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Solid lipid nanoparticles as a carrier of metformin for transdermal delivery

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Abstract

Worldwide prevalence of type 2 diabetes is increasing with alarming proportions. Metformin is the first-line oral antidiabetic drug of choice for the treatment of type 2 diabetes. The objectives of the present study were to develop Metformin solid lipid nanoparticles (M-SLN) and incorporate it in the transdermal patches. M-SLN was evaluated for Particle size, Zeta potential, Surface morphology by scanning electron microscopy (SEM), Transmission electron microscopy (TEM) and *In vitro- In vivo* release studies. Patches were evaluated by *Ex-vivo* skin permeation studies. M-SLN was prepared by solvent diffusion technique using propylene glycol (solvent), polymethacrylic acid (polymer) and Soya lecithin (lipid base). After doing the evaluation of the above mentioned pharmaceutical parameters, M-SLN was loaded in Methocel K100M transdermal patches. *Ex-vivo* skin permeation studies were conducted on male Wistar rat's skin using Franz-type diffusion Cells. The particle size of M-SLN varied among the formulation due to variation in the composition of formulations. Zeta potential of best formulation was found to be +27mV. SEM and TEM indicates discrete spherical structure without aggregation. Drug content was found to be 1.45mg/patch. The ex-*vivo* permeation studies indicate that the high cumulative amount of drug is permeated from M-SLNs. Our study proves the successful delivery of M-SLN from transdermal patch, and Histopathological studies confirmed that the M-SLN transdermal patch only provoked an acceptable modest inflammatory response. These results support the feasibility of developing transdermal metformin for human applications. Thus, transdermal delivery of M-SLN is a safe, painless and cost effective drug delivery system for diabetes patients.

Keywords: Solid lipid nanoparticles, Metformin, Transdermal patches, *In vitro-In vivo* studies, *Ex-vivo* studies, histopathological studies.

Introduction

Non-insulin-dependent diabetes mellitus (NIDDM or type 2 diabetes) constitutes a major risk factor for cardiovascular mortality and morbidity that is aggravated in turn by obesity, hyperlipidaemia and hypertension [1-4]. The prevalence of type 2 diabetes has reached epidemic proportions and is continuously increasing worldwide. As per global estimates predict that the number of cases were about 220 million in the year 2011, which represents a 50% increase in the prevalence compared to the year 2001, and prediction is that there will be about 370 million cases in the year 2030 [5,6]. India is going to be the Diabetes capital of world. This makes understanding of the pathogenesis of type 2 diabetes crucial in order to implement rational treatment strategies. Type 2 diabetes results from an insufficient compensatory insulin secretion to insulin resistance. Mostly insulin resistance is an early event due to environmental factors, obesity, and β-cell function decline a gradual but generally late event [7,8]. Main goals of management in patients with diabetes are to lower the incidence of degenerative complications and the risk of fatal or non-fatal health events, to improve quality of life, and to increase life expectancy [9].

Metformin hydrochloride lowers both basal and postprandial-elevated blood glucose in patients with type 2 diabetes [10,11]. Some high incidence of concomitant gastrointestinal symptoms, such as abdominal discomfort, nausea, and diarrhoea, may occur during the treatment [12-14]. Recent advances in nanoparticulate systems for improved drug delivery display a great potential for the administration of wide variety of active pharmaceuticals [15]. Many approaches have been used to enhance the penetration of drugs through skin. The role of these systems in the long-term treatment of diabetes, however, remains debatable [16]. Especially questionable are those methods involving physical or chemical disruption of the skin, which might cause chronic pathological changes. Transdermal route of drug administration have unique advantages drug bypass the first pass metabolism and reaches in the systemic circulation. Painless, noninvasive, and patient-friendly

application of patches offers good patient compliance and patches are also easy to remove in the event of hyperinsulinemia [17].

As an alternative carrier, the distinct feature of Solid lipid Nanoparticle (SLN) is that it integrates the benefits of traditional nanoemulsions and polymeric nanoparticles. SLN is by nature a special form of nanoemulsions wherein the matrix material is solid lipid (e.g. highly purified triglycerides, complex glyceride mixture, wax, etc.) instead of liquid lipid, i.e. oil. In comparison with nanoemulsions, SLN possesses good stability and is able to control the release of the incorporated drug. When compared with polymeric nanoparticles, the physiological lipids-made SLN is definitely better tolerated by the human body and its lipophilic nature helps it to penetrate the Skin [18-19]. The current study was undertaken to formulate Metformin solid lipid nanoparticles (M-SLN) employing solvent diffusion technique [18-19]. Animal studies were carried out of the Metformin solid lipid nanoparticles incorporated in Transdermal patches. In vitro release of drugs and histopathological evaluation of inflammation were investigated.

Materials and Methods

Materials

Polymethacrylic acid, Propylene glycol and Soya lecithin was purchased from Sigma Aldrich (CA,USA). Metformin was a kind gift from Ranbaxy Research Labs (New Delhi, India). Methocel (K100M), Acetone (HPLC grade) and Ethanol (HPLC grade) were purchased from Merck (Mumbai, India). Streptozocin was obtained from BioVision (PA, USA). Jolen hair removing cream (Jolen Inc. CA, USA) was used as chemical depilatory. Water used in all experiments was obtained from a Milli-Q Synergy 185 water purification system (Cedex, France). All other materials and reagents were of analytical or pharmaceutical grade, and used as received.

Experimental models

Male Wistar rats weighing 240±20 g (12-16 weeks) and Balb C mice 20-30 g (8 weeks) were obtained from the Experimental Animal Center of JSS College of Pharmacy, Mysore. All animals were fed with a standard laboratory diet and water. They were housed in a specific room at a temperature of 20–25 C and 50 ± 5% relative humidity under a 12-h dark/light cycle and acclimatized for l week before the start of experiment. All experimental procedures involving animals were approved by the Animal Ethical Committee of JSS University.

Preparation of Metformin - solid lipid Nanoparticles (M-SLN)

Nanoparticles containing Metformin were prepared using solvent diffusion technique [20-21]. Drug was dissolved in water, and then acetone (co-solvent) was added into this solution. Co-solvent was needed in order to make the inner phase more homogeneous. Then polymethacrylic acid (Polymer) and 150 mg of propylene glycol were dissolved in chloroform along with 3 mg soya lecithin (lipid base) and this solution was added to the drug solution to form dispersion. The dispersion was added to 10 ml of aqueous ethanol solution (70%). After 5 minutes of mixing, the organic solvents were removed by evaporation at 35 under normal pressure, nanoparticles were separated by using cooling centrifuge (10000 rpm for 20 min), supernatant were removed and nanoparticles washed with water and dried at room temperature in a desicator. By following the above mentioned procedure five other batches of nanoparticles ratio of 1:1, 1:2, 1:3, 1:4 and 1:5 were prepared and named F1, F2, F3, F4 and F5 respectively.

Preparation of Metformin transdermal patches

For preparation of transdermal patches Methocel K100M were used as a film forming agent [22-23]. The polymer was soaked overnight in water and then 50mg of prepared M-SLN were incorporated and mixed uniformly. Suspension was casted on a glass mould and after drying the patches were cut into small pieces and stored in between the sheet of wax paper in desiccator for further studies.

Particle size, zeta potential and surface morphology

Particle size and Zeta potential was determined using Photon correlation spectroscopy. Surface morphology (roundness, smoothness, and formation of aggregates) were studied by Scanning electron microscopy (SEM) and Transmission Electron Microscopy (TEM).

Photon correlation spectroscopy

Particle size was determined by photon correlation spectroscopy (PCS) which yields the mean particle size (Z-average), and the polydispersity index (PI), which measures the width of the size distribution. PCS was performed with Zetasizer Nano S (Malvern Instruments, Malvern, UK) at a detection angle of 173 , at 25 C. Samples were suitably diluted in ultrapurified water. Each value was measured in triplicate. The results are shown as mean ± standard deviation.

Scanning electronic microscopy (SEM)

The SEM analysis was performed in order to investigate the morphological characteristics of the SLN'S. Prior to analysis, the sample was diluted with ultrapurified water, placed on a double side carbon tape mounted onto an aluminium stud, and dried in a desiccator. Sample was then sputter coated with gold in order to make it conducting. SEM images were recorded on a Jeol, JSM 5310, (Tokyo, Japan) scanning electron microscope, with an acceleration voltage of 25 kV.

Transmission Electron Microscopy (TEM)

Transmission electron microscopy (TEM) was employed to evaluate the shape of the nanoparticles containing Metformin. A Philips CM 10 transmission electron microscope was used, and particle size was measured using NIH image software. Nanoparticle suspensions, at a concentration of 0.5% (w/v) of

nanoparticle, were sprayed on Formwar-coated copper grids and air-dried before observation.

Drug content

Drug content was determined by ultrafiltration-centrifugation method [24-25]. The ultrafiltration-centrifugation method was carried out through centrifugal filters (Amicon® Ultra-4, Millipore, Germany) with a 100 kDa molecular weight cut-off. Briefly, 1mL of M-SLN plus 1mL of methanol were placed into the upper chamber of the centrifuge (Kubota 1720 centrifuge), which was centrifuged at 4000 g for 90 min at 4 C. Methanol was added in order to dissolve crystals possibly present in the external phase of the M-SLN dispersion. The amount of free drug in the aqueous dispersion phase, collected in the outer chamber of the centrifugal filter after separation, was determined by UV-Vis spectrophotometry at 233 nm after suitable dilution.

Fourier Transform Infra-red Spectroscopy (FT-IR) analysis

In order to obtain information about the drug polymer interaction and stability of drug, the FT-IR spectra of pure Metformin and Polymethacrylic acid nanoparticles loaded with Metformin were recorded. Samples were prepared using potassium bromide pellet method. Then placed in the FT-IR device (Spectrum 400, PerkinElmer) and measured using four scans for each spectrum, with a resolution of 1cm ^{1} and a scan speed of 0.5. Spectra were collected between 4000 and 400cm ^{1}.

In-Vitro Studies

Drug content analysis

The patches (n=3) of specified area 3cm^2 were weighted and dissolved in 100ml methanol. The solution was filtered through membrane filter and drug content were analyzed by HPLC.

Preparation of skin for skin permeation studies

The Male Wistar rats were sacrificed and the hair in abdominal region was removed with depilatory, and examined for integrity using a lamp inspecting Method [26-29]. The subcutaneous fat and connective tissue were carefully removed by scalpel. Finally skin was rinsed with physiological saline and stored at -20 C in an aluminium foil.

Ex-vivo permeation study

The skin samples were mounted carefully on Franz-type diffusion Cells with the stratum corneum side up with an effective diffusion area of 1.72 cm [28-29]. The receiver compartments were filled with 10 ml of physiological saline to ensure sink condition. The diffusion cells were maintained at (37±0.5) C with stirring at 100 rpm throughout the experiment. 3 cm transdermal patch of Metformin was mounted onto skin surface 1 ml of the sample was collected from the medium at predetermined time Interval of (0, 1.7, 2.5, 4.5, 8.2, 10.7, 12.8, 24 hrs.) and replace same volume of fresh physiological saline. All the samples were filtered through a membrane filter and analysed by HPLC.

In-Vivo Studies

Preparation of animal for studies

Male Wistar rats were housed, with standard diet and water for three days, and then they were fasted overnight before experiment. Hair on the backside of the rats was removed with a depilatory cream. Prior to the day of the experiment, animals were divided into 3 groups (n=6) of normal and diabetes rats. The rats were treated as following

Group I- Placebo patch (control) prepared by Methocel without nanoparticles

Group II - Metformin oral administration contain 2mg drug

Group III-Transdermal patch (3 cm^2) contains Metformin nanoparticles

Induction of diabetes

Diabetes was induced by injecting 60 mg/kg of Streptozocin (STZ) dissolved in 0.1M citrate–citrate sodium buffer (pH 4.5) intra peritoneally in all the 3 groups [30-31]. After allowing the diabetic rats for stabilization over 72 hours. The blood glucose level was estimated by using Metformin transdermal patches. Blood samples were collected from the tail vein before and after treatment to determine blood glucose levels. These samples were taken at scheduled times of 0,2,4,8,10,12,24,36 & 48 hrs. Blood glucose levels were obtained by Using the once touch glucometer (Roche, Germany).

Statistical analysis

Results are expressed as mean ± SEM values. Statistical significances were evaluated using student t test. A value of $p<0.05$ was considered significant.

In vivo evaluation of M-SLN Transdermal patches for biocompatibility

Balb C mice were used to examine the biocompatibility of M-SLN Transdermal patches. After being sterilized by ^{60}Co-γ radiation, M-SLN Transdermal patches was subcutaneously applied on the back of the mice, respectively. The control group was applied with same Transdermal patch without M-SLN to make a comparison keeping similar environment. Individual mouse weight was taken daily prior to their scheduled sacrifice on days 1, 7, 14 and 21.The skin was cut and the applied site was exposed. Immediately after necropsy, skin tissues from the applied sites were retrieved and fixed in Bouin's solution for 7 days, and then washed by flowing water for 24 h. After being bisected into pieces with a thickness of 2 mm, all tissues were initially dehydrated in a graded series of alcohol and then embedded in paraffin. The transverse sections (4–5 μm thick) were prepared using rotator microtome and stained with hematoxylin and eosin dye for histopathological examination. The histological changes, such as acute–chronic inflammatory

symptoms, fibroblastic proliferation, foreign material deposits, and any other inflammation symptoms were evaluated through observation under a light microscope.

Results

Metformin nanoparticles with varying proportions of Metformin and polymethacrylic acid were prepared by solvent diffusion technique. The scanning electron microphotograph of Metformin nanoparticles, Shown in Figure.2 (a&b) and Transmission electron micrograph in Figure.3. It indicate that Metformin nanoparticles have a discrete spherical structure without aggregation. The particle size of nanoparticles varied some what among the formulation due to variation in the composition of formulations as shown in Table.1.

Zeta potential of best formulation was determined and it was found +27mV. Since there was a decrease of surface potential, it could be concluded that a part of drug was absorbed on the polymeric particles. The drug content was determined by centrifugation

Table1-Five batches of nanoparticles ratio of 1:1, 1:2, 1:3, 1:4 and 1:5 were prepared and named F1, F2, F3, F4 and F5 respectively

Formulation code	Drug : Polymer ratio	Drug Content* (%)	Particle Size *(nm)
F1	1:1	68.32±0.02	12±8
F2	1:2	74.3±0.08	225±5
F3	1:3	80.83±0.03	237±9
F4	1:4	94.62±0.02	242±5
F5	1:5	78.96±0.04	203±4

* Average of three preparation ± S.D

method and it was maximum in formulation F4. The nanoparticles exhibited an increase in drug content with an increased in the polymer ratio, up to particular concentration (1:4). A decrease in drug content was observed after that point due to the saturation capacity of polymer. In FT-IR study the characteristic peak due to pure Metformin has appeared in the spectra of nanoparticles without any markable change in the position (Figure.1). It indicated that there was no chemical interaction between Metformin and polymethacrylic acid.

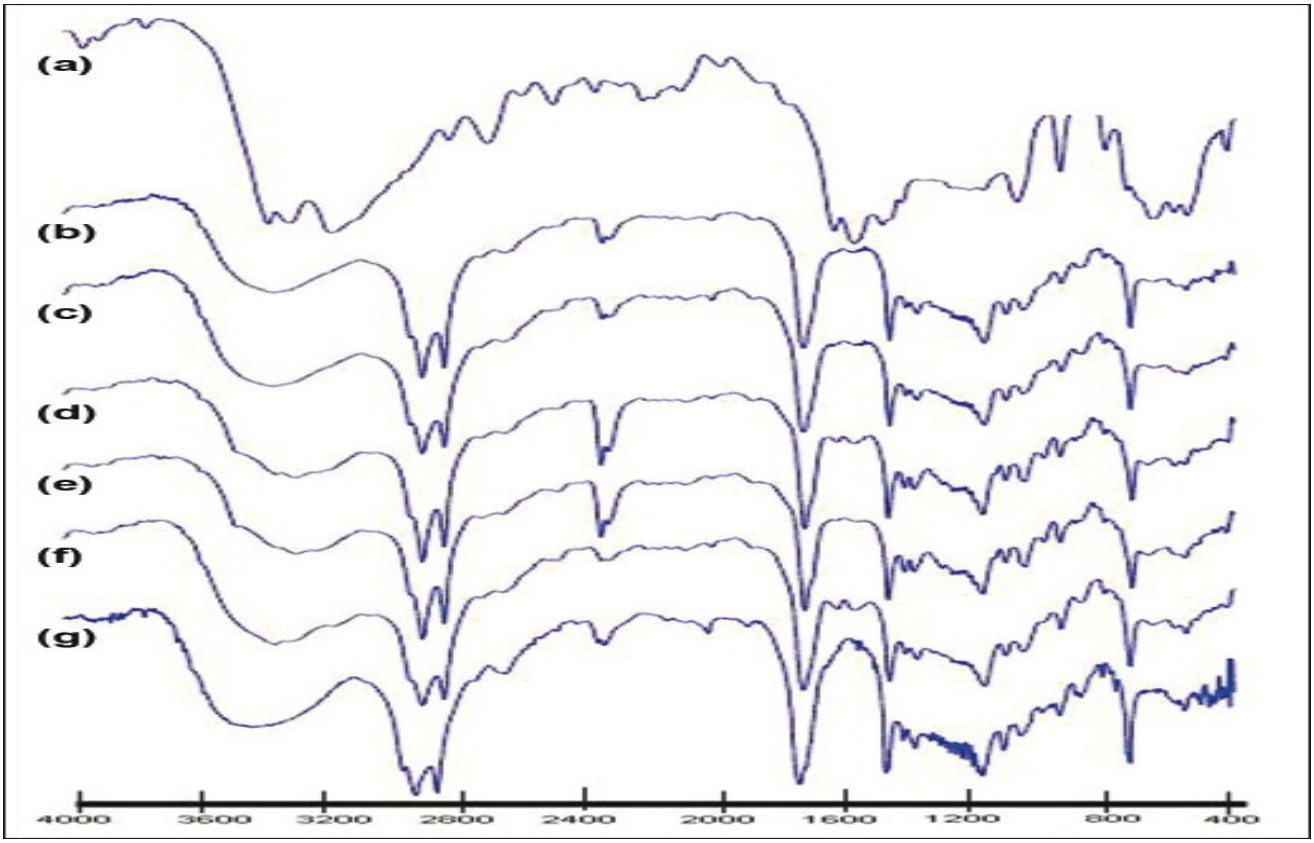

Figure: 1-FTIR spectra of (a) Metformin, (b) F1, (c) F2, (d) F3, (e) F4, (f) F5

Scanning Electron Microscopy (SEM)

SEM picture are shown in Figure. 2. (a&b). Non-aggregated microcapsules with almost spherical shape were obtained for all the formulations. Nanospheres did not have a smooth surface indicating that Metformin was drug present on the surface.

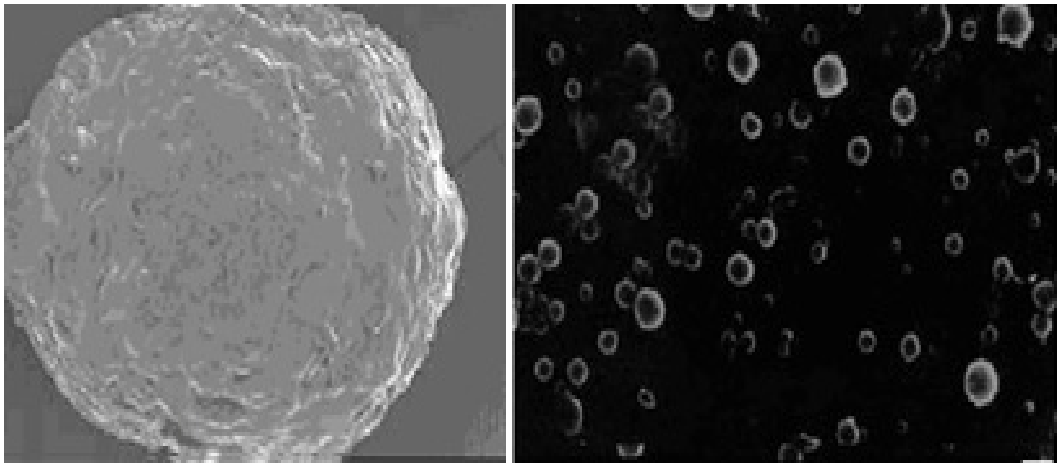

Figure: 2- Scanning electron microphotograph of Metformin nanoparticles
(a) At magnification 250 x (b) At magnification 40 x

Transmission Electron Microscopy (TEM)

To investigate Nanoparticle size and morphology further, microscopy studies were carried out using TEM analysis. TEM photomicrograph of the loaded nanoparticles are reported in Figure. 3 and confirm their previously ascertained sizes. Metformin loaded nanoparticles were spherical in shape with a rather uniform distribution.

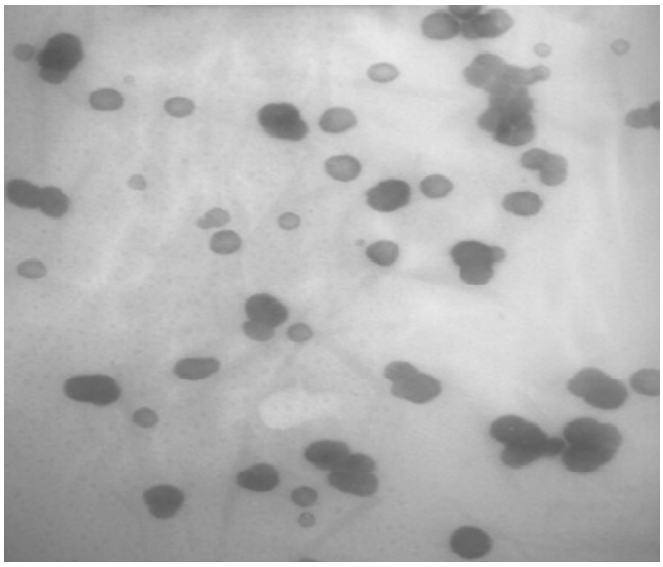

Figure:3- Transmission electron micrographs (TEM) of Metformin nanoparticles (46000X)

Loading evaluation of Solid lipid nanoparticles on transdermal patches

The solid lipid nanoparticles containing Metformin loaded in Methocel K100M transdermal patches. This polymer is highly hydrophilic, so the lipophillic M-SLN are not soluble and structure of M-SLN remains intact. The drug content of each patch was found to be uniform and ranged between 92.21 ± 0.12 to 96.00 ± 0.15 % (1.45mg/patch).

Ex-vivo skin permeation studies

Result of *ex-vivo* and *in-vivo* skin permeation of M-SLN from transdermal patches is shown in Figure 4. The cumulative amount of drug release from nanoparticle made by polymethacrylic acid and Soya lecithin 291 ± 2.16 µg/cm respectively .The release was shown in Figure 4.

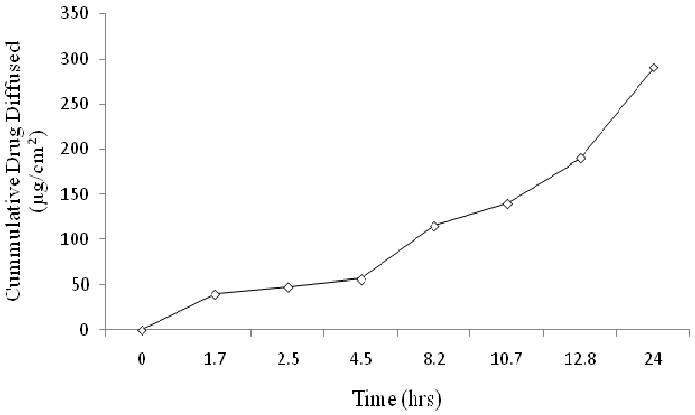

Figure:4- *Ex-vivo* diffusion studies showing the time course of cumulative drug diffusion

Hypoglycemic effects

The reports showed decreased blood glucose level by using transdermal patches loaded with M-SLN when compared with oral administration of Metformin (2mg) in both normal and diabetes rats. The time course profile of blood glucose response in rats were shown in Table 2.

The blood glucose level in normal rats drastically reduced in orally administered drug upon 10 hrs and was reported as 53.08 ± 0.18mg/ dl and 34.40 ±0.04 mg/dl for 48 hrs in the case of transdermal patches containing nanoparticles. In STZ induced diabetes rats, the blood glucose level gradually reduced up to 97.48 mg/dl at t=10 hrs from 330.67 mg/dl. Transdermal patches containing M-SLN produced maximum drop of blood glucose at 91.74 mg/dl at 48 hrs. Neither placebo patch applied showed hypoglycemic effect.

Table:2-Blood glucose level following Control, Oral and M-SLN Loaded patch in normal and diabetic rats

Normal rats (mg/dl)				*Diabetes rats (mg/dl)*		
Time (hrs)	Placebo Patch (Control)	Oral (2mg)	M-SLN loaded patch	Placebo Patch (Control)	Oral (2mg)	M-SLN loaded patch
0	86.17±0.12	86.02±0.34	85.98±0.24	335.67±0.02	330.67±0.10	336.67±0.10
2	84.33±0.32	69.60±0.82	79.16±0.76	333.33±0.02	184.42±0.32	300.18±0.62
4	84.17±0.22	67.12±0.96	76.00±0.14	335.10±0.14	128.33±0.40	260.27±0.02
8	84.92±0.53	59.42±0.16	70.18±0.38	337.67±0.12	111.60±0.62	248.00±0.22
10	84.04±0.72	53.08±0.22	68.66±0.38	331.45±0.98	97.48±0.60	211.92±0.60
12	84.86±0.24	71.18±0.42	64.20±0.72	339.10±0.22	227.10±0.34	195.68±0.02
24	84.10±0.41	75.92±0.44	51.38±0.34	339.48±0.62	260.87±0.10	111.34±0.06
36	83.98±0.92	76.64±0.10	39.42±0.04	338.62±0.74	258.44±0.22	107.62±0.88
48	84.62±0.74	77.06±0.04	34.40±0.04	339.09±0.02	268.19±0.02	91.74±0.02

Values are expressed as mean ± SEM, n=4, p<0.05.

In vivo evaluation of M-SLN Transdermal patches for biocompatibility

M-SLN Transdermal patches were subcutaneously applied on the back of the mice, respectively. The control group was applied with same Transdermal patch without M-SLN to make a comparison keeping similar environment. All the mice were healthy throughout the experiment period, as judged from the body weight experiments and pathological changes of the treated and control mice during the experiment. Animals were sacrificed for histopathological study at different time points. Fig.5 shows the representative histopathological changes noted at the application sites. It is observed that the inflammatory response (subacute response) characterized by increased permeability of capillaries and infusion of abundant lymphocytes occurred at the application site, which can be subacute host response sets in against the foreign material after 7 day of injection. The inflammation is increased and there are lots of neutrocytes and lymphocytes on day 7. However, new fibroblast cells begin to appear around the beam wall of the patch and fibroblast tissue could be observed (Fig. 5A). After 14 days, there are a few neutrocytes under the dermal layer and superficial layer, but a thick fibrous tissue is observed (Figure. 5B). After 21 days, a new and thin fibrous tissue is formed under the dermal layer and superficial layer of the muscularis and the histology is similar to that of normal skin (Figure. 5C). Although there are some signs of high inflammation in a few of the tissue samples but usually there is an indication of time-related healing process (i.e. time-related acute to subacute inflammatory reaction, associate with increased fibroplasia). Control group has also shown no inflammation.

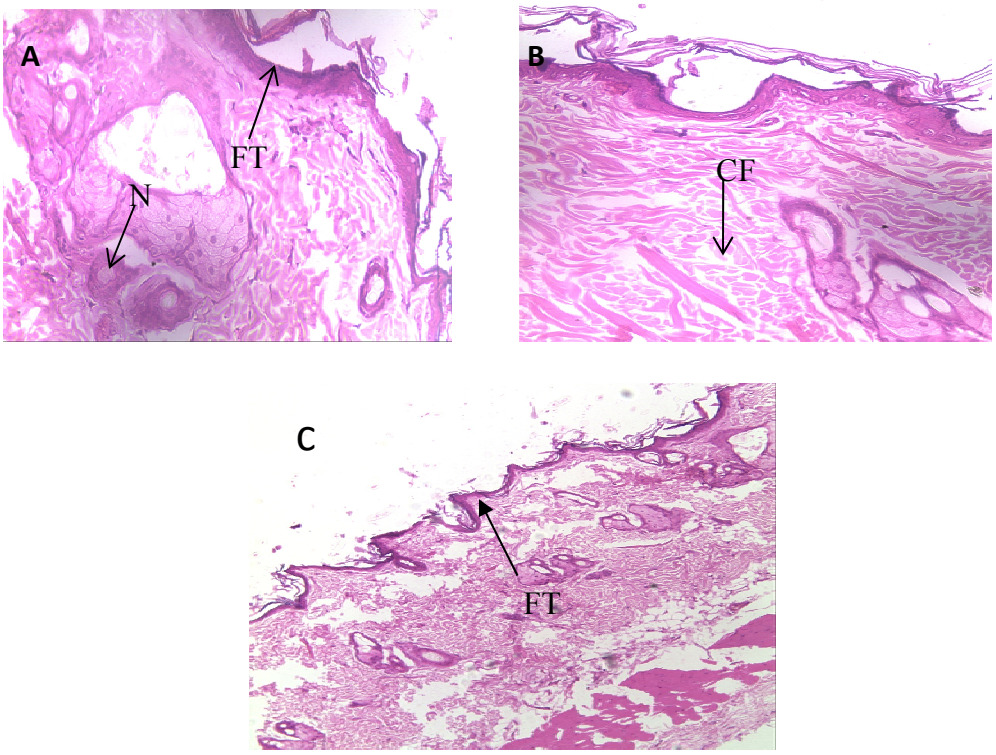

Figure: 5- Representative histopathological changes noted at the application sites on the 7th(A), 14th (B), 21th (C) (100 , N, neutrocyte; CF, collagenous fiber; FT, fibrous tissues).

Discussion

Overall results show that Metformin solid lipid nanoparticles incorporated in transdermal patch possess marked hypoglycaemic activity (when tested in fasted normoglycaemic rats) and antihyperglycaemic activity (by lowering the blood glucose levels in STZ-induced diabetic rats). Since transdermal delivery of Metformin solid lipid nanoparticles showed prominent result suggesting that nanoparticle could penetrate through stratum corneum, epidermis and dermis to reach the blood circulation. The penetration was due to ultrafine particles size of nanoparticles and its lipid content [19-21]. The phospholipids vesicles could penetrate rapidly when compared to other vesicles [15-16]. This study demonstrated the significant quantity of Metformin can be delivered into blood stream from a single transdermal nanoparticles patch over extended period of time. The *ex-vivo* permeation studies predicted that the high cumulative amount of drug permeated by using nanoparticles made by polymethacrylic acid. *In-vivo* experimentation proved that nanoparticles permeation from transdermal patch was more at 48 hrs compared to initial hours. It was reported that dropping of blood glucoselevel were prolonged by transdermal patches upto 48 hrs [22-24]. The slow and sustained hypoglycemic response could be due to slow release of drug from nanoparticles. In orally treated group, the hypoglycemic effect was reduced upto 10 hrs, which could be due to its short biological half life. The pharmacokinetic parameters obtained with transdermal nanoparticles patches were significantly ($p<0.05$) different from orally treated group. This could be a fast absorption and short half life [10]. Whereas nanoparticles through transdermal route showed slow release of drug from lipid vesicles and maintained peak plasma concentration over a prolonged period [24-26]. Transdermal nanoparticulate system would also protect the formulation from dehydration and accidental damage, leakage of drug from system. Metformin solid lipid nanoparticles incorporated in transdermal patch were devoid of unacceptable side effects even following chronic administration. On all accounts, no significant histological differences are observed between control and Transdermal patch applied tissue samples. An acceptable tissue reaction to polymeric patch should be temporary inflammatory responses. Thus, histopathological studies confirm that M-SLN Transdermal patches is biocompatible for use in drug delivery systems. There were no overt signs of toxicity and hepatotoxicity. In conclusion, our results demonstrate the use of Metformin solid lipid Nanoparticle in Transdermal patches for the

first time and show its therapeutic potential to be used as a cost effective safe mode of drug delivery systems.

Conclusion

We synthesized and characterized Metformin Solid lipid nanoparticles which were later incorporated in the transdermal patches. Metformin Solid lipid nanoparticles was prepared by the Solvent diffusion technique and easily incorporated in transdermal patches. Solid lipid nanoparticles system successfully delivered Metformin transdermally, as evidenced by a significant sustained decrease in blood glucose in normal rat and those with diabetes. The biocompatibility and initial inflammation of the M-SLN transdermal patches were evaluated from the histopathology of the tissue near the application site, which has shown that M-SLN transdermal patches have a well biocompatibility. These result support the feasibility of developing transdermal metformin for human applications. The transdermal delivery of solid lipid nanoparticles contained Metformin is safe, economic and continuous delivery for diabetes patients.

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Author's Contribution

Navneet Sharma: Conception and design
Sudha Rana: Acquisition of data, or analysis and interpretation of data
Hosakote G. Shivkumar: Final approval of the version to be published
Rakesh Kumar Sharma: Drafting the manuscript or revising it critically

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