INTERNATIONAL RESEARCH JOURNAL OF PHARMACY

www.irjponline.com

ISSN 2230 – 8407

Research Article

FORMULATION AND EVALUATION OF TRANSDERMAL PATCH OF PLUMBAGIN FOR ANTI-FUNGAL ACTIVITIES

Aman Sharma [1]*, Abhinav Agarwal [2]

[1] Raj Kumar Goel Institute of Technology (Pharmacy) 5th Km Stone Delhi Meerut Road, Ghaziabad, U.P., India

[2] Dean Academic and Associate professor, Raj Kumar Goel Institute of Technology (Pharmacy) Ghaziabad, U.P., India

*Corresponding Author Email: aman.soti96@gmail.com

Article Received on: 01/02/21 Approved for publication: 27/02/21

DOI: 10.7897/2230-8407.1202122

ABSTRACT

The objective of the current study is to improve the patient compliance and sustained drug release action by herbal medicine which can be achieved by developing alternative drug delivery system. The matrix type transdermal patches containing plumbagin were prepared by solvent evaporation method with different ratios of polymers (HPMC 50cps, PVP K29-32 and EUDRAGIT RS-100). In these matrix type transdermal patches, the PEG (Polyethylene glycol) was used as plasticizer and DMSO (Dimethyl sulfoxide) used as a penetration enhancer. The formulated patches were evaluated for physicochemical parameters like thickness, weight variation, % moisture content, % moisture uptake, % flatness, folding endurance and drug content. *In vitro* drug release studies were carried out by using the Franz diffusion cell. The cumulative % of drug released in 10 hours from the six batch formulations were 95.66%, 94.2%, 97.33%, 90.13%, 83.75% and 85.71%, respectively. On the basis of *in-vitro* drug release, formulation (HE-2) was found to be better than other formulation and these were selected for further evaluation such as anti-fungal activity and stability studies.

Keywords: Transdermal drug delivery system, anti-fungal activity, transdermal patches, formulation and evaluation of patches.

INTRODUCTION

Nowadays the herbal medicine has been a backbone for renovating the human body systems for early stages of human history. Herbal medicine has many beneficial effects such as non-invasive, cost effective, patient compliance, avoid hepatic first pass metabolism and they used in the treatment of many diseases. Herbal medicines have also disadvantage is that the entire process is very slow and it take too much time to act.[1,2] Transdermal drug delivery system is the topically administered medication in the form of patch which is applied on the skin to deliver a specific dose of medication via skin into the bloodstream at a predetermined and controlled rate.[3,4] The first transdermal patch (Scopolamine) was approved by FDA in 1979 for treatment of motion sickness.[5,6] Transdermal route is a potential mode of delivery of lipophilic drugs into the bloodstream. The success of topical delivery depends on the ability of the drug to permeate the skin in adequate quantities to obtain its desired therapeutic effects.[7-9]

Plumbagin (5-Hydroxy-2-methyl-1, 4-naphthoquinone) was obtained from the plant of *"Plumbago Indica"* as a Plumbaginaceae family. *Plumbago indica* is widely used in Ayurveda, Unani, Siddha and Homeopathy medicines. In the Plumbago indica, Plumbagin is the major bioactive compound of roots and it is used to treat many skin diseases.[10, 11]In the present study, the Plumbagin was used for the anti-fungal activities in the form of transdermal patch. Plumbagin is a naphthoquinone in yellow needle shape crystals and it is soluble in alcohol, chloroform, acetone, and benzene, and slightly soluble in hot water. The molecular weight of Plumbagin is 118.18 and melting point is 78ºC. Therefore, Plumbagin is an ideal drug for the transdermal drug delivery and the formulation of plumbagin transdermal patches by using different ratios of polymers (HPMC, PVP and Eudragit RS-100) polymers and PEG-400 as plasticizer and DMSO as enhancer. Evaluation of prepared transdermal patches was done by in-vitro drug release studies by using the Franz diffusion apparatus.

MATERIALS AND METHODS

Plumbagin (5-Hydroxy-2-methyl-1, 4-napthoquinone) was purchased from HiMedia Laboratories Pvt. Ltd, Mumbai. The other chemicals were obtained from authenticated manufacturers like HPMC 50 cps (CDH, New Delhi), PVP K29-32 (ACROS Organics), Eudragit RS-100 (Colorcon Asia Pvt. Ltd), PEG-400 (CDH, New Delhi), DMSO (CDH, New Delhi) and Methanol (Merck).

Characterization of plumbagin

Thin layer chromatography

1mg of Plumbagin is dissolved in chloroform and this solution was applied on Merck Aluminium pre coated plate with silica gel of 0.20mm thickness. The plate was developed using the mobile phase 10ml in different ratio of (Toluene: Ethyl acetate: Methanol) 8: 1: 1 and the plate was dried. This plate shows yellow coloured spot in day light and then sprayed with 10% alcoholic KOH, it shows magenta coloured spot.

Spectral analysis:

The standard plumbagin was spectroscopically analysed for confirmation of its structure. The instrumental spectral analysis such as:

- UV spectroscopy: Maximum absorbance peak was obtained at 518 nm
- IR Spectroscopy: Characteristic peaks appeared at 2967, 1646, 1608 and 752 cm $^{-1}$

Method for preparation of transdermal patches

Matrix type transdermal patches of plumbagin were prepared by the solvent evaporation technique. Weighted quantities of polymers (HPMC, PVP K29-32 and Eudragit RS-100) in different ratios were dissolved in 5ml of methanol. The drug was added in the polymeric solution along with plasticizer (Polyethylene glycol 400) and permeation enhancer (DMSO) and mixed to get a homogeneous mixture. This solution was poured into Petri plate and the evaporation of solvent was controlled by placed over an inverted funnel at the room temperature for 24 hours. After 24 hours the dried films were cut into 2x2cm^2 and kept in a desiccator for further used.

Physical evaluation of prepared medicated patches

1. Physical appearance

All the formulated transdermal films were visually inspected for colour, flexibility, smoothness and clarity.

2. Thickness

The dried films were cut specified area (4cm^2) and the thickness of films was measured at four different points using a digimatic caliper. Finally, the average thickness of film and standard deviation was observed.[12]

3. Weight uniformity

Four films were weighed of each batch individually by the digital weighing balance and the value of average weight and standard deviation was calculated.[13]

4. Percentage moisture uptake

The prepared films were weighed accurately and kept in the desiccators, containing saturated solution of potassium chloride, which maintains 84% relative humidity for 24 hours at room temperature. Next day these films were weighed again and calculated the average value of films.[14] Formula of % moisture absorption is:

% Moisture uptake= [Final Weight–Initial Weight/Initial Weight] x100

5. Percentage moisture content

The prepared films were weighed accurately and kept in the desiccators containing the anhydrous calcium chloride at room temperature for 24hours. Next day the films were reweighed and calculated the average weight of films.[15]

Formula of % moisture content is.

% Moisture content= [Initial Weight–Final Weight/Initial Weight] x100

6. Folding endurance

The specified area (4cm^2) of prepared film was cut equally and repeatedly folded at the same place until it breaks. The number of times of film was folded at the same place without breaking or cracking the film. It was giving the value of folding endurance.[16]

7. Percentage flatness

A transdermal film should not constrict with time and it should possess a smooth surface. For the determination of flatness, the three longitudinal strips were cut from each film, one from the center, one from the right side, and another one from the left side. The length of these three strips was measured and the variation in length due to non-uniformity of flatness was measured by determining percentage flatness, zero percent constriction equivalents to 100% flatness.[17]

% Constriction= [Initial length–Final length/Initial length] x100

8. Drug content

Prepared transdermal films were taken (4cm^2 areas) individually, cut into small pieces and taken in 100ml volumetric flask. These films were crushed and dissolved in 25ml of phosphate buffer pH 7.4. Finally, the solution was filtered and the absorbance the drug was determined UV-VIS spectrophotometer at 518nm against phosphate buffer pH 7.4 as a blank.

***In-Vitro* drug release studies of prepared patches**

In vitro drug release studies were conducted by using Franz diffusion cell (Receptor compartment capacity: 15ml). The excised rat skin as well as dialysis membrane were used to determine the permeation of drug. The dialysis membrane was cut and mounted between the donor and receptor compartment of the diffusion cell and then the prepared transdermal film (4cm^2 areas) was placed on the dialysis membrane. The receptor compartment of diffusion cell was filled with 15ml of phosphate buffer pH 7.4. The whole assembly was kept on a thermostatically controlled magnetic stirrer, and the solution in the receptor compartment was continuously stirred at a constant speed (100 rpm) using a magnetic beads and temperature was maintained at 37 ± 2^{o}C. The sample (5ml) was withdrawn at different time intervals and was replenished with an equal amount (5ml) of phosphate buffer at each sample withdrawal to maintain the membrane condition. Finally, the samples were analyzed by using UV–VIS spectrophotometer at 518nm after suitable dilution and the cumulative % drug release from the transdermal films was calculated.[18]

Anti-Fungal activity of prepared patch

The screening of anti-fungal activity of prepared patch was done by agar diffusion method and the plate was incubated at 37^{o}C for 48 hours. The selected patch containing plumbagin showed better anti-fungal activity when compared to standard amphotericin-b that is shown in Table 6.

Stability Study

The stability studies of selected (HE-2) patch showed that there was no significant change from its initial nature till the period of three months at room temperature; 40±2^{0}C & RH70±5% are shown in table 7.

RESULTS

Thin layer chromatography of plumbagin

The Rf value of plumbagin (0.78) shown in Table 1 and the magenta colour spot shown in figure 1.

Formulation details of prepared patch

The formulation details of prepared transdermal patches are shown in Table 2 and the medicated patches shown in figure 2.

Physical evaluation of prepared transdermal patches

The physical evaluation of transdermal patches is shown in Table 3 such as, weight variation, thickness, % moisture content, % moisture uptake, % flatness, folding endurance and drug content.

In-vitro drug release studies by Franz diffusion cell

The in vitro drug release studies by Franz diffusion cell are shown in Table 4. Cumulative % drug releases of prepared patches in phosphate buffer pH 7.4 are shown in Table 5 and the graph of drug release studies are shown in figure 3.

Table 1: TLC of Plumbagin

Sample	Plumbagin
Mobile Phase	Toluene: Ethyl acetate: Methanol
Ratio	(8**:**1**:**1)
Rf value	0.78
Spray reagent	10% Alcoholic KOH
Spot colour	Magenta colour

Table 2: Formulation details of prepared patch

Formulation -code	Polymer HPMC 50cps (mg)	PVP K 29-32 (mg)	Eudragit RS-100 (mg)	Drug in (mg)	Plasticizer (PEG-400) (ml)	Enhancer (DMSO) (ml)	Solvent Methanol (ml)
HP-1	270	30	-	15	0.1	0.06	5
HP-2	240	60	-	15	0.1	0.06	5
HP-3	210	90	-	15	0.1	0.06	5
HE-1	270	-	30	15	0.1	0.06	5
HE-2	240	-	60	15	0.1	0.06	5
HE-3	210	-	90	15	0.1	0.06	5

Table 3: Evaluation of prepared patches

S No.	Formulation Code	Weight Variation (mg)	Thickness (mm)	% Moisture Content	% Moisture Uptake
1	HP-1	58.2 ± 1.84	0.15 ± 0.016	4.12 ± 0.050	6.52 ± 0.034
2	HP-2	60.5 ± 3.98	0.16 ± 0.014	3.63 ± 0.062	7.12 ± 0.038
3	HP-3	54.4 ± 4.44	0.14 ± 0.018	4.22 ± 0.029	6.61 ± 0.042
4	HE-1	57.3 ± 0.90	0.18 ± 0.021	3.97 ± 0.053	5.35 ± 0.035
5	HE-2	59.5 ± 4.22	0.17 ± 0.025	3.99 ± 0.049	5.27 ± 0.031
6	HE-3	52.6 ± 3.45	0.13 ± 0.018	3.52 ± 0.026	4.93 ± 0.029

Code	Folding Endurance	Flatness %	Drug content (mg/4cm^2 area)
HP-1	>150	100	2.17 ± 0.029
HP-2	>150	100	2.07 ± 0.031
HP-3	>150	100	2.25 ± 0.018
HE-1	>150	100	2.23 ± 0.043
HE-2	>150	100	2.0 ± 0.045
HE-3	>150	100	2.38 ± 0.051

Table 4: In-vitro drug release studies by Franz diffusion cell

Dissolution media	Phosphate buffer 7.4
Membrane	Dialysis
Volume of receptor compartment	15ml
Volume pipette	5ml
Volume replaced	5ml of respective receptor medium
Set rpm	100 rpm
Temperature	37±2ºC
Equation	y = 0.078x
λ_{max}	518nm

Table 5: Cumulative % drug releases of prepared patches in phosphate buffer pH 7.4

Time in (Hr)	HP-1	HP-2	HP-3	HE-1	HE-2	HE-3
0	0	0	0	0	**0**	0
0.5	8.84	9.28	8.53	8.07	**7.76**	7.86
1	17.7	16.66	18.8	17.21	**17.51**	15.12
2	24.88	26.08	25.6	25.56	**25.27**	23.94
3	33.6	33.38	33.33	32.28	**31.97**	32.77
4	42.44	42.6	42.66	40.35	**39.6**	43.04
5	51.3	50.72	52	49.78	**48.73**	48.52
6	60.13	56.52	61.33	57.84	**56.34**	56.47
7	69.12	66.66	70	67.26	**64.41**	66.38
8	79.63	75.36	78.66	75.33	**71.57**	72.68
9	88.62	84.05	87.06	83.4	**77.96**	78.15
10	95.66	94.2	97.33	90.13	**83.75**	85.71

Table 6: Screening of anti-fungal activity of prepared (HE-2) patch

Zone of inhibition in (mm)		
Name of the microorganism	**HE-2 (area 4cm^2 patch) 2000µg/ml**	**Standard amphotericin B 1000µg/ml**
Candida albicans MTCC (183)	22	20

Table 7: Stability study of prepared HE-2 patch

Parameter	**Room temperature**	**40±2^{0}C & RH 70±5%**
Colour	Dark brown	Dark brown
Initial	No change	No change
End of 1st month	No change	No change
End of 2nd month	No change	No change
End of 3rd month	No change	No change
Texture	Smooth	Smooth
Initial	No change	No change
End of 1st month	No change	No change
End of 2nd month	No change	No change
End of 3rd month	No change	No change
Drug content	No change	No change
Initial	**2.0mg**	**2.0mg**
End of 1st month	1.98mg	1.97mg
End of 2nd month	1.96mg	1.94mg
End of 3rd month	1.93mg	1.92mg

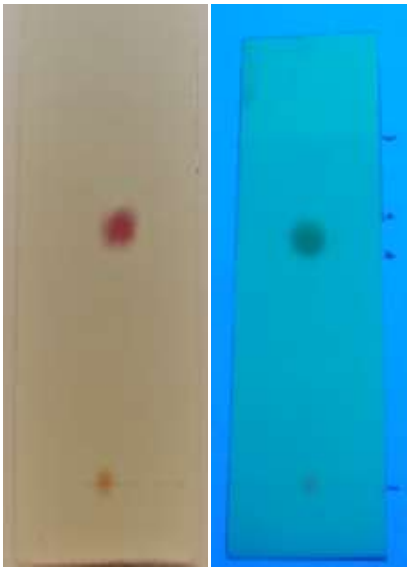

Fig.1: TLC of Plumbagin

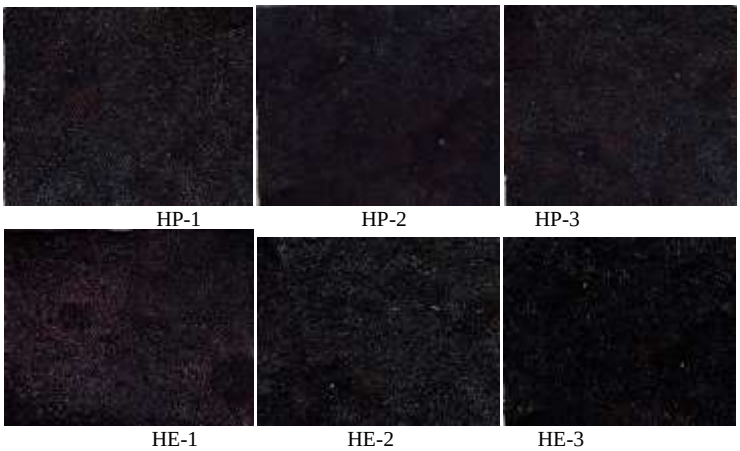

Fig. 2: Medicated films in different ratios of polymers by solvent evaporation technique

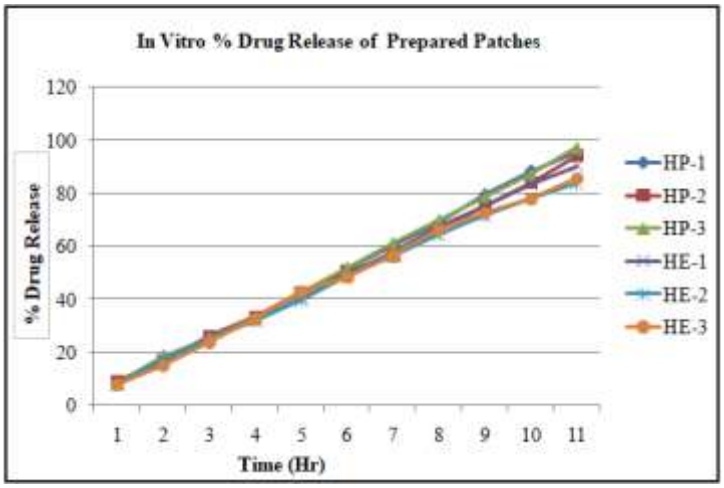

Fig. 3: Graph of in-vitro drug release studies of prepared patches

DISCUSSION

Six batches of (HP-1, HP-2, HP-3, HE-1, HE-2 and HE-3) medicated transdermal patches were prepared by solvent evaporation technique by using the different polymers such as HPMC 50cps, PVP K29-32 and Eudragit RS-100. Dimethyl sulfoxide was used as a penetration enhancer and Polyethylene glycol-400 used as a plasticizer. The prepared medicated patches were smooth, transparent, flexible and dark brown in colour. The thickness of patches was measured at four different points by using a digimatic caliper and it varied between 0.13±0.018mm to 0.18±0.021mm. The films prepared by HPMC/Eudragit RS-100 were relatively more flexible and transparent than HPMC/PVP. Percentage moisture content was found to be between 3.52±0.026 to 4.22±0.029 and % moisture uptake was found to be 4.93±0.029 to 7.12±0.038. The weight of patches was varied between 52.6±3.45mg to 60.5±3.98mg and the folding endurance was found to be >150. The drug content of prepared patches was analyzed by UV spectrophotometer in the range of 400 to 800nm and the maximum absorbance was obtained at 518nm and it varied between 2.00±0.045mg to 2.38±0.051mg.

In vitro drug release studies were carried out by using the Franz diffusion cell and the cumulative % of drug released in 10 hours from the six batch formulations were 95.66%, 94.2%, 97.33%, 90.13%, 83.75% and 85.71%, respectively. From the result of in vitro diffusion and physicochemical studies, formulation HE-2 (83.75%) was best formulation because it given the sustained drug release action. Thus, this patch was selected for further evaluation such as antifungal activity and stability studies. Antifungal activity was done by agar diffusion method and the plate was incubated at 37^{o}C for 48 hours. The results showed that the formulation HE-2 was highly inhibited the fungal growth around the patch when compared to the standard amphotericin-b. The stability study results of selected HE-2 patch, there was no significant change from its initial nature till the period of three months at room temperature, 40±2^{0}C & RH70±5%.

CONCLUSION

In the present work prepared matrix type transdermal patches were smooth, transparent, flexible and dark brown in colour. The medicated patches were prepared by using different ratios of polymers such as HPMC, PVP, and EUDRAGIT RS-100 by the solvent evaporation technique. The polyethylene glycol was used as a plasticizer and dimethyl sulfoxide was used as penetration enhancer. From the above results and discussion, it can be concluded that formulation (HE-2) showed the best result for their physicochemical evaluation and in vitro drug release studies because HE-2 patch given the sustained drug release action. This patch containing plumbagin showed better anti-fungal activity when compared to standard amphotericin-B. Thus, this transdermal patch may be beneficial for fungal infection but detailed preclinical and clinical studies are required to establish the use of plumbagin transdermal patches as anti-fungal formulation.

ACKNOWLEDGEMENT

The authors are thankful to HiMedia Laboratories Pvt. Ltd, Mumbai for providing the drug (Plumbagin) and also thankful to Raj Kumar Goel Institute of Technology, Ghaziabad for providing necessary facilities to carry out this research work with a wholehearted acceptance and encouragement.

REFERENCES

1. Aparna Raj, DK. Bhatt and Bhatt Kiran. Herbal and Medicinal plants of India. 1st ed. Shree publishers, New Delhi, 2007.
2. R. Keerthana Devi, R. Radha, N. Jayshree. Formulation and evaluation of Solasodine transdermal patches for Anti-Inflammatory activity. International Journal of Pharma Research and Review 2014; 3(4): 36-42.
3. Tanwar Himanshi and Sachdeva Ruchika. Transdermal drug delivery system: A review. International Journal of Pharm Sci. and Research 2016; 7(6): 2274-2290.
4. Padmaja Bookya, Raparla Ramakrishna, Sriramula Harikishan and Tarigopula Sunitha. Formulation development and evaluation of clopidogrel bisulfate transdermal patches. International Journal of Pharm Sci. and Research 2018; 9(1): 250-255.
5. Santus GC, Baker RW. Transdermal enhancer patent literature. Journal of Control Release 1993; 25: 1-20.
6. Dipen Patel, Chaudhary Sunita A., Parmar Bhavesh, Bhura Nikunj. Transdermal drug delivery system: a review. The Pharma Innovation 2012; 1(4): 66-75.
7. Upadhyay Gaurav, Verma Shubham, Nayyar Parvez, Kumar Pramod Sharma. Recent Trends in transdermal drug delivery system- a review. Advances in Biological Research 2014; 8(3): 131-138.
8. Richa Sachan and M. Bajpai. Transdermal drug delivery system- a review. International Journal of Research and Development in Pharmacy & Life Sci. 2013; 3(1): 748-765.
9. Kharat Rekha Sudam and Bathe Ritesh Suresh. A Comprehensive Review on: Transdermal drug delivery

systems. International Journal of Biomedical and Advance Research 2016; 7(4): 147-159.
10. Nayana S., Kapadia, Shalini A. Isarani and Mamta B. Shah. A simple method for isolation of Plumbagin from roots of Plumbago rosea. Pharmaceutical Biology. 2005; 43(6): 551-553.
11. Sharma Nisha and Kaushik Purshotam. Medicinal, Biological and Pharmacological aspects of Plumbago zeylanica (Linn.). Journal of Pharmacognosy and Phytochemistry 2014; 3(4): 117-120.
12. Keleb, E., Kumar Rakesh Sharma, Mosa Esmaeil, B., Abdalkadar Z aljahwi. Review on Transdermal Drug Delivery System. Design and Evaluation, International Journal of Advances in Pharmaceutical Sciences 2010; 1(3): 201-211.
13. Yuveraj Singh Tanwar, Chetan Singh Chauhan, Anshu Sharma. Development and Evaluation of Carvidilol Transdermal Patches. Acta Pharm. 2007; 57(2): 151–159.
14. Prabhu Prabhakara, Marina Koland. Preparation and Evaluation of Transdermal Patches of Papaverine Hydrochloride. International Journal of Research and Pharmaceutical Sciences 2010; 1(3): 259-266.
15. Mutalik S. Udupa N. Glibenclamide Transdermal Patches, Physicochemical, Pharmacodynamic and Pharmacokinetic Evaluations. Journal of Pharmaceutical Sciences 2004; 93 (6): 1557-1594.
16. Sankar V, Velrajan G, Palaniappan R and Rajasekar S. Design and Evaluation of Nifedipine Transdermal Patches. Indian Journal of Pharmaceutical Sciences 2003; 65(5): 510-515.
17. Pravin Gavali, Atul, Gaikwad, Radhika P.R, Sivakumar T. Design and Development of Hydroxypropyl Methylcellulose based polymeric film of Enalapril Maleate. International Journal of Pharmtech Research 2010; 2(1): 274-282.
18. Basavaraj K, Nanjwade, Kiran Suryadevara, Kella M.R and Sai Susmitha. Formulation and evaluation of transdermal patches of Ondansetron hydrochloride using various polymers in different ratios. Current Trends in Biotechnology and Pharmacy 2010; 4 (4): 917-921.

Cite this article as:

Aman Sharma and Abhinav Agarwal. Formulation and evaluation of transdermal patch of plumbagin for anti-fungal activities. Int. Res. J. Pharm. 2021;12(2):23-28.
http://dx.doi.org/10.7897/2230-8407.1202122

Source of support: Nil, Conflict of interest: None Declared

Disclaimer: IRJP is solely owned by Moksha Publishing House - A non-profit publishing house, dedicated to publishing quality research, while every effort has been taken to verify the accuracy of the content published in our Journal. IRJP cannot accept any responsibility or liability for the site content and articles published. The views expressed in articles by our contributing authors are not necessarily those of IRJP editor or editorial board members.