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Original Article

Skin targeted delivery of rutin-phospholipid complex: Patch formulation, *in vitro-in vivo* evaluation

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ABSTRACT

Oral bioavailability of Rutin is very low due to solubility issues and first pass effects. Rutin-phospholipid complexes (RN-P)s offer improved physicochemical properties favorable for drug delivery via the skin. The present work aims to develop sustained release polymeric patch containing RN-Ps to treat inflammation. Patch prepared by solvent evaporation method using polymers (Eudragit RL 100, PVP K30) and plasticizer (PEG 400). Prepared patch were studied for film properties. SEM and TEM images were used to study surface morphology. FE-SEM images of the patch taken after drug release and skin permeation. Sustained anti-inflammatory effect was established in carrageenan induced paw edema model. A 7 (seven) days skin irritation test on rabbit was performed. Optimized formulation gave 85.12 % drug release and 31.0 % cumulative permeation in 24 hours studies. Skin penetration and accumulation were found higher with RN-P than pure rutin. FE-SEM images showed that RN-Ps retain their structure after 24 hours exposure to physiologic environment. The test formulation gave 91.4 % inhibition of inflammation at 24 hours as compared to 39.58 % for standard. Skin irritation test revealed that the patches are safe for skin application. We conclude that polymeric transdermal patch able to deliver RN-Ps for sustained therapeutic effect useful in treating acute and chronic inflammation.

Key Words: Rutin, Phytosome; Phospholipid complex; Polyphenol; Sustained Release; Transdermal.

INTRODUCTION

NSAIDs are one of the most prescribed drugs in inflammation in the world wide though they have gastrointestinal side effects. The side effects of NSAIDs may be minimized through the administration of prostaglandin analogs and proton pump inhibitors [1]; however, these agents only diminish the clinical manifestations without inhibiting their causes. The administration of selective COX-2 inhibitors is limited by their high cost and by their increasingly recognized cardiotoxicity. Taking into consideration these side effects, it is appreciable to develop new strategies to allow their use in a safer context [2]. In the search for new strategies plant polyphenolic flavonoids were found to be most promising [3, 4]

The anti-inflammatory activity of Rutin was being well established in the world literature [5, 6, 7, 8, 9, 10] and found its application in the treatment of inflammatory conditions associated with excessive leukotriene production, such as rheumatoid arthritis and inflammatory bowel disease [11]. Rutin is the glycoside form of the aglycon quercetin and chemically it is Quercetin-3-rhamnosyl glucoside (Figure-1), is a low molecular weight polyphenolic compound that is widely distributed in vegetables and fruits [12, 13]. However rutin is practically insoluble or very less soluble in aqueous medium [14, 15, 16]. Lower solubility of rutin leads to its poor oral bioavailability [17, 18]. Poor solubility in non-toxic solvents also hampers pharmacological testing [19]. Apart from this poor absorption from GI tract is the major reason for its limited bioavailability [20]. The binding of a rhamnose or of a glucose-rhamnose moiety to the aglycone markedly depressed its absorption [21]. Yang and his coworkers reported that extensive conjugation metabolism of polyphenols occurred during the first pass through gut and liver. One of the reasons for poor absorption of rutin is its inability to diffuse

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through cell plasma membrane and it was absorbable only after being hydrolyzed into quercetin, which is absorbable [22]. Deglycosylation by b-glucosidases is an important process in the oral administration of quercetin glycosides in the gastrointestinal wall [23]. Only free flavonoids without sugar molecules are thought to be absorbed by the gut wall [24]. Thus oral absorption of glycosides, such as rutin, is delayed resulting in a lower bioavailability of glycosides than aglycone [25, 26].

The recent literature on rutin delivery therefore emphasized on designing novel drug delivery of rutin via the skin. Banjare and Ghillare [27] prepared polymeric nanoparticles of Rutin and reported that Rutin in colloidal carrier enhanced the drug penetration into the skin, and because of its lipoidal nature, the penetrated drug concentrates in the skin and remains localized for a longer period of time, thus enabling drug targeting to the skin. *Invitro* skin permeation studies on a pig ear model demonstrated that Rutin ethosome formulations were better able to permeate compared to pure Rutin [28]. Park, et al., [29] developed liposome-in-hydrogel complex system of Rutin to enhance transdermal permeation.

Lin and his group reported in their research findings that the transdermal route of quercetin glycosides greatly differed from the oral route. In their study no deglycosylation was found after examining the receptor medium used in skin permeation study. The glycosides with hydroxyl groups may form hydrogen bonds with ceramides in the epidermis. The multiple –OH groups, which present higher opportunities for hydrogen bonding. Partitioning from the aqueous solution to the SC thus increased [30]. Transdermal drug delivery system (TDDS) is one of the methods of novel delivery for sustained action of drugs. TDDS has got added advantages over oral administration, such as, less dosing frequency, ease of administration, patient compliance, avoidance of presystemic and hepatic first-pass metabolism and low incidence of side effects.

Phytosome® is a patented technology of Indena (Italy) wherein plant polyphenolics are complexed with phosphatidylcholine to improve bioabailability. In our previous work we developed Rutin-Phytosomes® and found better skin penetration than pure rutin [31]. The present work aimed to administer Rutin in Phytosomes® form via the skin for sustained treatment of inflammation. For skin delivery we proposed to formulate polymeric sustained release patch using Eudragit RL 100 and PVP K30, incorporated with Rutin-Phytosomes®. Such an approach of Rutin drug delivery had not been reported.

MATERIAL AND METHOD

Materials: Rutin (RN) was purchased from TCI Chemicals (India) Pvt. Ltd., Chennai. Phosphatidylcholine (PC) (Egg lecithin) was purchased from Sigma Aldrich, Bengaluru. Eudragit RL 100 (ERL), Poly vinyl Pyrrolidone K30 (PVP) were purchased from Yarrow Chem Products, Mumbai. Poly ethylene glycol 400 (PEG), methanol, dichloromethane, iso-propyl alcohol are all analytical grade and purchased from LobaChem, Mumbai.

Preparation of Rutin-Phospholipid complex (RN-Ps): RN-Ps was prepared by a method reported elsewhere [31]. Phosphatidylcholine and Rutin were taken in 1:1 molar ratio in the prepared phytosomes.

Formulation of transdermal patch: Polymeric transdermal patch was prepared by solvent casting method (Table 1). In a 10 ml beaker ERL and solvent was kept for 24 hours. Next day PVP and plasticizer (PEG) were added and mixed using magnetic stirrer for 15 minutes. The mixture was poured over the (4×3) cm^2 mold fabricated with 2 mm thick aluminum sheet into which aluminum foil was previously shaped to serve as backing membrane and left for drying at room temperature for 24 hours. The patches were dried in a hot air oven at 60^0 C for 2 hours and stored for future use in a desiccator (without any desiccant) to prevent moisture entry.

Animal study: All the animals used were treated according to the standard guidelines after getting approval from the Institutional Animal Ethical Committee vide reference number GIPS/IAEC/11/2013.

EVALUATION OF PATCH CHARACTERISTICS

Moisture content: Individual weights of the patches were noted and were kept in a desiccator at 40^0 C containing calcium chloride. At regular interval patches were weighed again and again till constant weight reached. The percentage of moisture content was calculated as a difference between initial and final weight with respect to initial weight [32].

Water vapor transmission rate: About 1 gm fused calcium chloride was taken in thoroughly cleaned and dried glass vials and the polymeric patches were fixed over the brim with the help of an

adhesive. Then the vials were weighed and stored in a humidity chamber at 85 % RH condition for a period of 24 hours. The vials were weighed after 24 h to note down the weight gain. Transmission rate was calculated as gm/cm^2/day [33].

Drug Content: Drug content was determined by taking 1 cm^2 patch cut out from three different places of the patch. The patch was dissolved in 2 ml of methanol and subsequently diluted with phosphate buffer pH 6.8 and filtered through 0.45µm filter paper. After appropriate dilutions, solutions were analyzed spectrophotometerically at 257 nm for Rutin against a blank prepared using a drug free patch [34].

Folding endurance: Folding endurance is a manual method of ascertaining the strength of a transdermal patch to withstand various stresses it receives, eg. movement of the body part to which applied. Folding endurance was measured by a reported method [35] in which the patch was repeatedly folded and unfolded at the same line till it broke. The number of times the patch can be folded without breaking gives the value of folding endurance.

In vitro Drug release study*:* Dissolution test apparatus (USP-II, Paddle Type) was used in the study. The transdermal patch was fixed over a glass slide from the backing membrane side of the patch using adhesive allowing drug release from one side. For the study 500 ml freshly prepared phosphate buffer pH 6.8 was used as medium. The patch along with the glass slide was dipped into the medium and adjusted to occupy center position of the vessel with the patch facing upward. During the release study the medium was maintained at 37.5 ± 0.5 º C and paddle speed was maintained at 50 rpm. At a predetermined time interval 5 ml samples were withdrawn and same quantity of fresh media was replaced. The 5ml sample was filtered through Whatman filter (0.45 µ). Drug concentration was quantified in a UV-visible spectrophotometer at 257 nm using drug free phosphate buffer (pH 6.8) as blank. After the release study, the patch under dissolution tester is removed carefully and stored in a desiccator for FE-SEM (Field emission scanning electron microscopy) study.

Ex Vivo Skin permeation study: For the study, a modified Franz diffusion cell with a diffusional area of 1.766 cm^2 was used. Rat abdominal skin excised after sacrificing the animal was used in the study. Skin hairs were shaved and subcutaneous fats were removed carefully. A round piece of the patch was cut out so as to accurately fit into the diffusion area. The round cut out of the patch was pressed over the stratum corneum side of the skin preparation after which the patch attached firmly with the skin because of the self-adhesive nature of the patch. The skin along with the patch was tightly tied to the donor compartment so that the dermis side faces the receptor compartment. The receptor compartment was filled with 32 ml phosphate buffer (pH 6.8) and maintained at 37 ± 0.5^{O}C under continuous stirring with a magnetic bar. From the receptor compartment 2 ml samples were withdrawn at predetermined time intervalsup to24 hours. Same volume of receptor compartment fluid was replaced after each sampling. Experiment was carried out in triplicate following the same procedure. Samples were analyzed in UV-Visible Spectrophotometer at 257 nm. The cumulative amount permeated at each time interval was calculated and a plot of cumulative amount permeated (Q, %) versus time (t, h) was constructed. The skin after 24 hours study was taken out of the diffusion assembly and was cut into small pieces and extracted with methanol by homogenizing in a tissue homogenizer. Aliquots of the extract were analyzed in the UV-Visible Spectrophotometer at 257 nm after suitable dilution with phosphate buffer (pH 6.8).

Also the patch used in the skin permeation experiment is removed carefully from the diffusion cell, washed the surface with distilled water and any droplets on the patch was soaked gently into tissue paper. The patch is then stored in desiccator for FE-SEM study.

Scanning electron microscopy (SEM): For SEM study a sample of the patch was fixed on a copper stab and dried in an oven at 70º C for 30 minutes and then placed in a Fine Coat Ion Sputter (JFC-1100). Inside the sputter a high vacuum was applied for 15 minutes and then coated with gold. Analysis was done on the coated sample in the JEOL (JSM 6360) Scanning electron microscope.

Transmission electron microscopy (TEM): A small sample of the patch was negatively stained with 2% uranic acid and placed on copper grid for TEM analysis in a JEOL (JEM 2100) Transmission electron microscope.

Field emission scanning electron microscopy (FE-SEM): Small section of the patches were fixed on copper grid and dried in hot air oven at 70˚C for 1 hour. Sample was fed into a coater (Emitech Sputter Coater) under which high vacuumwas applied and coated 1-2 nm layers with Pd/Au. Patch surface was photographed in a Carl Zeiss Sigma VP field emission scanning electron microscope.

In vivo anti-inflammatory study in carrageenan induced edema model: The anti-inflammatory activity and sustaining action of the RN-P loaded patches were evaluated using "carrageenan-induced hind paw edema" method developed by Winter et al., [36]. The animals, Wister rats weighing 180-220 g were divided into three groups, 6 (six) in each. Before the day of administration, rats were fasted overnight but were allowed access to water *ad libitum*. The areas to which patches will be applied were shaved 12 h before starting the experiments. Results of the anti-inflammatory study were compared in a one way analysis of variance followed by the least significant difference (LSD) as a post-hoc test was applied; using SPSS program version 9 software. The difference was considered as significant when $P<0.05$.

Group I (Control group): Paw edema was induced by injecting 0.1 mL of a 1% w/v homogeneous suspension of carrageenan in double-distilled water. The paw volume was measured immediately (0 h) and at 1, 2, 3, 4, 5, 6, 8, 10, 12 and 24 h after injection using a plethysmometer [37].

The amount of paw swelling with respect to initial volume was determined time to time. It is obtained by subtracting volume of injected paw at time '0' (V_0) from volume of injected paw at time't' (V_t) divided by volume of injected paw at time '0'.

$$\% \text{ Swelling} = (V_t - V_0/V_0) \times 100$$

Group II (Standard group): Treated similar to control group except that marketed diclofenac sodium gel (1%) in a quantity of 5mg/kg were applied one hour before sub plantar injection of carrageenan.

Group-III (Test group): Treated similar to control group except that RN-P containing patches (equivalent to 20 mg rutin) were applied to the shaved area one hour before subplantar injection of carrageenan. Percent (%) inhibition of edema in treated groups was calculated against the control group using the following formula % Inhibition = [% Swelling (control) - % Swelling (drug) / % Swelling (control)] ×100

Skin Irritation test: Skin irritation of the patch was evaluated by Draize method of scoring [38]. Albino rabbits weighing 1.5 to 2.0 kg were used in this study, divided into three groups containing 4 in each group. They were housed in cages under controlled temperature and light conditions. They were fed a standard laboratory diet and had access to water *ad libitum*. The dorsal surface of the rabbits was cleared and the hair was removed by shaving (Fig: 2). The skin was cleared with rectified spirit. Rabbits of the Group I received plain patch (without drug) and Group II received RN-P patch, were applied to the shaved skin of rabbits and secured using USP adhesive tape (Johnson & Johnson limited, Mumbai). An aqueous solution of formaldehyde (0.8% v/v) was applied as standard irritant in the Group III. Its effect was compared with the test. The animals were observed for any sign of erythema and edema for a period of 7 days and scored in the scale 0 – 4.

Histological Study: Full thickness rats abdominal skin without subcutaneous tissue were exposed to RN-Ps containing patch using modified Franz diffusion cells for 24 h. The exposed region was dehydrated using ethanol, replaced ethanol with 1:1 mixture of xylene and cedar wood oil, embedded in paraffin for 30 minutes under vacuum at 60º C for fixing, subjected to paraffin vertical sections (Thermo Scientific Microm HM 340 E Rotary Microtome, Germany), stained with haematoxylin and eosin and finally mounted on glass slide with DPX [39]. These samples were then observed under Trinocular Advanced Research Microscope with Image Analysis System (Nikon ECLIPSE 80i, USA) and compared with the control sample that was free of exposure to drug.

RESULTS AND DISCUSSION

Moisture content and water vapor transmission rate: The results of moisture content studies suggest that the patches are hygroscopic and such patches are difficult to handle and care should be taken in their storage (Table 2). As the total polymer weight increased (300-600 mg) the moisture content of the patches increased. Also the patches with higher PVP content showed higher affinity of moisture (ERL: PVP in 50:50 and 60:40 ratios). Such behavior according to Rajabalaya, et al., [40] is due to hydrophilic nature of PVP. All patches containing ERL and PVP in 70:30 ratio were found to contain less moisture (A3, B3, C3 and D3). The water vapor transmission (WVT) rate was affected by the thickness which is related to the total polymer weight. As polymer weight increased from 300 mg (A1, A2 and A3) to 600 mg (D1, D2 and D3) WVT gradually decreased. Also the ERL and PVP content affected the WVT which decreased in the order of 50:50>60:40>70:30 ratios of ERL: PVP.

Drug Content: All patch formulations showed average drug content of more than 98 % (Table 2) except some patches (A1, A2, A3 and B1). The low drug content of these patches may be due to the drug loss in handling caused by very high sticking.

Folding endurance and flatness: The folding endurance of the films was affected by total

polymer weight used in the patch. The patches A1, A2 and A3 showed the lowest folding endurance which contained 300 mg total polymer. Formulation B1 and B2 too showed similar results containing 400 mg total polymer. These patches do not have sufficient mechanical strength to maintain structural integrity. Formulation B3 containing ERL and PVP in 70:30 ratio showed folding endurance value of 33± 3.92. All other formulations containing 500 mg (C1, C2 and C3) and 600 mg (D1, D2 and D3) total polymer weight exhibited considerably higher folding endurance (Table-2). When plasticizer content kept constant (20% w/w) and polymer ratio (ERL:PVP) varied, folding endurance found in the order of 50:50> 60:40> 70:30. The more the content of ERL, less is the folding endurance. Results indicated that the patches will not break and maintained their integrity with general skin folding when applied.

In vitro drug release: Drug release study was carried out on selected formulation, which contained ERL and PVP in 70:30 ratios. Formulation DR which contained 100 mg pure RN and rest of the formula same with C3 was also selected for release study to compare the release pattern of free drug with patches containing rutin complexes. The sorting of the patches for release study was based on the results of physicochemical evaluation of the patch. Formulation DR showed least drug release of 39.36 ± 2.68 % after 24 hours, which is thought to be primarily because of high hydrophobicity of rutin. In case of B3 complete drug release occurred at 12 hours (Table 5). It is hypothesized that 400 mg total polymer is unable to form matrix carrying 314 mg RN-P (equivalent to 100 mg rutin) to produce sustained release. Sustained drug release was observed for C3 (85.12 ± 2.84 %) and D3 (63.92 ± 3.98%) in the 24 hour release study. Further relatively low drug release in case of D3 suggests that 600 mg total polymer is a much higher quantity for 314 mg RN-P (Fig3). Therefore, 500 mg total polymer is found to be the optimum quantity of polymer for sustained drug release.

Effect of plasticizer on patch properties: Formulation E1, E2, E3 and E4 were prepared to state on plasticizer (PEG) role. After optimizing the total polymer required which was found to be 500 mg, plasticizer content was varied as 15%w/w, 25% w/w, 30%w/w, and 35% w/w in E1, E2, E3 and E4, respectively. Rest of the formulae was same with C3 except that it contains 20%w/w of PEG. Modi et al., [41] advocated that physicochemical properties of the patches have direct relation with plasticizer level. Percent moisture content of the patches were 1.42 ± 0.61% (C3), 1.26± 0.17% (E1), 1.38± 0.64% (E2), 1.44 ± 0.49% (E3), 1.53 ± 0.73%(E4) (Table 2). Here it can be stated that PEG do not play any major role in % moisture content of the transdermal patches fabricated with ERL and PVP and as discussed earlier PVP content is mainly responsible for moisture content of the patches. Water vapor transmission (WVT) rate of the patches increased proportionally with plasticizer level which may be due to increased permeability of the patches with increase in plasticizer from 15%-35% w/w. WVT rate in $gm/cm^2/day$ of the formulations noted as 0.0018 (E1), 0.0024(C3), 0.0029 (E2), 0.0036(E3) and 0.0075(E4) (Table 2). Folding endurance which is a measure of mechanical strength was affected by plasticizer content. The number of folds to break the patch increased with increase in plasticizer content and counted as 40 (E1), 49 (C3), 55 (E2), 58 (E3) and 61(E4) (Table 2). The percent cumulative drug release was also found to be affected by plasticizer content of the patches to a lesser extent. After 24 hours drug release was calculated as 82.33 ± 2.92% (E1), 85.12 ± 2.84 % (C3), 87.66 ± 3.88 (E2), 90.34 ± 3.06 (E3) and 94.29 ± 3.14 (E4). The increased drug release-time profile of the patches with increase in plasticizer from 15-35%w/w of total polymer may be due to the possibility that PEG increases the aqueous penetration into the patches and relaxes the polymer chains more effectively thus increasing the rate of diffusion through the polymeric matrix (Fig4). From the physicochemical and drug release study it was confirmed that PEG when used in 20%w/w of total polymer weight (ERL and PVP in 70:30 ratio) exhibits most satisfactory patch properties. These studies suggest that plasticizer has important role in some of the patch properties fabricated from ERL and PVP.

Ex vivo skin permeation study: Skin permeation study was carried out on pure drug and patch formulation DR, C3, D3 and E4. Skin permeation study with pure drug showed that at the end of 24 hours only 13 ± 1.12 % of RN was present in the receptor fluid (Fig5). Here it was confirmed that rutin is a less soluble and less permeable drug. Patch formulation DR containing free rutin also showed similar results where the % cumulative permeated was only 11.5 ± 1.84 %. There was not much difference in drug permeation between free rutin and formulation DR and the lower permeation from DR may be due to the less drug release from polymeric patch as was found in *in vitro* drug release study. Whereas a higher cumulative permeation of 19 ± 3.42, 31 ± 2.32 and 34.5 ± 2.74 % was found for D3, C3and E4, respectively, where RN-P (1:1) was incorporated in the patch. The results show higher permeability of RN-Ps, where phosphatidylcholine played a major role in bringing the Rutin molecules via the lipophilic

stratum corneum to epidermal-dermal site and gradually passing the viable dermis which is hydrophilic in nature. Rutin as well as RN-P were lipophilic [31]; do not find easy passage through the viable dermis, which may be the reason of lower cumulative (%) permeation after 24 hours. This increases the possibility of accumulation of Rutin either in free form or as complex at the epidermal dermal site beneath the stratum corneum, as it was reported in earlier works that phyto-phospholipid complexes enhance the passage through the outer lipophilic horney layers [42, 43]. The skin extract obtained from the skin used for permeation study of free Rutin and formulation DR revealed that they contain 17±1.06 % and 15 ± 0.8 % Rutin, respectively. Whereas the skin extract obtained from permeation study using C3, D3 and E4 showed 46 ± 1.33%, 37 ± 2.42% and 49± 2.69% Rutin content, respectively. The results suggest that Rutin phytosomes® better able to penetrate the highly impermeable stratum corneum than free Rutin. Retention of this higher quantity of Rutin will be available for slow passage through the viable dermis and prolonged anti-inflammatory effect at superficial as well as deep skin and adjacent muscular tissues and bone interlocks for getting relief in arthritis, rheumatism, athletic aches, etc.

The relatively less skin permeation (19 ± 3.42 %) and skin retention (37 ± 2.42%) of D3 can be related to its slow release from polymeric patch (63.92 ± 3.98% at 24 hour) (Fig-3). E4 showed highest skin permeation and skin retention which may be due to higher PEG content as reflected in the *invitro* drug release study (94.29 ± 3.14). But some of the patch properties were compromised by E4 viz. WVT rate and moisture uptake (Table-2). Patch C3 exhibited most satisfactory physicochemical attributes, drug release, skin permeation and skin retention.

Scanning electron microscopy: RN-Ps appeared as clusters of imperfect spheres in the SEM photographs taken after dispersing in isopropyl alcohol (Fig 6-A). These structures were intact in the polymeric patch as can be seen in the photographs (Fig 6-B, C) where numerous similar RN-P particles are evenly distributed mostly beneath the patch surface. Also it was read that the patch surface is homogenous without any visible cracks and depressions.

Transmission electron microscopy: The vesicular structures of phytosomes can be seen in the TEM photographs (Fig 7-A), which resulted from the physical shields of the two long aliphatic chains around the active polyphenolic principle [44].The spherical disc like structures of RN-Ps are very evenly dispersed in the polymeric patch (Fig 7-B, C, D).

FE-SEM images of the patch after in vitro release and ex vivo skin permeation study: Images obtained shows the structural integrity of R-NPs after the 24 hours *invitro* drug release (Fig 8-A, B and C) and *exvivo* skin permeation (Fig 8-D and E) experiments. Also the polymeric patch provides sufficient mechanical strength for sustaining the release of RN-Ps from the matrix. Multiple depression spots and pores on the patch surface are the places from where the RN-P particles have depleted. Clusters of RN-Ps and larger particles mostly remain bound to the polymer network (Fig 8- B) after long time exposure to release media and skin. Smaller groups and single spherical particles find easy to get released by formation of permanent pores in the patch because of the thermodynamic activity of the RN-P particles. Polymer erosion in very few regions can be seen in the patches applied to rat skin (Fig 8- D and E), but not in the patches used in drug release study.

In vivo* anti-*inflammatory study by paw edema model: RN-P containing patch C3 was selected based on satisfactory results of *in vitro* drug release and *exvivo* skin permeation study. Injection of carrageenan resulted in a significant increase in inflammation in non-treated control rats (Group I) (swelling 110 ± 5.42 % at 10 hour and 38.4 ± 3.48 % at 24 hours) (Table 3). In the rats of group II (standard) highest inflammation of 59.56 ± 4.72 % was observed after 10 hours which was reduced to 23.2 ± 4.58 % after 24 hours. Rats of the group III (test) showed 3.3 ± 2.18 % swelling after 24 hours which is significantly lower than control and standard groups ($P<0.05$) (Figure 9). The standard drug application (Group II) produced 52.0 % inhibition of inflammation at 2 hours and 39.58 % after 24 hours (Table 3). The results suggest that the drug action is faster with early lag time (less than 2 hour) but the conventional diclofenac sodium gel failed to give long lasting inhibition. In the test group RN-P containing patch started to act slowly which is because of slow drug release from the polymer matrix (50.8 % inhibition at 4 hour). Also the test formulation was able to give significant inhibition ($P < 0.05$), at 24 hour (91.4 %) compared to standard (39.58 %). From the study it was concluded that RN-Ps being lipophilic get deposited in the epidermal-dermal site from where drug was slowly released to give sustained anti-inflammatory effect.

Skin irritation study: The results obtained in skin irritancy study revealed that the plain patch and RN-P containing patch showed a skin irritation score (erythema and edema) of less than 1 (Table

4). From the Draize method of scoring, the control animals showed moderate to severe erythema and slight edema, whereas the test animals showed only very slight erythema and edema in some animal. Compounds producing scores of 2 or less are considered non-irritant [45]. Hence the RN-P containing polymeric patch is non-irritable to skin and safe for therapeutic use.

Histological observations: The control group exhibited an intact morphology throughout the full-thickness skin (epidermis 28 ± 5.34 µm, keratin layer 5.80 ± 2.41 µm). SC layer was intact and lied adjacent to the topmost layer of the epidermis (Fig-10). Lin, et al., [30], reported the presence of lymphocytes and macrophages in the dermis of Rutin treated rat skin. Such inflammatory cells in the present study not observed. The complexation of rutin with phosphatidylcholine removed any local irritation and inflammation of rutin molecules as PC is also present in all cells. After exposure to the RN-P containing patch skin appendages were found in normal status, the SC integrity did not significantly differ from the control.

CONCLUSION

Rutin due to its poor oral bioavailability necessitates the development of novel carrier such as Phytosomes® for efficient drug delivery. Rutin when presented as phospholipid complex (RN-Ps) (Phytosomes®) are better soluble and permeable than free rutin. Drug loaded polymeric patch are convenient means of sustaining drug application. The physicochemical, *in vitro* release and skin permeation study of patches containing Rutin-Phytosomes® revealed that drug permeates the keratinized horney layer and remain localized for a longer period of time, thus enabling drug targeting to the dermis and feasibility of using rutin for sustained effect in both acute and chronic inflammatory conditions. Phospholipid-complexes of rutin formed by weak bonding and enwrapping the rutin molecules by phosphatidylcholine, the structures such obtained are stable at physiologic thermodynamic environment of drug release and skin permeation, as evident from the FE-SEM images. Results of the skin irritation test on rabbit and histological studies revealed that the RN-Ps containing polymeric patches are safe for skin application. The present work is a successful attempt and the prepared RN-Ps containing polymeric patch can be used for obtaining relief in acute and chronic inflammation like arthritis.

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Table-1: Formulation of polymeric patch

Formulation & Polymer ratio	**Ingredients (mg)**						
	Rutin	**Rutin Phytosome (1:1)**	**Eudragit RL 100**	**PVP K30**	**PEG 400 (% w/w)**	**Isopropyl alcohol**	**Total Polymer weight**
A1 (50:50)	--	214	150	150	20	4 ml	300
A2 (60:40)	--	214	180	120	20	4 ml	
A3 (70:30)	--	214	210	90	20	4 ml	
B1(50:50)	--	214	200	200	20	4 ml	400
B2(60:40)	--	214	240	160	20	4 ml	
B3(70:30)	--	214	280	120	20	4 ml	
C1(50:50)	--	214	250	250	20	4 ml	500
C2(60:40)	--	214	300	200	20	4 ml	
C3(70:30)	--	214	350	150	20	4 ml	
D1(50:50)	--	214	300	300	20	4 ml	600
D2(60:40)	--	214	360	240	20	4 ml	
D3(70:30)	--	214	420	180	20	4 ml	
E1(70:30)	--	214	350	150	15	4 ml	500
E2(70:30)	--	214	350	150	25	4 ml	
E3(70:30)	--	214	350	150	30	4 ml	
E4(70:30)	--	214	350	150	35	4 ml	
DR (70:30)	100	--	350	150	20	4 ml	

Table-2: Results of Moisture Content, Water Vapor Transmission, Drug Content and Folding Endurance studies. All data presented are average ± SD, n=3.

Formulation	% Moisture content	Water vapor transmission ($gm/cm^2/day$)	Drug content (%)	Folding endurance
A1	2.64 ± 0.58	0.0236 ± 2.11	94.50	22 ± 4.55
A2	2.09 ± 0.41	0.0211 ± 2.08	93.44	25 ± 4.03
A3	1.23 ± 0.33	0.0176 ± 2.09	96.87	23 ± 4.18
B1	3.32 ± 0.26	0.0105 ± 2.31	97.25	23 ± 4.51
B2	2.18 ± 0.17	0.0086 ± 1.33	98.18	23 ± 3.67
B3	1.65 ± 0.37	0.0053 ± 1.44	98.46	33 ± 3.92
C1	3.44 ± 0.44	0.0058 ± 2.0	98.74	54 ± 4.72
C2	3.03 ± 0.34	0.0059 ± 1.45	99.14	51 ± 3.69
C3	1.42 ± 0.61	0.0024 ± 1.18	99.06	49 ± 2.22
D1	4.54 ± 0.27	0.0043 ± 1.75	97.82	41 ± 4.63
D2	3.36 ± 0.38	0.0037 ± 1.08	98.78	40 ± 3.44
D3	2.73 ± 0.21	0.0011 ± 1.21	98.50	36 ± 2.07
E1	1.26 ± 0.17	0.0018 ± 1.85	98.90	40 ± 1.85
E2	1.38 ± 0.64	0.0029 ± 1.44	99.30	55 ± 1.57
E3	1.44 ± 0.49	0.0036 ± 1.08	99.23	58 ± 2.33
E4	1.53 ± 0.73	0.0075 ± 1.42	98.17	61 ± 1.65
DR	1.22 ± 0.51	0.0031 ± 1.33	99.60	51 ± 1.74

Table-3: Paw edema data of rats obtained by carrageenan injection and after treatment

Groups	**% Swelling (edema volume with respect to initial volume) ± SD, n=6**											
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr	8 hr	9 hr	10 hr	12 hr	24 hr
Control	39.2 ± 2.17	43.0 ± 0.78	49.2 ± 4.35	53.1 ± 5.11	61.6 ± 5.24	69.1 ± 3.86	78.0 ± 5.08	88.4 ± 4.22	101.0 ± 2.44	110.0 ± 5.42	92.0 ± 4.66	38.4 ± 3.48
Standard	28.1 ± 1.66	20.6 ± 1.82	24.5 ± 1.56	35.8 ± 3.39	37.0 ± 3.66	41.7 ± 4.74	44.4 ± 4.38	47.0 ± 5.39	58.66 ± 4.45	59.56 ± 4.72	53.0 ± 3.84	23.2 ± 4.58
Test	39.0 ± 1.86	37.5 ± 0.92	30.4 ± 3.76	26.1 ± 3.64	27.2 ± 1.22	28.0 ± 1.28	28.5 ± 3.50	29.2 ± 2.22	30.0 ± 3.05	27.6 ± 4.83	18.4 ± 3.98	3.3 ± 2.18
% Inhibition of inflammation												
Standard	28.3	52.0	50.2	32.5	32.3	39.6	43.0	46.8	41.9	45.8	42.39	39.58
Test	0.51	10.7	38.2	50.8	55.8	59.4	63.4	66.9	70.2	74.9	80	91.40

Table-4: Score for skin irritation study on rabbit calculated after 7 days

Rabbit No	Plain Patch (Group I)		RN-P containing patch (C3) (Group II)		Formalin (0.8% v/v) (Group III)	
	Erythema	Edema	Erythema	Edema	Erythema	Edema
1	0	1	0	0	2	1
2	0	0	2	0	2	2
3	1	0	1	1	3	1
4	1	1	0	0	1	1
Score ± SD	0.5 ± 0.57	0.5± 0.57	0.75 ± 0.95	0.25 ± 0.5	2 ± 0.85	1.25 ± 0.5

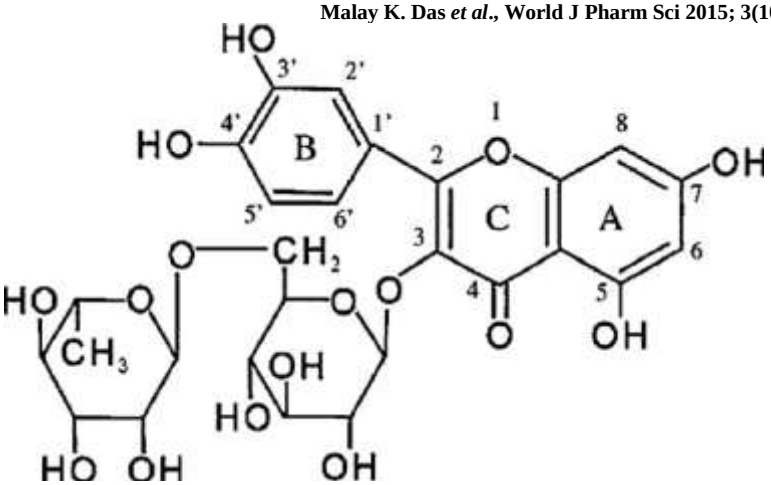


Fig-1: Structure of Rutin

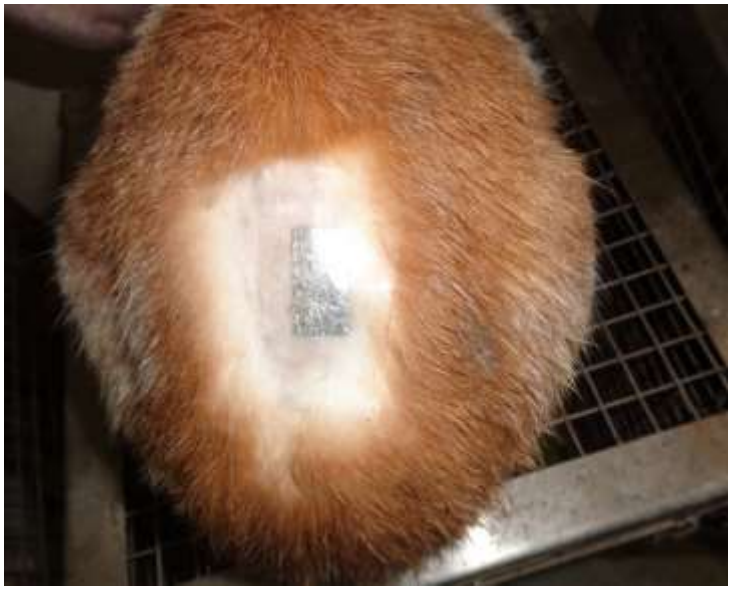


Fig-2: Patch application on back side of rabbit after shaving and cleaning the skin

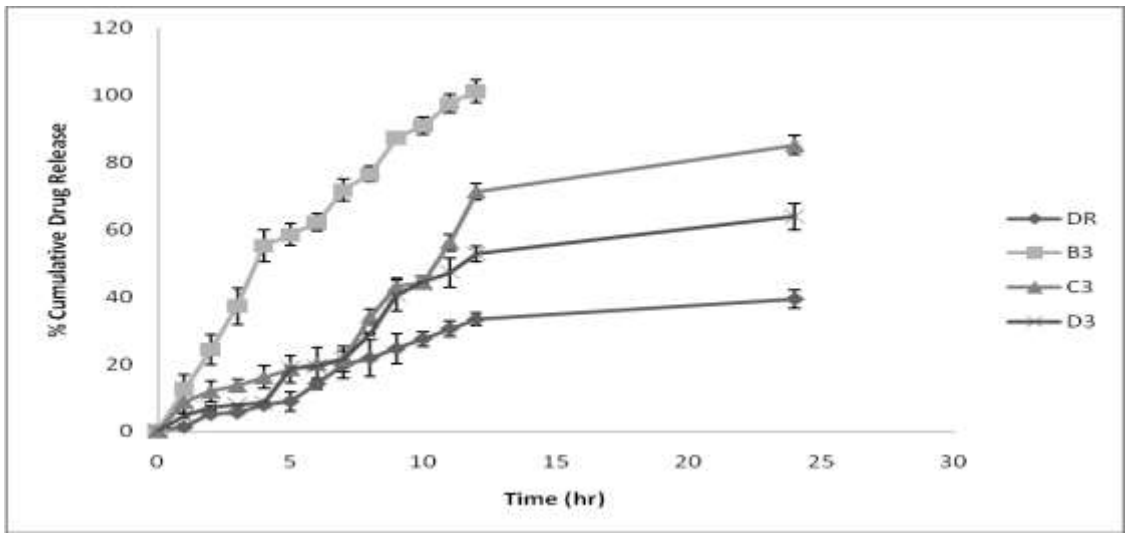


Figure-3: *In vitro* drug release profile of RN from polymeric patch (Result ± SD, n =3).

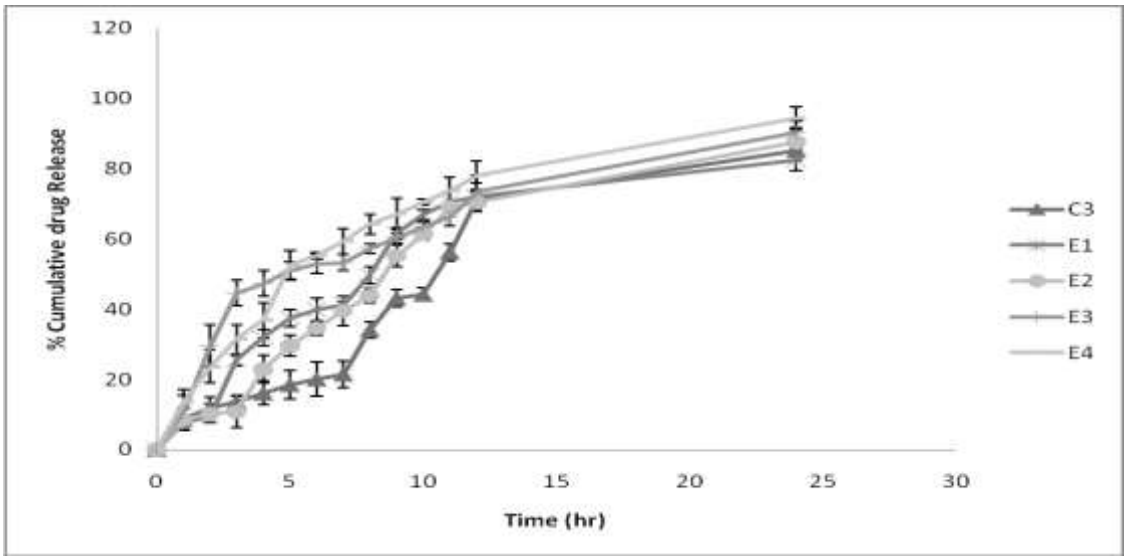


Figure-4: Effect of plasticizer on drug release (result ± SD, n =3)

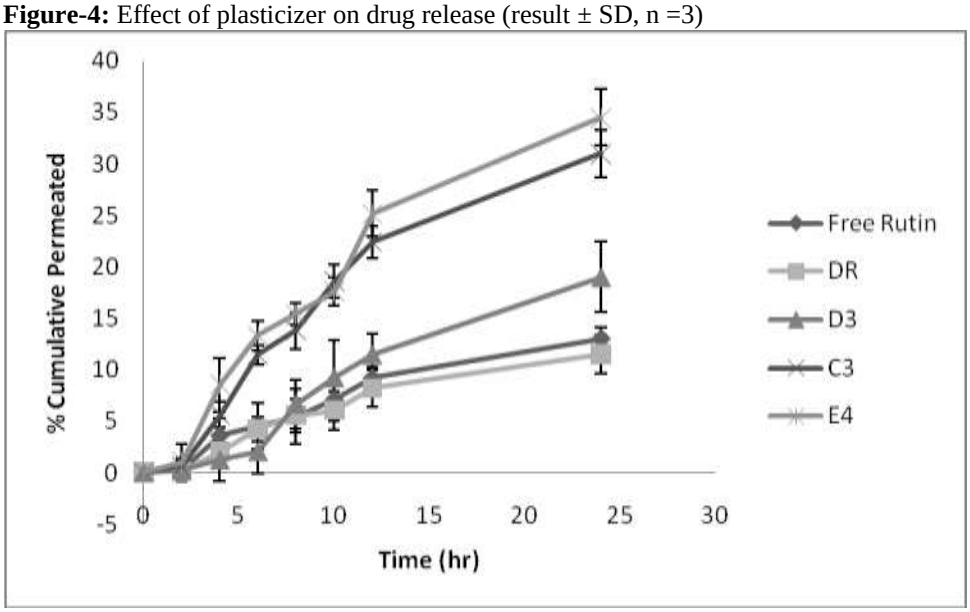


Fig-5: Results of *ex vivo* skin permeation study using rat abdominal skin (Results ± SD, n=3).

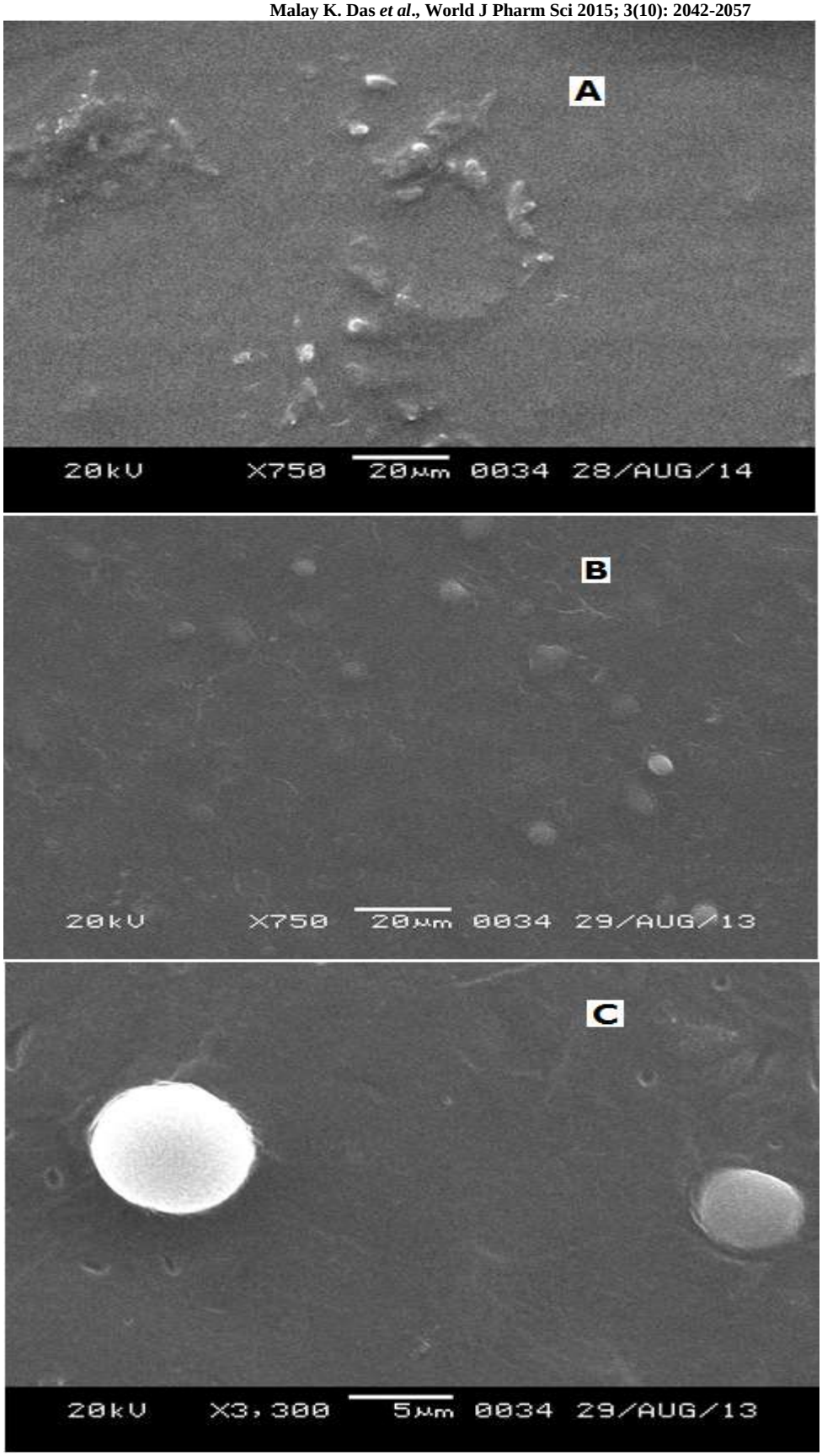


Fig 6: SEM photographs of Rutin-phytosomes (A), patch surface containing rutin-phytosomes (B, C).

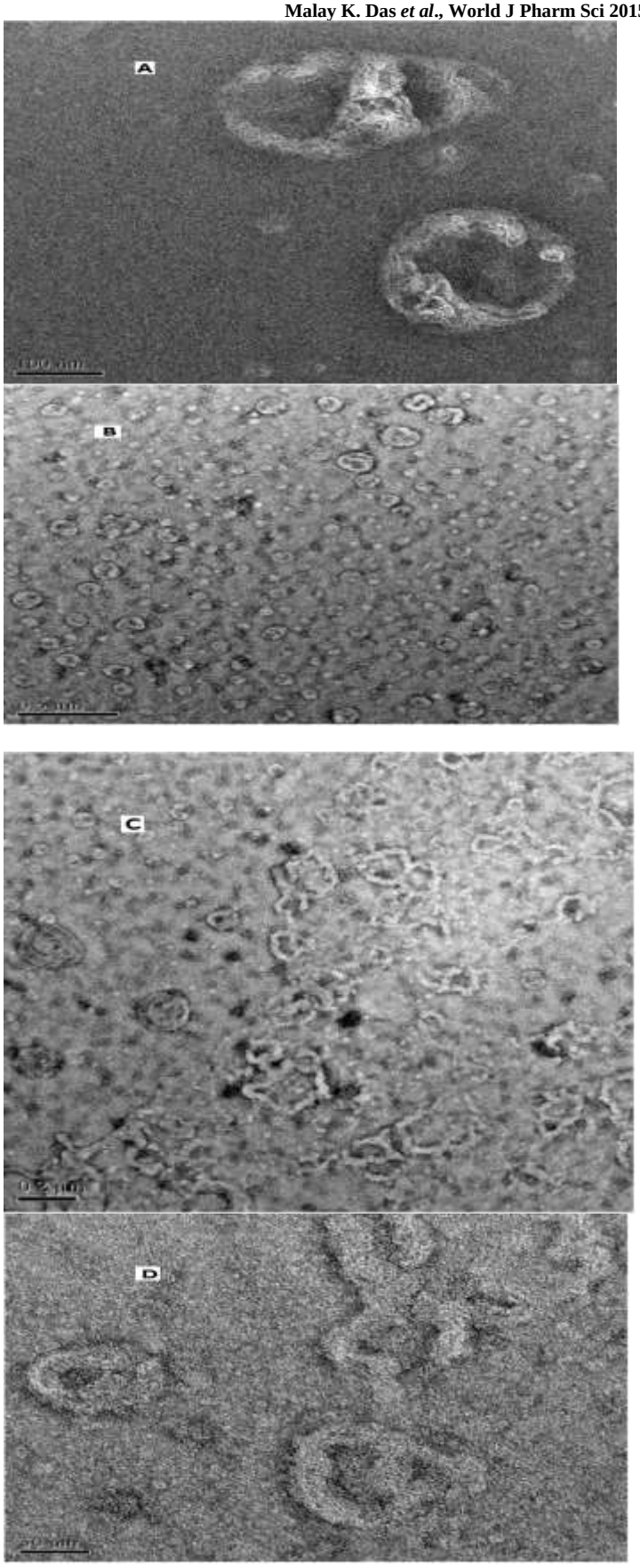
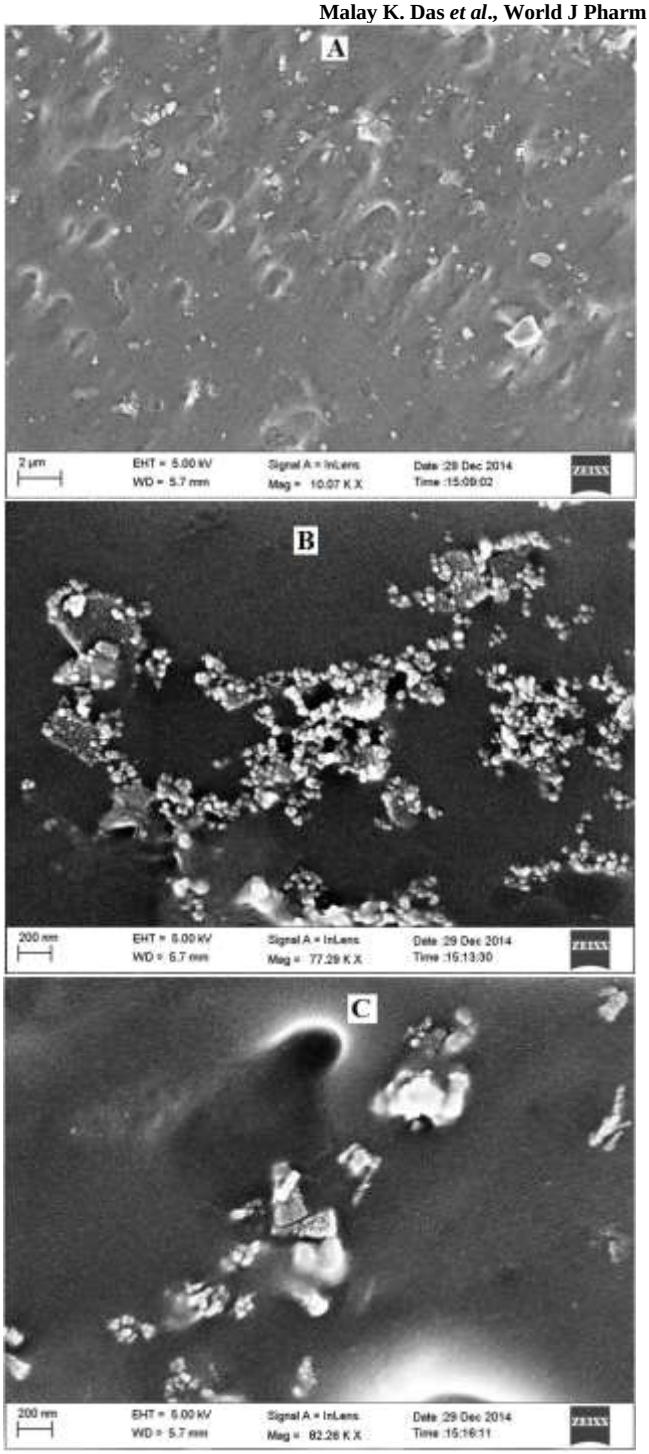


Fig-7: TEM photograph of RN-Ps (A), RN-Ps containing polymeric patch C3 (Fig B, C and D).


A
2 µm
EHT = 5.00 kV
WD = 5.7 mm
Signal A = InLens
Mag = 10.07 K X
Date :29 Dec 2014
Time :15:09:02
B
200 nm
EHT = 5.00 kV
WD = 5.7 mm
Signal A = InLens
Mag = 77.29 K X
Date :29 Dec 2014
Time :15:13:30
C
200 nm
EHT = 5.00 kV
WD = 5.7 mm
Signal A = InLens
Mag = 82.26 K X
Date :29 Dec 2014
Time :15:16:11

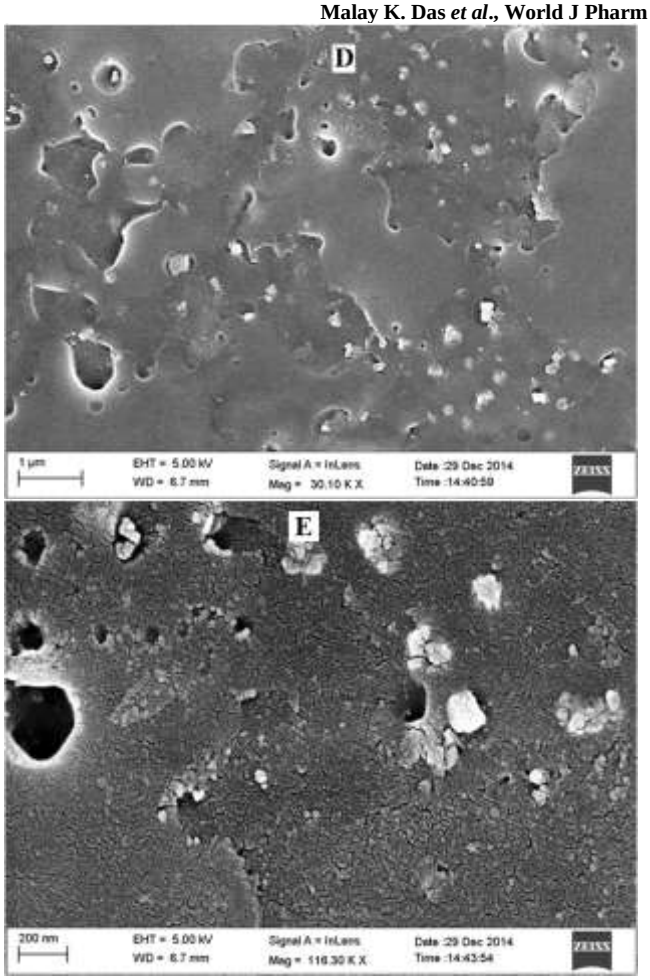


Fig 8: FE-SEM images of RN-P containing patch taken after *in-vitro* drug release study (A, B and C), and FE-SEM images of RN-P containing patch taken after *ex vivo* skin permeation study (D and E).

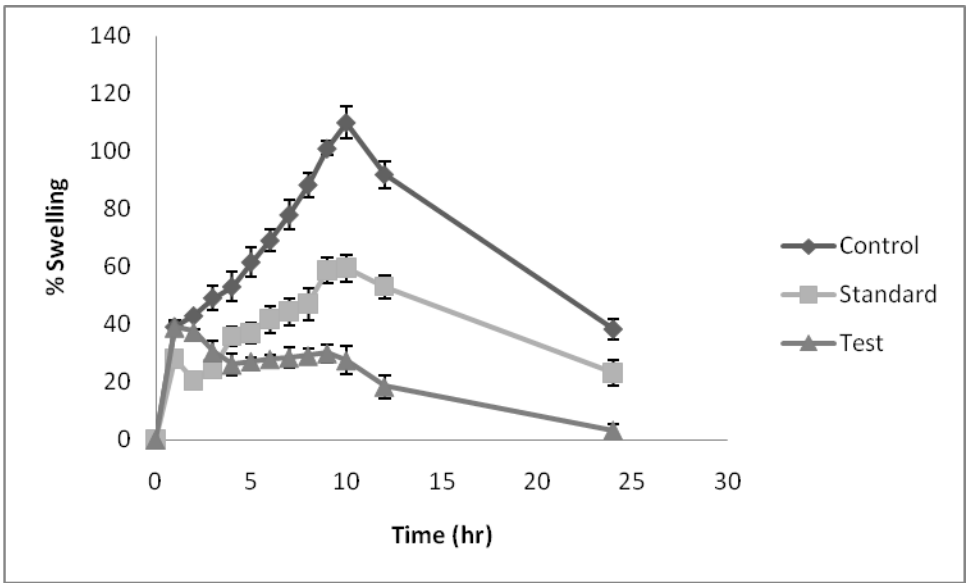


Figure-9: Paw swelling after carrageenan injection (control) and drug treatment (standard and test). Data points are average of 6 observations ± SD.

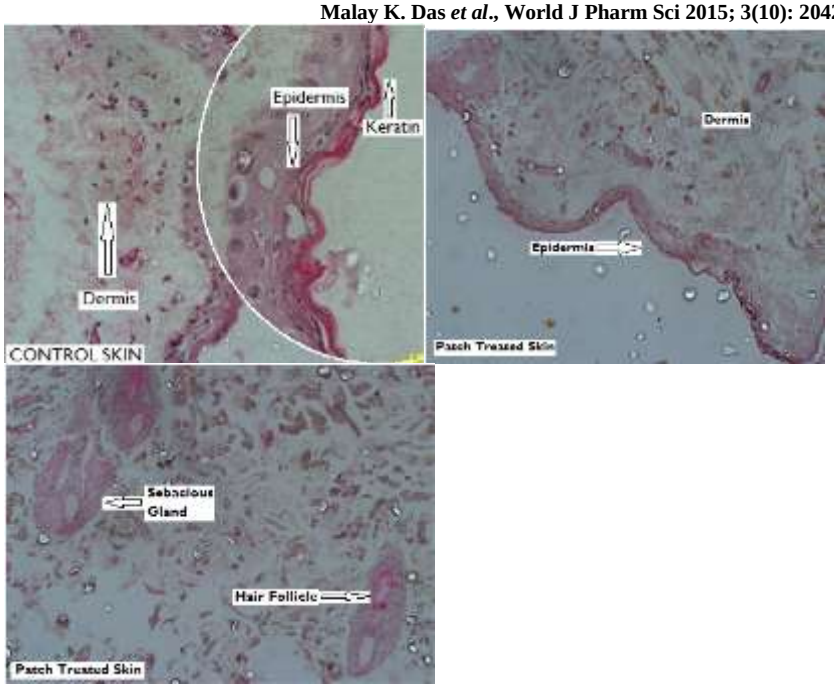


Fig-10: Histological photomicrograph of the control and patch treated rat skin

REFERENCES

1. Chan FK. Primer: managing NSAID-induced ulcer complications–balancing gastrointestinal and cardiovascular risks. Nat Clin Pract Gastroenterol Hepatol 2006; 3: 563–73.
2. Carrasco-Pozo C et al. Differential protective effects of quercetin, resveratrol, rutin and epigallocatechin gallate against mitochondrial dysfunction induced by indomethacin in Caco-2 cells. Chemico-Biological Interactions 2012; 195: 199–205.
3. Materska M. Quercetin and its derivatives: Chemical structure and bioactivity-A review. Pol J Food Nutr Sci 2008; 58(4): 407-13.
4. Ushida Y et al. Endothelium-dependent vasorelaxation effect of rutin-free tartarybuckwheat extract in isolated rat thoracic aorta. J Nutr Biochem 2008; 19: 700–707.
5. Selloum L et al. Anti-inflammatory effect of rutin on rat paw oedema, and on neutrophils chemotaxis and degranulation. Exp Toxicol Pathol 2003; 54(4): 313-18.
6. Obied HK et al. Bioactivity and analysis of biophenols recovered from olive mill waste. J Agric Food Chem 2005; 53: 823-37.
7. Wonhwa L et al. Barrier protective effects of rutin in LPS-induced inflammation in vitro and in vivo. Food and Chemical Toxicology 2012, 50: 3048–55.
8. Zeilhofer H, Brune K. Analgesic strategies beyond the inhibition of cyclooxygenases. Trends Paharmacol Sci 2006; 27(9): 467-74.
9. Karki R et al. Extract of buckwheat sprouts scavenges oxidation and inhibits pro-inflammatory mediators in lipopolysaccharide-stimulated macrophages (RAW264.7). J Integr Med 2013; 11(4): 246-52.
10. Bouriche H et al. Effect of *Cleome arabica* leaf extract, rutin and quercetin on soybean lipoxygenase activity and on generation of inflammatory eicosanoids by human neutrophils. Prostaglandins Leukot Essent Fatty Acids 2005; 72: 195-201.
11. Appleton J. Evaluating the Bioavailability of Isoquercetin. Natural Medicine Journal 2010; 2(1): 1-6**.**
12. Rosane WI et al. Development of a biosensor based on gilo peroxidase immobilized on chitosan chemically cross linked with epichlorohydrin for determination of rutin. J Pharm Biomed Anal 2006; 41: 366–72.
13. Mahmoud AM. Influence of rutin on biochemical alterations in hyperammonemia in rats. Exp Toxicol Pathol 2012; 64: 783–89.
14. The United States Pharmacopeial Convention. USP Material Safety Data Sheet (MSDS). http://www.cymitquimica.com/uploads/products/45/pdf/1606503-msds.pdf (Accessed on June 24, **2013**).
15. Khalifa TI, Muhtadi FJ, Hassan MMA. Rutin. In: *Analytical Profiles of Drug Substances*, FLOREY K, ed; Academic Press, New York, **1983**; pp. 623-75.
16. Zhong L et al. Rutin in different media: Determination of equilibrium solubility. http://anpaper.com/medical/rutin-in-different-media-determination-of-equilibrium-solubility.html (Accessed on June 23, 2013).
17. Miyake K et al. Improvement of solubility and oral bioavailability of rutin by complexation with 2-hydroxypropyl -beta- cyclodextrin. Pharm Dev Technol 2000; 5(3): 399-407.
18. Pedriali CA et al. The synthesis of a water-soluble derivative of rutin as an antiradical agent. Quím Nova 2008; 31(8): 2147-51.

19. Lukac M et al. Solubilisation of griseofulvin and rutin in aqueous micellar solutions of gemini and heterogemini surfactants and their mixtures. Eur J Pharm Sci 2011; 44: 194–99.
20. Kerem Z et al. Regev-Shoshani G. Protection of lipids from oxidation by epicatechin, trans-resveratrol, and gallic and caffeic acids in intestinal model systems, J Agric Food Chem 2006; 54:10288–93.
21. Morand C et al. Respective bioavailability of quercetinaglycone and its glycosides in a rat model. Biofactors 2008; 12(1-4): 169-74.
22. Yang C-Y et al. Bioavailability and Metabolic Pharmacokinetics of Rutin and Quercetin in Rats. Journal of Food and Drug Analysis 2005; 13(3): 244-50.
23. Kim MR et al. Antioxidative effects of quercetin-glycosides isolated from the flower buds of *Tussilagofarfara* L. Food Chem Toxicol 2006; 44: 1299–1307.
24. Hollman PCH, Katan MB. Absorption, metabolism and health effects of dietary flavonoids in man. Biomed. & Pharmacother 1997; 51: 305-10.
25. Cermak R et al. The bioavailability of quercetin in pigs depends on the glycoside moiety and on dietary factors. J Nutr 2003; 133: 2802–07.
26. Reinboth M et al. Oral bioavailability of quercetin from different quercetin glycosides in dogs. Br J Nutr 2010; 104:198–203.
27. Banjare L, Ghillare N. Development of biocompatible nanoparticles for sustained topical delivery of Rutin. Int J Pharm Biol Arch 2012; 3(2): 326-32.
28. Dhiman A, Singh D. Development, characterization & *in vitro* skin permeation of rutin ethosomes as a novel vesicular carrier. International Journal of Biomedical Research 2013; 4(10): 559-66.
29. Park SN et al. Preparation of quercetin and rutin-loaded ceramide liposomes and drug-releasing effect in liposome-in-hydrogel complex system. Biochem Biophys Res Commun 2013; 435(3): 361–66.
30. Lin CF et al. Anti-inflammatory activity and percutaneous absorption of quercetin and its polymethoxylated compound and glycosides: The relationships to chemical structures. Eur J Pharm Sci 2012; 47(5): 857-64.
31. Das MK, Kalita B. Design and evaluation of phyto-phospholipid complexes (phytosomes) of Rutin for transdermal application. J App Pharm Sci 2014; 4(10): 51-7.
32. Ramesh G et al. Development of Nitrendipine Transdermal Patches: In vitro and Ex vivo Characterization. Current Drug Delivery 2007; 4: 69-76.
33. Patel HJ et al. Design and evaluation of amlodipine besilate transdermal patches containing film former. Int J Pharm Res Dev 2009; 7: 1-12.
34. Pathan IB, Setty CM. Comparison of the effect of Essential Oils on the permeation of Diclofenac Diethylamine through various barriers. Acta Pharmaceutica Sciencia 2008; 50: 219-28.
35. Sankar V et al. Design and evaluation of nifedipine transdermal patches. Ind J Pharm Sci 2003; 65: 510-15.
36. Winter CA et al. Carrageenan induced oedema in the hind paw of rat as an assay for anti-inflammatory activity. Proc Soc Exp Biol Med 1962; 111: 544–47.
37. Özgüney IS et al. Transdermal Delivery of Diclofenac Sodium Through Rat Skin From VariousFormulations. AAPS PharmSciTech 2006; 7(4): 39-45.
38. Draize JH et al. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J Pharmacol Exp Ther 1944; 82: 377-79.
39. Kong M et al. Investigations on skin permeation of hyaluronic acid based nanoemulsion as transdermal carrier. Carbohydrate Polymers 2011; 86: 837-43.
40. Rajabalaya R et al. Studies on the Effect of Plasticizer on *in vitro* Release and *Ex Vivo* Permeation From Eudragit E 100 Based Chlorpheniramine Maleate Matrix Type Transdermal Delivery System. J Excipients and Food Chem 2010; 1(2): 3-12.
41. Modi C. Effect of components (polymer, plasticizer and solvent) as a variable in fabrication of diclofenac transdermal patch**.** J Pharm Bioallied Sci 2012; 4(1):S57–S59.
42. Semalty A, Semalty M, Rawat MS. Development and characterization of novel herbal drug delivery system (Phytosomes) of xanthones isolated from *Swertia alternifolia* for improving bioavailability, In: *Science and Technology in Uttarakhand*, Dobhal R, Kotlia BS, eds; Macmilan, New Delhi , **2008**; pp. 247–53.
43. Chen Y et al. Preparation of curcumin-loaded liposomes and evaluation of their skin permeation and pharmacodynamics. Molecules 2012; 17: 5972-87.
44. Bombardelli E, Mustich G. Bilobalide phospholipid complex, their uses and formulation containing them. US patent EPO-275005, **1991.**
45. Panchaxari DM et al. Design and characterization of diclofenac diethylamine transdermal patch using silicone and acrylic adhesives combination. DARU J Pharm Sci 2013; 21(6): 2-14.