. Initially around the year 1965, the concept of “percutaneous absorption” was suggested by Stoughton. Later, based on this theory, a nascent form of transdermal drug delivery system was developed and named, Transderm Scop (Baxter). This formulation was officially approved in 1979 by the Food and Drug Administration and used to prevent nausea and vomiting associated with sea travel [2].

The percutaneous permeation of drugs is evaluated from different aspects by various techniques. Conventionally, permeation studies and physical characterization, such as solubility and partitioning studies are used. Other techniques, including Fourier-Transform infrared spectroscopy and differential scanning calorimetry are introduced, which can help to elucidate the penetration enhancement mechanisms. Very recently, FT-IR micro spectroscopy has also been introduced. Quantitatively, the drug permeation can be measured by the drug flux *in vitro* [3-6], the drug flux (drug absorption) *in vivo* [7-9], or the resulting drug response *in vivo* [10]. This article focuses on one of the most widely used *in vitro* methods, the diffusion cell method.

2. DIFFUSION CELL METHOD

The standard *in vitro* method for measuring drug permeation across the skin is the diffusion cell method. Other employed methods include attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy permeation and tape stripping.

2.1. The setup:

Two basic designs of the diffusion cells are available: the static (nonflowing) vertical or side by side cells and the flow-through (in line) cells [10-12]. A widely used type of vertical static cells is Franz diffusion cell (**Figure 1**) which is composed of a donor and a static receiver compartment between which the skin sample or the diffusion barrier is inserted. The donor compartment contains the drug formulation while the receiver solution, to which the drug is diffused. Samples of the receiver solution are withdrawn at specified time intervals and analysed to measure the amount of the diffused drug. Each withdrawn sample is replaced by a fresh receiver solution to provide sink conditions.

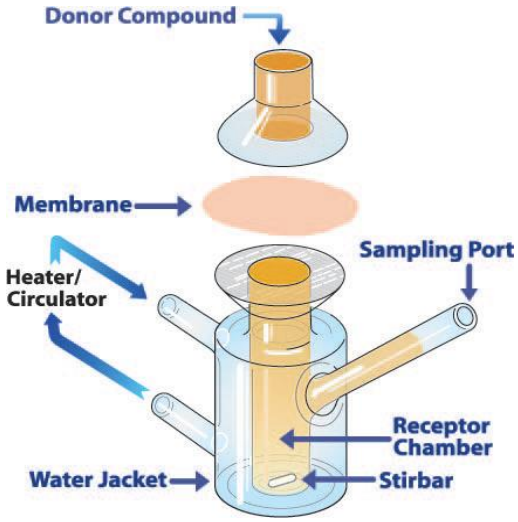


Figure 1: Illustration of Franz diffusion cell (www.permegear.com)

2.2. The diffusion Barrier:

It is essential to find an appropriate medium that will act as the diffusion barrier. Human skin acts as the best medium due to its relevance [13]. Isolated stratum corneum sheets and epidermis with proper thickness may be used for this purpose (**Figure 2**). They hold major advantages because of good mechanical stability. But their high enzymatic metabolic activity limits their use in the diffusion cell [14, 15].

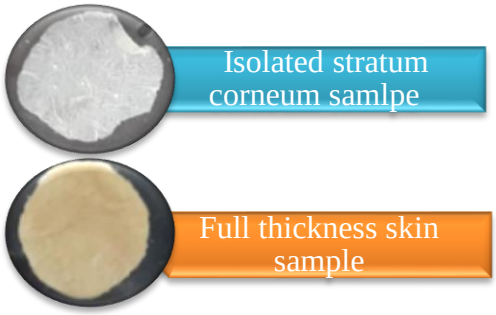


Figure 2: Human isolated stratum corneum sheet and full thickness skin disc.

Another limitation of using human skin as a diffusion medium is low availability. Therefore, alternatives have been designed that is either derived from animal skin or created artificially [16]. The permeation of drugs through these skins are generally higher than that of human skin [17].

Some options of animal skin that can be used as a diffusion medium are derived from pig, mouse, rat, guinea pig, rabbit [18], and monkey. Among these, experiments carried out with pig skin show most relevance in data when compared with human skin which makes it most desirable [19-22]. Mouse, rat and guinea pig skin generally show higher permeation rates than human skin [23-26]. On the other hand, shed snake skin shows comparable or slightly lower permeation rates than human skin [27, 28].

Models based on artificial membranes were introduced in an attempt to overcome the problem of high variability in the permeability of the animal skin due to species variation, age and variations in the anatomical site from where the skin was initially collected [29-31]. The artificial membranes show lower variability which is a big advantage but this system also comes with some disadvantages. The absence of pores in the simple lipid structure membranes is a major disadvantage. Also, when experiments are conducted with artificial membranes of higher complexity, they must be supported with filters [32]. With time, various modifications have been implemented to

eliminate the limitations. These modified membranes have silicones and poly(2-hydroxyethyl methacrylate) embedded within their structure. Such modifications are advantageous as it allows drugs to permeate through lipid layer [33, 34].

2.3. Data treatment and calculation of the diffusion process parameters:

To obtain the rate of drug diffusion, first the different parameters of the diffusion process have to be collected. For that, the amount of drug that successfully reacted the receptor compartment with a certain period of time must be noted. It has to be kept in mind that this value is valid only if the drug passes through an appropriate diffusion medium. From this, if the drug that passes through one unit of surface area in one unit of time is noted, the Steady State Flux (J_{ss}) can be obtained. Furthermore, one can obtain the flux (presented in quantity.cm^{-2}.h^{-1}) by plotting the cumulative diffused amount in the receptor medium per unit surface area of the barrier Q (quantity unit/cm^2) versus time t(h) then obtaining the slope of the linear part of the plot (**Figure 3**). This is in accordance with Fick's first law [35], which states that:

$$J = \frac{D_m K C_v}{L} \quad (1)$$

Where:

J: the rate of drug diffusion per unit surface area of the diffusion barrier (i.e. the slope)

D_m: the diffusion coefficient of the drug in the diffusion barrier.

K: the drug partition coefficient between the diffusion barrier and the vehicle.

C_v: the concentration of the drug in the vehicle.

L: the diffusion path length in the diffusion barrier.

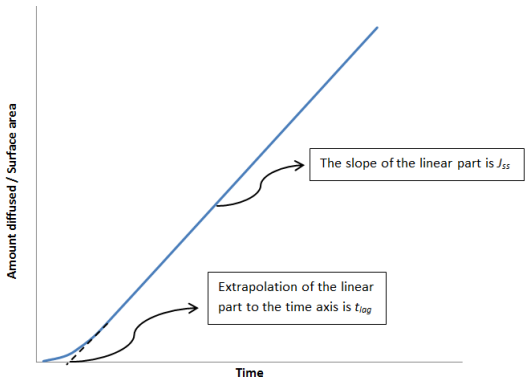


Figure 3: The diffusion plot showing how to obtain J_{ss} and t_{lag}

The lag time t_{lag} is the time needed to establish equilibrium drug concentration within the diffusion barrier. To estimate D_m, t_{lag} is determined from the intercept of the linear part of the plot with the time axis by extrapolation (**Figure 3**). Knowing L, the following equation is used [36]:

$$t_{lag} = \frac{L_2}{6D_m} \quad (2)$$

If D_m is obtained, then K can be easily calculated from Equation 1.

3. REFERENCES

1. *Pathan, I. B., & Setty, C. M. (2009). Chemical penetration enhancers for transdermal drug delivery systems. Tropical Journal of Pharmaceutical Research, 8(2).*
2. *Allen, L., & Ansel, H. C. (2013). Ansel's pharmaceutical dosage forms and drug delivery systems. Lippincott Williams & Wilkins.*
3. *Kim, C. K., Kim, J. J., Chi, S. C., & Shim, C. K. (1993). Effect of fatty acids and urea on the penetration of ketoprofen through rat skin. International journal of pharmaceutics, 99(2-3), 109-118.*
4. *Koyama, Y., Bando, H., Yamashita, F., Takakura, Y., Sezaki, H., & Hashida, M.*

(1994). Comparative analysis of percutaneous absorption enhancement by d-limonene and oleic acid based on a skin diffusion model. Pharmaceutical research, 11, 377-383.

5. *Shah, H. S., Tojo, K., & Chien, Y. W. (1992). Enhancement of in vitro skin permeation of verapamil. Drug development and industrial pharmacy, 18(13), 1461-1476.*
6. *Williams, A. C., & Barry, B. W. (1991). Terpenes and the lipid–protein–partitioning theory of skin penetration enhancement. Pharmaceutical research, 8, 17-24.*
7. *Doucet, O., Hagar, H., & Marty, J. P. (1991). Enhancement of indomethacin percutaneous absorption effect of 2-n-nonyl-1, 3-dioxolane. STP Pharma sciences, 1(1), 89-93.*
8. *Leopold, C. S., & Lippold, B. C. (1995). Enhancing effects of lipophilic vehicles on skin penetration of methyl nicotinate in vivo. Journal of pharmaceutical sciences, 84(2), 195-198.*
9. *Takayama, K. (1991). Terpenes as percutaneous absorption promoters. STP Pharma sciences, 1, 83-88.*
10. *Barry, B. W. (1983). Dermatological formulations: percutaneous absorption.*
11. *Frantz, S. W. (1990). Instrumentation and methodology for in vitro skin diffusion cells in methodology for skin absorption. Methods for skin absorption.*
12. *Tojo, K. (1987). Design and calibration of in vitro permeation apparatus. Transdermal controlled systemic medications.*
13. *Sekkat, N., & Guy, R. H. (2001). Biological models to study skin permeation. Pharmacokinetic optimization in drug research, 155-172.*
14. *Bronaugh, R. L., & Maibach, H. I. (2023). In vitro percutaneous absorption: principles, fundamentals, and applications. CRC Press.*
15. *Tauber, U. (1989). Drug metabolism in the skin: advantages and disadvantages. Transdermal Drug Delivery: Developmental Issues and Research Initiatives ed. NewYork: Marcel Dekker, 99-112.*
16. *Haigh, J. M., & Smith, E. W. (1994). The selection and use of natural and synthetic membranes for in vitro diffusion experiments. European journal of pharmaceutical sciences, 2(5-6), 311-330.*
17. *Williams, A. C., & Barry, B. W. (2012). Penetration enhancers. Advanced drug delivery reviews, 64, 128-137.*
18. *Katz, D., & Touitou, E. (1991). Enhanced skin permeation of prazosin by transcutol-oleic acid mixture. In Proc. Inf. Symp. Controlled Release Bioact. Mater (Vol. 18, pp. 533-534).*
19. *Bhatia, K. S., & Singh, J. (1996). Pig ear skin as a model for predicting percutaneous absorption in man. Pharmacy and Pharmacology Communications, 2(6), 275-276.*
20. *Klain, G. J., Bonner, S. J., & Bell, W. G. (1986). The distribution of selected metabolic processes in the pig and human skin. In Swine in biomedical research (Vol. 1, pp. 667-671). Plenum New York.*
21. *Meyer, W., Schwarz, R., & Neurand, K. (1978). The skin of domestic mammals as a model for the human skin, with special reference to the domestic pig1. In Skin-drug application and evaluation of environmental hazards (Vol. 7, pp. 39-52). Karger Publishers.*
22. *Gray, G. M., & Yardley, H. J. (1975). Lipid compositions of cells isolated from pig, human, and rat epidermis. Journal of lipid research, 16(6), 434-440.*
23. *Dick, I. P., & Scott, R. C. (1992). Pig ear skin as an in-vitro model for human skin permeability. Journal of Pharmacy and*

Pharmacology, 44(8), 640-645.

24. *Pendlington, R. U., Minter, H. J., Sanders, D. J., & Howes, D. (1998). Comparative in vitro percutaneous absorption of 2-hydroxyacids. Perspect Percutan Penetrat A, 6, 107-109.*
25. *Roberts, M. E., & Mueller, K. R. (1990). Comparisons of in vitro nitroglycerin (TNG) flux across Yucatan pig, hairless mouse, and human skins. Pharm Res, 7(6), 673-6.*
26. *Sato, K., Sugibayashi, K., & Morimoto, Y. (1991). Species differences in percutaneous absorption of nicorandil. Journal of pharmaceutical sciences, 80(2), 104-107.*
27. *Itoh, T., Magavi, R., Casady, R. L., Nishihata, T., & Rytting, J. H. (1990a). A method to predict the percutaneous permeability of various compounds: shed snake skin as a model membrane. Pharmaceutical research, 7, 1302-1306.*
28. *Itoh, T., Xia, J., Magavi, R., Nishihata, T., & Rytting, J. H. (1990b). Use of shed snake skin as a model membrane for in vitro percutaneous penetration studies: comparison with human skin. Pharmaceutical research, 7, 1042-1047.*
29. *Guy, R. H. (2010). Transdermal drug delivery. Drug delivery, 399-410.*
30. *Dupuis, D., Rougier, A., Lotte, C., Wilson, D. R., & Maibach, H. I. (1986). In vivo relationship between percutaneous absorption and transepidermal water loss according to anatomic site in man. Journal of the Society of Cosmetic Chemists, 37(5), 351-357.*
31. *Schalla, W., & Schaefer, H. (1982). Mechanism of penetration of drugs into the skin. Dermal and transdermal absorption, 41-59.*
32. *Matsyzaki, K., Imaoka, T., Asano, M., & Miyajima, K. (1993). Development of a model membrane system using stratum corneum lipids for estimation of drug skin permeability. Chemical and pharmaceutical bulletin, 41(3), 575-579.*
33. *Morimoto, Y., Hatanaka, T., Sugibayashi, K., & Omiya, H. (1992). Prediction of skin permeability of drugs: comparison of human and hairless rat skin. Journal of pharmacy and pharmacology, 44(8), 634-639.*
34. *Tomomi, H., Masami, I., Kenji, S., & Yasunori, M. (1992). Prediction of skin permeability of drugs. II. Development of composite membrane as a skin alternative. International journal of pharmaceutics, 79(1-3), 21-28.*
35. *Higuchi, T. (1960). Physical chemical analysis of percutaneous absorption process from creams and ointments. J Soc Cosmet Chem, 11, 85-97.*
36. *Friend, D. R. (1992). In vitro skin permeation techniques. Journal of controlled release, 18(3), 235-248.*

Wound Healing and The Role of SCUBE 1 in Various Pathogenesis

Ayesha Shahid and M.P. Chopra*
Department of Pharmaceutical Sciences and Technology,
Birla Institute of Technology, Mesra, Ranchi-835215, Jharkhand, India.
*Corresponding Author: mpchopra@bitmesra.ac.in

A B S T R A C T

The process of wound healing is a combination of a lot of underlying mechanism that carry out ideal execution of numerous processes, cell types, molecular mediators, and structural changes. Despite all the four basic phases of wound healing, there are many factors that work in sequential manner so as to fasten the process of repair. The cellular and biochemical event corresponds to the various stages of healing such as haemostasis, inflammatory phase, proliferative phase and remodelling where in all these attributes supports continuous restoration of damaged surface. Moreover, when an injury occurs, the process of coagulation goes simultaneously with vasoconstriction involving the activation of platelets and the destruction of fibrinogen. Recent studies implicated that upon activation and stimulation of platelets, a new adhesive surface associated protein SCUBE 1 is expressed in vascular endothelium that play vital roles in many pathogenesis. Based on data findings SCUBE 1 is identified as a prominent biomarker of many thrombosis related conditions including Hypertension, acute coronary syndrome, Diabetes retinopathy and embolism.

Keywords: Biomarker, Endothelium, Platelets, SCUBE 1, Wound Healing.

1. INTRODUCTION

Wound healing is a coordinated dynamic process consisting of sequential events that work in an efficient manner to heal the damaged tissue. The cellular aspects of healing in response to injury maintains the haemostasis promoting prevention of internal chronic damage [1]. Each sequential phase consists of characteristic feature that initiate and stimulates transition from one step to another. Furthermore, it is extensively seen that a delayed or impaired wound healing is associated with some local and systemic factors. Local factors include those criteria that directly influence the nature of wound. It consists of poor oxygenation or hypoxia condition, infections, and venous insufficiency. Systemic factors include all those conditions that have impact on the overall state of physiology and health condition of the patients. It consists of sex, age, diabetes, medication, obesity and intake of alcohol.

Wound healing consists of four basic phases, namely haemostasis, inflammation, proliferation and remodelling [2]. All the phases rely on optimal and accurate functioning of a number of metabolic factors and elements that govern the repair process. An imbalance in any of the molecular mediators results in uncoordinated interaction between different components often resulting in impaired wound healing [3].

2. CELLULAR EVENTS INVOLVED IN WOUND HEALING

2.1. Haemostasis:

Haemostasis is the first stage of wound healing. It is the foremost step after vascular damage that is involved in reducing the blood flow. It serves as a means of preserving the circulatory system and protecting it from damage,

ensuring that the critical organs continue to operate normally despite the wound. The haemostasis maintains its cellular events through a series of coordinated occurrences of vasoconstriction, primary haemostasis and secondary haemostasis [4].

The principal mechanism that begins is vasoconstriction that aims to stop the blood flow. Simultaneously primary haemostasis takes place through formation of platelet plug. The platelet is a crucial component for this process, involving fibrinogen which is a major part of the matrix [5]. This step is further preceded by secondary haemostasis where there in the activation of cellular cascade of releasing clotting factors from platelets and thereby providing a scaffold for potentiating further phase of healing. It results in the transformation of soluble fibrinogen into the insoluble strands that make up the fibrin mesh. Fibrinogen, which is produced by liver hepatocyte cells, is carried throughout the bloodstream. Although fibrinogen is also found in platelets, it is not converted into the fibrin fibres that are essential for the formation of a blood clot [6]. The coagulation cascade is triggered by intrinsic and extrinsic pathways in conjunction with hemostatic events to prevent excessive blood loss. This causes platelets to aggregate and aids in clot formation.

2.1.1. Vasoconstriction

Vascular constrictions occur immediately after damage to stop bleeding from ruptured microvasculature. The vascular smooth muscle carries out this function through the activation of vasoconstrictors such endothelin, which is generated from the damaged endothelium. [7]. Furthermore, vasoconstriction is regulated by circulating catecholamines, epinephrine, norepinephrine, and prostaglandins generated by damaged cells. The platelets secrete a substance called platelet-derived growth factor (PDGF), which selectively activates mesenchymal cells, especially the smooth muscles in artery walls, causing contraction. Blood flow begins when the wound's hypoxia and acidity rise, and the initial reflexive contraction only momentarily stops the bleeding due to the muscle's passive relaxation. In order to maintain the regulation of vasoconstriction and to stop the bleeding over a long period of time a number of mediators such as bradykinin, fibrinopeptide, serotonin, and thromboxane A2 are activated.

2.1.2. Platelet Plug Formation

Platelets, also known as thrombocytes are cells that circulate in blood. Often involved in coagulation cascade, the platelets cells move close to the endothelial cells during the haemostasis phase. Thus, the intact endothelial cell monolayer, shows anti-thrombotic capabilities where it produces nitric oxide, prostacyclin, and negatively charged heparin-like glycosaminoglycans that inhibit platelet activation, adhesion, and aggregation [8][9]. On encountering an injury, the blood vessel gets ruptured. This leads to exposure of subendothelial matrix which along with platelets is found to activate the signalling pathway via G protein coupled reaction. This mediates the release of von Willebrand that is known to adhere the platelets ultimately leading to platelet plug formation [10].

The outside-in signal ling pathway is then activated, which boosts platelet activity and modifies the actin cytoskeleton. Filamentous actin is the most abundant protein in platelets, constituting around 40% of the cellular protein when the cells are in the resting condition and more than 70% when they are active. Actin conformation transforms a platelet's shape from a free-floating disc to a rounded form, and finally into a cell containing lamellipodia and pseudopodia [11] and thereby seals the blood artery by contracting and adhering to the Extra Cellular Matrix (ECM). There are intracellular granules within plasma membrane which secrete many substances needed for haemostasis. Along with many cell surface receptors required for

platelet aggregation namely glycoprotein, integrin which together form platelet plug. This plug present in platelet is often involved in healing that release different growth factors and cytokines [12]. It is seen that the first hour is involved in the release of platelets factors following the release for seven days due to paracrine effects.

2.1.3. Coagulation

The coagulation cascade often referred as secondary haemostasis, is the step involving a series of factors mediating the activation of clotting factors. Coagulation complexes can be formed on platelets and then activated [13]. The intrinsic and extrinsic pathways, both of which are activated by exposure of the subendothelial matrix, are the basic coagulation processes that activate factor X. Prothrombin is transformed into thrombin after factor X is activated by either pathway, and thrombin then cleaves fibrinogen into fibrin. Factor XIII covalently crosslinks fibrin to generate an aggregated platelet plug that eventually forms the thrombus, a permanent secondary hemostasis plug. In the next phases of healing, the thrombus also serves as a temporary wound matrix for the infiltration of more cells. [14].

2.2. Inflammatory phase:

Inflammatory phase is the second phase of wound healing occurring immediately after haemostasis phase [15]. It begins as soon as the wound is healed and transudate—a mixture of water, salt, and protein—leaks from the broken blood vessels. This results in localized edema. In addition to reducing bleeding, inflammation also prevents infection. The migration of healing cells to the wound site is made possible by the fluid engorgement. During the inflammatory phase, bacteria, germs, and damaged cells are removed from the wound area. As a result, the wound immediately activates transcription-independent pathways. They comprise purigenic molecules, gradients of reactive oxygen species (ROS), and Ca^{2+} waves [16]. Within the first few minutes after damage, there is an increase in intracellular Ca^{2+} at the wound borders, which spreads to the wound centre simultaneously with time. Initiating the recruitment of immune cells to the location of the wound occur by cytokines and growth factors, which attract a lot of neutrophils. Grass-like white blood cells called neutrophils phagocytose germs and other objects. They release proteases, reactive oxygen species (ROS), and cytokines including monocyte chemoattractant protein 1(MCP-)1, tumour necrosis factor (TNF)-, and interleukin 1(IL-1) to draw monocytes to the site of the lesion. Over the many phases of growth, neutrophils produce distinctive granules, each of which contains a distinct combination of antimicrobial substances designed to perform a particular function [17].

Proteases are crucial for both antimicrobial activity and the destruction of the ECM and basement membrane, which enables neutrophils to exit blood vessels and penetrate the damaged tissue. During the inflammatory phase, monocytes are drawn to the wound site where they differentiate into macrophages. In addition to their phagocytic function, macrophages also secrete proinflammatory molecules to amplify the inflammatory response. Yet, more importantly, macrophages secrete pro-proliferative molecules, which will start the subsequent stages of recovery.

Moreover, pro-inflammatory macrophages produce matrix metalloprotease MMPs, enabling them to break down thrombus and the ECM to speed up their movement. In addition to being bactericidal, macrophages also carry out efferocytosis, which is vital in removing spent neutrophils within three to four days following injury [18]. Studies report that improper neutrophil clearance often precipitates a prolonged inflammatory state and nonspecific tissue damage. Macrophages secrete a wide range of cytokines and growth factors, such as platelet derived growth factor (PDGF), transferring growth factor (TGF), fibroblast growth factor

(FGF), insulin like growth factor 1 (IGF-1), TNF, IL-1, and IL-6. These soluble mediators can either induce angiogenesis or attract and activate fibroblasts, which produce, deposit, and arrange the new tissue matrix. Many cytokines, such as growth factors involved in the migration, proliferation, and formation of new connective tissue and vascular beds inside the wound, are produced by the macrophage. [19]. The ability of the macrophage to produce and release cytokines over time ensures that the process of tissue repair will continue. A sign that the inflammatory phase is about to end and the proliferative phase is starting is the lack of neutrophils and a decline in the number of macrophages in the wound.

2.3. Proliferative phase:

At the proliferative stage of wound healing, collagen and extracellular matrix-based new tissue is added to the wound site. The wound contracts during the proliferative phase as new tissues are formed [20]. In order for the newly formed connective tissue also known as granulation tissue to be healthy and obtain adequate oxygen and nutrients, a new network of blood vessels also needs to be built. A significant number of fibroblasts, granulocytes, macrophages, blood vessels, and collagen bundles are combined to form granulation tissue, which replaces the temporary wound matrix created during haemostasis and partially restores the structure and function of the injured skin. In response to cytokines and growth factors, including as PDGF, transforming growth factor (TGF), and basic fibroblast growth factor bFGF, produced by platelets and macrophages in the wounds, fibroblasts play a crucial part in the creation of the granulation tissue, migrating mostly from the surrounding dermis to the wound. The fibrocytes (progenitor cells contributing to formation of collagen) move to areas of skin damage and aid in healing not only by providing a portion of the fibroblasts needed to close the wounds but also by releasing cytokines, chemokines, and growth factors, acting as antigen-presenting cells, and promoting angiogenesis [21][22]. Fibroblasts migrate into the provisional wound matrix, where they multiply and form proteinases such as matrix metalloproteinases (MMPs) that break down the provisional matrix. Local microvascular endothelial cells (ECs), which line the inner surface of blood vessels, are activated during angiogenesis. In the granulation tissue, ECM is broken down by activated ECs as they proliferate, move, establish new cell-cell junctions, and branch out to generate new capillaries. In the granulation tissue, ECM is broken down by activated ECs as they proliferate, move, establish new cell-cell junctions, and branch out to generate new capillaries.

Angiogenesis is mostly regulated by a number of additional endothelial cell receptors [23]. Endothelial cells possess glycoprotein receptors including P-selectin and E-selectin in response to injury and also the presence of chemokines in the microenvironment, promotes leukocyte adherence and infiltration into the skin. Intercellular adhesion molecule-1 (ICAM) and vascular cell adhesion molecule-1 (VCAM)-1, which stop leukocyte migration, are also upregulated by endothelial cells [24].

The wound contracts during the proliferation phase, and myofibroblasts play a vital role in this process [25]. When fibroblasts are stimulated, they start to express smooth muscle (SM actin) and develop into myofibroblasts. The elements of the ECM that eventually replace the temporary matrix are created and deposited by these myofibroblastic cells. Due to the production of -SM actin in microfilament bundles or stress fibres, these cells have contractile capabilities and are crucial to the maturation and contraction of the granulation tissue [26].

2.3.1. Re-epithelialization

After cutaneous injury, re-epithelialization is the procedure that restores an unbroken epidermis [27]. Re-epithelialization often entails the

following processes: the proliferating keratinocytes that are used to augment the moving and migrating epithelial tissue and the migration of epidermal keratinocytes from wound margins. Most experts agree that the breakdown of cell-cell and cell-substratum connections is the first step in the process by which keratinocytes complete the goal of re-epithelialization [28]. Following this, basal and a fraction of suprabasilar keratinocytes begin to polarise and migrate over the temporary wound matrix. Then, a portion of keratinocytes that are nearby but outside the wound bed go through mitosis. In order to re-establish the epidermis functioning, the newly generated epidermis gets multi-layered and the process of differentiation-specific gene products takes over the machinary. During morphogenesis two specific multiprotein complexes i.e., Desmosomes (junction of attachment of intercellular epithelial cells) and hemidesmosomes (junction of attachment of basement membrane and epithelial cells) connect keratinocytes to the ECM of the underlying basement membrane and to one another, respectively. These associations need to be broken down after the epidermis is being injured in order to allow keratinocytes to go to the border of the wound and take part in the re-epithelialization procedure. Keratinocyte migration and proliferation are regulated by a number of growth factors and proteins. It is known that the transcription factors Slug and protein kinase C (PKC) reduce the adhesiveness and increase the motility of keratinocytes [29]. Keratinocyte migration is regulated by a variety of variables, including cytokines, chemokines, integrins, growth factors keratins, matrix metalloproteinases (MMPs), and extracellular macromolecules. MMPs are also discovered to be widely expressed by migrating keratinocytes around the borders of wounds, enabling these cells to pass through the fibrin barrier and over the granulation tissue.

2.4. Remodelling:

The last stage of the healing process for wounds is remodelling phase, where granulation tissue production takes place simultaneously. The growth of new epithelium and scar tissue is the main goal of the remodelling phase [30]. The collagen fibres are broken down and realigned by fibroblasts during the remodelling stage. The fibril packing density in each collagen fibre is shown to rise when fibroblasts realign and deposit collagen fibres, resulting in collagen fibres with bigger diameters and superior mechanical properties. The initial, disorganised collagen matrix is changed into a highly ordered collagen matrix whose structure nearly resembles that of the native tissue by realigning collagen fibres. The restored collagen matrix may be able to recapture up to 80% of the tensile strength of the original tissue [31]. The collagen that was put down during proliferation is eventually replaced by a more stable interwoven type III collagen as the wound remodels, which also results in a decrease in the water content of the wound. During wound contraction and the production of scars, the levels of connective tissue and capillaries are decreased. Most blood vessels, fibroblasts, and inflammatory cells vanish from the wound area as a result of apoptosis, emigration processes, or other unidentified cell death mechanisms. A scar with fewer cells is formed. The fibroblasts of the granulation tissue, also known as myofibroblasts, later undergo phenotypic shift and start to momentarily express smooth muscle actin. When the wound heals, many kinds of proteolytic enzymes are produced by cells in the wound bed. These enzymes function to remodel the extracellular matrix proteins at various points in the healing process [33]. Serine proteases and matrix metalloproteinases (MMPs) are two of the most significant groups. Collagen III in granulation tissue gradually decreases and collagen I eventually takes its place [32]. The inhibitors of MMPs known as tissue inhibitors of metalloproteinases, (TIMPs) stops additional

degradation of ECM [34]. Apoptosis causes a decrease in macrophage and fibroblast numbers, and angiogenesis stops as the remodelling phase progresses. The remodelling phase results in the formation of new tissue with a high tensile strength, a small number of cells, and little vascularization. Myofibroblast and fibroblast apoptosis may have a significant role in reducing excessive scarring and speeding up wound healing. Neovessels are pruned during remodelling to produce stable, well-perfused blood vessels that can regain equilibrium and to produce quiescent endothelial cells.

3. SCUBE 1, THE NOVEL PLATELET ADHESION MOLECULE

SCUBE1, or Signal peptide CUB domain (complement protein C1r/C1s, Uegf and Bmp1) and EGF (epidermal growth factor)-like domain containing protein 1, is a member of the SCUBE family, characterized by cell surface glycoproteins with nine EGF-like repeats, a signal peptide at the beginning, and a CUB domain at the end [35,41]. It holds significance in vascular biology, being expressed in endothelial cells and platelets. [36]. The presence of the SCUBE genes in humans, mice, and zebrafish shows that these proteins are evolutionarily conserved and may have a vital biological purpose [37]. Throughout mouse embryogenesis, it has been discovered that SCUBE genes are primarily expressed in a range of developing tissues, including the dermomyotome, the central nervous system, limb buds, the gonads, and the digital mesenchyme.

In inactive platelets SCUBE1 molecules reside within alpha granules but translocate to the platelet surface post thrombin activation, contributing to thrombus formation. SCUBE1 is a new adhesive molecule that mediates platelet-matrix contact and ristocetin-induced platelet agglutination, according to molecular and biochemical investigations. Ristocetin induces platelet agglutination, which is a fluid-phase analogue of platelet-subendothelial matrix adhesion. This is done by altering the structure of von Willebrand factor thereby facilitating the binding of von Willebrand factor to platelet glycoprotein Ib which is a mechanoreceptor involved in platelet adhesion.[39]. An in-vitro study showed that serum proteases cleaved the secretory version of SCUBE1 to release the carboxyl-terminal CUB from the amino-terminal region of the EGF-like repeats. In fact, recent research has shown that the amino-terminal 9 copies of the EGF-like repeats serve as an adhesive module to mediate the contacts between platelets and the platelet matrix as well as amongst platelets. The EGF-like repeats 7-9 are adequate for reciprocal and lateral contacts between SCUBE1 in homophilic adhesions, while the N-terminal repeats play a Ca^{2+}-dependent role in adhesion formation [40]. A conserved configuration of six cysteine residues that create three pairs of disulfide bonds defines the EGF-like motif. A structural component frequently seen in extracellular proteins that mediate protein-protein and protein-carbohydrate binding interactions is the Ca2+-binding EGF-like repeat. Moreover, they play a crucial role in a wide range of functions including cell adhesion, receptor-ligand interactions, cell fate determination, blood coagulation, cholesterol uptake, and extracellular matrix structure maintenance

[36]. The presence of the SCUBE genes in humans, mice, and zebrafish shows that these proteins are evolutionarily conserved and may have a vital biological purpose [37]. Throughout mouse embryogenesis, it has been discovered that SCUBE genes are primarily expressed in a range of developing tissues, including the dermomyotome, the central nervous system, limb buds, the gonads, and the digital mesenchyme.

In inactive platelets, these molecules are kept in alpha granules. They are translocated to the platelet surface after thrombin activates them, and thus are released as tiny, soluble particles, being integrated into thrombus. SCUBE1 is a new adhesive molecule that mediates platelet-matrix

contact and ristocetin-induced platelet agglutination, according to molecular and biochemical investigations. Ristocetin induces platelet agglutination, which is a fluid-phase analogue of platelet-subendothelial matrix adhesion. This is done by altering the structure of von Willebrand factor thereby facilitating the binding of von Willebrand factor to platelet glycoprotein Ib which is a mechanoreceptor involved in platelet adhesion.[39]. An in-vitro study showed that serum proteases cleaved the secretory version of SCUBE1 to release the carboxyl-terminal CUB from the amino-terminal region of the EGF-like repeats. In fact, recent research has shown that the amino-terminal 9 copies of the EGF-like repeats serve as an adhesive module to mediate the contacts between platelets and the platelet matrix as well as amongst platelets. The EGF-like repeats 7-9 are adequate for reciprocal and lateral contacts between SCUBE1 in homophilic adhesions, while the N-terminal repeats play a Ca^{2+}-dependent role in adhesion formation [40]. A conserved configuration of six cysteine residues that create three pairs of disulfide bonds defines the EGF-like motif. A structural component frequently seen in extracellular proteins that mediate protein-protein and protein-carbohydrate binding interactions is the Ca^{2+}-binding EGF-like repeat. Moreover, they play a crucial role in a wide range of functions including cell adhesion, receptor-ligand interactions, cell fate determination, blood coagulation, cholesterol uptake, and extracellular matrix structure maintenance

3.1 SCUBE 1 level in different pathogenesis:

3.1.1. Acute Ischemia and the Role of SCUBE 1

The platelets play a major role in acute coronary syndrome and acute ischemia stroke, where in there is rupture of plaque leading to platelet activation and ultimately leading to formation of thrombus. In acute coronary syndromes and acute ischemic stroke, plasma SCUBE1 levels is markedly increased. In cases of acute thrombotic illness, plasma SCUBE1 may serve as a biomarker of platelet activation. In the same investigation, researchers have found that individuals with unstable angina had higher SCUBE1 levels. Plasma SCUBE1 may also be helpful in differentiating between an acute stroke and a transient ischemic event. Important biomarkers include SCUBE1, which can detect changes in unstable plaques during ischemia. The diagnosis of pulmonary embolism may benefit from using plasma SCUBE1 levels because of its high specificity for the condition. Along with cardiac troponins, plasma SCUBE1 as a biomarker of platelet activation may help to strengthen the diagnosis of Unstable Angina and distinguish it from other acute chest pain causes. Moreover, plasmaSCUBE1 may be helpful for risk classification of individuals with acute large-vessel atherothrombotic stroke as well as for separating acute stroke from transient ischemic attack [42].

3.1.2. Hypertension and the role of SCUBE 1

Hypertension is a disorder that involves a risk factor of atherothrombotic complications where the central role is played by endothelium. It has been observed that soluble CD40L (sCD40L), a member of the tumour necrosis factor family released into the bloodstream by activated platelets, is present at higher levels in patients with hypertension (HT). sCD40L [43] is one of the markers that predicts cardiovascular events (CVEs) in healthy adult populations. Using sCD40L as a platelet activation maker, the researchers discovered an elevated amount in our hypertensive patient group, which is in line with earlier studies. There have been suggestions that this increase could be related to issues with atherosclerotic vascular disease. Researchers hypothesised that higher SCUBE1 in hypertensive people was connected to platelet activation because of the positive connection

between sCD40L and SCUBE1. In hypertensive patients, hyperlipidemia was one of the variables affecting SCUBE1 expression. This has been demonstrated to cause an increase in platelet activation and a propensity for atherothrombotic problems in both in vitro and clinical trials. A link between high SCUBE1 and hyperlipidemia is supported by the positive correlation between Low Density Lipoprotein and SCUBE1 and the association between SCUBE1 and Low-Density Lipoprotein at linear regression analysis A common illness linked to platelet activation is hypertension. Early detection of HT-related thrombotic problems and the development of new antiplatelet medications for use in therapy and prevention will be aided by knowledge of the mechanism of platelet activation and the detection of indicators of that activation [44].

3.1.3. Diabetic retinopathy and the role of SCUBE 1

Significant functions in thrombosis, angiogenesis, and vascular biology are played by SCUBE1 gene expression levels. SCUBE1 levels are also known to be linked to inflammation and angiogenesis. Blocking angiogenesis is vital for the treatment of diabetic retinopathy (DR) [45] since it is a key pathophysiological mechanism. SCUBE1 may be employed as an early diagnostic marker in patients who develop DR because serum SCUBE1 levels were found to be considerably greater in patients who acquired DR compared to those who did not. Researchers have found that, in addition to VEGF, the SCUBE1 molecule may have a role in the pathogenesis of DR. For patients who do not benefit from therapy strategies that reduce VEGF levels, treatment plans that lower SCUBE1 levels or combine medications can be established [46].

3.1.4. Embolism and the role of SCUBE 1

Human platelets were stimulated to take part in platelet concentrating by SCUBE-1, which was mostly expressed on their surface. This led to the development of embolism. According to reports, SCUBE-1 can be employed as a diagnostic for embolic disorders and is a coagulation marker linked to damage to vein endothelial cells. Moreover, venous thrombosis risk is increased by SCUBE-1 gene mutation. The production of SCUBE-1 in megakaryocytes/platelets has been found to contribute to arterial thrombosis in the absence of EC-derived SCUBE-1 and its sticky EGF-like repeats [47]. In vivo thrombus development depends on these repeats for crosslinking and stabilizing platelet aggregation. The potential for SCUBE-1 to identify clinical condition hazards associated with thrombosis may be implied by its involvement in thrombosis. Platelet endothelial aggregation receptor-1 (PEAR-1), a putative receptor for SCUBE-1, has been linked to Pulmonary Embolism in prior research [48]. According to these results, SCUBE-1 may be employed as a potential coagulation-related marker for the evaluation of PE [49].

3.1.5. Crimean-Congo hemorrhagic and the role of SCUBE 1

Crimean-Congo hemorrhagic fever (CCHF) is caused by a virus belonging to the Bunyaviridae family that is spread by ticks [50]. The illness can present with a modest clinical course or a severe profile that includes potentially deadly hemorrhaging. Patients usually report fever, even if severe instances can also exhibit petechiae, ecchymosis, hematemesis, melena, hematuria, gingival bleeding, and vaginal hemorrhage. exhaustion, generalised discomfort, myalgia, nausea, and vomiting. The mononuclear phagocyte system and endothelial cells are the primary targets of the CCHF virus. Massive haemorrhages caused by thrombocytopenia and endothelial dysfunction have an especially high mortality rate. For replenishment of blood and blood products, administering supportive care, and setting up an intensive care condition, haemorrhage prediction is crucial [51]. SCUBE1

levels were assessed using an ELISA kit. The output was given in ng/ml. Human SCUBE1 typically has a minimum detectable dosage of less than 0.16 ng/ml. When patients first arrived at the hospital, their SCUBE1 levels were over 292 ng/ml, and this might predict potential bleeding with 67.7% sensitivity and 80% specificity. Increasing SCUBE1 levels might make it easier to foresee death. SCUBE1 levels above 310 ng/ml in a study reliably predicted mortality with a 71.4% sensitivity and a 72.5% specificity. Elevated SCUBE1 levels may also help with mortality prediction. In order to guarantee intensive support treatment after the first day of hospitalisation, disease prognosis prediction may be crucial. SCUBE1 levels may be a reliable biomarker for CCHF patients who present to the hospital in order to predict their prognosis and degree of condition [52,53].

3.1.6. Acute mesenteric ischemia and the role of SCUBE 1

Acute mesenteric ischemia is a potentially fatal vascular condition that needs to be identified quickly in order to effectively reestablish mesenteric blood flow, stop bowel necrosis, and save the patient's life [54]. The prognosis is based on the particular pathologic findings and depends on the variety of underlying causes. In acute mesenteric ischemia, platelet activity is essential [55]. SCUBE-1 plasma concentration is upregulated in response to platelet stimulation. In the absence of a proper diagnosis and course of treatment, acute mesenteric ischemia is an uncommon emergency situation that has the potential to be lethal. This is because intestinal necrosis frequently develops before a diagnosis is made. Because laboratory results are nonspecific, suspicion of mesenteric embolism is at this time the most crucial. Currently no single biochemical marker has emerged as sufficiently accurate for the early identification of patients with acute mesenteric ischemia. Two hours of acute mesenteric ischemia resulted in a sharp increase in SCUBE-1. Following the second hour of ischemia, this increase accelerated and kept rising. These findings suggest that SCUBE-1 levels could be used to detect acute mesenteric ischemia early [56].

3.1.7. Haemodialysis and the role of SCUBE 1

The thrombotic consequences myocardial infarction (MI) and vascular access thrombosis are common in individuals getting haemodialysis treatment [57]. According to certain research, haemodialysis results in a prothrombotic process and increases platelet activation and the coagulation cascade in blood samples taken after hemodialysis even though heparin has been administered [58]. sCD40L belongs to the class of tumour necrosis factors. sCD40L was initially found on CD4 T cells, but in more current history, it has also been found on activated platelets. The hemodialysis patient population has high levels of sCD40L, which have been linked to cardiovascular disease and death in several studies. Haemodialysis treatment activates platelets, as shown by a post-hemodialysis increase. Despite the lack of an ischemic event, SCUBE1 levels are high in the group of hemodialysis patients pre- and post-hemodialysis, where they are known to be prone to thrombotic problems. SCUBE1 levels may be elevated in this patient group despite the absence of an acute ischemia event for a number of reasons. SCUBE1 levels in this patient group may have enhanced due to thrombocyte activation given that sCD40L levels and SCUBE1 had a positive connection. Chronic inflammation is well-known to exist in the haemodialysis patient population. It's possible that this inflammation led to an increase in SCUBE 1 level [59].

3.1.8. Overt and Subclinical Hyperthyroidism and the role of SCUBE 1

Hyperthyroidism is a condition in which the thyroid gland secretes excess number of thyroid hormones. The presence of increased

triiodothyronine (T3) and/or thyroxine (T4) levels along with low or suppressed thyroid stimulating hormone (TSH) levels is referred to as overt hyperthyroidism (OHyper) [60]. Furthermore, there is convincing evidence that the vascular endothelium is impacted and activated by hyperthyroidism. Increased levels of vascular cell adhesion molecules and activator inhibitor type-1 (which are also linked to enhanced platelet activation) are found to be enhanced in OHyper [61]. It has been found that OHyper is connected to an elevated risk of cardiovascular morbidity and mortality. The elevated incidence of cardiovascular disease (CVD) observed in OHyper patients may be caused by increased platelet plug formation. Low or suppressed TSH with normal T3 and T4 levels indicates subclinical hyperthyroidism (SHyper). Usually, these people have little to no hyperthyroidism symptoms. In addition, patients with SHyper are more likely to get atrial fibrillation (AF), nonfatal Cardio Vascular Disease, and heart failure [62]. Subclinical and overt hyperthyroidism are linked to serious long-term consequences. In the initial stages of hyperthyroidism, SCUBE1 levels may be investigated as a predictor of vascular damage. Elevated SCUBE1 levels are indicative of greater platelet activation and potential endothelial dysfunction, which may increase the risk of atherosclerotic and atherothrombotic consequences in both SHyper and OHyper patients [63].

4. CONCLUSION

Skin injuries have a common natural mechanism for repair and healing despite the variety of their causes. This process is a complex series of physiological events that, when brought on by an injury, are intended to restore and eventually heal the skin. No matter what its cause or how it manifests, a wound weakens the tissue and disturbs the environment in its immediate area. They may have an unintentional or intentional cause, or they may be the outcome of a disease process. The timely and ideal execution of numerous processes, cell types, molecular mediators, and structural components are necessary for successful wound healing. Throughout different stages of the repair, different cells predominate making the healing process coordinated and synchronized.

SCUBE1, new platelet endothelial adhesion molecule, are stored in an inactive form in alpha granules of platelets. According to a recent study, platelets have higher levels of SCUBE1 expression than endothelial cells do. The enhanced level of SCUBE1 itself is an indication of major pathological condition that serves as a biomarker. In summary it is evident that SCUBE 1 has significant biological role in unravelling its increased expression in many pathogeneses. Further many clinical studies are also needed to identify the sensitivity of this novel biomarker.

5. FUTURE SCOPE

In the present review the role of SCUBE 1 is identified as a novel biomarker in different pathogenesis. Plasma SCUBE 1 can be now used as a reliable diagnostic assessment in order to clinically measure the level in varied etiology. The mechanistic role in platelet activation as well as in vascular conditions make it a promising molecule in future clinical research to resolve further complications associated with the diseased conditions. SCUBE1 has to be explored more in order to understand its functions in thrombosis, hemostasis, and the aetiology of cardiovascular disorders. It can be regarded as an innovative effective biomarker to prompt future detailed study in terms infectious diseases that affect platelets and endothelial cells. Still further research findings and clinical implications needs to be explored in order to confirm its sensitivity and specificity.

7. REFERENCES

1. *Doughty, D. B., & Sparks-Defriese, B. (2007). Wound-healing physiology. Acute and Chronic*

Wounds: Current Management Concepts. St Louis, MO: Mosby/Elsevier, 307-336.

2. *Clark, R. A. (1996). Wound repair: overview and general considerations. The molecular and cellular biology of wound repair, 3-50.*
3. *Reinke, J. M., & Sorg, H. (2012). Wound repair and regeneration. European surgical research, 49(1), 35-43.*
4. *Pool, J. G. (1977). Normal hemostatic mechanisms: a review. The American journal of medical technology, 43(8), 776-780.*
5. *Rumbaut, R. E. (2010). Platelet-vessel wall interactions in hemostasis and thrombosis, 2(1), 1-75.*
6. *Tennent, G. A., Brennan, S. O., Stangou, A. J., O'Grady, J., Hawkins, P. N., & Pepys, M. B. (2007). Human plasma fibrinogen is synthesized in the liver. Blood, 109(5), 1971-1974.*
7. *Berk, B. C., Alexander, R. W., Brock, T. A., Gimbrone Jr, M. A., & Webb, R. C. (1986). Vasoconstriction: a new activity for platelet-derived growth factor. Science, 232(4746), 87-90.*
8. *Spaet, T. H., & Zucker, M. B. (1964). Mechanism of platelet plug formation and role of adenosine diphosphate. American Journal of Physiology-Legacy Content, 206(6), 1267-1274.*
9. *Golebiewska, E. M., & Poole, A. W. (2015). Platelet secretion: From haemostasis to wound healing and beyond. Blood reviews, 29(3), 153-162.*
10. *Pradhan, S., Khatlani, T., Nairn, A. C., & Vijayan, K. V. (2017). The heterotrimeric G protein Gβ1 interacts with the catalytic subunit of protein phosphatase 1 and modulates G protein–coupled receptor signaling in platelets. Journal of Biological Chemistry, 292(32), 13133-13142.*
11. *Fox, J. E., & Phillips, D. R. (1981). Inhibition of actin polymerization in blood platelets by cytochalasins. Nature, 292(5824), 650-652.*
12. *Steed, D. L. (1997). The role of growth factors in wound healing. Surgical Clinics of North America, 77(3), 575-586.*
13. *Schenone, M., Furie, B. C., & Furie, B. (2004). The blood coagulation cascade. Current opinion in hematology, 11(4), 272-277.*
14. *Davie, E. W., Fujikawa, K., & Kisiel, W. (1991). The coagulation cascade: initiation, maintenance, and regulation. Biochemistry, 30(43), 10363-10370.*
15. *Szpaderska, A. M., & DiPietro, L. A. (2005). Inflammation in surgical wound healing: friend or foe?. Surgery, 137(5), 571-573.*
16. *Lansdown, A.B. (2002). Calcium: a potential central regulator in wound healing in the skin. Wound repair and regeneration, 10(5), 271-285.*
17. *Stallmeyer, B., Kämpfer, H., Kolb, N., Pfeilschifter, J., & Frank, S. (1999). The function of nitric oxide in wound repair: inhibition of inducible nitric oxide-synthase severely impairs wound reepithelialization. Journal of Investigative Dermatology, 113(6), 1090-1098.*
18. *Su, Y., & Richmond, A. (2015). Chemokine regulation of neutrophil infiltration of skin wounds. Advances in wound care, 4(11), 631-640.*
19. *Diegelmann, R. F., Cohen, I. K., & Kaplan, A. M. (1981). The role of macrophages in wound repair: a review. Plastic and reconstructive surgery, 68(1), 107-113.*
20. *Nissen, N. N., Polverini, P., Koch, A. E., Volin, M. V., Gamelli, R. L., & DiPietro, L. A. (1998). Vascular endothelial growth factor mediates angiogenic activity during the proliferative phase of wound healing. The American journal of pathology, 152(6), 1445.*
21. *Goldman, R. (2004). Growth factors and chronic wound healing: past, present, and future. Advances in skin & wound care, 17(1), 24-35.*
22. *Baum, C. L., & Arpey, C. J. (2005). Normal cutaneous wound healing: clinical correlation with cellular and molecular events. Dermatologic surgery, 31(6), 674-686.*
23. *Eilken, H. M., & Adams, R. H. (2010).*

Dynamics of endothelial cell behavior in sprouting angiogenesis. Current opinion in cell biology, 22(5), 617-625.

24. *Goffin, J. M., Pittet, P., Csucs, G., Lussi, J. W., Meister, J. J., & Hinz, B. (2006). Focal adhesion size controls tension-dependent recruitment of α-smooth muscle actin to stress fibers. The Journal of cell biology, 172(2), 259-268.*
25. *Schultz, G. S., & Wysocki, A. (2009). Interactions between extracellular matrix and growth factors in wound healing. Wound repair and regeneration, 17(2), 153-162.*
26. *Tomasek, J. J., Gabbiani, G., Hinz, B., Chaponnier, C., & Brown, R. A. (2002). Myofibroblasts and mechano-regulation of connective tissue remodelling. Nature reviews Molecular cell biology, 3(5), 349-363.*
27. *Barrientos, S., Stojadinovic, O., Golinko, M. S., Brem, H., & Tomic-Canic, M. (2008). Growth factors and cytokines in wound healing. Wound repair and regeneration, 16(5), 585-601.*
28. *Coulombe, P. A. (1997). Towards a molecular definition of keratinocyte activation after acute injury to stratified epithelia. Biochemical and biophysical research communications, 236(2), 231-238.*
29. *Raja, K. S., Garcia, M. S., & Isseroff, R. R. (2007). Wound re-epithelialization: modulating keratinocyte migration in wound healing. Frontiers in bioscience-landmark, 12(8), 2849-2868.*
30. *Baum, C. L., & Arpey, C. J. (2005). Normal cutaneous wound healing: clinical correlation with cellular and molecular events. Dermatologic surgery, 31(6), 674-686.*
31. *Clark, R. A. (1993). Regulation of fibroplasia in cutaneous wound repair. The American journal of the medical sciences, 306(1), 42-48.*
32. *Reinke, J. M., & Sorg, H. (2012). Wound repair and regeneration. European surgical research, 49(1), 35-43.*
33. *Levenson, S. M., Geever, E. F., Crowley, L. V., Oates III, J. F., Berard, C. W., & Rosen, H. (1965). Healing of rat skin wounds. Annals of surgery, 161(2), 293.*
34. *Visse, R., & Nagase, H. (2003). Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. Circulation research, 92(8), 827-839.*
35. *Yang, R. B., Ng, C. K. D., Wasserman, S. M., Colman, S. D., Shenoy, S., Mehraban, F., ... & Topper, J. N. (2002). Identification of a novel family of cell-surface proteins expressed in human vascular endothelium. Journal of Biological Chemistry, 277(48), 46364-46373.*
36. *Wu, B. T., Su, Y. H., Tsai, M. T., Wasserman, S. M., Topper, J. N., & Yang, R. B. (2004). A novel secreted, cell-surface glycoprotein containing multiple epidermal growth factor-like repeats and one CUB domain is highly expressed in primary osteoblasts and bones. Journal of Biological Chemistry, 279(36), 37485-37490.*
37. *Grimmond, S., Larder, R., Van Hateren, N., Siggers, P., Morse, S., Hacker, T., ... & Greenfield, A. (2001). Expression of a novel mammalian epidermal growth factor-related gene during mouse neural development. Mechanisms of Development, 102(1-2), 209-211.*
38. *Yang, R. B., Ng, C. K. D., Wasserman, S. M., Colman, S. D., Shenoy, S., Mehraban, F., ... & Topper, J. N. (2002). Identification of a novel family of cell-surface proteins expressed in human vascular endothelium. Journal of Biological Chemistry, 277(48), 46364-46373.*
39. *Berndt, M. C., Ward, C. M., Booth, W. J., Castaldi, P. A., Mazurov, A. V., & Andrews, R. K. (1992). Identification of aspartic acid 514 through glutamic acid 542 as a glycoprotein Ib-IX complex receptor recognition sequence in von Willebrand factor. Mechanism of modulation of von Willebrand factor by ristocetin and botrocetin. Biochemistry, 31(45), 11144-11151.*
40. *Tu, C. F., Yan, Y. T., Wu, S. Y., Djoko, B., Tsai, M. T., Cheng, C. J., & Yang, R. B. (2008).*

Domain and functional analysis of a novel platelet-endothelial cell surface protein, SCUBE1. Journal of Biological Chemistry, 283(18), 12478-12488.

41. *Tu, C. F., Su, Y. H., Huang, Y. N., Tsai, M. T., Li, L. T., Chen, Y. L., ... & Yang, R. B. (2006). Localization and characterization of a novel secreted protein SCUBE1 in human platelets. Cardiovascular research, 71(3), 486-495.*
42. *Dai, D. F., Thajeb, P., Tu, C. F., Chiang, F. T., Chen, C. H., Yang, R. B., & Chen, J. J. (2008). Plasma concentration of SCUBE1, a novel platelet protein, is elevated in patients with acute coronary syndrome and ischemic stroke. Journal of the American College of Cardiology, 51(22), 2173-2180.*
43. *Novo, S., Basili, S., Tantillo, R., Falco, A., Davì, V., Novo, G., ... & Davì, G. (2005). Soluble CD40L and cardiovascular risk in asymptomatic low-grade carotid stenosis. Stroke, 36(3), 673-675.*
44. *Ozkan, G., Ulusoy, S., Menteşe, A., Karahan, S. C., & Cansız, M. (2013). New marker of platelet activation, SCUBE1, is elevated in hypertensive patients. American journal of hypertension, 26(6), 748-753.*
45. *Wang, W., & Lo, A. C. (2018). Diabetic retinopathy: pathophysiology and treatments. International journal of molecular sciences, 19(6), 1816.*
46. *Lin, Y. C., Chao, T. Y., Yeh, C. T., Roffler, S. R., Kannagi, R., & Yang, R. B. (2017). Endothelial SCUBE2 interacts with VEGFR2 and regulates VEGF-induced angiogenesis. Arteriosclerosis, thrombosis, and vascular biology, 37(1), 144-155.*
47. *Wu, M. Y., Lin, Y. C., Liao, W. J., Tu, C. F., Chen, M. H., Roffler, S. R., & Yang, R. B. (2014). Inhibition of the plasma SCUBE1, a novel platelet adhesive protein, protects mice against thrombosis. Arteriosclerosis, thrombosis, and vascular biology, 34(7), 1390-1398.*
48. *Chen, Q. H., Lin, D., Zhou, J., & Deng, G. (2016). Role of signal peptide-Cub-Egf domain-containing protein-1 in serum as a predictive biomarker of outcome after severe traumatic brain injury. Clinica Chimica Acta, 456, 63-66.*
49. *Xiao, L., Wang, M., Yang, S., Li, S., Huang, Q., Xu, L., ... & Fu, Y. (2022). The diagnostic potential of plasma SCUBE-1 concentration for pulmonary embolism: A pilot study. The Clinical Respiratory Journal.*
50. *Whitehouse, C. A. (2004). Crimean–Congo hemorrhagic fever. Antiviral research, 64(3), 145-160.*
51. *Ergonul, O., Celikbas, A., Baykam, N., Eren, S., & Dokuzoguz, B. (2006). Analysis of risk-factors among patients with Crimean-Congo haemorrhagic fever virus infection: severity criteria revisited. Clinical microbiology and infection, 12(6), 551-554.*
52. *Gönen, İ. (2011). Clinical and laboratory findings of patients with Crimean-Congo hemorrhagic fever in the emergency department at hospital admission. Journal of Microbiology and Infectious Diseases, 1(01), 1-4.*
53. *Menteşe, A., Yilmaz, G., Sümer, A., Arslan, M., Karahan, S. C., & Köksal, I. (2013). The diagnostic and prognostic significance of SCUBE1 levels in Crimean-Congo hemorrhagic fever. International Journal of Infectious Diseases, 17(11), e1042-e1045.*
54. *Oldenburg, W. A., Lau, L. L., Rodenberg, T. J., Edmonds, H. J., & Burger, C. D. (2004). Acute mesenteric ischemia: a clinical review. Archives of internal medicine, 164(10), 1054-1062.*
55. *Altinyollar, H., Boyabatli, M., & Berberoğlu, U. (2006). D-dimer as a marker for early diagnosis of acute mesenteric ischemia. Thrombosis research, 117(4), 463-467.*
56. *Turkmen, S., Mentese, S., Mentese, A., Sumer, A. U., Saglam, K., Yulug, E., ... & Gunduz, A. (2013). The value of signal peptide-CUB-EGF domain-containing protein 1 and oxidative stress parameters in the diagnosis of acute mesenteric ischemia. Academic Emergency*

Medicine, 20(3), 257-264.

57. *Herzog, C. A., Ma, J. Z., & Collins, A. J. (1998). Poor long-term survival after acute myocardial infarction among patients on long-term dialysis. New England Journal of Medicine, 339(12), 799-805.*
58. *Milburn, J. A., Cassar, K., Ford, I., Fluck, N., & Brittenden, J. (2011). Prothrombotic changes in platelet, endothelial and coagulation function following hemodialysis. The International Journal of Artificial Organs, 34(3), 280-287.*
59. *Ulusoy, S., Ozkan, G., Menteşe, A., Yavuz, A., Karahan, S. C., & Sümer, A. U. (2012). Signal peptide-CUB-EGF domain-containing protein 1 (SCUBE1) level in hemodialysis patients and parameters affecting that level. Clinical biochemistry, 45(16-17), 1444-1449.*
60. *Osman, F., Gammage, M. D., Sheppard, M. C., & Franklyn, J. A. (2002). Cardiac dysrhythmias and thyroid dysfunction-The hidden menace?. The Journal of Clinical Endocrinology & Metabolism, 87(3), 963-967.*
61. *Burggraaf, J., Lalezari, S., Emeis, J. J., Vischer, U. M., De Meyer, P. H. E. M., Pijl, H., & Cohen, A. F. (2001). Endothelial function in patients with hyperthyroidism before and after treatment with propranolol and thiamazol. Thyroid, 11(2), 153-160.*
62. *Cooper, D. S., & Biondi, B. (2012). Subclinical thyroid disease. The Lancet, 379(9821), 1142-1154.*
63. *Erem, C., Civan, N., Coskun, H., Mentese, A., Suleyman, A. K., Altay, D. U., ... & Deger, O. (2016). Signal peptide-CUB-EGF domain-containing protein 1 (SCUBE 1) levels in patients with overt and subclinical hyperthyroidism: effects of treatment. Clinical Endocrinology, 84(6), 919-924.*

Standardization of an Ayurvedic Formulation using Modern Analytical Techniques following ICH Guidelines

Anwesh Bhowmick and Manik Ghosh*
Department of Pharmaceutical Sciences and Technology,
Birla Institute of Technology, Mesra, Ranchi-835215, Jharkhand, India
*Corresponding Author: manik@bitmesra.ac.in

A B S T R A C T

Standardization of any pharmaceutical product (allopathic, herbal or ayurvedic) means confirmation of its identify and determination of its quality and purity. It is conducted by following the guidelines given in International Conference on Harmonization. But due to the absence of any regulatory guidelines for standardization of herbal or ayurvedic formulations, we need to develop an analytical method for standardizing which can be done by analyzing their organoleptic, physical, microscopic and phytochemical properties. Synthetic drugs have clearly defined Active Pharmaceutical Ingredients which directly relate to its therapeutic effectiveness and qualitative assurance ideating about its stability and thus have specific guidelines and regulations that pertain to it. But in case of herbal or ayurvedic drugs, there is no specific Active Pharmaceutical Ingredients as it is a mixture of several phytoconstituents that possess therapeutic efficacy, thus threatening its overall shelf life when affected by any one or even a combination of factors like temperature, light, pH, humidity, CO_2, and O_2.

So, in this short research work we will be discussing about the modern analytical techniques being utilised for standardizing ayurvedic finished formulations, shed light on the progress of our ongoing research in this field, instruments utilized so far for this purpose, provide insights of certain peculiar observations that we have come across until now and also give a brief into some upcoming studies to be conducted.

Keywords: Efficacy, Phytoconstituents, Regulatory, Standardization, Therapeutic.

1. INTRODUCTION

Dating back as far as the early 2nd Century BC, Ayurveda came into existence and immediately plants started being used as a rich source of biologically active molecules with significant efficacy, potency and possessed a huge potential to treat various diseases. Since then, numerous ayurvedic formulations started being commercialized and even in modern times, when their allopathic counterparts are ruling the medical and health sectors because of their impeccable safety and efficacy, there is still a population who believes that their trusty *asava, avaleha, arishta, churna* or *lehya* can treat it all. But without confirming the identity, quality and purity just like any modern allopathic drug, the World Health Organization (WHO) wouldn't let these products be used worldwide. Thus, standardization parameters for crude drug evaluation and their corresponding finished products have been laid down. Moreover, specific regulatory guidelines of various countries have been formed for commercialization of them outside its origin country as well. [1]

Now, to be compliant with those regulatory guidelines, a number of chromatographic analytical techniques in the form of Thin-Layer Chromatography (TLC), High-Performance Thin Layer Chromatography (HPTLC), Ultra-Performance Liquid Chromatography (UPLC), and Gas Chromatography (GC) have been utilized.

Meanwhile spectrophotometric techniques like UV-Visible Spectrophotometry (UV-Vis), Nuclear-Magnetic Resonance Spectroscopy (NMR), Mass Spectrophotometry (MS) and Fourier-Transform Infrared Spectroscopy (FTIR), has also garnered great utility. [1]

Recent innovations have bought about more sensitive techniques of analysis such as Liquid Chromatography-Mass Spectroscopy (LC-MS) and Gas Chromatography-Mass Spectroscopy (GC-MS) have also been commercialized for even better analytical interpretation and documentation.

In this particular project, we've also used few of these techniques like TLC and HPTLC for the quantitative identification of the required phytoconstituents present in our formulation. For instance, in case of *Mehari Churna*, one of the formulations being considered, its main phytoconstituents consist of ascorbic acid, gallic acid and curcumin which were analysed and verified to be present in the main ingredients that collectively form the product, i.e.; amla, jamun and turmeric respectively

2. MATERIALS

The main raw materials and chemicals utilized for the initial study of phytoconstituent detection and identification are dried amla fruit (*Phyllanthus emblica*), dried jamun seeds (*Syzygium cumini*) and turmeric rhizomes (*Curcuma longa*), procured from a local store in Ranchi, who specializes in selling these products. All powdered reference standards like Ascorbic Acid, Gallic Acid and Curcumin were procured online from Yucca Enterprises, Mumbai. The methanolic sample extracts of Amla (Ascorbic Acid), Jamun (Gallic Acid) and Turmeric (Curcumin) were made in-house inside our laboratories with most of the chemical tests utilizing Methanol, Ethanol, Ethyl Acetate, Toluene, Acetic Acid/ Glacial Acetic Acid, Formic Acid, Dichloromethane and Chloroform, all procured from the chemical store of the institute and sourced from Rankem (Avantor Performance Materials India). Glasswares like beaker, volumetric flask, covered TLC chamber, capillary tube, iodine flask, round bottom flask, soxhlet apparatus, condenser, were also procured from the institute chemical store. The silica pre-coated aluminium TLC plates were ordered online on Amazon from a local distributor who specializes in providing plates like these. Originally, they were imported from Germany.

3. METHODOLOGY

3.1 Size Reduction and Size Separation:

The work started with grinding all raw ingredients into small pieces using a large mortar & pestle and then fine grinding them inside a mixer grinder. Post grinding, we undertook sieving for size uniformity by utilizing a size 72 sieve, due to the absence of a size 80 one mentioned in our references. [7, 8]

After size separation we stored the powdered raw materials in separate food grade plastic containers, in a cool and dark place to avoid too much environmental exposure.

3.2. Extraction Process:

Extraction process of all ingredients begun after following necessary referential information, where extraction of ascorbic acid was undertaken by maceration. Meanwhile, curcumin from turmeric and gallic acid from jamun were to be extracted using soxhlation.

After some hit and trials, with other solvent systems, eventually maceration of amla was done using 100 ml of 95% methanol mixed with 25 gm of powdered amla for a period of 7 days at normal room temperature in a 250 ml iodine flask [2, 3, 8]. 25 gm each of jamun and turmeric were subjected to soxhlation at 50-60° C using 200 ml of methanol for a max 48 hours beyond which they were starting to dry with only clean methanol running the siphon tube. [4, 5, 8]

All methanolic extracts were collected in 100 ml beakers, lidded with aluminium foil and kept in a cool, dark and dry place.

3.3. Phytoconstituent Detection & Identification:

Preliminary phytoconstituent detection and identification were conducted using TLC for which both the reference standards and extracts were run in parallel by spotting them in precoated silica alumina plates using capillary tubes [2, 3, 4, 8].

In this case, separate TLCs were performed of Vitamin-C Tablets + Methanolic extract of Amla, Gallic Acid Powder + Methanolic extract of Jamun, Curcumin Powder + Methanolic extract of Turmeric for finding out the suitable solvent system that will be utilized for chromatographic fingerprinting in HPTLC. Additionally, we also ran Vitamin-C tablets along with Methanolic extract of Amla, Gallic Acid Powder as a confirmation to check whether ascorbic acid and gallic acid is present in Amla.

4. RESULTS & DISCUSSIONS

An optimal solvent system was finalised and numerous TLCs were done in the process. For Vitamin-C Tablets + Methanolic extract of Amla TLCs the solvent systems that were selected for Chloroform: Acetic Acid: Ethyl Acetate (5:4:1) and Ethyl Acetate: Formic Acid: Glacial Acetic Acid (5:3:2) **(Figure 1 (I))**

Then we shifted focus to Gallic Acid Powder + Methanolic extract of Jamun TLCs which had various ratios of Toluene, Ethyl Acetate & Formic Acid giving minutely different separation of bands. So, we selected Toluene: Ethyl Acetate: Formic Acid (3:2:1) because it was showing more prominent band separation. **(Figure 1(III))**

For Curcumin Powder + Methanolic extract of Turmeric, the hunt for the ideal solvent system was less cumbersome as we already had a short-list from where we could permutate and combine solvents to crack the one needed. Among them, dichloromethane: methanol (9:1) and chloroform: methanol (9.2:0.8) gave crisp bands. **(Figure 1(II))**

But eventually we finalized Toluene: Ethyl Acetate: Formic Acid: Methanol (4:3:2:1) to be our solvent system as all six samples i.e.; the three references and the three samples showed acceptable band separation even though we had three completely different ones initially due to tailing, and other problems.

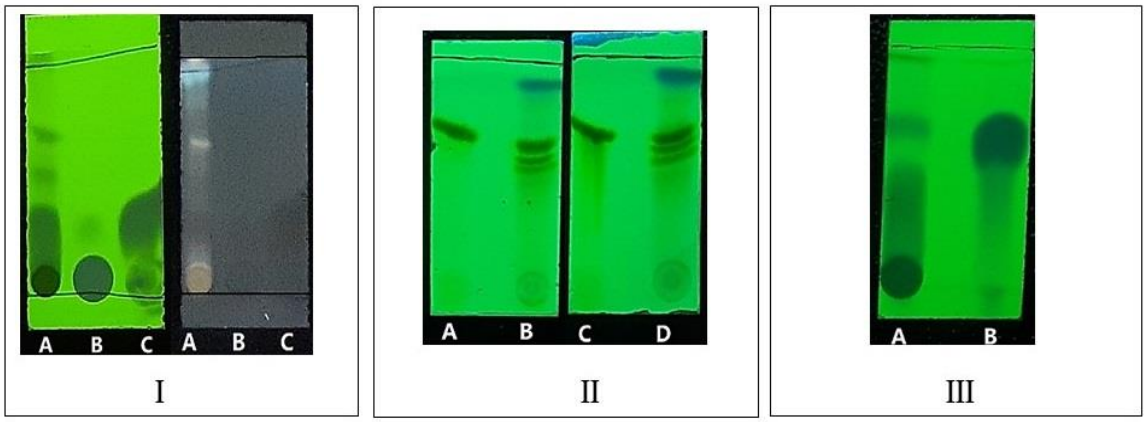


Figure 1: TLC plate visualization of different samples. **I: (A)** Amla extract, **(B)** Vitamin C tablets**, (C)** Gallic acid in the solvent systems Ethyl acetate: Formic acid: Glacial acid::5 :3: 2; **II:** Turmeric acid and curcumin powder in different solvent systems **(A, B)** in Dichloromethane: Methanol::9: 1 and **(C, D)** in Chloroform: Methanol::9.2: 0.8; **III: (A)** Jamun extract, **(B)** Gallic acid in solvent systems Toluene: Ethyl acetate: Formic acid::3: 2: 1.

After method optimization, we started searching for a common solvent system for all 6 samples and then we finalized Toluene: Ethyl Acetate: Formic Acid: Methanol (4:3:2:1) as mentioned before.

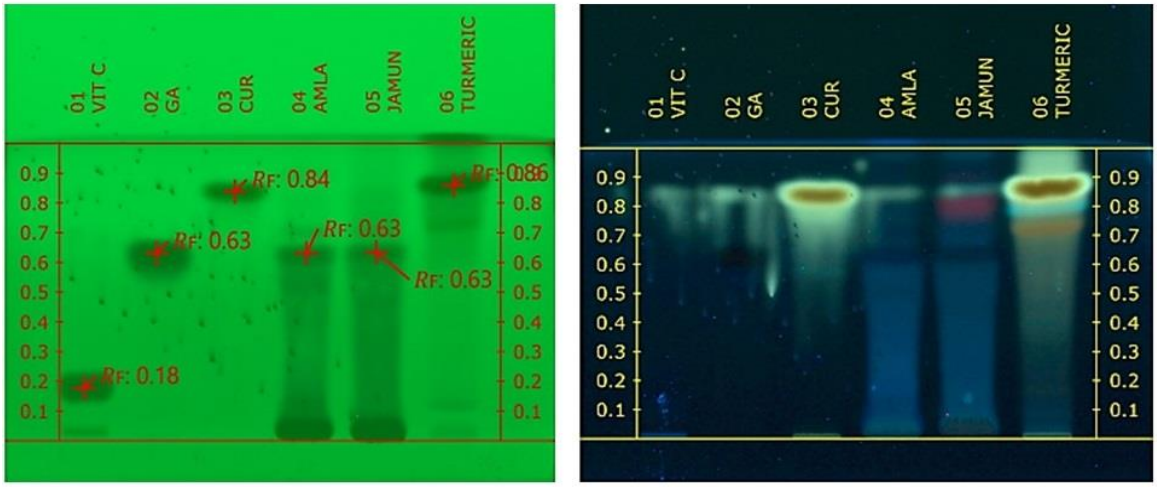


Figure 2: HPTLC studies of the 6 samples (3 references and 3 experimental samples). **I:** Visualization in short wavelength of UV (in 254nm) and **II:** Visualization in long wavelength of UV (in 366nm).

Even though we got acceptable results with respect to understanding the presence of gallic acid in both the amla and jamun extracts; curcumin in turmeric extract. But strangely, ascorbic acid couldn't be found in the amla extract as its Rf value didn't match with any component in the amla extract. This is because they are present in trace amounts and often below the Limit of Detection (LOD). So, we decided to repeat it once again later.

On the flip side, the Rf value of curcumin 0.84 came close to the Rf value of turmeric 0.86 just as said in many references confirming its presence, gallic acid matched exactly with amla and jamun at Rf value of 0.63 confirming its presence in both of them. **(Figure 2)**

Then we found out the lambda maxes during out HPTLC studies of all compounds to quantitatively evaluate the presence of each phytoconstituent and the results were also acceptable as has been represented in Table 1 given below:

Table 1: Comparison of Lamda Max (λ_{max}) values of all reference compounds and their corresponding single compounds

Sl. No.	References	Theoretical λ_{max} (nm)	Samples	Practical λ_{max} (nm)
1	Ascorbic acid (Rf: 0.18)	295 (pH 7.0)	Amla Extract	ND
2	Gallic Acid (Rf: 0.63)	280-283	Jamun Extract (Rf: 0.63)	282
3	Curcumin (Rf: 0.84)	425	Turmeric Extract (Rf: 0.86)	425

($\lambda_{max:}$ Lambda Max; ND: Not detected)

As is visible in Table 1, ascorbic acid's theoretical lambda max doesn't match the lambda max of amla extract received. Gallic Acid Powder's lambda max has a range within which precisely, lambda max of Jamun extract comes in. Curcumin and turmeric's lambda max matches precisely and doesn't leave any doubt about its presence.

In between all of these though other novel unknown compounds were also detected at each of the compound's lambda maxes creating room for further research and discovery.

5. FUTURE SCOPE

Work to be done is now concentrated only to forced-degradation of each compound according to the ICH guidelines. [9] This comprises of the following mentioned below:

- **Thermal Degradation** (thermal stress conditions generated for 40-80°C for 1-2 months)
- **Oxidative Degradation** (0.1–3% of Hydrogen Peroxide is used as a common initiator for oxidative forced degradation studies and is conducted at 40°C for 1–7 days)
- **Photolytic Degradation** (samples are exposed to UV or fluorescent radiations of wavelength 300-800 nm)
- **Hydrolytic Degradation** (samples are treated with 0.1N Hydrochloric Acid or Sulphuric Acid or 0.1N Sodium Hydroxide at 50–60°C and study is conducted for 7 days)

6. ACKNOWLEDGEMENTS

The authors would like to extend their heartfelt gratitude towards the Head of Department (HOD) of Department of Pharmaceutical Sciences & Technology, Central Instrumentation Facility, BIT Mesra, Ranchi for providing all the required support to carry out the review-cum-research work.

7. REFERENCES

1. *Das, C., Ghosh, G., Bose, A., & Das, D. (2019). Analytical methods for standardization of Ayurvedic Asavas and Aristas; A review. Indian Journal of Pharmaceutical Sciences, 81(3), 396-405.*
2. *Raghu, V., Platel, K., & Srinivasan, K. (2007). Comparison of ascorbic acid content of Emblica officinalis fruits*

determined by different analytical methods. Journal of Food Composition and Analysis, 20(6), 529-533.

3. *Chakraborthy, G. S. (2009). Quantitative estimation of ascorbic acid by HPTLC in different varieties of amla. Journal of Young Pharmacists, 1(1), 82.*
4. *Das, A., Bharath, M., Jeevanantham, M., Kumar, S. M., & Bindhu, R. P. (2018). Phytochemical Screening and Antimicrobial activity of Syzygium cumini (Jamun) seed Extract. Research Journal of Pharmacy and Technology, 11(9), 4096-4100.*
5. *Ashraf, K., Mujeeb, M., Ahmad, A., Amir, M., Mallick, M. N., & Sharma, D. (2012). Validated HPTLC analysis method for quantification of variability in content of curcumin in Curcuma longa L (turmeric) collected from different geographical region of India. Asian Pacific Journal of Tropical Biomedicine, 2(2), S584-S588.*
6. *Pawar, H., Karde, M., Mundle, N., Jadhav, P., & Mehra, K. J. M. C. (2014). Phytochemical evaluation and curcumin content determination of turmeric rhizomes collected from Bhandara District of Maharashtra (India). Med. Chem, 4(8), 588-591.*
7. *Abdel-Kader, M. S., Salkini, A. A., Alam, P., Alshahrani, K. A., Foudah, A. I., & Alqarni, M. H. (2022). A High-Performance Thin-Layer Chromatographic Method for the Simultaneous Determination of Curcumin I, Curcumin II and Curcumin III in Curcuma longa and Herbal Formulation. Separations, 9(4), 94.*
8. *The Ayurvedic Pharmacopoeia of India (2018). New Delhi: Ministry of Health and Family Welfare, Department of Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homeopathy. Government of India, 2(2), 45-120.*
9. *International Conference on Harmonization. (2003). Q1A (R2), Stability testing of new drug substances and products. In International Conference on Harmonization. Geneva: International Conference on Harmonization.*

Instructions to Authors

PHARMBIT is an official scientific journal and annual publication of the Pharmaceutical Society of Department of Pharmaceutical Sciences, Birla Institute of Technology, Mesra, Ranchi. The journal is devoted to publishing review and research articles in pharmacy and the related disciplines of pharmaceutical education. PHARMBIT is abstracted in Natural Science Database, ProQuest, USA; Index Copernicus, Poland; Google Scholar, CiteFactor, USA making our publications International.

The articles submitted for publication are subjected to iThenticate - Originality Check & Peer Review before publication. Manuscripts will be subjected to peer review process to determine their suitability for publication provided they fulfill the requirements of the journal. After reviewer's comments, the revised manuscript should be submitted by e-mail in MS Word. There is no publication fee.

Submission of a manuscript to PHARMBIT for publication implies that the same has not been either published or under consideration for publication in another journal. The author should confirm during submission of the manuscript with the help of a cover letter.

PREPARATION OF MANUSCRIPTS

Manuscripts should be concisely written to highlight the findings of the article. Manuscripts should be a MS word document with 1.0 space and a 1.0-inch margin on both sides.

Research Papers should not exceed 8-10 pages (4,000 to 5,000 words) and Review Articles should not exceed 12-15 pages (6,000-7,000 words). Please note that the page limit mentioned before includes references.

All the pages should be numbered consecutively and the matter should be arranged in the following order: Title, Name of authors, their designation along with address, Abstract, Keywords, Introduction, Materials and Methods, Results, Discussion or Results and Discussion, Future scope, Acknowledgements, Conflict of Interest, and References.

More detail regarding the arrangement of the article is present in the Template.

ABSTRACT and KEYWORDS - Abstracts should briefly reflect all aspects of the study and should primarily highlight the novelty of the study. Do not cite any literatures and use abbreviations (except standardized abbreviations) in the abstract. The abstract should not exceed 300 words.

The abstract has to be followed by 4 to 5 keywords related to the topic which has to be arranged in an alphabetical format.

INTRODUCTION – The Introduction should lead the reader to the importance of the study; tie-up published literature with the aims of the study and clearly state the rationale behind the investigation. The hypothesis of the study should also be mentioned in this section of the manuscript.

MATERIALS AND METHODS – This section should discuss in brief about the methodology adapted to gather the data for the study.

Articles focusing on reviews (of any kind), this section should talk about the method adapted to search for the appropriate articles. The search strategy should be mentioned (like the nature of the articles selected, the databases from which the articles were selected, etc.).

RESULTS – All findings presented in tabular or graphical form shall be described in this section. The data should be statistically analyzed and the level of significance stated.

DISCUSSIONS – This section should follow results, deal with the interpretation of results, convey how they help increase current understanding of the problem and should be logical.

The entire section consisting of the different results and the discussion of results can also be combined into one subheading and named "**Results and Discussions.**"

FUTURE SCOPE – This section should clearly state the future scope of the study and how author thinks the research field can develop in the future.

ACKNOWLEDGEMENTS – This section should be given after the text and not in the form of footnotes.

CONFLICT OF INTEREST – The authors should mention if there is any conflict of interest regarding the study. If not then they can write "The authors declare no conflict of interest."

REFERENCES – The **APA style of referencing** is applicable for PHARMBIT. So, the authors should follow the journal recommended format and check the references BEFORE SUBMITTING the article. References should be numbered consecutively in the order in which they are first mentioned in the text (*not in alphabetic order*). Identify references in text, tables, and legends by numerals in third bracket.

SUBMISSION:

Authors are required to submit their manuscript along with a cover letter from the author. The submission has to be by e-mail at the address, pharmbit@bitmesra.ac.in.

Note: The Editor does not claim any responsibility, liability for statements made and opinion expressed by authors.

Dr. Manik Ghosh
Chief Editor, PHARMBIT,
Department of Pharmaceutical Sciences and Technology,
Birla Institute of Technology, Ranchi, Jharkhand.
Website: www.bitmesra.ac.in

- **GIVEN BELOW IS THE TEMPLET OF PHARMBIT**
- **PLEASE DO NOT CHANGE THE FONT, PARAGRAPH OR PAGE SETTINGS**
- **TYPE OR PASTE AFTER THE GIVEN SENTENCE ONLY.**
- **AFTER PASTING, CLICK ON MERGE FORMATTING FROM THE DROP-DOWN LIST.**
- **REFERENCES MUST BE AS PER JOURNAL'S GUIDELINES.**
- **ADD A COVER LETTER FROM THE GUIDE IN A DIFFERENT WORD FILE AND ATTACH IT ALONG WITH THE SUBMISSION.**

Title of the Article

Author Name[1], Author Name[2,*]
1. Email id, Department, and Institute address
2. Email id, Department, and Institute address
*Corresponding Author: xyz@example.com

A B S T R A C T
Type or Paste abstract after this sentence and then delete this sentence. After pasting, click on Merge formatting from the drop down list at the end of the pasted text.

Keywords: Type or Paste key words after this sentence and then delete this sentence. After pasting, click on Merge formatting from the drop down list at the end of the pasted text (arrange in alphabetical order).

1. INTRODUCTION

Type or Paste Indroduction after this sentence and then delete this sentence. After pasting, click on Merge formatting from the drop down list at the end of the pasted text.

Next Paragraph: Type or Paste Introduction after this sentence and then delete this sentence. After pasting, click on Merge formatting from the drop down list at the end of the pasted text.

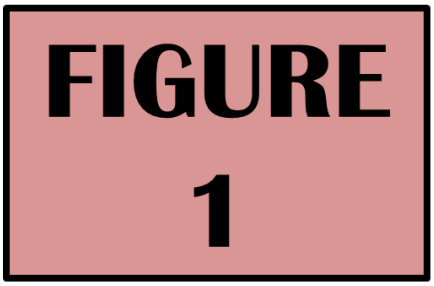


Figure 1: Name of the figure.

2. MATERIALS AND METHOD

Write or Paste after this sentence and then delete this sentence. After pasting, click on Merge formatting from the drop down list at the end of the pasted text.

Next paragraph: Type or Paste Introduction after this sentence and then delete this sentence. After pasting, click on Merge formatting from the drop down list at the end of the pasted text.

2.1. Subheadings:

Type or Paste the content at the end of this sentence and then delete this sentence. After pasting, click on Merge formatting from the drop down list at the end of the pasted text**.**

2.1.1. Smaller sections

Type or Paste the content at the end of this sentence and then delete this sentence. After pasting, click on Merge formatting from the drop down list at the end of the pasted text.

Next Paragraph: Type or Paste the content at the end of this sentence and then delete this sentence. After pasting, click on Merge formatting from the drop down list at the end of the pasted text.

2.1.2. Smaller sections

Type or Paste the content at the end of this sentence and then delete this sentence. After pasting, click on Merge formatting from the drop down list at the end of the pasted text.

Next Paragraph: Type or Paste the content at the end of this sentence and then delete this sentence. After pasting, click on Merge formatting from the drop down list at the end of the pasted text.

2.2. Subheadings:

Type or Paste the content at the end of this sentence and then delete this sentence. After pasting, click on Merge formatting from the drop down list at the end of the pasted text.

Next Paragraph: Type or Paste the content at the end of this sentence and then delete this sentence. After pasting, click on Merge formatting from the drop down list at the end of the pasted text.

3. RESULTS

Write or Paste after this sentence and then delete this sentence. After pasting, click on Merge formatting from the drop down list at the end of the pasted text.

Next paragraph: Type or Paste the content at the end of this sentence and then delete this sentence. After pasting, click on Merge formatting from the drop down list at the end of the pasted text

3.1 Subheadings:

Write or Paste after this sentence and then delete this sentence. After pasting, click on Merge formatting from the drop down list at the end of the pasted text.

Next paragraph: Type or Paste the content at the end of this sentence and then delete this sentence. After pasting, click on Merge formatting from the drop down list at the end of the pasted text**.**

3.2. Subheadings:

Write or Paste after this sentence and then delete this sentence. After pasting, click on Merge formatting from the drop down list at the end of the pasted text.

Next paragraph: Type or Paste the content at the end of this sentence and then delete this sentence. After pasting, click on Merge formatting from the drop down list at the end of the pasted text.

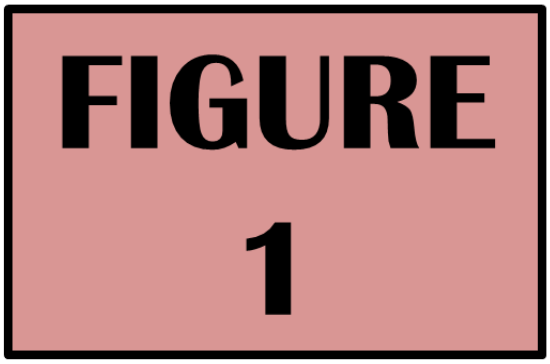


Figure: Name of the figure

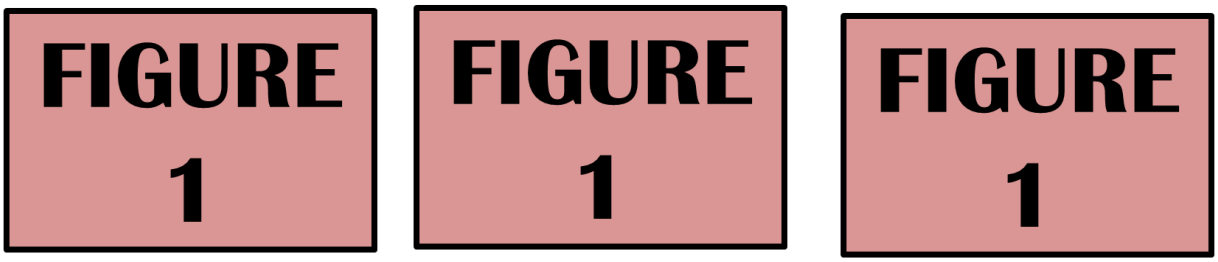


Figure: Name and description of figure

Table 1: Name of Table 1

Data	Data	Data	Data	Data
Data	Data	Data	Data	Data
Data	Data	Data	Data	Data
Data	Data	Data	Data	Data
Data	Data	Data	Data	Data
Data	Data	Data	Data	Data
Data	Data	Data	Data	Data
Data	Data	Data	Data	Data

(Table footnotes)

4. DISCUSSION

Write or Paste after this sentence and then delete this sentence. After pasting, click on Merge formatting from the drop down list at the end of the pasted text.

Next Paragraph: Type or Paste the content at the end of this sentence and then delete this sentence. After pasting, click on Merge formatting from the drop down list at the end of the pasted text.

5. CONCLUSION

Write or Paste after this sentence and then delete this sentence. After pasting, click on Merge formatting from the drop down list at the end of the pasted text.

Next Paragraph: Type or Paste the content at the end of this sentence and then delete this sentence. After pasting, click on Merge formatting from the drop down list at the end of the pasted text.

6. FUTURE SCOPE

Write or Paste after this sentence and then delete this sentence. After pasting, click on Merge formatting from the drop down list at the end of the pasted text.

Next Paragraph: Type or Paste the content at the end of this sentence and then delete this sentence. After pasting, click on Merge formatting from the drop down list at the end of the pasted text.

7. ACKNOWLEDGEMENT

Write or paste the content at the end of this sentence and then delete this sentence. After pasting, click on Merge formatting from the drop down list at the end of the pasted text.

8. CONFLICT OF INTEREST

Write or paste the content at the end of this sentence and then delete this sentence. After pasting, click on Merge formatting from the drop down list at the end of the pasted text.

8. REFERENCES

References should be done in the **APA format**.

1. *Zhang, H.-Z., Zhao, Z.-L., & Zhou, C.-H. (2018). Recent advance in oxazole-*

based medicinal chemistry. European Journal of Medicinal Chemistry, 144, 444–492.

2. *Turchi, I. J. (1981). Oxazole chemistry. A review of recent advances. Industrial & Engineering Chemistry Product Research and Development, 20(1), 32–76.*
3. *Goulart, L., Silva, A.G., Shiampi, K.B., Patrício, K.C.M., Barbosa, M.V.J. and Alves, S.T. (1998) Síndrome de sheehan: Relato de caso. Revista Universitária Alfenas, 4, 63-65.*
4. *Guardia, O.G., Herrera, E.S., Brealey, A.G. and Hao, C.K.C. (2011) Sheehan's syndrome in Costa Rica: Clinical experience with 60 cases. Endocrine Practice, 17, 337-344.*

PHARMBIT is the annual scholarly publication of the Department of Pharmaceutical Sciences and Technology, BIT Mesra, published on behalf of the Pharmaceutical Society.

For more information contact:

The Chief Editor at

pharmbit@bitmesra.ac.in or visit

www.bitmesra.ac.in

http://tinyurl.com/pharmbit

The latest issues of the journal are available online at:

www.bitmesra.ac.in

http://tinyurl.com/pharmbit

BIRLA INSTITUTE OF TECHNOLOGY
MESRA, RANCHI, INDIA

SUBMIT YOUR MANUSCRIPTS TODAY