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THE EFFICACY AND SAFETY ASSESSMENT OF TOPICAL CONTAINING EXTRACT OF *PIPER NIGRAM* AND *DICTAMNUS DASYCARPU TURCZ* FOR VITILIGO IN VIVO AND VITRO

Dr. R. Rachana Devendra*, Dr. Sheelpriya R. Walde and Dr. Ramteke Devendra S.

*Associate Professor, Dept. of Kaumarbhritya, Shri Ayurved Mahavidyalaya, Nagpur.
Professor, Dept. of Quality Assurance, Gurunanak College of Pharmacy, Nagpur.
Professor, G. H. Raisoni Institute of Business Management, Nagpur.

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***Corresponding Author**
Dr. R. Rachana Devendra
Associate Professor, Dept of Kaumarbhritya, Shri Ayurved Mahavidyalaya, Nagpur.

ABSTRACT

Vitiligo is the depigmentation disorder and recently it is also identified as a autoimmune disorder, in which antigens produced by melanocytes(including those released from damaged melanocytes) can be recognized by antigen-specific immune effecter cell including cytotoxic T-cell(CD8+), T-Helper cells(CD4+) and β-cells. The piperine from piper nigram linn. has shown a strong repigmentation capacity. The three phenolic glycoside from Dictamnus dasycarpu turz, has already been reported for it selectively show remarkable activity of inhibiting the proliferation of T-cells(CD8+). Both the drugs were separately use for the treatment of vitiligo, but never in a combination. The isolated phytoconstituents were formulated into o/w, PEG1000 and Bees wax type of cream and all the evaluation of cream were carried out; such as colour, pH, and viscosity, skin irritation in vitro and in vivo studies. All the evaluation parameters are found within limits and result found satisfactory. Formulation containing, combination of both the extracts have shown to be non irritant during draize skin irritation test and the drug release profile, in-vitro study shows the satisfactory result.

KEYWORD: Vitiligo, piper nigram Linn, Dictamnus dasycarpus turz, Topical cream.

INTRODUCTION

Vitiligo is an autoimmune disease. It is an acquired skin depigmenting disorder characterized by the loss of of melanocytes, from basal layer of the epidermal and the matrix portion of the hair bulb. Vitiligo is defined clinically by expanding areas of well circumscribed, milky

white, cutaneous macules on the skin surface due to the destruction or inactivation of epidermal melanocytes.[1] It's also affects the psychology and social status of the patient.[2-5] Vitiligo is classified as segmental & non-segmental vitiligo[6-8], further non segmental includes generalised, universal, focal, acrofacial and mucosal vitiligo. White patches are more common in area where the skin is exposed to the sun. People with vitiligo often have hair that turns gray early. Those with dark skin may notice loss of colour inside their mouths.[1]

The prevalence of vitiligo is likely less than 1% but varies based on region, females usually acquires the disease earliar than males.[9] Studies found that the prevalence in China, India and Denmark have 0.93%, 0.05% and 0.38% respectively.[10-12] In India, Gujrat is considered to have the highest prevalence in the world, at about 8.8%.[13] Men & women are equally affected[12,14], but women are more likely to seek treatment.[15,16] The mean age of onset is earlier in those with a positive family history[17,18], which ranges from 7.7% to more than 50%.[17,16,19-23] Vitiligo is generally more prevalent in significantly more prevalent in young women (30 years of age) than young men.[24,12,25,26]

Autoimmune hypothesis of melanocyte destruction is further appreciated by the current clinical practice of vitiligo managment; all the nonsurgical vitiligo treatment with proven efficacy is based on immunosuppression. Thus future investigations in vitiligo etiology would focus on finding the exact condition that trigger and sustain this melanocyte –specific autoimmune responce.[27]

Women are more severely affected with respect to quality of life, being more likely to be depressed about their appearence and more likely to internalize stigmatization and attribute an internal cause. Psycological effects appear to be more prominent when visible body areas are affected, psycological intervention should be offered as a way of improving coping mechanisms in patients with vitiligo.

There are several treatment options available to vitiligo patients. Most treatments are intended to restore pigment to the skin. Treatment options generally fall into several categories[12]: UVB phototherapy[6], PUVA phototherapy[6], transplanting melanocytes, Dapsone & vitamin B6, Tacrolimus, Cosmetics, Topical corticosteroids & Depigmentation.[28] Current treatment which use of photosensitisers (eg. psorlens) with UVA

radiation (PUVA), corticosteroids or skin grafting have low sucess rates are generally accompaneid by unpleasent side effects.

Certain plant remedies, usually administered as mixtures of hearbs or extracts, particularly those used in traditional Chinese medicine and Indian Ayurvedic medicine, have been employed for the treatment of vitiligo for a long time and in many cases have given positive result in small scale studies. Herbs like Psoralea corylifolia L and Vermonia anthelmintica wild. Centratherum anthelminticum kuntze are well known for their use in this disease. Psoralens, which are employed in modern PUVA and khellin in KUVA therapy, were originally derived from plant sources (Psoralea corylifolia L and Ammi visnaga respectively) used in traditional remedies for vitiligo. However these therapies rely on the use of UV irradiation for their efficacy, which is associated with the etiology of skin cancer.[29]

Studies shows that use of extract piperine in vitiligo by reducing the effect of UV radiation and also in avoiding side effects. Topical formulation was found to be stable throughout the shelf life[30], and induced marked pigmentation responce with clinically better result than UVR alone.[31] Root bark Extract of Dictamus dasycarpus Turcz is widely used in China, Japan and Korea against eczema, pruritis & urticaria which is also demonstrated anti-allergic and anti-inflammatory effect.[32] Thus this efforts was taken to study the efficacy and safety of topical cream containing extract of *Piper nigrum* and *Dictamnus dasycarpu turcz* for vitiligo.

MATERIALS AND METHODS

Collection and Authentification of drug

Black pepper were collected from the local market and authenticated. The authenticated sample of dried root bark of Dictamnus dasycarus turcz was procured from U.S which is indigenous to South Korea.

The piperine & dictamnus dasycarus turcz was extracted[32] by Soxhlation method[30,33] by using 95% ethanol and reflux method 60% aqueous acetone as a solvent respectively and identified[34] by Dragendroff’s, Hager’s, Mayer’s & Wagner’s reagent test; TLC[35,36], Infrared spectromeroscopy[30,37], UV spectral analysis.[30]

Formulation of cream[38]

1) The o/w cream was prepared from piperine and dictamnus dasycarpus turcz extract.

Sr. No.	Material Name	Scale
1.	Piperine	1%
2.	dictamnus dasycarpus turcz	12%
3.	Stearic acid	60mg
4.	White petrolatum jelly	145mg
5.	Mineral oil	116mg
6.	Lanolin	10mg
7.	Cetearyl alcohol	20mg
8.	Distilled water	q.s
9.	Triethanolamine	14 mg
10.	Perfume	q.s

2) The piperine and dictamnus dasycarpus turcz extract was formulated by using PEG1000 and Bees wax as a creame base.

Sr. No.	Material Name	Scale
1.	Piperine	1%
2.	dictamnus dasycarpus turcz	12%
3.	PEG1000 : Bees wax	1:1 (gm)

Evaluation of Cream: Evaluation of cream was done by physical evaluation method (after feel, type of smear, removal)[39,40,41], pH[42], Homogeneity[41], Viscosity[39], Irritancy test[43], Drug content uniformity[44], and Drug release.[30]

Study of Irritancy: The experiment was carried out using six adult male rabbits weighing about 1.5 to 2.5 kg to test for the skin irritation. Test animal were kept in a limited access rodent facility with enviromental condition at temperature of $25\pm2\ ^{0}$ c, a humidity of 60-90% RH and 12 hr light/12hrs dark cycle. Animals were provided ad-labium access to commercial rabbit diet and drinking water.

The area of the back of each rabbit was shaved prior to experiment and divided into two marked areas. The one marked area of respective animal was used for the topical application of developed cream, while remaining area was considered as blank sample for testing skin irritation. The test cream was applied to the shaved area of approximately $6cm^2$ of skin. Treated site were covered by gauze and the back of animal was wrapped by non occlusive bandage. The animals were then returned to their cages. After 24 hrs the bandage and test material were removed and 1 hrs later the sites were examined for skin irritation. Observation of the sites was done at 24 hrs after application and repeated at 48 hrs and 72 hrs thereafter.

The reaction, defined as erythema and edema were evaluated according to the scoring system of skin reaction. Control marked area on animals were prepared in the same manner and 0.5 gm of cream without drug were applied and observation were made similar to the test animals. The score of primary irritation (SPI) was calculated for each rabbit as the following. Scoring for erythema and edema at 24, 48, and 72 hrs were summed and divided by the number of the observation for the treated sites. The SPI for the control sites were calculated in the same manner.

SPI for each rabbit = erythema and edema grade at 24,48, and 72 hrs

Number of observation

Reaction	Score
Erythema	
No erythema	0
Very slight erythema	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema(beet redness) to eschar formation	4
Edema	
No edema	0
Very slight edema	1
Well defined edema(edges of the area well defined by define raising)	2
Moderate edema(raising approx. 1 mm)	3
Severe edema (raised than 1mm and extended beyond the area of exposure)	4
Total possible score for primary irritation	8

The difference between the summation and the SPI scores of 6 animals from the treated site and the control site were calculated and used for primary Irritation Index (PII) determination. The PII was calculated as the arithmetical mean of the SPI values of the six rabbits. The irritation degree was categorized as negligible, or slight, moderate or severe irritation based on the PII.

Category	Primary Irritation Index
Negligible	0-0.4
Slight irritation	0.5- 1.9
Moderate irritation	2- 4.9
Severe irritation	5- 8

PII = SPI(test) – SPI(base) / number of animal

Study of drug release: The in-vitro diffusion studies of the cream were performed by using dialysis membrane (Sigma Inc. MO, USA; dry, unwashed, pre-cut and open-ended; fiat

width: 35mm; inflated diameter, 21mm; length: 30mm). The membrane soaked in phosphate buffer pH 6.8 for 6-8hrs was clamped carefully to one end of the hollow glass tube of dialysis cell (2.3cm diameter, 4.16cm^2 area). As receptor compartment for the study 25ml of phosphate buffer was taken in a beaker, which was used as receptor compartment for the study. 1 gm of each formulation was spread uniformly on the membrane. The donor compartment was kept in contact with the receptor compartment and the temperature was maintained at 37±0.5^{0}c. the solution on the receptor side were stirred by externally driven Teflon-coated magnetic bars. At pre determined time intervals, 1ml of solution from the receptor compartment was pipette out and immediately replaced with 1ml phosphate buffer solution. The drug concentration of the receptor fluid was determined spectrophotometrically at 203nm and 340nm against appropriate blank. The amount of drug permeation of all the formulation was calculated.

OBSERVATIONS AND RESULT

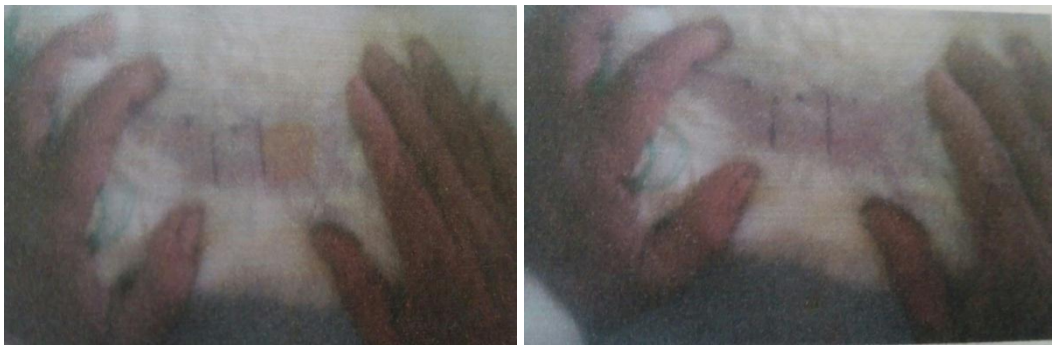
Table No. 1: Physical evaluation of formulation.

Sr. No.	Parameters	F-1	F -2
1.	After feel	Smooth	Smooth
2.	Color	Brown	Yellow
3.	Homogeneity	Good	Good
4.	Type of smear	Non greasy	Non greasy
5.	Removal	Easy	Sticky
6.	pH	7.26*	6.86*

(* the values are expressed in average of three determination)

Table No. 2: Skin irritation test.

Sr. No.	Group	Animal	Score	Interpretation Erythema	Interpretation Edema
1.	Control	Rabbit	0	Nil	Nil
2.	Blank	Rabbit	0	Nil	Nil
3.	Test	Rabbit	0	Nil	Nil



(a) Application **(b) After 24 hrs**

(c) After 48 hrs **(d) After 72 hrs**

Figure No. 1: Skin Irritation Test.

Table No. 3: Drug Content.

Sr. No.	Formulation	%Drug content	% Drug Content
		Release of piperine (340.5nm)	Release of dictamnus dasycarpus turcz (203nm)
1.	F1(340.5nm)	91.5366+ 1.37	77.7533±1.21
2.	F2 (203nm)	86.1233±0.87	65.86±0.57

Table No. 4: Drug Release: In vitro drug release profile.

Sr. No.	Time (hr.)	%cdr F1 (340.5nm)	%cdr F1 (203nm)	%cdr F2 (340.5nm)	%cdr F2 (203nm)
1.	0	0	0	0	0
2.	30	5.278	5.569	5.356	4.85
3.	60	11.330	11.587	11.381	10.194
4.	90	18.028	17.946	17.789	15.852
5.	120	26.899	24.764	24.429	22.229
6.	150	37.790	32.153	31.603	28.875
7.	180	48.940	40.075	39.555	36.734
8.	210	60.590	48.834	47.899	45.356
9.	240	72.800	58.545	56.847	54.517
10.	270	85.530	69.365	66.546	64.442
11.	300	98.690	80.902	76.546	74.546

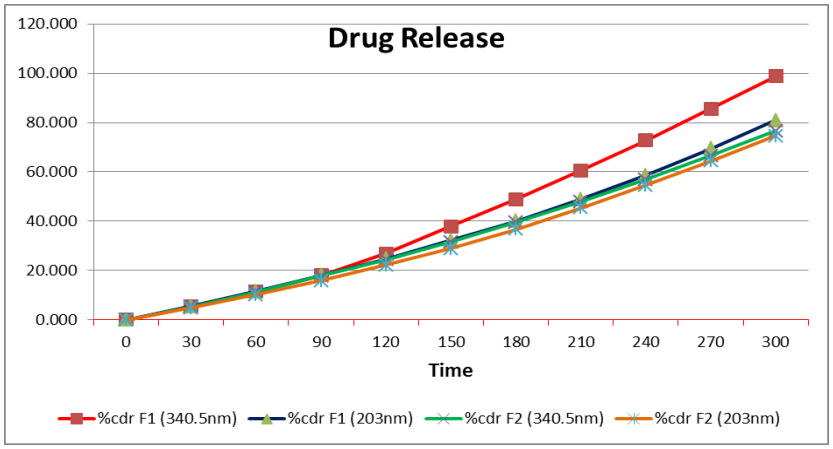


Figure No. 2: Graphical representation of In vitro drug release.

CONCLUSION

Vitiligo is the depigmentation disorder and recently it is also identified as a autoimmune disorder, in which antigens produced by melanocytes(including those released from damaged melanocytes) can be recognized by antigen-specific immune effecter cell including cytotoxic T-cell(CD8+), T-Helper cells(CD4+) and β-cells. The piperine from piper nigram linn. has shown a strong repigmentation capacity. The three phenolic glycoside from Dictamnus dasycarpu turz, has already been reported for it selectively show remarkable activity of inhibiting the proliferation of T-cells(CD8+). Both the drugs were separately use for the treatment of vitiligo, but never in a combination.

The piperine from *piper nigram Linn* and dasycarpusides A&B, three phenolic glycosides[1. 2-methoxy-4hydroxymethyphenol,1-O-α-rhamnopyranosyl-(1”-6’)-β-glucopyranoside; 2. 2-methoxy-4-acetylphenol,1-O-α-rhamnopyranosyl-(1”-6’)-β-glucopyranoside; 3. 2-methoxy-4-(8-hydroxyethyl)-phenol,1-O-α-rhamnopyranosyl-()1”-6’)-β-glucopyranoside] from *Dictamnus dasycarpus turz* were determined.

The isolated phytoconstituents were formulated into o/w & PEG1000 and Bees wax type of cream and all the evaluation of cream were carried out; such as colour, pH, and viscosity, skin irritation in-vitro and in-vivo studies. All the evaluation parameters were found within limits and result found satisfactory. Formulation containing, combination of both the extracts have shown to be non irritant during draize skin irritation test and the drug release profile in vitro study shows the satisfactory result.

Future Scope: Vitiligo is a serious skin problem which affects the social and mental status of a person. In the present attempt, a cream was developed with the combination of both the extract having the antivitiligo activity, this formulation can be helpful in the future treatment of vitiligo and will be proved by clinical experimental study.

REFERENCES

1. http://www.niams.nih.gov/Health_Info/ Vitiligo/ vitiligo_ff.pdf as on 17/7/13.
2. Harsh Mohan, textbook of pathology, Jaypee brothers, medical publishers pvt. Ltd, New delhi, 1990; 46-47.
3. Vitiligo, http://www.herbsandcures.com/2013/may as on 17/7/13.
4. Vitiligo treatment, http://pushpakaran.shoutpost.com- / archives/2013/may as on 17/7/13.

5. Shajil E.M, Chaterjee S., Agrawal D., Bagehi T. & Begum D.R. vitiligo pathomachanisum and genetic polymorphism of susceptible genes, Indian journal of experimental biology, 44, 2006, pg 526- 539.
6. http://en. Wikipedia.org/wiki/vitiligo as on 17/7/13.
7. Huggins RH, Schwartz RA, Janniger CK. Vitiligo, Acta dermatovenerologica alpine, Pannonia, et Adriatica, 14, 2005; 137-42, 144-5.
8. Haldar RM, et al, vitiligo. In: Wolff k, et al. Fitzpatrick's Dermatology in general Medicine, 7th ed. New York, N. Y, McGraw- Hill professional, 2007.
9. Ali Alikhan, Lesley MF, Meaghan D, and Vesna petronic-Rosic, MSccBerwyn and Chicago Illinois; and New York, Vitiligo: A comprehensive overview, 2011; 473-490.
10. Lu T, Gao T, Wang a, Jin Y, Li Q, Li C. Vitiligo prevalence study in Shaanxi Province, China. International Journal of Dermatology, 2007; 46: 47-51.
11. Howitz J, Brodthagen H, Schwartz M, Thomsen K. Prevalence of vitiligo. Epidemiological survey on the Isle of Bornholm, Denmark. Archives of Dermatology, 1977; 113: 47-52.
12. Das SK, Majumdar PP, Chakraborty R, Majumdar TK, Haldar B. Studies on Vitiligo. I. Epidemiology profile in Calcutta India. Genetic Epidemiology, 1985; 2: 71-78.
13. Dwivedi M, Laddha NC, Shajil EM, Shah BJ, Begum R. The ACE gene I/D Polymorphism are not associated with generalized vitiligo susceptibility in Gujarat population. Pigment cell melanoma research, 2008; 21: 407-408.
14. Dogra S, Prasad D, Handa S, Kanwar AJ. Late onset vitiligo: a study of 351 patients in benin city, Nigeria. International journal of dermatology, 2005; 44: 193-6.
15. Onunu AN, Kubeyinje EP. Vitiligo in the Nigerian Aferican: A study of 351 patients in benin city, Nigeria. International journal of dermatology, 2003; 42: 800-2.
16. Haldar RM, taliaferro SJ, vitiligo In: wolf k, goldsmith, Katz S, Gilchrest B, Paller A, Lefell D, editors. Fitzpatrick's dermatology in general medicine. New York: McGraw-Hill, 2008; 72.
17. Zang Z, Xu SX, Zhang FY, Yin XY, Yang S, Xiao FL, et al. The analysis of genetic and associated autoimmune disease in Chinese vitiligo patients. Archives of dermatology research, 2009; 301: 167-73.
18. Alkhateeb A, Fain PR, Thody A, Bennett DC, Spritz RA. Epidemiology of vitiligo and associated autoimmune diseases in Caucasian probands and their families. Pigment cell research, 2003; 16: 208-14.

19. Sehgal VN, Rege VL, Mascarenhas F, Kharangate VN. Clinical pattern of vitiligo amongst Indian, international journal of dermatology, 1976; 3: 49-53.
20. Alzolibani A. Genetic epidermiology and heritabilioty of vitiligo in the qassim region of Saudi Arabia,. Acta dermatovenerogica alpina pannonica adriatica, 2009; 18: 119-25.
21. Handa s, Kaur I, vitiligo: clinical finding in 1436 patients, international journal of dermatol, 1999; 26: 653-7.
22. Jarallah JS, Al- Sheikh OA, El- Shabrawy M, Al- Wakeel MA, vitiligo: epidemiology and clinical pattern at king Khalid university hospital. Annals Saudi medical, 1993; 13: 332-4.
23. Al Robaee AA, Assessment of quality of life in Saudi patients with vitiligo in a medical school in qassim province, Saudi Arabia. Saudi medical journal, 2008; 28: 1414-7.
24. Kyriakis KP, Palamaras I, Tsele E, Michailides C, Terzoudi S. Case detection rates of vitiligo by gender and age. International Journal of Dermatology, 2009; 48: 328-9.
25. Handa S, Dogra S, epidermiology of childhood vitiligo, a study of 625 patients from North india. Paediatric dermatol, 2003; 20: 207-10.
26. Jaisankar TJ, Baruah MC, Garg BR, vitiligo in children, international journal of dematol, 1992; 31: 621-3.
27. http://www.qaigen.com/ product/genes%20and %20pathway %20 details.aspx? pwid=47
28. Haldar RM, johnathan L, Chappell, vitiligo update, 2009; 86-92.
29. Raman A, Lin Z, Robert CH, Venkatasamy R, 2002, Treatment of skin condition, U.S. Patent, 6, 346, 539, B1.
30. Vinod KR, Santhosha D, Anbazhagan S. Formulation and evaluation of piperine cream-A new herbal dimensional approach for vitiligo patients, international journal of pharmacy and pharmaceutical science, 2011; 3: 29-33.
31. Fass L, Venkatasamy R, Hider RC, Young AR, and A. Soumyanath, In vivo evaluation of piperine and synthetic analoguesas potential treatments for vitiligo using a sparsely pigmented mouse model, british journal of dermatology, 2008; 158: 941-950.
32. Hyungwoo Kim, Miyoung Kim, Hanna Kim, Guem San Lee, Gun An, Su in Cho. Anti-inflammatory activity of Dictamnus Dasycarpus turz, root bark on allergic contact dermatitis induced by dinitroflurobenzene in mice, Journal of Ethonopharmacology, 2013; 1-27.
33. Dr. G. Devala Rao, Practicle Natural and pharmaceutical chemistry, birla Publication Pvt. Ltd, pg. 13-14.
34. Dr. Khadabadi, Dr. Deore, Bhaviskar BA, Experimental Pathopharmacognosy, A Comprehensive guide, Nirali Prakashan, pg 3.

35. Indian Herbal Pharmacopoeia, Revised New Edition, 2002; 317-325.
36. Stahl E. Thin layer chromatography a laboratory handbook, Academic press, New York, 1969, 2nd edition 52-105.
37. Ikan R, Natural products, A laboratory guide, New York, Academic, 1969; 233-236.
38. Niazi SK, Handbook of pharmaceutical manufacturing formulation semisolid product, 4: 186.
39. Khan s. Development and standardization of turmeric creame by HPTLC, International Journal of Biomedical and Advance Research, 2010; 01.
40. Rajvansh Anurag, Sharma Shalini, Khokra Sukhbir Lal, Sahu Ram Kumar, Jangde Rajendra. Formulation and Evaluation of Cyperus Rotundus and Cucumis Staivas based Herbal face cream, Pharmacologyonline, 2011; 2: 1238-44.
41. Vijayalakshami A. Development and Evaluation of Anti-Acne products from Terminalia Arjuna bark. International Journal of chemical technology Research, 2011; 3: 320-327.
42. Kuntal Das, Raman Dang, Manjunath UM, Ugandar RE, Lalitha BR. Evaluation for safety assessment of formulated vanishing cream containing aqueous stevia extract for topical application. Indian Journal of novel Drug delivery, 2012; 4: 43-51.
43. More BH, Sakharwade SN, Tembhurne SV, Sakarkar DM. Evaluation for skin irritancy testing of developed formulation containing extract of butea monosperma for its topical application, International Journal of toxicology and applied pharmacology, 2013; 10-13.
44. Shivhare UD, Jain KB, Mathur VB, Bhusari KP, Roy AA. Formulation development and evaluation of Diclofenac gel using water soluble polycryalamide polymer, Digest Journal of nonmaterial and biostructure, 2009; 4: 285-290.